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POST COVID-19 COMPLICATIONS

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Introduction

Post-COVID-19 complications have emerged as a silent killer. Those who recovered from the disease are now grappling with complications and unable to get treatment due to the absence of any dedicated post-COVID-19 care centre. Several people, including those who stayed in home isolation / quarantine, reportedly developed critical complications. The virus may remain in the body up to 3 months after diagnosis. This may mean some people get a second positive test result even after their recovery, although this does not indicate the virus is still transmissible. Fibrosis of lungs, cardiac arrest, renal failure, neurological problems and diabetes are among the most common complications observed.

Organs affected by COVID-19

Organs are affecting most of the body systems including heart, lung, kidney, skin, and brain functions. Multiorgan effects can also include conditions that occur after COVID-19, like multisystem inflammatory syndrome (MIS) and autoimmune conditions.

Lung or Respiratory complications

Most of the COVID-19 patients recover completely except some minor complications such as cough and shortness of breath. However, a certain proportion of patients have excessive lung damage, and some of them develop pulmonary



fibrosis. Patients may suffer with post viral bronchial hyper responsiveness symptoms of which resemble asthma.

Cardiac complications

Post covid cardiac complications like acute coronary syndrome (ACS), Acute MI (stroke), dysrhythmias, persistent hypotension, infective myocarditis have been reported. Heart damage or cardiac muscle injury complication is seen after several days of recovery. The infection from COVID-19 can lead to inflammation in various parts of the body resulting in the weakening of the heart muscle, abnormal heart rhythm and may cause blood clots in blood vessels. Complications like Myocarditis or an inflammation of the heart, leads to problem in blood pumping , causing the narrowing of the arteries, triggering high BP, and thus leaving one susceptibility to a heart attack.

Brain disease

Acute Necrotizing Encephalopathy (ANE) is an immune mediated disease which is usually seen after infection with mycoplasma, influenza A and herpes simplex virus. However, it was recently reported in post covid patients. Corona virus binds to ACE receptors which are abundant in glial cells and arterial smooth cells in the brain, that is why neurological manifestations are seen in covid 19 and also post covid complications. COVID 19 with Cytokine storm leads to immune mediated damage with predilection to Central nervous system, with focal seizure, hemiparesis, irritability, fluctuating consciousness. Irritability and intolerance with feeds, shortness of breath, hypotension. As both are immune mediated and are due to post covid complications they tend to be similar.

Mucormycosis

Mucormycosis is a fungal infection that is seen in an immune compromised host. It presents itself as a respiratory or a skin infection and is mainly caused due to exposure to Mucoromycetes molds, by breathing in the affected mold spores in air also referred to as a sinus (pulmonary) exposure.

When the infection spreads to the brain through

- Nasal route it is called as Rhino Cerebral Mucormycosis
- Lungs it is called as Pulmonary Mucormycosis
- Skin it is called Cutaneous Mucormycosis
- Bloodstream it is called Disseminated Mucormycosis





Mucormycosis

The predisposing factors for Mucormycosis can be uncontrolled diabetes mellitus, immune suppression by steroids, prolonged ICU stay. For a patient affected with Mucormycosis it is advised to control Hyperglycemia, monitor Blood glucose levels, post COVID-19 discharge and also in diabetics which uses steroids. By using clean and sterile water for humidifiers during oxygen therapy, and antibiotics/ antifungals must be taken regularly. It is always better to pay attention to the warning signs and symptoms. Never consider all the cases with blocked nose as cases of bacterial sinusitis, particularly in the context of immune suppression/COVID-19 patients on immunomodulators. Do not lose crucial time to initiate treatment for Mucormycosis, if untreated, can cause death.

Symptoms

- Fatigue
- Difficulty breathing
- Joint pain
- Chest pain
- Brain fog, including an inability to concentrate and impaired memory
- Loss of taste or smell
- Sleep issues
- Mood changes
- Changes in period cycles

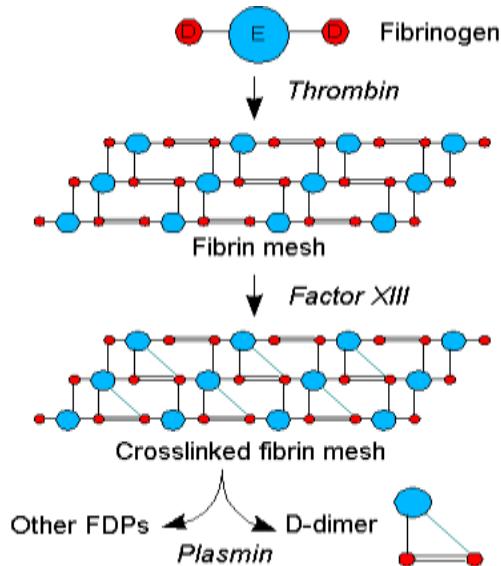
Diagnosis

D-dimer Test

D-dimer test is a blood test that can be used to help rule out the presence of a serious blood clot. D-dimer is a Fibrin Degraded Protein (FDP), a small protein fragment present in the blood after a blood clot is degraded by Fibrinolysis. It is so



named because it contains 2D fragments of the fibrin protein joined by a cross linking molecule. D-dimer levels are used as a predictive biomarkers for the blood disorder, disseminated intravascular coagulation and in the coagulation disorders associated with COVID 19 infection. A four-fold increase in the protein is an indicator of poor prognosis in people hospitalized with COVID19.



It has Fibrinogen with its one *E* domain and two *D* domains, acted upon in cascade, by the enzymes

- Thrombin - To create a *mesh of fibrin* protofibrils
- Factor13- To *crosslink the fibrin mesh* (linking protofibril D domains)
- Scaffold - For clot formation
- Plasmin - Whose action in fibrinolysis which produces fibrin degradation products (*FDPs*) the smallest of which are *D-dimers*, protein fragments with one *E* and two crosslinked with *D* domains from an original fibrinogen.

Units	Non-pregnant adult	First trimester	Second trimester	Third trimester
mg/L or µg/mL	<0.5	0.05 - 0.95	0.32-1.29	0.13-1.7
µg/L or ng/mL	<500	50 - 950	320-1920	130-1700
nmol/L	<2.7	0.3 - 5.2	1.8-7.1	0.7-9.3



Treatment

- **Remdesivir** - An antiviral which has been shown to shorten the recovery time needed in some hospitalized patients.
- **Dexamethasone** - A corticosteroid used to prevent or reduce inflammation in hospitalized patients with severe illness who need supplemental oxygen.
- **Tocilizumab** - A biological therapy used to reduce inflammation in hospitalized patients with severe illness requiring oxygen delivery through a high-flow device, invasive mechanical ventilation or ECMO, if used in addition to *dexamethasone*.

Precautions

- Vaccination
- Monitor your symptoms
- Stay home
- Keep safe distance
- Wear mask
- Sanitization



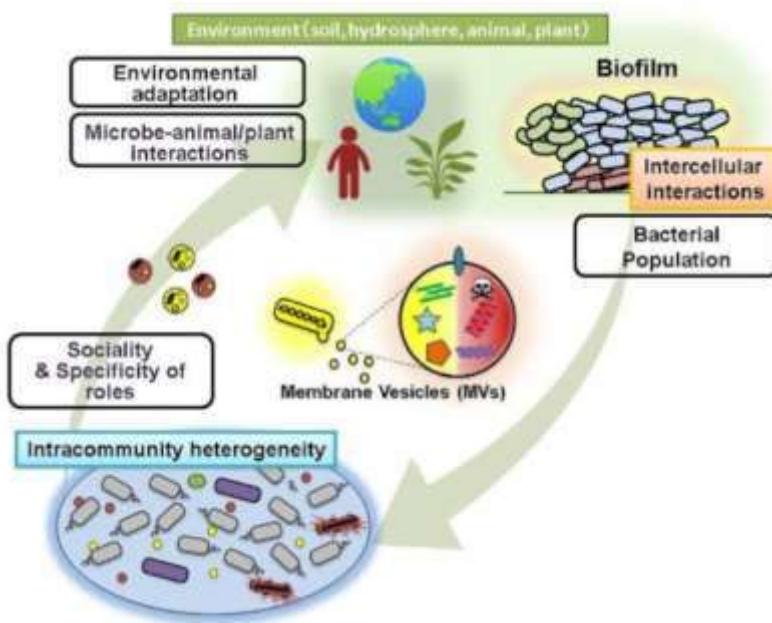
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BACTERIAL POPULATION AND THEIR INFECTION

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Bacterial population is very dangerous to humans and also the other living organisms. Because they can impair thyroid function. It can also affect thyroid in fresh water animals like fish, amphibians even altering gonad development in some animals. Normally a bacterial population is a group or a set of microorganisms. They are originated from discharge of untreated or poorly treated domestic or industrial waste. The main source of the bacterial population is intreated or poorly treated water. Sewer overflows are a major cause of bacterial population.



Some other key potential sources of faecal bacteria are the animal waste from agricultural operations, waterfowl and urban wildlife. Bacterial populations are occurred variety of areas like water, air and also soil.

Bacterial Pollution in Water

Many coliforms are enter into the streams or rivers via direct deposition of waste in the water and run off from areas with high concentration of animals. Runoff from Woodlands, specific tanks and sewage plants may also cause an increase in coliform and other bacterial population. Fecal Coliform are bacteria whose presence can indicate water contaminated by human and animal waste. These bacterial population makes water contamination and then thereby they cause short term health effects like cramps, nausea, diarrhoea, headaches and more they may also possess a greater risk for people with severely weak immune systems elderly, young children and infants

Bacterial Pollution in Air

Bacterial pollution is occurred not only in water nowadays they also occurred in air. Some Bacteria that can be identified in indoor air they are *Micrococcus* and *Corynebacterium*. The most common genera of bacteria found in indoor air they are *Streptococcus*, *Staphylococcus aureus* and *Clostridium*. Bacterial polluted air possesses a significant threat to human health like cardiovascular disease including allergies and obstructive lung disease.

Bacterial Pollution in Soil

Bacteria perform many important ecosystem services but also many bacteria in soil that can cause problems in people such strains of *Nocardia* which causes Nocardiosis an infection of lungs or whole-body legionella causes legionnaires disease and clostridium cause tetanus. Some bacteria can produce poisonous toxins which can be fatal if the spores of such bacteria are inhaled or ingested via wound. Such bacterial infections that can make people sick and some most bacteria won't hurt humans less than 1 %. Mostly antibiotics are usual treatment for the bacterial causing infections. Some microbial contaminants can be removed by water treatment coagulation and filtration process. Disinfection has proven effective against bacteria and other organisms.



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INSULIN

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Hormones

Hormones are chemicals that coordinate different functions in your body by carrying messages through your blood to your body. Human body secrets about 50 different types of hormones.

Pancreas

Pancreas plays an essential role in converting the food into energy for the body is cell. The pancreas has two main functions: An Exocrine function that helps in digestion and an Endocrine function that regulates blood sugar. The endocrine component of the Pancreas consists of islet cells (islets of Langerhans) that create and release important Hormones directly into the bloodstream. Two of the main pancreatic hormones are Insulin, which acts to lower blood sugar, and Glucagon which acts to raise blood sugar.

Islets of Langerhans

Islets of Langerhans was named by German physician Paul Langerhans discovered in 1869. The pancreatic islets constitute 1 – 2 % of the Pancreas volume and receive 10 – 15 % of its blood flow. The Pancreatic islets are arranged in density routes throughout the human pancreas, and are important in the metabolism of Glucose.



Types of Cells

- a) Alpha Cells producing glucagon
- b) Beta cells producing insulin and amylin
- c) Delta Cells producing somatostatin
- d) Epsilon cells producing ghrelin
- e) PP Cells (Gamma cells or F cells) producing pancreatic polypeptide

Insulin

Insulin discovered by Dr. Fredrick Banting and Charles Best in 1921 from dog pancreas they received Noble Prize in 1932. The word Insulin derived from Latin word Insula. Insulin is a polypeptide hormone. It is secreted by Beta cells in the Islets of Langerhans of Pancreas. It regulates the metabolic activity of carbohydrates, fats and protein. It is an Anabolic hormone. Insulin secretes about 45 – 50 unit daily by human pancreas. Biological half-life of insulin is 5 – 6 min. Normal value 20 - 30 units/ml. Insulin chemical Structure was given by sanger in 1956. Human hormone contains 51 amino acids arranged in 2 polypeptide chains. Chain A = 21AA, chain B = 30 AA.



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THE CYTOKINE STORM IN COVID-19

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Cytokine storm is generally used to maladaptive release of Cytokine in response to infection and other stimuli. Some evidence shows that, during the Coronavirus Disease 2019 (COVID-19) epidemic, severe deterioration in some patients has been closely associated with dysregulated and excessive cytokine release. It includes loss of regulatory control of proinflammatory cytokine production.

Coronaviruses are single-stranded, positive-strand RNA viruses belonging to the Coronaviridae family, Nidovirales order. It serves as messenger RNA (mRNA), allowing the translation of replicase/transcriptase and viral structural proteins. The replicase/transcriptase genes account at the 5'-end RNA sequence and are composed of two overlapping Open Reading Frames (ORFs): ORF1a and ORF1b. The ORFs encode 16 non-structural proteins. The remaining RNA sequence encodes four classical viral structural proteins, namely, spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein. Coronaviruses can infect a variety of host species, including birds, humans and some other vertebrates. These viruses mainly cause respiratory and intestinal infections and induce a variety of clinical manifestations. So, now the question is arrived what's the function cytokine storm in it? It has long been believed that cytokines play a key role during viral infection. A rapid and well-coordinated innate immune response is the first line of defence against viral infection. The production of IFN-I or IFN- α/β is the main natural immune defence response against viral infections, and IFN-I plays an antiviral role in the early stages of viral infection. The patients with mild to moderate symptoms have higher serum cytokine and chemokine levels. The rapidly increased



cytokines and chemokines attract many inflammatory cells, such as neutrophils and monocytes, resulting in excessive infiltration of the inflammatory cells into lung tissue and thus lung injury. Local excessive release of cytokines is the decisive factor that induces this pathological change and clinical manifestation. In COVID-19, the inflammatory cytokine storm is closely related to the development and progression of ARDS (Acute Respiratory Distress Syndrome). The serum levels of cytokines are significantly increased in patients with ARDS, and the degree of increase is positively correlated with mortality rate. Glucocorticoids are mainly used in critically ill patients suffering inflammatory cytokine storm in the early stage. A too early administration of glucocorticoids inhibits the initiation of the body's immune defence mechanism, thereby the viral load is increasing and ultimately leading to adverse consequences. Therefore, glucocorticoids are mainly used in critically ill patients suffering inflammatory cytokine storm. Beside this, IFN- λ may be an ideal treatment. IFN- λ firstly activates epithelial cells and reduces the mononuclear macrophage-mediated proinflammatory activity of IFN- $\alpha\beta$. In addition, IFN- λ inhibits the recruitment of neutrophils to the sites of inflammation. SARS-CoV and MERS-CoV mainly infect Alveolar Epithelial Cells (AEC). IFN- λ activates the antiviral genes in epithelial cells, thereby exerting antiviral effects without overstimulating the human immune system. At the time of cytokine storm, the three most important cytokines in the IL-1 family are IL-1 β , IL-18, and IL-33. From some studies, it gets to know that if we inhibit the IL-1 β , it can lead to reduce the cytokine storm. IL-6 antagonists can suppress the immune system function. Serum IL-6 level is significantly increased in severely ill patients with COVID-19. So, we can use the antagonists of IL-6. Except these, TNFs (Tumour Necrosis Factor) act as key inflammatory factors that trigger a cytokine storm. Neutralization of TNF activity or loss of TNF receptor provides protection against SARS-CoV-induced morbidity and mortality.

Chloroquine inhibits the production and release of TNF and IL-6, which indicates that chloroquine may suppress the cytokine storm in patients infected with COVID-19. Chloroquine together with phosphate has been used for treatment. Sphingosine-1-phosphate (S1P) is a signal lysophospholipid promotes cytokine synthesis and secretion. If we can inhibit the signal transduction of Sphingosine-1-Phosphate receptor in respiratory endothelial cells modulates pathogenic inflammatory responses, we can get a solution of the treatment also. Another one is stem cell therapy. Mesenchymal Stem Cells (MSC) not only have the potential of self-renewal and multidirectional differentiation, but also have strong anti-inflammatory and immune regulatory functions. MSC can inhibit the abnormal activation of T lymphocytes and macrophages, and induce their differentiation into



regulatory T cell. Therefore, MSC are expected to make it an effective method for the treatment of COVID-19. So, in these ways, we can prevent the cytokine storm. It is difficult to eliminate infections successfully without inflammation as inflammation is an important as well as essential part of effective immune response. SARS-CoV-2 induces excessive and prolonged cytokine/chemokine responses in some infected individuals, known as the cytokine storm. Cytokine storm causes ARDS or multiple organ dysfunction. It leads to physiological deterioration and death. Timely control of the cytokine storm in its early stage through such means as immunomodulators and cytokine antagonists, as well as the reduction of lung inflammatory cell infiltration, is the key to improving the treatment success rate and reducing the mortality rate of patients with COVID-19.



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ANTIBIOTICS

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Antibiotics

Antibiotics are medicines that fight bacterial infections in people and animals. They work by killing the bacteria or by making it hard for the bacteria to grow and multiply. Antibiotics that kill bacteria are called "Bactericidal". Bacteriostatic drug used to prevent the growth of bacteria. Bacteriostats do not kill bacteria. Antibiotics destroys or inhibits the growth of other pathogenic microorganisms and is used in the treatment of external or internal infections. Antibiotics should only be prescribed to treat health problems that are not serious but are unlikely to clear up without antibiotic such as acne.

History of Antibiotics

Alexander Fleming discovered Penicillin, the first natural product Antibiotic in 1928. He observed that *Penicillium* molds produced a diffusible extract that had antibacterial activity against *Staphylococci*. Taking antibiotics for colds and other viral illnesses doesn't work and it can create bacteria that are harder to kill. Taking antibiotics too often or for the wrong reasons can change bacteria so much that antibiotics don't work against them. This is called bacterial resistance or antibiotic resistance. Penicillin was the first effective antibiotic that could be used to kill bacteria. This meant that cures for serious illnesses were possible even if the patient had the disease. Penicillin had been discovered during the 19th century, but it was Alexander Fleming who first realised its great importance in 1928.



How do Antibiotics enter the Body?

When we swallow an antibiotic pill or liquid, it enters our digestive tract and is absorbed into the blood stream just as nutrients are from food. From there, it circulates throughout the body, soon reaching its target area, where pathogenic bacteria are causing an infection. The word antibiotic means “against life.” Any drug that kills germs in our body is technically an antibiotic. Shall we see some of the antibiotics how it works in our body: Tetracycline works by binding specifically to the 30S ribosome of the bacteria, preventing attachment of the aminoacyl tRNA to the RNA-ribosome complex. It simultaneously inhibits other steps of the protein biosynthesis. Tetracycline antibiotic is used to treat infections caused by bacteria including pneumonia and other respiratory tract infections; certain infections of skin, eye, lymphatic, intestinal, genital and urinary systems; and Chloramphenicol is an antibiotic that works by stopping the growth of bacteria. This medication treats only bacterial eye infections. It will not work for other types of eye infections. A fluoroquinolone is an antibiotic that destroys bacteria by interfering with its DNA replication. Early generation fluoroquinolones hamper bacterial DNA synthesis during replication primarily by inhibiting DNA gyrase, one enzyme required for bacterial (but not human) DNA replication.

Advantages of Antibiotics

- Antibiotics treats many diseases, selectively toxic to microorganisms.
- It is also used to treat infectious diseases in animals.
- It helps the body fight off infections or other bacterial issues.
- Antibiotics are fast acting some will begin working within a few hours.

Disadvantages of Antibiotics

- It can react with other medications.
- It can cause side effects like vomiting, diarrhoea, loss of appetite, feeling sick, itching skin rash, life threatening allergic reaction.
- When used appropriately, they quickly and effectively eliminate infections, causing us to feel better in a matter of days. However, when used to treat other health conditions, antibiotics are not only ineffective but can be harmful to our overall health.



Conclusion

Antibiotics clearly saves life. Poor prescribing of antibiotics are putting patients at unnecessary risk. Every time an antibiotics is prescribed. Make sure an indication, dose and expected duration are part of the prescription antibiotics are the only drug where use in one patient can impact the effectiveness in another. If everyone does not use antibiotics well, we will all suffer the consequences



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EVOLUTION OF INFECTIOUS DISEASE: RESISTANCE OF IMMUNE SYSTEM

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Evolution is the change in genetic frequency and it plays an important role in many infectious diseases. Evolution of infectious disease goes way back to the primitive earth. Vaccine escape in Influenza, Drug resistance in HIV and virulence evolution in Marek's disease are all examples of this evolutionary process. The emergence of the Human Immunodeficiency Virus (HIV) in the early 1980s demonstrates that infectious diseases pose a major problem for human health all over the world and that newly arising infectious agents such as SARS Cov-19 can be especially devastating. Since the population of human species on Earth has crossed the threshold of 6 billion in 1999, the opportunity for various infectious agents that enter the human host has only increased. Although, there have been advances in molecular biology and medicine, our resources to combat these diseases are limited. Even HIV, for which no vaccine is available, comes under this category.

About one or two hundred years ago a great crack split the super-continent where the Mid- Atlantic Ridge is today. Our present day New and Old world started moving apart at the rate of a few centimeters a year, taking with them all the parasites and their host, as well as the insects, intermediate host and so on that existed at that time .This must have had a profound effect on present day infections. Rapidly increasing population, the spread of agriculture, urbanization, mass movement of peoples and the coming of the industrial age are also some of the major



reasons for the evolution of infectious disease. An example of evolution is the roundworm *Ascaris* which has been recovered from eight locations in prehistoric Europe. The pinworm *Enterobius* and the hookworm *Ancylostoma* have all been found in the Pre-columbian New World and must have been carried there during the Ice age but *Ascaris* has not been found. This suggests that *Ascaris* evolved in man after the Ice age.

Diseases are caused by parasites in organisms. Such disease-causing pathogens may or may not be suppressed by fever. Fever causes metabolic processes in the body to speed up with temperature and hence pathogens multiply quickly within a given period of time. The host would have only limited options to deal with this threat. Suppressing the fever mechanism would do more harm than good to the host. The host may be able to develop a mechanism to identify fever-resistant and fever-susceptible pathogens but pathogens evolve rapidly.

Fever in mammals could either control or enhance the pathogenic behaviour but this may not be the same case for all mammals, for example, when rabbits and mice are kept in warm environments, they are better able to survive the infection. Mammals kept in cold environments have a higher chance of death because their high metabolic rates at cold temperatures drain resources that could otherwise be used to fight the virus. For example, rabbits treated with fever-suppressing drug controls *Haemophilus influenzae* and *Streptococcus pneumoniae* when artificially heated. Therefore, the overall net effect of fever may be positive or negative, depending on the association between pathogen and host.

The human immune system is essential for our survival in a world full of potentially dangerous microbes. The immune system has been partially moulded by evolution to respond efficiently to acute infections, to adapt to pregnancy and to transmit protection to infants, and is adapted to cope with many chronic infections lasting for decades. Assuming an absence of a major selective pressure on humans beyond reproductive age, we may have to pay for genetic traits selected to ensure early-life fitness by the later development of immunological phenotypes such as chronic inflammation. Massive ageing and advanced longevity are very recent phenomena occurring in an optimized environment. As proposed by Hayflick, ageing may be an artefact of civilization, and hence changes in the ageing immune system might just be a consequence of evolutionary unpredicted antigenic exposure over the lifetime of an individual.





The understanding of evolution as a major player in pathogens for causing diseases as well as in humans for selective resistance helps us in understanding diseases better and gives us a better chance of alleviating their effects.

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ALZHEIMER'S - A LETHAL DISEASE

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Alzheimer's is one among the most known diseases. It's a progressive disease that mainly affects the brain. It is a neurological condition during which the death of brain cells induces memory loss & interrupts mental functions & cognitive disease. It was first characterized in 1906 by a German Psychiatrist and Pathologist Dr. Alois Alzheimer. Its common type of dementia, one among the deadly diseases.

The origin of Alzheimer's starts with malfunctioning of brain proteins. These proteins spread and accumulate in and around brain cells (affecting them) in abnormal way. There are thousands of protein present inside the brain. Between them amyloid and tau have critical role. Amyloid (larger protein) build the cluster & forms large deposits known as plagues. Plagues have toxic effect on neurons & disrupt their transmission. Tau protein, responsible for neurons core support and transport system. They alter shapes & arrange them into neurofibrillary tangles, disturbing the transport system. But the look for causes isn't fully acknowledged. It is strongly believed the causes may be in collaboration of genetics, lifestyle and environmental factors.

Since Alzheimer is a progressive disease, it scales from mild to severe. Mild Alzheimer's (initial stage) - spread memory problem & cognitive difficulties affects the entorhinal cortex & hippocampus [the inner region of the temporal lobe]. Moderate Alzheimer's - affects the cerebral cortex [outer layer of neural tissue of the cerebrum of the brain], associated in consciousness, emotion, reasoning, language & emotion. Severe Alzheimer's - brain starts to shrink due to plagues & tangles which present throughout the brain. In severe cases it can leads to death.



The symptom starts with mild and get worse over time. Some common are: cognitive deficits, problem with recognition, spatial awareness, speaking, reading and writing, behavior or personal changes, memory loss. Apathy, depression, disorientation were some common symptoms.

Inevitable risk factors of Alzheimer are age, carrying certain gene, family history with AD, even gender plays a crucial role, sometimes any injury in head may stimulate, studies say even down syndrome and other factors like High Cholesterol Level (HCL) and High Blood Pressure (HBP) may also raise the risk. These risk factors cannot be avoided.

A single test cannot suspect Alzheimer's. There are numerous procedures done to diagnose Alzheimer's. Tests may include: inspecting the person's overall health, tests like memory, cognitive, language and problem solving are done to evaluate a person's potential to ponder and recollect. Neurological function test and some basic tests like blood and urine carried out to spot the root of the illness. As AD mainly affects brain, scanning them is obligatory processed by Computed Tomography (CT), Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI) and Genetic testing (to estimate DNA to identify genetic difference).

Treatments cannot cure Alzheimer's completely, but still ease the progression of symptoms and ameliorate standard of life of a person and people around them. Medications also can reduce symptoms and aid to improve the standard of life. The drugs used were Cholinesterase, Galantamine Donepezil, Memantine and Rivastigmine.



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ANTIBACTERIAL ACTIVITY OF *Moringa oleifera* AGAINST BACTERIAL PATHOGENS

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Moringa oleifera is the most widely cultivated species of a Monogeneric family, the Moringaceae. It is commonly known as "Drumstick". It has an impressive range of medicinal uses with high nutritional value. Various parts of moringa acts as cardiac and circulatory stimulants, possess antitumor, antipyretic, anti-inflammatory, antiulcer, antidiuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal and are being employed for the treatment of different ailments in the indigenous system of medicine. Moringa and other medicinal plant has a long history of been traditionally used as a cure of illness such as cough, cold, asthma, nausea, fever, vomiting and diarrhoea. In recent times, the use of plants as a source of novel compounds to combat microbial infections has gained prominence. But with development in science and technology, advancement has been made in the medicinal field with the discoveries of many natural and synthetic drugs. The necessity to search for plant-based antimicrobials is increasing due to high cost, reduced efficacy and increased to conventional medicine. The leaves of *Moringa oleifera* can be eaten fresh, cooked or stored as dried powder for many months without refrigeration and reportedly without loss of nutritional value. The leaves are considered to offer great potential for those who are nutritionally at risk and may be regarded as a protein and calcium supplement.



Moringa species are well documented plant herbs due to their extraordinary nutritional and medicinal properties. *Moringa oleifera* and *Moringa stenopetala* are the most widely cultivated species. They are known to be anti-helminthic, antibiotic, detoxifiers, immune builders and have been used to treat malaria. The disinfectant efficacies of different concentrations of the seed extract of *M. oleifera* and *M. stenopetala* on the above microorganisms are not known. There is need therefore to establish the disinfectant efficacies of Moringa species extracts for recommendation in their processed form as an alternative to chlorine in water purification.

Plant based medicines are widely used and form an integral part of primary health care in many developing countries across the globe. Recently plants have been explored to obtain crude natural extracts for testing and further refinement to develop effective antimicrobial drugs. In India there are different systems of medicine practiced like Ayurveda, Siddha, Unani and local health traditions, which utilize a large number of plants or herbs for the treatment of human diseases.

Fresh leaves of *Moringa oleifera* free from diseases were collected and then washed thoroughly 2-3 times with tap water and once with sterile water. 20 g of fresh leaves was finely chopped and added to 100 mL of distilled water and stirred at 60°C for 30 mins. After boiling, the mixture was cooled and filtered through Whatman No.1 filter paper.

The *Moringa oleifera* shows utmost action versus *Escherichia coli*, *Enterobacter* sp., *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi A*, *Staphylococcus aureus*, *Streptococcus mutans* and *Bacillus subtilis*. The agar well diffusion method, 5mm size wells were cutted in cultural strain swabbed plates. Then, 5µl of plant extract (methanol and water extract) was added in the well by using micropipette. Then plates were incubated at 37 °C for 24 hours. After incubation period the zone of inhibition was measured. A well was prepared in the plates with the help of a cork-borer (6 mm).

The present study methanol and water extract of *Moringa oleifera* showed antibacterial activity against tested organisms. The comparison between the methanol and water extract of the plant recorded significant zone of inhibition activities against these two tested bacterial strains. The maximum zone of inhibition in water extract for *Staphylococcus aureus* (19 mm) and *Escherichia coli* (16 mm) and methanol extract of *Moringa oleifera* showed the maximum zone of inhibition for *Staphylococcus aureus* (15 mm) and minimum for *Escherichia coli* (10 mm), *Moringa oleifera* was found to be active against *Staphylococcus aureus* and *Escherichia coli*.



9

MUSHROOMS: A SOURCE OF VITAMIN D

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Edible mushroom has long been not only as a delicacy, but also as a source of nutrition in human diets. Mushrooms have been found to be rich sources of protein, lipids, amino acids, glycogen, vitamins and mineral elements. Oyster and other edible mushrooms have been recognized as a source for vitamin D since 1994. The two main dietary forms of vitamin D are D₂, which is found in fungus and yeast, and D₃, which is found in animals. A mushroom's vitamin D level is affected by postharvest processing, particularly exposure to sunlight. UV-exposed mushrooms contain significant levels of vitamin D, according to the US Department of Agriculture. Researchers found that artificial UV light technologies were similarly effective for synthesizing vitamin D in fresh mushrooms as mushrooms exposed to natural sunlight in a complete safety assessment, and that UV light has a long history of safe use for vitamin D generation in food.

Mushrooms that have been sun-dried and exposed to UV radiation may be a good source of vitamin D₂. When exposed to UV radiation, ergosterol in the mushroom cell wall is converted to pre-vitamin D₂, which is then thermally isomerized to ergocalciferol, also known as vitamin D₂. Fresh button mushrooms produce considerable levels of Vitamin D₂ When exposed to noon sunlight for 15 - 120 min, frequently in excess of 10 µg/100 g FW, which approaches the daily vitamin D intake suggested in many countries. The majority of fresh retail mushrooms sold in the UK, Europe, North America, Australia and New Zealand, particularly the button mushroom, are grown in atmospherically controlled growing rooms, then harvested and transported in refrigerated transport to markets and retail outlets. The National Nutrient Database of the United States Department of



Agriculture lists shiitake, white button, and oyster mushrooms as all containing less than 1 µg/100 g FW of Vitamin D₂.

Exposing mushrooms to particular, controlled quantities of UV light through a fluorescent UV lamp or a pulsed UV lamp is an efficient approach to produce physiologically relevant amounts of Vitamin D₂. Mushrooms will produce vitamin D₂ in response to UV exposure both throughout the growing phase and post-harvest. When Fresh mushrooms are exposed to UV radiation source in post-harvest, they produce considerable levels of Vitamin D₂, typically reaching 40 µg/g dried mass (DM). UV-B radiation (280 - 315) is the most effective wavelength for stimulating the production of vitamin D₂ in mushrooms.

Mushrooms not only produce vitamin D₂ but also produce vitamin D₃ and vitamin D₄. Vitamin D₂ and vitamin D₃ are structurally very similar. The only exceptions are that vitamin D₂ has a double bond between carbons 22 and 23 and a methyl group on carbon 24. Vitamin D₄ is structurally similar to vitamin D₃ and a methyl group on carbon 24 of the vitamin D₃ side chain. Vitamin D₄ is produced from the UV irradiation of its precursor, 22,23-dihydroergosterol (provitamin D₄). Provitamin D₄ found in several mushroom species including crimini, portabella, enoki, shiitake, maitake, oyster, morel and chanterelle. Previtamin D₄ was detected in UV-irradiated mushrooms with a retention time of 6.2 min in straight phase and its existence was confirmed by chromatography once it was converted to vitamin D₄. Previtamin D₃ was detected in UV-irradiated mushroom with a retention time of 6.3 min in straight phase and its existence was confirmed by chromatography when it was converted to vitamin D₃.

Mushrooms have gained popularity as functional foods or as a source of compounds that can be extracted and used in food. Vitamin D is an essential vitamin for human health and plays a vital role in the regulation and maintenance of calcium homeostasis. Vitamin D deficiencies have also been linked to an increased risk of cancer, hypertension, autoimmune diseases, and diabetes. Ergosterol, the precursor of vitamin D₂ involved in triggering programmed cell death in host cells. Ergosterol, a vital component of the fungal cell membranes, plays a fundamental role in membrane fluidity and integrity while acting as a drug target of several antifungal agents.

Further research on the potential culinary applications of mushroom extracts enriched in ergosterol and vitamin D₂ is a field that requires further work. Despite the potential of ergosterol and vitamin D₂ from mushrooms in the production of anticancer and anti-inflammatory drugs, further *in vivo* mechanistic research is





needed for future clinical trials. As novel drug development entails large resources and time, the approach of combining pre-existing drugs with natural product-based adjuvants to increase their efficiency is promising and economical. Therefore, studies investigating the possible synergistic interactions of ergosterol and vitamin D₂ with currently available anticancer and anti-inflammatory medications should yield useful information for future pharmaceutical applications.

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POTASSIUM SOLUBILIZING ACTINOMYCETES: UNEXPLORED BUT VIABLE OPTION FOR SOIL FERTILITY

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Potassium (K), is an essential nutrient and a major constituent within all living cells and major macronutrients in plant growth and development. Potassium is the most abundant nutrient constituting about 2.5 per cent of the lithosphere. The available potassium status in Indian soils has been categorized into three classes where 21 per cent districts fall in low, 51 per cent in medium and 28 per cent in high potassium content soils. K is present in several forms in the soil, including mineral K, non-exchangeable K, exchangeable K, and solution K. Depending on soil type, from 90 to 98 % of soil mineral K is in various forms like feldspar (orthoclase and microcline) and mica (biotite and muscovite). Only 1 to 2 % soil potassium source is called the exchangeable or readily available potassium and is found on the cation exchange sites or in the soil solution. In total soil major portion of potassium exists in insoluble K minerals and very little potassium becomes available to plants.

Certain groups of microorganisms including bacteria, fungi and actinomycetes are known to solubilized potassium minerals into soluble form which can be utilized by the plants. Microbial inoculants that are able to dissolve potassium



from mineral and rocks have influence on plant growth and have both economic and environmental advantage.

Numerous studies have documented the release K from K bearing minerals. The potassium solubilizing bacteria (KSB) and fungi reported to solubilize K-bearing minerals and convert the insoluble K to soluble forms of K available to plant uptake. Hence KSB can be an effective alternative to chemical fertilizers. Many microorganisms such as *Acidithiobacillus ferrooxidans*, *Paenibacillus spp.*, *Bacillus mucilaginous*, *Bacillus extroquens*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus* etc. have capacity to solubilize K minerals (e.g., biotite, feldspar, illite, muscovite, orthoclase, and mica). Some species of rhizobacteria are capable of mobilizing potassium in accessible form from insoluble K. There are considerable population of K solubilizing microorganisms in soil and rhizosphere. The mechanism of Potassium solubilization is through the production of organic acids, acidolysis, polysaccharides, complex lysis, chelation, and exchange reactions.

Actinomycetes are aerobic, spore forming, Gram-positive and free-living bacteria with aerial mycelia. Actinomycetes found in soil are *Actinomyces meyeri*, *Actinomyces israelii*, *Nocardia brasiliensis*, *Nocardia asteroides*, *Streptomyces coelicolor*, *Streptomyces scabies*, etc. Actinomycetes play important ecological roles in soil nutrient cycling and are recently being regarded as plant growth promoting rhizobacteria. They favor plant growth directly through several mechanisms including Nitrogen fixation, P-solubilization, 1-aminocyclopropane-1-carboxylate deaminase production, indole acetic acid production, siderophore production, chitinase production and several cell wall-degrading enzymes etc.

Actinobacteria solubilized K minerals, can quickly conserve our existing resources and avoid environmental pollution. Many potassium solubilizing bacteria and fungi reported to show significant increase in germination percentage, seedling vigor, plant growth, yield, and K uptake in plants under greenhouse and field conditions. However, the applications of Actinomycetes as Potassium solubilizers or Plant growth promoters in agriculture field are relatively less explored. As per available data potential of actinomycetes for Potassium solubilization is yet poorly explored. The importance of Actinomycetes in soil is well established. They are sporulating organisms can survive for longer period in adverse environmental condition. This property can be exploited in developing long lasting effective actinomycetes inocula for potassium solubilization.



Potassium solubilizing actinomycetes can act as an alternative and viable technology to solubilize insoluble K into soluble form, their application in agricultural practice is not revealed yet because of several factors including lack of awareness about bio-fertilizers amongst the farmers; slow influence of the K bio-fertilizer on crop yield; less interest in scientific community on the development of K bio-fertilizer technologies; unavailability of proper and well equipped culture collection banks and deficiency in technology in respect to carrier suitability and product formulations. Therefore, there are immense possibilities to use actinomycetes as K-bearing rock materials solubilization and potassium solubilizing biofertilizers for further increasing the production of crops. Overall, the multifunctional property of actinomycetes makes them unique and their potentials are yet to be fully exploited.



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MARINE BACTERIAL EXOPOLYSACCHARIDES AS A BIOSORBENT FOR HEAVY METAL MERCURY REMOVAL.

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Mercury is a highly toxic metal whose concentration is increasing at an alarming rate in the environment day by day. The various detrimental anthropogenic activities like manufacturing of pesticides, batteries, explosives, electronics, catalysis, chloroalkali chemicals, thermometers, dental preparations and fluorescent and ultraviolet lamps are responsible for increasing the level of mercury in the environment. Degassing from the earth's crust constitutes one of the most abundant natural sources of mercury pollution in the environment that accounts for approximately 100,000 tons per year. However, manmade sources contribute about 20,000 tons of Hg each year. Hg pollution is considered to be a warning to the universe as it is the most toxic within all metals. Immoderate exposure to Hg has detrimental effect on health and can damage the central nervous system and immune system as well as some important organs of body like the brain, heart, kidneys, and lungs. Minamata incident triggered awareness about the hazardous effect of mercury among the people. The most usual form of the organic mercury is methyl mercury



(MeHg), which is the crucial source of organic mercury existing in the ecosystems. MeHg is readily delivered by water into the aquatic ecosystems. Due to its low water solubility and relatively high lipid solubility, MeHg is easily taken up by lower organisms.

Marine bacteria are known to produce number of bioactive compounds amongst which exopolysaccharides have immense applications. Exopolysaccharides (EPS) are high-molecular weight polymers consisting mainly of carbohydrates. EPS occur as either capsular polysaccharides in which the polymers are covalently bound to the cell surface or slime polysaccharides that remain loosely attached to the cell surface. EPS produced by marine bacteria are play a role in the defense against very extreme physicochemical conditions of marine environment such as low or high temperature, high pressure, low nutrient availability, high salt and heavy metal. EPS can be used as stabilizers, gelling agents, adhesives, thickening agents, emulsifying agents, flocculants and flushing agents, with indispensable characteristics and enormous applications of EPS for the bioremediation of heavy metal have received considerable attention in the past two decades. EPS mediated heavy metal removal is an eco-friendly, sustainable and cost effective method compared to other ordinary methods. Biosorption is the uptake of heavy metal by any biological material by different physico-chemical processes such as ion exchange, sorption, complexation, chelation and micro-precipitation. Biosorption is a surface phenomenon without any involvement of microbial metabolism. EPS can be used as a biosorbent for biosorption of mercury from surrounding environment. EPS mediated biosorption occurs by interaction between positively charged metal ions and negatively charged EPS and cell surfaces. EPS from marine bacterium has been of special interest given to their higher uronic acid content which imparts them enhanced anion nature. Furthermore the adsorption of heavy metals by EPS is non-metabolic, energy independent and can be caused by interaction between metal cations and negative charge of acidic functional groups of EPS. The acidic characteristics of the EPS is proved by the presence of phosphate and carboxylate groups which have a huge potential to bind with the positively charged metal ions present in the polluted environment. EPS can be environmentally safe and cost effective bioadsorbent. Additionally EPS do not generate toxic byproducts, both live and dead cell bound EPS can be used for metal adsorption. Due to too many significant applications nowadays EPS mediated heavy metal removal has become popular aspect of bioremediation.



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ENVELOPE BIOSYNTHESIS IN *Escherichia coli*

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Cell envelope consists of cytoplasmic membrane, cell wall and capsule which is essential for bacterial growth, environmental adaptation and drug resistance. Cell envelope biosynthesis is a complex process which involves enzyme reactions that take place in cytoplasm and outer side of cytoplasmic membrane. Cell envelope biosynthesis has been studied extensively in *Escherichia coli* using Synchronous culture by membrane elution technique. *E.coli* is a gram negative bacilli which can cause several diseases such as Pneumonia and Diarrhea. The protein in the envelope of *Escherichia coli* with molecular weight of about 7,500 is covalently attached to the Peptidoglycan (PG). The Lipoprotein (LPP) exists in the *Escherichia coli* envelope fraction not only in bound form but also in free form, not covalently attached to the peptidoglycan. LPP provides only covalent crosslink between the OM (Outer membrane) and the PG through its N-terminus and C-terminus. The OM-PG covalent bridge and length of LPP are important for transmission of stress signals. The rate of synthesis showed as a stepwise pattern with a discrete doubling in rate in the first half of the cycle. The SDS – PAGE and autoradiography revealed that the great majority followed the pattern of bulk measurements, with increase in rate of synthesis early in cycle. Envelope synthesis using Synchronous culture demonstrated that (i) No post synthetic modification of polypeptides was deleted and (ii) About 70/- of newly synthesized 76,000 molecular weight protein was lost from envelope during succeeding generation.



The outer leaflet of OM composed of Glycolipids, principally Lipopolysaccharide (LPS). LPS is a molecule which is responsible for endotoxic shock associated with septicemia. There are about 100 OM lipoproteins in *Escherichia coli*, and the functions of most are not known. The OM is essential for the survival of *Escherichia coli*, but it contains only a few enzymes, such as Phospholipase. The active site of all of these enzymes is located in the outer leaflet or it faces the exterior of the cell. LPS plays a critical role in the barrier function of OM. Glucosamine disaccharide, a polysaccharide core and an extended polysaccharide chain called as O – antigen. Pathogenic *Escherichia coli* are classified by the antigenic properties of their O – antigen and the major protein component of the Flagella.

A protein called PBPIB helps to produce new cell wall material. Another complex called Tol system provides energy needed for outer membrane. Here, proteins interact with PBPIB during cell division in *Escherichia coli*. Many experiments found that a protein called CpoB interacts with both PBPIB and the Tol system. However, CpoB is disrupted, cell wall production and movement of outer membrane are no longer coordinated, leading to the death of the bacteria.

To this end, physiological and biochemical evidence indicates that the *Escherichia coli* envelope can sense protein over-expression in cytoplasm and react by modulating the abundance of some membrane proteins, with possible consequences on the membrane traffic of small solutes. Likewise, the membrane lipids may act as a second stress sensor responsive to the aggregation state of the recombinant protein and further contribute to changes in cellular exchanges in the environment.



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DNA PLASMID BASED VACCINE: PROGRESS AND PROSPECTS IN SARS- CoV-2 VACCINE

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DNA vaccination is an alternative to traditional vaccines. Discovered in the 1990s, these vaccines have gained scientists' interest across the globe due to their ability to elicit humoral and cellular immune responses. DNA vaccines work by injecting genetically engineered plasmid containing the DNA sequence encoding the antigen(s) against which an immune response is sought, so the cells directly produce the antigen, thus causing a protective immunological response.

The basic working principle behind DNA vaccines involves using a DNA plasmid that encodes for a protein that came from the pathogen, in this case, SARS-CoV-2. Plasmid DNA (pDNA) is inexpensive, stable, and safe, allowing the non-viral platform to be considered a good option for gene delivery.

Overall, there are 250 vaccines in development to combat the coronavirus pandemic. Of these, 181 are in preclinical development, while 69 are undergoing clinical evaluation. Among the vaccines in clinical evaluation, ten vaccines are DNA-based. Among these, one vaccine is in the phase 3 trial, the ZyCoV-D vaccine by Zydus Cadila.



Zydus Cadila, a leading Indian pharmaceutical company, has applied to the Drug Controller General of India (DCGI) for emergency use authorisation of ZyCoV-D, after interim analysis shows ZyCoV-D to have a primary efficacy of 66.6% for symptomatic reverse transcription PCR-positive cases. If approved, ZyCoV-D will be the world's first DNA vaccine to protect against SARS-CoV-2 infection.

The company's DNA vaccine works by injecting genetically engineered plasmids that trigger the body to produce the spike protein of Covid-19, eliciting the production of antibodies to protect against the virus. ZyCoV-D is administered in three doses. The vaccine is also needle-free. Instead doses are given using a device that delivers fluid through a spring-powered jet that penetrates the skin, and could reduce the side effects.

The plasmid DNA platform provides ease of manufacturing with minimal biosafety requirements (BSL-1) unlike Bharat Biotech's Covaxin that requires high biosafety level of BSL-3.

Some vaccines, such as the Pfizer and Moderna Covid-19 vaccines, must be kept at extremely cold temperatures, making transport and storage more difficult. ZyCoV-D, on the other hand, is stored at 2-8°C and has shown good stability at 25°C for at least three months. This thermostability means Zydus Cadila's vaccine can be transported and stored easily, meaning a reduction in vaccine wastage.

ZyCoV-D has already demonstrated a robust immunogenicity, tolerability and safety profile in the adaptive Phase I/II clinical trials carried out in 2020 and early 2021. The vaccine has been tested on almost 30,000 participants across Phase I, II and III studies, and is the first vaccine in India to be tested on 12 to 18-year-olds. Around 1,000 participants were enrolled in the adolescent age group, among whom the vaccine was found to be safe and well tolerated, with similar findings in the adult population. No moderate cases of Covid-19 were observed in the vaccine arm of the study after administration of the third dose, suggesting 100 % efficacy for moderate disease after the dosing course is complete. Although, there are many advantages with using DNA plasmid based vaccine but "every rose has its thorn". Risk of affecting genes controlling cell growth ,integration of the plasmid DNA into the host genome and adverse immunopathological effects are some of the problems with using DNA based vaccine .

Despite initial concerns that they might integrate into patients' genomes, DNA vaccines have proven remarkably safe; for instance, making them ideal in



cancer immunotherapy or for vaccinating people with weakened immune systems. DNA-based vaccine approaches have become a reality due to the easy handling of DNA plasmids and straightforward manufacturing and stability of highly purified DNA preparations. The DNA plasmid based vaccine are cost-effective and are being touted for effectiveness and ability to produce more quickly than traditional, protein vaccines.



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IMMUNIZATION

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Immunization is a process by which the individual immune system gets fortified against an agent (immunogen). Immunization is done through vaccines. A vaccine is a pharmacological compound that improve immunity to a particular disease. When a disease-causing bacteria or a virus invades the human body, the immune system recognizes the material as foreign, by detecting the protein portion of invading organism known as ANTIGENS. Vaccines affect the immune system, primarily through B-LYMPHOCYTES and T-LYMPHOCYTES. Before exposure, the immune system contains both lymphocytes with potential to respond to a unique antigen. Many antigens require a combined response of both B-LYMPHOCYES and T-LYMPHOCYTES. This form of immunity is known as T-CELL-DEPENDENT. Less commonly an antigen causes the stimulation of B-LYMPHOCYTES For antibody production without the help of T-LYMPHOCYTES. The vaccine administration causes the immune system to response. Initially the foreign material is phagocytized, and broken down by Macrophages. The resultant peptide is created by breaking down the protein and supplied to surface of Antigen Presenting Cells (Ex Dendritic cells). These are presented by molecules called MAJOR HISTOCOMPATIBILITY COMPLEX (MHC). It is MHC1 and MHC2. This presentation stimulate the action of inflammatory mediators, including Cytokines, interferons, which further stimulate other response. The T-CELLS recognize antigen and stimulate corresponding B-LYMPHOCYTES to get differentiated into PLASMA B-CELLS which produce Antibodies and MEMORY B-CELLS. The antibody performs various function such as intracellular digestion of bacteria,



inactivation of toxins, prevention of bacterial adhesion. In response to vaccine the first antibody such as IgM is produced and IgG will appear for following weeks.

Since the time of ancient Greeks, it has been recognized that people who have recovered from Plague, Smallpox, Yellow fever, and other various infectious diseases rarely contract the diseases again. The first scientific attempt on artificial immunization were made in late 18th century by Edward Jenner (1749-1823): a country doctor from Brekley Gloucestershire, England. Edward Jenner investigated the basis of widespread belief of English peasants that anyone who has Vaccinia (Cowpox) never contracted Small pox. Yet most English Milkmaids, who were readily infected with cowpox, had clear skin because cowpox was a relatively mild infection that left no scars. It was on May 14, 1796, that Jenner extracted the contents of pustule from the arm of Cowpox-infected Milkmaid Sarah Nelmes, inoculate into arm of eight year old boy James Phipps. As Edward Jenner expected, immunization with Cowpox virus cause only mild symptoms in boy. When he subsequently inoculated the boy with smallpox the boy show no symptoms to the disease. Edward Jenner then inoculates large to his patients with Cowpox pus, as did other physicians in England and other European countries. Further immunization work was done by Louis Pasteur (1822-1895). Lois Pasteur discovers that if cultures of Chicken cholera bacteria (*Pasteurella multocida*) were allowed to age for 2 or 3 months the bacteria produce only a mild attack of Cholera of when inoculated into chickens. somehow old culture had become less pathogenic (Attenuated) for chickens. He then found that fresh culture of bacteria failed to produce cholera in chicken that had been previously inoculated with old, attenuated cultures. By 1800 practice known as vaccination was started in America, and by 1805 Napoleon Bonaparte had ordered all French soldiers to be vaccinated. To honor Jenner work with Cowpox, Pasteur gave the name VACCINE to any preparation of weakened pathogen that was Used to immunize against infectious diseases.

Vaccines were eventually developed against most of the epidemic diseases that had plagued Western Europe and North America (Diphtheria, Measles, Mumps, Polio and Whooping cough).Vaccines for the other diseases were not emerge until the later part of 19th century, when largely through a process of trial and error, methods for inactivating and attenuated microorganisms were developed and vaccines were produced .Indeed, towards the end of 20th century it began to seem that the combination of vaccines and antibiotics would temper the problems of microbial infections. Nevertheless, vaccination is one of most cost-effective way of disease prevention.



Classification of Immunization

Types of Artificial Methods of immunity: (i) Active Immunization and (ii) Passive Immunization. Active Immunization is defined as protection of susceptible individual from communicable diseases by administration of vaccines. It is a preparation from infectious agents to provide immunity. It may consist of preparation of Killed microorganisms: living, weak microbes, inactivated bacterial toxins, Recombinant vectors, DNA vaccines that are administered to induce immunity artificially. Passive immunization is defined as artificially acquired passive immunity produced by injecting an animal with preformed antibodies that are produced in another animal or *in vitro*. This immunization is routinely administered to expose certain microbial pathogen which cause diseases.



Figure - 1: Types of Immunization



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RENEWABLE ENERGY AND AGRICULTURE

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Introduction

At times, people used to produce renewable energy by growing corn to make ethanol. But now, more number of farmers is increasing their income by harvesting the wind that blows across the land to make electricity. Renewable energy and farming are a better combination. Wind, solar and biomass energy provides a longer term source of income. This renewable energy helps us to replace other fuels or as a "cash crop". Renewable energy helps to reduce pollution, global warming and dependence on important fuels. And it also helps us to increase the rural income and provides employment for youth.

Wind Energy

Farmers used wind power to pump water and generate electricity. Wind developers have introduced large wind turbines on the farm and provide power to electric companies and consumers. At ancient times people barely choose lands with alternative sources. But nowadays, as the lifestyle differs, it is a lot tougher to earn a living from traditional farming so people started introducing several alternative energy sources and introduced wind turbines.

How Wind Energy Helps Farmers?

Farmers and ranchers can lease the land to wind developers; use the wind energy produced or can be used by themselves. Small wind generators, about 400



watts to 40 kilowatts, can meet the entire requirement of a farm. Electric wind generators are more efficient compared to old water pumping fan bladed windmills."NET METERING" enables farmer to get the most out of their wind turbines.

When the turbines produces more power than the requirements the extra flows back to the electricity system for others to use and if it is less, the meter spins forward as normal. At the end of month or year, farmer pays the net consumption or electric company pays for net production. Wind energy are also used for grinding grains and legumes.

Solar Energy

Solar energy is frequently used renewable energy all over the world. Earlier solar energy was used to grind grain to flour and pump water from wells. Windy and solar areas are used mainly for raising crop and livestock and are also used for lands to host wind turbines and solar – sun tracking units. Solar energy can supply and supplement many energy requirements in agriculture. Some of the applications of solar energy technologies in agriculture are:

Crop and Grain Drying

Using of sun to dry crops and grains are one of the oldest method in agriculture. It is the cheapest and simplest technique that allows the crop to dry naturally under the sun after they are harvested. The major disadvantage is that it gets affected by wind, rain, rodents, dust etc. The basic components of solar dryer are an enclosure or shed, drying trays or racks and a solar collector. Solar collector are as a glazed box with dark colored interior to absorb the solar energy that heats air. The solar collector heats the air inside, moves either by natural convention or by forced fan.

Greenhouse Heating

Greenhouse is a major modern technology that helps to make a major profit in agriculture. Solar greenhouse, are designed to utilize the solar energy both for heating and lighting. Solar greenhouse has thermal mass to collect and store solar heat energy and insulation to retain the heat for use during the night and on cloudy days. A solar greenhouse reduces the use of fossil fuels for heating. Passive solar greenhouse is cost effective and helps the farmer for better growth of plant and optimum yield.



Solar Photovoltaic System

Photovoltaic water pumping systems are well suited for water supply in remote areas. Solar photovoltaic technology, the solar radiation is converted into electricity by a device called solar cells. This is a eco -friendly technology. Spy pumping system helps to lift water for drinking and irrigation without any damage to the environment. These pumps can be installed in bore wells, tanks, cisterns or rivers. DC floating pumps are suitable for wide range of flow and head situations.

Hydroenergy

Hydroelectricity is produced by the flow of water that passes through the turbine, turning the blade. These turbines are connected to the generator and the energy is converted to electricity. The amount of electricity produced depends upon the quantity of water passing out through the turbine and the height from which the water falls. Greater the flow, greater the electricity. Hydropower is a clean, renewable source of energy. It provides inexpensive electricity and produces no pollution. Hydroelectric system varies in size and application. Micro hydroelectric plants are the smallest in hydroelectric systems. They can be used for processing machines, small farms and communities. Large hydroelectric system can be used to large amount of electricity and they can be used to power large communities and cities.

Water use for Irrigation

Agriculture is by far the largest water use at global level. Irrigation of agricultural land is about 70 %. In several developing countries, irrigation is about 95 % of usage and plays a major role in food production and food security. Water used for agriculture comes from natural and other alternative sources. Natural sources are rainwater and surface water. These are sustainable way. An alternative source includes irrigation water, reuse of municipal waste and drainage water.

Conclusion

The only realistic solution to the problem is to find sources of renewable energy to replace today's dwindling supplies of affordable and usable fossil energy. Solar energy is the only source of truly renewable energy. Windmills, falling water, solar collectors, and photovoltaic cells are all sources of renewable solar energy. However, we need to be realistic about the extent to which energy from agriculture can replace our current use of fossil energy. While the energy experts may not agree on specific quantities or percentages, the overall limits on energy from agriculture are fairly basic and straightforward.



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MUCORMYCOSIS

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Mucormycosis is a type of fungal disease which is also known as Zygomycosis. It is usually caused by a group of molds called as Mucormycetes. Mucormycosis mainly affects people with weekend immune system, diabetes and non-diabetic Covid 19 patients. It presents itself as either a respiratory or a skin infection. It occurs initially at the site of skin trauma and spread to other areas. Signs of the fungus include cough, fever, headache, blackened skin tissue, redness, swelling etc. Mucormycosis is diagnosed by looking at a tissue sample in the lab. Phlegm or nasal discharge is also collected for suspected sinus infection. It is treated by surgical debridement which involves cutting away all infected tissue. Some antifungal medications like Amphotericin B, Posaconazole and Isavuconazole are also given. Fungal disease recovery greatly depends on early diagnosis and treatment. The basic principles of Mucormycosis treatment include risk stratification for severity of the diseases, intense attempts for early clinical and laboratory diagnosis, timely initiation of an effective antifungal therapy and when feasible control of the underlying medical condition. In a study of 70 patients with Mucormycosis, delayed antifungal therapy for less than 6 days after diagnosis resulted in a 2-fold increase in mortality rate, compared with early treatment. In Pulmonary Mucormycosis, surgical treatment along with appropriate systematic antifungal therapy has been shown to significantly improve survival compared to antifungal therapy alone. The diagnosis and treatment of this fungal disease found to be very challenging. Clinical approach lacks sensitivity and specificity.



Five different types of documented Mucormycosis includes, Rhino- Orbito-Cerebral Mucormycosis (ROCM), Cutaneous Mucormycosis (CM), Gastrointestinal Mucormycosis (GM), Renal Mucormycosis (RM) and Pulmonary Mucormycosis (PM). ROCM is the most common form and is often seen in patients with uncontrolled diabetes mellitus. Solid organ transplant, corticosteroid therapy, chronic kidney disease and intravenous drug use were also found as risk factors in ROCM cases. Cutaneous Mucormycosis often occurs after trauma and can be seen in the immunocompetent host. The major predisposing factor in CM is penetrating trauma and other risk factors include intramuscular infection in sub-optimal healthcare facility, contaminated dressings, burns, natural disasters, animal bites etc. Gastrointestinal Mucormycosis is the most difficult disease to diagnose and commonly seen in patients with malnutrition or undergoing peritoneal dialysis. Chronic alcoholism, malnutrition, peritoneal dialysis and use of Broad spectrum antibiotics are the major risk factors. Site of infection includes large intestine, small intestine, stomach, oesophagus. Renal Mucormycosis has been seen in patients with fever, flank pain, hematuria. CT and ultrasound are helpful in the early diagnosis of RM. Pulmonary Mucormycosis is the second most infection mostly occurs in patients with hematological disorders and transplant recipients. Post-pulmonary tuberculosis is one of the risk factors for PM. The diagnosis of PM remains as challenging.

Link between Covid 19 and black fungus infection is increasing the risk in India. Covid 19 treatment make the immune system vulnerable to other infections, including black fungus. With the risk in black fungus cases, India faces a shortage of treatments in the face of two epidemics. People who recovered from Covid 19 died from Mucormycosis and there are calls by India's health authorities to declare a Mucormycosis epidemic. In addition, Oxygen support for people with severe Covid 19 can cause drying of the nasal cavity and further increase the risk of fungal infection.

On a final note, the diagnosis of Mucormycosis should be suspected in any diabetic patient with neutropenia with rhino-orbitofrontal brain or lung unimproved by appropriate antibiotic therapy. Other locations are less characteristic. Diagnosis is suspected on clinical and radiological features and confirmed by mycological and pathological examination. Treatment consists of Amphotericin B combined with surgery. ROCM is an aggressive invasive fungal infection which tends to affect patients with a history of diabetes, chronic steroid use and immunosuppression. Overall prognosis of Mucormycosis in ICU remains poor, especially in patients with hematological malignancies. A therapeutic strategy including curative surgery was the main factor associated with survival.



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THE COMPOSITION AND STRUCTURE OF BIOFILM

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More than 99 % of the microbes inhabiting on earth are able to form a biofilm and hence this is the most stable nature of all microorganisms. The capacity to form biofilm allows the microbes to coexist in micro niches of different physiological conditions. In the natural environment microbes have the tendency of forming a coating around the particles of soil and at times they may also form a thin film at water sediment inter faces.

Biofilm structure and its surrounding are very important in understanding the mechanism of interaction between the microbes and its nearest environment. The trace elements particularly the metal ions present in the vicinity of the biofilm interact chemically, physically as well as biologically. All these three process ultimately leads to the distribution, internalization; immobilization, accumulation and remobilization of the metals which is defined by the sorption properties of the biofilm. The sorption property is defined and determined by the various ligands found on the matrix of the biofilm and the pH of the biofilm. One of the most important factors for biosorption by the biofilm is the redox potential (Eh) conditions around the cell in comparison to the bulk of the liquid. The various organic functional groups which have the tendency of complexing along with the micro environment of the biofilm matrix within the biofilm interacts physico-chemically



with the aqueous phase where in are found the metal that are going to interact with the biofilm.

The composition of the biofilm can be described as heterogeneous matrix defines by extracellular polymeric substances within which the microbes are immobilized. According to Fleming 1995 and Southerland 2001a, a general overall composition of a typical microbial biofilm is defined in table 2.1 Out of four major components EPS forms the outer most interactive face with the absorbing metal ions. Chemically EPS are very complex and mainly constituted by a matrix of macro molecules consisting of polysaccharides and proteins, however, at times, nucleic acids, lipids, or certain humic substances may also be found in them. The components of EPS readily provide cationic groups, anionic groups, a polar group and groups capable of forming hydrogen bonds. Some of the important physicochemical property displayed by the components of EPS includes electrostatic interactions, hydrogen bonds and dispersion forces. The carboxyl, phosphoric, amine and hydroxyl group provide the necessary charges that enable EPS to sequester or adsorb minerals or nutrients or even at times toxic metals. The outer membrane lipopolysaccharide, lipoteichoic acids and the cytoplasmic membrane also play roles in biosorption by various means including reductive precipitation of metals by enzymic transformation and metal precipitation. Similarly the components of peptidoglycan are also important source of polar interactions.



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ROLE OF HALOPHILIC AND HALOTOLERANT PGPRs FOR SUSTAINABLE AGRICULTURE IN SALINE SOIL

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The coverage of Earth's surface with soil varies from place to place. There are several different classes of soil depending on how the soil is formed and where it is located. Some modern agricultural practices leave soil exposed to the number of elements and increase the risk of soil erosion leading to loss of soil productivity. Salinity is the term used to describe the condition when soluble salts get accumulated in the solution (soil or water). Salinity of water and soil are the most important factors limiting the production of crops. When salts accumulate in soils, problems arise due to two main reasons: the soil become less permeable, and the salt also damages or kills the plants.

India has around 6.727 million hectares area, of which 2.956 million hectare area is salt affected. Nearly 75 % of this salt-affected soils in the country exist in the state of Gujarat (2.23 million hectare). Various anthropogenic activities, progressive growth of population, industrialization and urbanization, irrigation of agricultural



land with saline water, inadequate irrigation management, waste waters from pesticide manufacturing industries, pharmaceuticals, herbicides, oil and gas recovery processes are principal causes for increasing salinity of soils. Moreover, with the increasing population of the planet and occurrence of saline fields worldwide use saline soil and water for agriculture will be mandatory in the near future.

Effect of Salt on Plant Growth

Generally, soil salinity negatively affects the plant growth through various mechanisms listed below.

Effect	Manifestations
Osmotic stress	During stress, water intake by root becomes difficult which results in reduced transpiration rate, stomatal closure, water retention, and increased rate of senescence of older leaves.
Nutrient imbalance	Soil salinity causes nutrients deficiency in plant occurring in saline soils, as it becomes difficult to take phosphorus because phosphate ions precipitate with Ca^+ ions and become unavailable for plant utilization.
Decrease in photosynthesis	Salt stress damages the photosynthetic capacity of the plant by harmful effects on PSII and PSI resulting in the decreased photosynthetic efficiency of plants.
Effect on reproductive	Salt stress inhibits microsporogenesis, floral development, causes ovule abortion, embryo arrest and senescence of fertilized embryos, it also induces apoptosis in some reproductive tissues.
Ion toxicity	Mobilization of essential macronutrients in plants is affected by abundance of Na^+ and Cl^- ions in the rhizospheric region. Excessive accumulation of Na^+ and Cl^- ions in the rhizosphere induces competitive absorption of certain mineral ions in the roots, along with competition and translocation to tissue-specific localization. Intake of salts by plants beyond their tolerance leads to storage of ions in the intercellular spaces, which causes dehydration and death of plant tissues.

To overcome these problems and for the fulfilling the ever-increasing food demand in an environment-friendly manner, there is a need of exploring more useful alternative strategies for sustainable agriculture in saline soils. The Plant Growth Promoting Bacteria (PGPB) plays an important role in promoting healthy plant growth and provide defense mechanisms under unfavorable conditions. They



commonly occur in the rhizosphere, stimulate plant growth and are termed as plant growth promoting rhizobacteria (PGPR).

Halophiles are “salt-loving” organisms represented by archaea, bacteria, and eukarya for which their main characteristic is their salinity requirement. Halotolerance is the adaptation of microorganisms to conditions of high salinity. Halophiles and halotolerant Plant Growth Promoting Bacteria (PGPB) alleviate salt stress and give improved plant growth and yield. They have several mechanisms to tolerate salt stress. These bacteria survive under salt stress conditions because they modify the osmolality of the rhizosphere. These modified osmolytes are made available for utilization of plant, act as free radical scavengers, a regulator of the photosynthetic apparatus and stabilizer of subcellular structures and positively affect the plant growth. The additional mechanisms of ion homeostasis, improvement of nutrient uptake (N_2 fixation, solubilizing of P, K, and Zn) production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (that removes salt stress and ethylene from the rhizosphere), Indole acetic acid (IAA), Siderophores and exopolysaccharides mitigate soil salinity stress and improve agricultural yield. Thus, halophiles and halotolerant plant growth-promoting (PGP) can be considered as sustainable and a cost-effective solution for improving agriculture crop yield in saline soils.



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KOMBUCHA: A MAGICAL HEALTH ELIXIR

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What is Kombucha?

Fermentation of sugared tea with a symbiotic culture of acetic acid bacteria and yeast (tea fungus) also known as SCOBY, yields kombucha tea which is consumed worldwide for its refreshing and beneficial properties on human health. Bacteria and yeast involved in Kombucha are *Bacterium gluconicum*, *Acetobacter xylinum*, *Saccharomyces ludwigii*, and *Saccharomyces apiculatus*.

Leathery pancake, we call as SCOBY, that stands for Symbiotic Culture of Bacteria and Yeast. The SCOBY is sometimes called the mushroom, because it resembles the smooth, thick body of a mushroom.

What to do with extra SCOBY after using in Kombucha?

- To improve plant growth
- The culture pulls circulation to the surface of skin which regenerates the skin cells.
- The acidic pH. Of the culture sloughs dead skin cells and leaves soft and smooth skin.

Ingredients to make Kombucha

- Distilled water and reverse osmosis water can also be used to brew Kombucha.
- Kombucha require real tea (*camellia sinensis*) for both minerals and nitrogen.
- Sugar: Any kind of cane sugar is acceptable for kombucha.



- SCOBY: A small piece of SCOBY.
- Cover: Cheese cloth, muslin or paper towel

Health Benefits of Kombucha

- Kombucha teas has been granted a great deal of recognition for helping with a variety of health problems includes skin problems, digestion, concern to hair loss and high blood pressure or cholesterol.
- Contains glucosamine's which ease joint pain and help prevent arthritis.
- Cellulose from kombucha would be remotely effective at absorbing silica dust from inside lungs.
- Improved digestion, mental clarity, and mood stability and noted for eliminating the symptoms of fibromyalgia, depression, anxiety, etc

Composition of Kombucha Tea

- *Gluconic acid*: Impedes the progression of viral infections; can dissolve gallstones.
- *Hyaluronic acid*: A component of connective tissue.
- *Vitamins* - B1 (Thiamine); B2 (Riboflavin); B3 (Niacin); B6 (Pyroxidine); B12 (Folic acid) and Lactic acid.
- *Usnic acid*: A substance with strong Antibacterial and Antiviral properties.
- *Several Probiotic strains* - Loaded with Probiotics this helps the digestive system.
- *Glucaric acid* - Helps to prevent Cancer.
- *Theanine* - Helps to ease stress by increasing Serotonin.
- *Antioxidants* - Strengthen the Immune system.



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THE ROLE OF OUR GUT MICROBIOME IN DEVELOPING OBESITY

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The human gut houses approximately 100 trillion bacteria, 10 times the number of eukaryotic cells in our body and representing 150 times unique genes than our own genome. Our gut microbiota is basically a dynamic entity which begins colonizing during, and immediately after, birth. By 2016, about 100 different phyla had been identified. In adults, approximately 90% of the microorganisms belong to phyla Bacteroidetes and Firmicutes; some members of Actinobacteria and Proteobacteria are also seen. The three most dominant enterotypes found are *Bacteroides*, *Prevotella* and *Ruminococcus*.

The composition of gut microbiome varies based on geographical location and also depends on an individuals' diet. For example, a vegan or a vegetarian diet can significantly alter gut microbiota as compared to omnivores. The people living on such restrictive diets show a decrease in their *Bacteroides*, *Bifidobacterium* and *Enterobacteriaceae* species, as well as *Escherichia coli*. The ratio of Firmicutes: Bacteroidetes also varies with age; this ratio is 0.4 for infants, rises to 10.9 in adults and further decreases in the elderly population to about 0.6.

The intestinal epithelium is symbiotic with the intestinal innate immune system, and together they cooperate in interactions between the gut microbiota and the host. The gut innate immune system is mainly composed of the epithelial cell barrier and immune cells (Neutrophils and NK cells). Defects in this cell barrier can



lead to a paracellular influx of luminal antigens and toxins, resulting in a gut-sourced systemic inflammation. Increased inflammation is associated with enhanced weight gain, which again leads to more inflammation, trapping the patient in a vicious cycle. Several intrinsic and extrinsic factors can perturb the gut microbiota, causing a shift from normobiosis (normal, healthy gut microbiota) to dysbiosis (imbalanced gut microbiota). Dysbiosis is followed by an onset of obesity.

Obese individuals have been observed to exhibit differences in their gut microflora as compared to lean, healthy individuals. These patients have a reduced count of Bacteroidetes. Increase in *Lactobacillus*, *Leuconostoc* and *Pediococcus* species further predispose one towards developing obesity. Fewer *Bacteroides thetaiotaomicron* bacteria are found in the gut of obese patients, along with an associated increase in their serum glutamate concentration. Experiments involving mice proved that the transfer of these bacteria, as well as bariatric surgery, were reported to alter the gut microbiome by increasing *Bacteroides thetaiotaomicron* numbers. This reduced serum glutamate levels and improved certain metabolic parameters, including insulin resistance.

Certain gut microorganisms are capable of degrading polysaccharides at rates exceeding that of their hosts, leading to the production of SCFAs such as acetate, propionate and butyrate. In normal, lean adults, this can provide 80 - 200 kcal energy per day. However, dysbiosis (eg. 20 % increase in Firmicutes and consequently, 20 % decrease in Bacteroidetes) can result in the production of 150 kcal of excess energy on a daily basis. The increased amount of SCFAs, therefore, puts such individuals at a risk for developing obesity. This happens because of two reasons, the first one being that SCFAs are crucial energy substrates for the host, resulting in excessive bodily energy harvest in the host that is subsequently stored as fat. SCFAs also act as ligands for specific GPCRs – Gpr41 and Gpr43 – that promote energy absorption and/or triglyceride synthesis, further influencing immune and inflammatory responses. Studies in mice show that Gpr41 +/+ mice are significantly fatter than their recessive mutants, as the former produce GLP-1, GLP-2 and peptide YY. PYY is a hormone secreted by the intestines that negatively regulates satiety, gut motility and energy expenditure. Gpr41 -/- genotype corresponds with reduced expression of PYY, so the mice remain lean. The same results can be seen for Gpr43 +/+ and Gpr43 -/- mice, confirming that Gpr43 is also an inhibitor of energy expenditure and triglyceride synthesis. Hence +/+ mice remain fatter than -/-, even if the latter is fed on a fat-rich diet.



Gut bacteria also affects bile acid metabolism, reducing the number of bacteria that confer protection against obesity. Bile acids act as ligands of the FXR and TGR5, which might be involved in the secretion of gut hormones and glucose and lipid metabolism.

Individuals that consume a fat-rich diet have increased levels of plasma LPS, as such a diet induces colonization of the gut by gram negative bacterial cells (having LPS cell walls). An excess of LPS (metabolic endotoxaemia) can lead to inflammation of the gut, hepatic and adipose tissues. LPS can elicit an innate immune system response linked to adiposity and de novo triglyceride synthesis. A fat-rich diet might alter the intestinal microbiota. This may lead to a decrease in IAP activity and an increase in TLR4 activation in the gut wall, resulting in subsequent increase in luminal LPS and gut permeability. Higher degree of gut permeability can increase plasma levels of LPS, resulting in hyperphagia and obesity. By binding to TLR4 and its co-receptors, LPS triggers a cascade of responses, ultimately resulting in the release of pro-inflammatory molecules that interfere with the modulation of glucose and insulin metabolism.

Gut microbiota affect expression of obesity related genes in host. ANGPTL4/FIAF overexpression can stimulate triglyceride metabolism, reducing adipose tissue weight. It is a circulating lipoprotein lipase inhibitor expressed in the intestine. Some gut epithelial microbiota are capable of selectively inhibiting it, causing adipocyte triglycerides to accumulate. Such microorganisms even downregulate expression of AMPK that further inhibits fatty acid oxidation.

Obesity is a serious health problem in both developing and developed countries and is associated with other complications including type 2 diabetes, ischemic heart disease, hypertension, obstruction, sleep apnea and even cancer. The human gut microflora heavily influence host metabolism and play a significant role in the development of obesity. The composition of gut microbiome depends on factors including location, diet and age. In addition, intake of too many antibiotics is harmful to the gut microflora and reduces its diversity. Prebiotics, probiotics and symbiotics are the ways to go in order to replenish and maintain a healthy gut microenvironment.



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DNA FINGERPRINTING

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The DNA is a Deoxyribonucleic acid, where it is a hereditary material. The genetic material of all living organisms and chemically called as nucleic acid. DNA contains sugar, phosphoric acid and nitrogenous bases. Watson and crick designed the structure of DNA in 1953. The genetic information in DNA is converted to characteristics features of living organisms like colour of the skin and eye, height, intelligence, ability to metabolise particular substance, ability to withstand stress, susceptibility to disease and ability to produce certain substances. The segment of DNA that contains information for a protein is known as gene. DNA is transmitted from parents to off springs and then transfer genetic information from one generation to another.

The DNA Finger Printing is the method in which variable elements are isolated and identified within the base pair sequence of DNA. The DNA Fingerprinting was invented in 1984 by sir Alec Jeffreys after he realised to detect variations in human DNA, in the form of minisatellites. DNA finger printing is a technique that simultaneously detects lots of minisatellites in the genome to produce a pattern unique to an individual. A sample of cells such as skin, hair and blood cells are first obtained and then DNA is extracted from the cells and purified. The DNA Finger printing is a laboratory technique used to establish a link between biological evidence and suspect in a criminal investigation. The DNA sample taken from an investigation crime area is compared with suspected DNA sample. If the two DNA profiles match, then the evidence shows the suspect. Conversely, if two DNA profiles do not match then evidence cannot have come from the suspect. The DNA



fingerprinting has a tremendous impact on criminal and justice system. It also plays an important role in paternity. There are various methods for analysis of DNA to establish if two samples are the same or different. This is referred as DNA fingerprinting. For example, two cloned pieces of DNA can be studied in laboratory to determine if they have portions in common, and thus overlap with one another. In a different setting, such as a crime scene, DNA samples can be collected and analyzed to determine if they match DNA samples obtained from suspects of that crime. If two DNA samples have the same fingerprint, then there is a very high statistical likelihood that they came from the same person. Such an approach can be used to establish paternity. DNA analysis is one of the evidence which is not subjected to any change in an individual life time, which aids in proper identification of a person. Although, 99.9 % of human DNA arrangements are the similar in every individual, sufficient of the DNA is dissimilar that is likely to differentiate one separable from another, unless they are monozygotic twins. DNA profiling uses repetitive orders that are extremely adaptable called Variable Number Tandem Repeats (VNTRs) in Specific Short Tandem Recurrences (STRs).

DNA Fingerprinting Technique

Blood sample of suspect person is collected. The DNA is extracted from the blood cells of the suspected person. DNA is cut into fragments by a restriction enzyme. The DNA fragments are separated into band electrophoresis in an agarose gel. The DNA band pattern in the gel is transferred to a nylon membrane by a technique known as southern blotting. The radioactive probe is prepared. The DNA probe binds to specific DNA sequences in the membrane. Excess DNA probe is washed off. At this stage, the radioactivity probe is bound to DNA pattern on the membrane. X-ray film is placed next to membrane to detect the radioactive pattern.

Applications of DNA Fingerprinting

The DNA fingerprinting has wide range of applications. It plays an important role in identification of missing or dead people. The most paternity cases require DNA fingerprinting. The microbial forensics uses the DNA fingerprinting. (The bacteria present on human skin provides information required for forensic analyst regarding the individual host and geographical location) Kinship analyses are frequently used in paternity cases. The preparation of DNA profiles from skeletal remains can be essential in the personal identification of missing persons and victims of mass disaster. Examination of non-human DNA can provide proof for wildlife crime investigation. Biological evidentiary clues such as semen, saliva and swabs are important in DNA fingerprinting and aids in identification of suspect. Individual should go through DNA analyses to rescue cases such as establishing parentage,





illegal immigration, human trafficking. DNA fingerprinting is also helpful in identifying ancestry of an individual. DNA fingerprinting has been successfully applied to plants to develop genetic profiles. It has become an important tool in diverse fields of plant population research, e.g. breeding system, estimating of selfing rates, paternity and maternity.

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SECRETION OF INSULIN IN RESPONSE TO DIET AND HORMONES

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Insulin is a hormone made by an organ located behind the stomach called the Pancreas. The pancreas is a complex gland active in digestion and metabolism through secretion of digestive enzymes from the exocrine portion and hormones from the endocrine portion. Each pancreatic islet is composed of α , β , δ , ϵ and PP cells; these are primarily endocrine (hormone secreting) cells, containing numerous secretory granules with stored hormone molecules, ready for release upon receipt of the appropriate stimulus. The hormone insulin was first isolated in the 1960's Dorothy Hodgkin defined its tertiary structure. During translation of preproinsulin from its mRNA, the N-terminal signal peptide is cleaved to yield proinsulin.

The proinsulin molecule is a single chain polypeptide containing both the B-chain and the A-chain. In proinsulin, two chains are connected by C-peptide, which is cleaved to release C-peptide and the remaining insulin molecule, which contains the A and B-chains connected via two disulfide bonds. Although insulin and C-peptide are coreleased from cell secretory vesicles into circulation, only insulin is biologically active in regulating blood glucose. C-peptide, however, can serve as a useful clinical and research measure of endogenous insulin production, in patients receiving exogenous insulin injections. Insulin is then released from the pancreas into the bloodstream so that it can reach different parts of the body. Insulin has many effects but mainly it controls how the body uses carbohydrates found in certain types of food. Carbohydrates are broken down by the human body to produce a type of





sugar called glucose. Glucose is the main energy source used by cells. Insulin allows cells in the muscles, liver and fat (adipose tissue) to take up this glucose and use it as a source of energy so they can function properly. The Insulin is an anabolic hormone that promotes Glucose uptake, Glycogenesis, Lipogenesis, and Protein synthesis of Skeletal muscle and Fat tissue through the Tyrosine Kinase Receptor Pathway.

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COVID-19 VACCINES

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A COVID-19 vaccine is a vaccine intended to provide acquired immunity against severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus that causes Coronavirus Disease 2019 (COVID-19). On 11th March 2020, World Health Organization (WHO) Director - General, Dr. Tedros Adhanom Ghebreyesus, declared to the world that COVID-19 could be characterized as a pandemic. At the time, there were no COVID-19 vaccines available. As countries across the world fell into lockdown the full impact of the pandemic was only beginning to be felt on millions of lives, and billions of livelihoods, around the world. The COVID-19 vaccines are widely credited for their role in reducing the spread, severity, and death caused by COVID-19. Hope came in the form of vaccines as researchers across the globe raced to develop safe and effective COVID-19 vaccines. By the end of the year, thanks to one of the largest responses to a health crisis from the scientific community in history, we had not one, but several vaccines ready for rollout. COVAX is now hard at work delivering these vaccines worldwide.

In September 2020, approval and commencement of distribution of a vaccine by the first quarter of 2021 was planned. The first recipients were to be 30 million health workers directly dealing with COVID patients. On 1st January 2021, the Drug Controller General of India (DCGI) approved emergency use of the Oxford-AstraZeneca vaccine (local trade name "Covishield"). On 2nd January, the DCGI



also granted an interim emergency use authorization to BBV152 (trade name "Covaxin"), a domestic vaccine developed by Bharat Biotech in association with the Indian Council of Medical Research and National Institute of Virology. This approval was met with some concern, as the vaccine had not then completed phase 3 clinical trials. Due to this status, those receiving Covaxin were required to sign a consent form, while some states chose to relegate Covaxin to a "buffer stock" and primarily distribute Covishield. COVID-19 vaccination rolled out in AIIMS, New Delhi, India on 16th January 2021.

India began its vaccination program on 16th January 2021, operating 3,006 vaccination centers on the onset. Each vaccination center will offer either Covishield or Covaxin, but not both. Nearly, 165,714 people were vaccinated on the first day of availability. Difficulties in uploading beneficiary lists at some sites caused delays. In the first three days, 631,417 people were vaccinated. Of these, 0.18 % reported side-effects and nine people (0.002 %) were admitted to hospitals for observation and treatment. Within those first days, there were concerns about low turnout, due to a combination of vaccine safety concerns, technical problems with the software used, and misinformation.

The first phase of the rollout involved health workers and frontline workers including police, paramilitary forces, sanitation workers, and disaster management volunteers. The second phase of the vaccine rollout covered all residents over the age of 60, residents between the ages of 45 and 60 with one or more qualifying comorbidities, and any health care or frontline worker that did not receive a dose during phase 1. Online registration began on 1 March via the Arogya Setu app and Co-WIN ("Winning over COVID-19") website. Amid the beginnings of a major second wave of infections in the country, vaccine exports were suspended in March 2021. The DCGI issued a standard emergency use authorization to Covaxin on 11 March 2021. From 1 April, eligibility was extended to all residents over the age of 45. On 8th April, Indian government called for four-day *Teeka Utsav* ("Vaccine Festival") from 11 to 14th April, with a goal to increase the pace of the program by vaccinating as many eligible residents as possible. By the end of the *Utsav*, India had reached a total of over 111 million vaccine doses to-date. The third phase, Sputnik V approval, On 12th April, the DCGI approved Russia's Sputnik V vaccine for emergency use in India. A phase 3 trial had been conducted in the country in September 2020, which showed 91.6 % efficacy. The local distributor Dr. Reddy's Laboratories stated that it planned to have the vaccine available in India by late-May 2021.



On 19th April, it was announced that the next phase of the vaccine program would begin on 1st May, extending eligibility to all residents over the age of 18. Registration for the next phase began on 28 April; a single-day record of nearly 13.3 million people registered. Due to supply issues, several states, including Delhi, Gujarat and Madhya Pradesh announced that they would delay their wider rollouts of vaccines to later in the month. The initial shipment of 150,000 Sputnik V doses arrived on 1st May, and began to be administered on 14th May. An estimated 156 million doses are expected between August and December; initially, doses will be sourced from Russia, but domestic production is expected to begin by August 2021.

On 25th May, India exceeded 200 million vaccine doses administered in total. On 3rd June, the Ministry of Health and Family Welfare pre-ordered 300 million doses of a potential fourth vaccine, Corbevax, which is undergoing phase 3 clinical trials. By June 23 about 78 percent of vaccines had been administered via walk-in registration. As of 28 June 2021, India has administered 329,029,510 doses overall, including first and second doses of the currently-approved vaccines. Scientists around the world are working faster than ever to develop and produce vaccines that can stop the spread of COVID-19.

Leading COVID-19 Vaccines across the World

Name of the Vaccine	Company and Country where the vaccine is developed
Covaxin	Bharat Biotech, India
Covishield	Serum, India (AstraZeneca's Vaxzevria jab)
Sinopharm	Beijing, Wuhan
Sinovac	China
Gamaleya (Sputnik V)	Russia
Moderna, Novavax, Johnson & Johnson	United States
Oxford-AstraZeneca	Sweden, UK
Pfizer-BioNTech	Germany, US



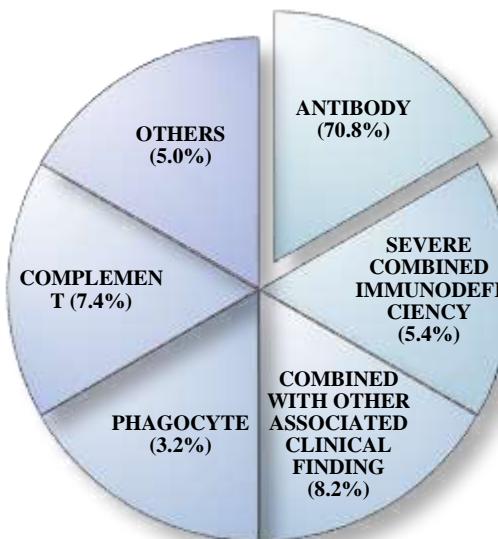
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PRIMARY IMMUNODEFICIENCY DISEASES

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Primary immunodeficiencies constitute a large group of diseases, including more than conservatively defined hereditary disorders affecting development of the immune system, its function, or both. These diseases have a wide spectrum of clinical manifestations and laboratory findings. The spectrums of patients with PID are classified according to a modified WHO classification as shown in below given Figure.



Distribution of Primary Immunodeficiency Diseases



PIDs is been diagnosed based on clinical manifestations and haematological/biochemical laboratory tests. The clinical phenotypes of patients are heterogeneous; therefore, the accurate identification of an underlying PID may not always be possible. The appropriate diagnosis of PIDs is crucial for initiating proper management and reducing infection-associated morbidity and mortality, and therefore, detection of specific protein abnormalities or significant genetic variants is crucial. Particularly, molecular tests are essential for identifying the structural abnormalities of genes, thus enabling accurate diagnosis. However, genetic tests are time-consuming, laborious and costly.

Flow Cytometry (FCM) is a diagnostic tool used for both Immunophenotyping and functional assays of Immune cells in real time. FCM has been used to assess functional characteristics linked to PIDs. Thus, it can be an initial screening test for rapid diagnosis in a spectrum of patients suspected of having PIDs. For example, the absence of T cells, Natural killer (NK) cells or both in Severe Combined Immunodeficiency (SCID), absence of B cells in a Gammaglobulinemia, or markedly reduced Dihydrorhodamine (DHR) in X-linked chronic granulomatous diseases (XL-CGD) provide very significant evidences of PIDs and should draw the immediate attention of clinicians to start therapeutic intervention while awaiting results of confirmatory genetic tests. Considering the reports from major databases, antibody deficiencies are the most common PID, which comprise more than half of all patients. Other well-defined immunodeficiencies, combined T- and B- cell immunodeficiencies, and phagocytes defects are also relatively common. Among them, Common Variable Immunodeficiency (CVID) seems to be the most common symptomatic PID.

The exact prevalence of PIDs in the general population is unknown. Although the overall prevalence of PIDs had been estimated to be 1 per 10,000 individuals, excluding asymptomatic IgA deficiency, recent reports indicated a higher prevalence of PIDs worldwide; this prevalence may differ among different ethnic groups and countries, while the discovery of new PIDs, infectious and otherwise, may necessitate a revision of previous estimates of the frequency of PIDs in the general population. There is no single system of classification of the large and heterogeneous group of primary immunodeficiencies that suffices for every educational or clinical purpose. One may distinguish, for example, antibody or humoral immune defects, combined immunodeficiencies (affecting both specific humoral and cellular immunity), phagocytic cell defects, complement deficiencies, and other defects of innate immunity or immune dysregulation.



The PID results in an ineffective immunological balance between the patient and environment. Thus, interventions to bias this balance toward host defense and away from allowing for pathogen success should be considered a general goal. In some instances of PID, specific holes in host defense can be filled through therapeutic intervention, while in others treatments are more directed at globally reducing susceptibility to infection. It can be critical to the well-being of the patient to strive towards striking this balance as perfectly as possible, while maintaining the general health of the patient and their family. As the variety of treatments and management options available to patients affected by the different diagnoses is often specific to a particular diagnosis, this section is only focused upon more general concepts of the expert care applied to and range of options available for PID patients. Essential general issues in the care of PID patients that can help create an effective structure to prevent and contend with disease morbidity include educating the patient about their diagnosis, insuring general health maintenance, and providing continuity in sub-specialty care.



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GUT MICROBIOTA AND SEROTONIN

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The human body consists of 10-100 trillion symbiotic microbial cells (even outnumbering our own human cells), collectively known as the human microbiota. Primarily, this population resides in the gut. Although host-associated microbes are presumably acquired from the environment, the composition of the microbiota, especially in the gut, is surprisingly different from free-living microbial communities. An analysis of bacterial diversity from free-living communities in terrestrial, marine, and freshwater environments as well as communities associated with animals suggests that the vertebrate gut is an extreme. In contrast, bacterial communities from typically extreme environments, such as acidic hot springs and hydrothermal vents, are similar to communities in many other environments. This suggests that coevolution between vertebrates and their microbial symbionts over hundreds of millions of years has selected for a specialized community of microbes that thrive in the gut's warm, eutrophic, and stable environment. The four dominant bacterial phyla in the human gut are Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Most bacteria belong to the genera *Bacteroides* sp., *Clostridium* sp., *Faecalibacterium* sp., *Eubacterium* sp., *Ruminococcus* sp., *Peptococcus* sp., *Peptostreptococcus* sp., and *Bifidobacterium* sp..

Gut microbiota play a fundamental role in promoting various homeostatic functions such as digestion and nutrition (including development of gut function), immunity, metabolism, etc. They might also play a role in obesity and bowel diseases. As a part of their metabolic role, gut bacteria salvage calories, produce short chain fatty acids, produce arginine and glutamine, synthesize Vit. K and folic acid, as well as participate in drug metabolism. These microbes also prevent colonization by



pathogenic bacteria, by competing for nutrients and producing a variety of substances that inhibit or kill potential pathogens. Immunomodulation includes stimulation of IgA production, promoting anti-inflammatory cytokines and down regulating pro-inflammatory cytokines and also induce regulatory T cells.

It has also been found that gut bacteria also produce hundreds of neurochemicals that are used to regulate physiological and mental processes such as learning, memory and mood. Indigenous bacteria manufacture and regulate more than 95% of the host's serotonin (5-hydroxytryptamine, 5-HT), which influences both mood and the GI activity. The gastrointestinal tract contains much of the body's serotonin. The microbes play a critical role in this mechanism, Indigenous bacteria promote 5-HT biosynthesis from Enterochromaffin cells (ECs) which are a type of enteroendocrine cells. They modulate neuron signaling in the Enteric Nervous System (ENS). This serotonin acts in synergy with other digestive hormones and regulates gastrointestinal reflexes. Serotonin is an essential modulator of mucus secretion and sensory transduction.

Bacteria in the gut produce metabolites which signal the colonic ECs which in turn increase tryptophan expression and 5-HT biosynthesis (tryptophan being the precursor of serotonin) after which serotonin is secreted, increases uptake by platelets, leading to increased stimulation of myenteric neurons. The microbiota regulates blood 5-HT levels, and could impact development or function of 5-HT producing ECs. This synthesis/secretion of 5-HT is via a direct signaling of a soluble, bacteria modulated factor. The host's serotonin relies on bacteria that interact with the host to produce serotonin i.e., via stimulation of host intestinal cells to produce serotonin.

Microbiota dependent 5-HT impacts host physiology, modulating GI motility and platelet function. GI motility is the movement of food from the mouth, through the pharynx, stomach intestines and is responsible for digestion. Serotonin enhances platelet pro-coagulant properties (5HT is released to promote coagulation) and activation during platelet tissue factor uptake. Depletion of serotonin content in platelets partially impairs their functionality. 5-HT causes contraction of blood vessels; aggregating platelets release serotonin, evoke vasoconstrictor responses. Platelets secrete serotonin to promote homeostasis and distribute to various body sites. Indigenous microbiota also modulates hippocampal levels of 5-HT, revealing the role of microbiota in regulating the brain serotonergic system.



Gut derived 5-HT regulates diverse functions such as enteric motor and secretory reflexes. It initiates peristaltic reflexes, activates afferent neurons, and transmits information to the CNS. Regulation of immune responses include contribution to innate and adaptive responses via 5-HT receptors present in the immune system, also aid communication with the brain. Bone development regulation also takes place, peripherally 5-HT inhibits bone formation, while in the brain, it has a positive and dominant effect, enhancing bone formation.

Tryptophan hydroxylase (Tph) is the enzyme that catalyzes the first step in synthesis of serotonin. It exists in two forms, Tph1 and Tph2. These isoenzymes mediate non neuronal and neuronal biosynthesis of 5-HT. In the CNS, it is produced in the raphe nuclei located in the brain stem. In the ENS, secretion takes place by ECs. Gut microbiota require host Tph to up-regulate peripheral 5-HT. In culture, 5-HT is reported to stimulate growth of *Enterococcus faecalis*, *E. coli*, and *Rhodospirillum rubrum*. These processes impose further questions such as, whether members of microbiota alter host 5-HT biosynthesis to, in turn, support colonization, growth and resilience of particular gut microbes, does the bacteria mediated increase in serotonin impact cellular immune responses. This gives us a pathway to explore additional implications on microbially induced 5-HT on host health, mental health, and disease.



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MICROORGANISMS AND THEIR PRODUCT IN CANCER THERAPY AND PREVENTION

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It is widely recognized that many bacteria can fight a variety of human disease and indeed the American Academy of microbiology convened an interesting meeting in 2014 in San Diego on the topic 'Bugs as Drugs' emphasizing the important role that bacteria play in fighting various diseases. The recent emphasis in this regard involve the role of human microbiome comprising of bacteria, archaea, fungi and protozoa, whose number is 10 fold higher than the human cell themselves. Many such gut bacteria have been implicated in immune modulation and protection of the human body from attack by external pathogens. However, the disease fighting role of pathogenic bacteria goes back more than 100 years when in 1892-93, William coley in New York city's Memorial Hospital observed that bacterial infection of his cancer patients often led to Tumor regression. Since then, many efforts have been made and are continually being made to use genetically- modified bacteria to fight cancer, but only with limited success in the clinical trials because of the elimination of the cancer fighting bacteria by the patient's immune system. Our product of pathogenic bacteria such as *Pseudomonas aeruginosa*. One such cancer fighting protein, azurin has shown significant Tumor regression in mice. Since protein are designated as biologics and thus requiring undergoing stringent regulation by the USFDA for clinical trials, a company CDG. Therapeutics Inc., has used a fragment of azurin termed p28, a peptide of 28 amino acids for both pre-clinical and phase-I clinical trials. P28 showed no toxicity in a variety of animals, whereupon the FDA approved a phase-I trials of p28, in 15 stage-Iv cancer patients with solid tumor such as melanoma, colon, surcoma, prostate and prancreae. These tumors were resistant to



all conventional drugs and the patients were terminally ill with a life expectancy of about 6 months. When administered through intravenous injections, p28 demonstrate very little toxicity but significant beneficial effects including partial and complete regression of these drug resistant tumors in 4 patients. Encouraged by such result, the National Cancer Institute (NCI) sponsored a second phase-1 trial in 11 major hospitals in the US in pediatric brain tumor patients in October, 2013. That trials has been ongoing for more than 2 years suggesting that p28 not only demonstrated acceptable toxicity but significant regression of the tumors in some patients. Indeed it is important to note that the USFDA has approved on December 02, 2015, the designation of azurin- p28 as an orphan drug for the treatment of brain tumor glioma. Another company Amrita Therapeutic in India has developed similar bacterial peptide as potential anticancer drugs, indicating the role that bacterial protein/ peptide can play in cancer therapy.



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IMMUNE RESPONSE OF BATS TOWARDS ZOONOTIC VIRUSES

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Bats are source of high viral diversity and high-profile zoonotic viruses worldwide. Notably, all of the outbreaks of emerging viral diseases, like Hendra, Nipah, Marburg and Ebola virus diseases, Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) as well as the Pandemic of Corona virus disease (COVID-19), have been linked to bats. In the aggregate studies, greater than 15 virus families have been identified in at least 200 species of 12 bat families around the world. The adaptive evolution has shaped the host defense system of bats to balance defense and tolerance. The bat immune system appears to be uniquely adapted for the control of viral infection without the manifestation of Disease or Pathology.

The key, researchers found is the ability of bats to limit inflammation. Bats do not react to the infection with the typical inflammatory response that often leads to pathological damage. In humans, while the inflammatory response helps fight infection when properly controlled, it has also been shown to contribute to the damage caused by infectious diseases. The natural ability of bats to dampen inflammation caused by stress and infection may be a key mechanism underlying their long lifespan and make them unique reservoir of rapidly reproducing and highly transmissible viruses.



One key trick of many bats' immune system is the hair-trigger release of a signaling molecule called interferon-alpha, which tells other cells to "man the battle stations" before a virus invades. Bats respond to RNA virus infections by inducing a robust IFN response while controlling an exaggerated pro-inflammatory response, thus limiting virus-induced immunopathology as observed in humans infected with these viruses. The effect of inducing the IFNs in human cells may help us identify the role played by the IFNs in limiting replication of zoonotic bat-borne viruses. Innate signaling pathways are being extensively investigated in human and rodent cells and recent studies have discovered the existence of similar pathways in bats.

Many standard elements of adaptive immune system of bats, including multiple immunoglobins, antibody responses, interleukins and other cytokines and cell mediated T-cell responses have been studied to understand immune response of bats. The antibodies that arise in bats in response to virus infection may control viruses via mechanism that is independent of virus neutralization. At genomic level bats appear to have a much larger repertoire of germ line genes encoding immunoglobulin variable, diversity and joining segments than humans, which indicates that they rely more on their germ line repertoire to respond to infections. The genomes and transcriptomes of at least 18 bat species are currently available, providing important insights into the evolution of their immune system and antiviral immunity. With COVID-19 pandemic outbreak originating from bats, it is now more important than ever to focus time and interest into bats as viral reservoirs to gain an understanding of their immunology to reduce the emergence of new viruses from bats and manage their spread in future.



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EXPLORING THE GUT-MICROBIOME

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The human microbiota is a specific community of microorganisms inhabited inside and on the human body which includes bacteria, viruses, fungi, protozoa, and archaea. The womb of a mother is primarily a sterile environment devoid of any other living organisms. However, as a kid enters the world, it is covered by an invisible layer of friendly microbes from the mother's birth canal. These little creatures invade the human body during birth or shortly thereafter and remain there throughout life which is commonly referred to as the normal flora of the body, they are our body armour shields and play a vital role in protecting our general well-being and health. Not all of the bacteria in our bodies are harmful some of them are useful to us in some manner. Many regions of the human body have normal flora, including the skin, respiratory tract, urinary tract, and digestive tract. Bacterial flora in normal person includes *Streptococcus* spp., *Cutibacterium* spp., *Haemophilus* spp., *Staphylococcus* spp., *Anaerobes*, *Enterococcus* spp., *Lactobacillus* spp., *Candida* spp. and so on. Whereas certain parts of the body, such as the brain, lungs, and circulatory system, are meant to be sterile. Our human body consists of 90 % bacterial cells and just 10 % human cells, indicating that we are more microbes than a human. These microbes proliferate to become a three-pound microscopic organism within our gut that weighs just like our brain, known as the gut microbiome.

Gut-microbiome research has been carried out extensively around the world, including at universities such as the Universities of Calgary, Universities of Colorado Boulder, and Universities of California San Diego, which have contributed greatly to the field of the gut microbiome. The gut microbiome, as described by



molecular scientist Joshua Lederberg, is the collection of bacteria and their genetic material found in the gastrointestinal system. Our gut is made up of a long tube that runs from our mouth to our back passage. The central nervous system and the enteric nervous system are linked by a bidirectional axis, often known as the gut-brain axis. This gut-brain axis in the simple term refers to the relationship between the gut and the brain. Even the complicated interaction between neuro-immuno-endocrine mediators is mediated by this axis. As a result of this phenomenon, the human gut is frequently referred to as the "second brain". It enables the brain to regulate our intestine, and eventually, all of the gastrointestinal tract's cells are influenced by the gut microbiome.

The digestive system is made up of four phyla: *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*. The human diet has a significant impact on the gut microbiota. Eating plenty of vegetables, fruits, whole grains, proteins, and fibre is the core of a nutritious diet. But nowadays modern human diet contains less nutritional fibres and more processed carbs, causing our gut microbiome to change our mental health. These processed foods are highly addictive and stimulate our brain's dopamine (also known as a feel-good hormone) regions, which are associated with pleasure and satisfaction but can cause inflammation throughout the body and brain, perhaps contributing to mood disorders such as anxiety and depression. It can be resolved by establishing healthy eating habits and consuming sufficient amounts of fruits, nuts, whole grains, and fermented foods to promote the growth of beneficial bacteria. The gut microbiome can produce a variety of metabolites and bioproducts that are required to protect the host's and gut's health. Numerous gut resident bacteria, known as probiotics, are protective against cancer progression. According to an ongoing study, probiotics are being researched for their capacity to treat dysbiosis in patients who are receiving chemotherapy or radiotherapy. The positive benefits of anti-cancer immunotherapy may be enhanced by gut inhabitant species.

Many studies and strategies have been used to improve microbiota over the last decade, including faecal microbiota transplantation, prebiotics, probiotics, a healthy diet, and a healthy lifestyle, all of which have been demonstrated to encourage microbiota function, which will be utilized in the future to improve brain and mental wellbeing as well as the diagnosis and control of disorders linked with it. Faecal Microbiota Transplantation (FMT) is a treatment in which the recipient's gut receives filtered faeces from a healthy donor via nasogastric or nasoduodenal tube, colonoscopy, enema, or capsule. This medication has been demonstrated to be effective and used to treat gastrointestinal illnesses such as *Clostridium difficile* colitis. Dystopia, chronic tiredness, fibromyalgia, Parkinson's disease, multiple sclerosis,



obesity, insulin resistance, metabolic syndrome, and autism are among the conditions for which FMT is being investigated. In recent FMT studies, it was discovered that the bacteria that was introduced was a multi-drug resistant strain that was responsible for the patient's death. In another case, after receiving *Escherichia coli*, a sick patient developed extended spectrum-beta-lactamases, which are resistant to a wide spectrum of medicines already available. To some extent, microbial therapies like FMT can help with illness prevention and therapy, but there are still a lot of unknowns. Understanding the beneficial and harmful microorganisms, as well as the appropriate use of these microbial therapies, are issues that should be worked on to ensure patient safety and therapeutic efficacy. The gut flora is essential for nutrition, immunity, and effects on the brain and behaviour, therefore this is one of several areas of research.



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INFLUENCE OF GUT MICROBIOME AS A THERAPEUTIC AGENT FOR CANCER

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Introduction

The microorganisms that colonize and proliferate in the gut are called gut microbiota or gut microbiome. The human body is colonized by several microbial population, among which greatest richness appears to be in gut. It comprises a variety of bacteria, fungi, and archaea. It has been documented that these gut microbiomes play a crucial role in pathogenesis and manifestation of diseases. They also influence the health and immune response of the body towards infection and the treatment it undergoes.

Cancer has been recorded as the second largest cause of deaths across the globe. Few microbes are known to cause cancer; and few are involved in oncogenesis process and induce inflammation or immunosuppression which leads to cancer. On the contrary some microbes have shown immunomodulatory effect and promote cancer therapies. Analysis of interaction between gut microbiota and immune system have shown their ability of enhancing anti-tumor responses through different ways. The anti-tumor activity is observed with certain composition of microbiota.

Dysbiosis could takes place during the time of infection, affecting homeostasis and in disturbance in various species population. This might intend to increase the inflammation which in turn enhances the metastasis of tumors. Hence, it becomes necessary to maintain microflora that exhibits anti-tumor activity during treatment



for effective cancer therapy. The microflora effects different types of cancer therapies. Few therapies are explained below - fecal microbiota transplant, chemotherapy, prebiotics, probiotics, synbiotics, antibiotics administration, traditional Chinese treatments etc.

Fecal Microbiota Transplant (FMT)/Stool Transplantation

In this method, healthy stool is received from a healthy donor and is transplanted into the patient in the form of processed stool. The process adds healthy bacteria into the microflora of the patient. The technique is used to modulate the gut microbiota in cases of dysbiosis and to clear infections caused by *Clostridium difficile*. Illustrating here few studies that responded to modulation of microbiome. On Ruminococcaceae modulation, it was found that the activity of Cytosine-Guanine oligonucleotides (CpG-ODNs) increased, which enhanced the tumour antigen presenting capacity. This when introduced to patient along with chemotherapy, boosted tumour antigen presenting capacity, induced proinflammatory response at the site of action and activated dendritic cells, that either inhibits further growth of existing tumours or dissolve tumours.

When *Bifidobacterium adolescents* was introduced in patients and in mice model, programmed cell Death protein-1 (PD-1) blockage increased. This led to increase in immunity by activation of dendritic cells followed by increase in CD8+ T cells. If the PD-1 pathway is not blocked, it leads to activation of its ligands, PD-L1 and PD-L2, following which cell proliferation is induced and anti-tumour activities are degenerated. (Matson *et al.* 2018)

Prebiotics

As per WHO, Prebiotics are non-viable mixture of cells that have the ability to modulate the composition of microbiota in the administered patient. Prebiotics of different forms have proven to be therapeutic via increasing healthy gut microflora or supplementing with therapeutic agents. Oats consumption provides avenanthramide-C. This compound on metabolism by gut microflora exhibits anti-inflammatory and anti-proliferative property reducing the tumour progression. Hops plants which are used in beer production contain prenyl flavonoids. These on augmentation by the microflora, have shown anticancer effects in colorectal cancer (CRC) study models.

Probiotics

Probiotics are live microorganisms, which when consumed impart a profound impact on health. These have shown significant benefits in CRC suppression. Probiotic cocktails of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and



Bifidobacterium infantum have shown to increase beneficial bacteria. The cocktail if enriched with oligofructose and maltodextrins can reduce the population of *Pseudomonas*, *Congregibacter*, *Clostridium*, *Escherichia*, *Helicobacter* and increase healthy microbes such as *Prevotella* and *Oscillibacter*. These microbes release metabolites that are anti-inflammatory in nature which further reduces metastasis and tumour growth.

Synbiotics

These are mixture of probiotics and prebiotics. Use of synbiotics during the neoadjuvant therapy for oesophageal cancer has proven that the gut microflora turns healthy and beneficial. Dysbiosis caused during chemotherapies could lead to disorders and development of adenocarcinomas, thus using synbiotics could play an effective role in preventing this.

Antibiotics

If antibiotics used are of broad spectrum, they are known to deplete the gut microflora and if administered at higher dose (Example - Penicillin) have shown to induce gastric, oesophageal, and intestinal cancer. When enterotoxigenic *Bacteroides fragilis* population was treated with Cefoxitin, it was observed that growth of colon adenoma and progression of IL-17A dependent carcinogenesis was reduced.

Conclusion

Microflora is a very diverse community present in our body. They play an important role in pathogenesis and treatment. Much focus is needed to unravel the mechanisms for effective therapy and discover novel techniques to incorporate them in diets and consume as supplements. Based on the type of therapies, there is a lot of scope to discover new targets, diagnostic and prognostic biomarkers employing human gut microbiome to treat cancer. Administering the required microorganisms along with chemotherapy and immunotherapy is a promising method in controlling cancer.



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PROBIOTIC - THE REPLACEMENT OF ANTIBIOTIC

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Probiotic are microbial food supplement which are beneficiary for the host as it improves the intestinal microbial balance. Clinical studies suggest that probiotic helps in immune system, prevention of allergic diseases, GI tract of inflammatory diseases and cancer prevention. They are used to improve lactose intolerance and prevent Diarrhea. The use of non-digestable oligosaccharide (prebiotic) can fortify microbes of intestine and stimulate their growth.

Pathogens developed resistance to antibiotic at a rate much faster than the discovery of new antimicrobial compound. Many reports have shown that multi drug resistance bacteria isolated from boilers spread diseases amongst humans. Many European countries are planning to ban the inclusion of antibiotic feed. There are various benefits of probiotic such as some species help in intestinal angiobenefits, Establishment of immune system, processing of fats and potentially even long life. Probiotic helps to restored microbial numbers inside the body because many dies or are excreted daily in stool. Many gets died due to the use of antibiotic.

Probiotics can reduce the risks of antibiotic which reduce superinfection in the vaginal gut. It also secretes antibacterial substances that lower bacterial population at distant mucosal site and disrupts biofilm, making it easier for antibiotic to function. It also enhances mucosal immunity which help in irradiation of the organism at the mucosal site.



Many evidence has shown that Probiotic can prevent diarrhea or vaginal infection. Probiotics can be substitute for the microbes that have been destroyed by the Antibiotic. This can be done by number of mechanism, including indirectly reducing excess electrolyte release, acting on the physiological process of gut mobility, signaling down the regulation of toxin released by *Clostridium difficile* or inhibiting growth of yeast and other opportunistic pathogen. Some probiotic *Lactobacillus* strains may inhibit or kill intestinal pathogen, including viruses and down regulate toxin release in *Escherichia coli*. As well as *Staphylococcus* exotoxin. In some cases like, *Escherichia coli* 0157:H7, antibiotic are not a treatment option because they cause the release of toxin and thus, if probiotic organism function in vivo in the same way as suggested by in vitro experiment. *Helicobacter pylori* were randomly assigned to its probiotic therapy or a placebo during *Helicobacter pylori* antibiotic treatment. After 3 weeks later, no significance differences in individual symptoms were found between the two groups.

Probiotic are safe for the majority of the population, but side effects can occur. The more common side effects are temporary increase in gas, bloating, constipation. Some people may get allergy due to probiotic supplement. For intake of transgenic probiotic, a person should be well known about human microbiota and should take probiotic in an adequate manner.



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ROLE OF MICROBIOLOGIST IN FIGHTING AGAINST COVID - 19

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"Always trust a microbiologist because they have the best chance of predicting when the world will end".

As we all know, Covid19 is caused by SARS CoV2 which is a virus belonging to the Coronaviridae family. The method to diagnose Covid19 infection is to detect its RNA in a respiratory sample. This method is known as RT-PCR. It involves collection of a nasopharyngeal and/or an oropharyngeal swab in a suitable viral transport media followed by Extraction and Amplification of its Genetic material (RNA) which leads to its detection.

Microbiologists and Microbiology Laboratory Technicians are at the forefront of this Diagnostic process. New research has said that clinical microbiology is critical to understanding COVID-19, with laboratories studying the coronavirus globally. Microbiologists are playing a crucial role as a frontline worker along with other essential professionals during the current COVID-19 outbreak. Working tirelessly to provide reliable and timely diagnosis of SARS CoV2 infection. In the pandemic time, their routine work starts with collection and careful handling of samples with suspected SARS COV2 infection, processing of these samples with adequate quality control and delivering accurate and timely test results. The positive results are communicated to the clinical team as soon as possible. This is quite helpful in designing the best treatment plan for admitted patient's microbiologist.



Microbiologist plays a crucial role with the following activities:

- Establishing a Viral testing laboratory
- Conductance of Polymerase Chain Reaction (PCR) tests
- Specimen handling and transportation
- Guidance on specimen collection and best diagnostic tests
- Confirming diagnosis of Covid-19
- Assisting in development and production of Covid-19 vaccine and antiviral therapies
- Assisting in developing rapid and confirmatory covid-19 diagnostic tests with high specificity and susceptibility.

The outbreak of COVID-19 has taught the importance of hand- hygiene to all. Today, all the hospitals have adapted to frequent disinfectants of the premises, multiple accessibilities to hand wash, proper method to handle PPE and proper disposal of the waste. Microbiologists are also doing pandemic related educational webinars for healthcare staff and community. This is to increase awareness about facts related to SARS CoV2 infection and various infection prevention measures. There is huge responsibility lies on Medical Microbiologists and improved sense of teamwork inside their laboratories and outside with other clinical team continues to drive their spirited response to the crisis.



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CURB LIFE STYLE DISEASES TO PREVENT FUTURE PANDEMICS

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We all have become familiar with the word Pandemic as we are in the midst of one now. Even though vaccines have been found we still are unsure about complete eradication of the infection and prolongation of its effect. But now the question arises, is this the end of the pandemic or are we going to face any future ones? And will they only be comprised of communicable disorders or are there any other threats? Aim of this article is to answer these questions and discuss some solutions to prevent them.

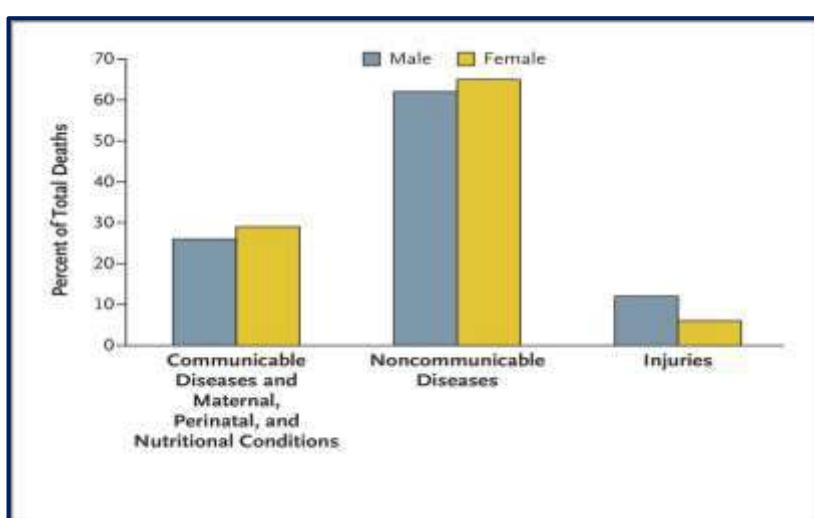


Figure - 1: Percentage of Deaths due to NCDs



Rapid rise in the percentage of total deaths, due to Non-communicable Diseases (NCD) are observed in every region in the world (Refer Figure – 1). NCDs include cardiovascular diseases, cancer, chronic respiratory diseases and diabetes. The causes of these diseases are linked to four ‘Modifiable Behavioural Risk Factors’ which includes - Tobacco use, Unhealthy diets, Physical inactivity and Harmful use of alcohol. Moreover, the rapid global spread of these risk factors is driven by forces that encompasses emergence of unplanned urbanization assisted by the globalization of the production, marketing and sales of harmful foods, beverages, alcohol, and tobacco products. Importantly, NCDs are not merely a problem of high-income countries; their impact is universal, as NCD-related deaths already occur mostly in low and middle-income countries

Vulnerable and socially disadvantaged people are more susceptible to these risk factors due to prolonged exposure to harmful products such as tobacco, unhealthy dietary practises and have limited access to health services. This proves the association of NCDs with socioeconomic status. Universality and scale of the problem make NCDs a pandemic phenomenon that requires a powerful international and domestic approach. Interventions for this approach should include both population and individual level activities, and this can be brought about by linking health science with law and policy on national and international levels. Laws can target consumers by creating incentives and disincentives that directly shape consumer behaviour (e.g. taxes and subsidies) or by facilitating behavioural changes (e.g. nutritional).

Domestic level interventions that have been identified as a good value for money involves regulations and taxations on tobacco and alcohol (general bans, age-requirements), promotion of healthy foods through transformed marketing policies, including improved packaging of commercial products, and revised fiscal strategies. Commercial products can be reformulated to reduce salt, saturated fats, and sugar consumption, and health literacy can be promoted by educating at-risk populations, so that they recognise the importance of healthy options. The same is true for the interventions aimed at individuals along with cardiometabolic risk assessment and management, early detection and effective treatment of major NCDs. Successful policy mediations are needed to be embedded in multisectoral approaches spanning various policy sectors on international level. For instance, WHO’s Framework Convention on Tobacco Control (FCTC) brings together 181 parties to address the ‘devastating health, social, economic and environmental consequences of tobacco use and exposure to tobacco smoke’ and many treaties are modelled on this for other risk factors such as Alcohol Control framework and Global Convention on healthy



diet. Amalgamation of such treaties in global market is vital to control and deplete risk factors of NCDs. Coordination between health science and law is crucial to identify and develop more effective regulations and policy options, in particular to bring out the 'Best Practices'. This process will be catalysed if co-operation of societies increases in research. Thus, to lessen the impact of NCDs on individuals and society, a comprehensive approach is needed requiring all sectors including health, finance, transport, education, agriculture, planning and others to collaborate to reduce the risks associated with NCDs, and promote interventions to prevent and control them.



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ROLE OF NUTRIENTS IN BACTERIAL BIOSURFACTANT PRODUCTION AND EFFECT OF BIOSURFACTANT PRODUCTION ON PETROLEUM HYDROCARBON BIODEGRADATION

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Why we need to study Biodegradation?

- Hydrocarbons are highly hydrophobic in nature and oil permeation into the soil makes the degradation process really difficult, because hydrocarbons have less water solubility and get attached to soil particles, resulting in the decreased bioavailability to microorganisms and limiting the mass transfer rate in biodegradation.
- Oil-derived pollutants enter in environments through leaks during drilling, transport and storage, discharge of water at offshore oil exploration and combustion of fossil fuels.
- In soil these organics scarcely degrade, causing damage to human wellbeing, ruining environment and imposing threats to animal and plant species.
- Due to insolubility of the hydrocarbon components the bioavailability of pollutant remains an important factor.
- Biodegradation of hydrocarbon is the application of bacterial strains capable of producing biosurfactants.



Biosurfactants

- Biosurfactants were first discovered as extracellular amphiphilic compounds in hydrocarbon fermentation.
- Bacterial biosurfactant synthesis is an important characteristic, particularly in various hydrocarbon contaminated sites.
- The role of microbial biosurfactants is to enhance the natural reduction process and intensify the extent of hydrocarbon biodegradation.
- Despite of having many attractive features, like no toxicity, renewability of resources, ease in production, high activity and natural degradability, the role of biosurfactants is commercially very insignificant.

Materials and Methods with Results

i) Screening of Bacteria for Hydrocarbon utilization

- Take soil sample from petroleum contaminated site.
- Inoculate it into Nutrient agar plate
- Add 1 % Diesel oil into Nutrient agar plate
- Observe the Hydrocarbon utilization after 13 days incubation period

ii) Drop Collapse Test

- Seven μl of Diesel oil was added in each well of Microtiter plate.
- Plate was equilibrated for 60 min at 30 °C.
- Then, 20 μl culture of each strain was added.
- Then observed the results for Collapse test.

iii) Inoculum preparation and Biosurfactant production

- Inoculate each strain in 50 ml NB medium and incubate for 24 hrs. Then strains were washed thrice using Saline.
- Bacterial suspension of 10^8 cells/ml were prepared.
- Inoculating 1 ml of 10^8 cells/ml in 50 ml of each medium supplemented with 2 % Diesel oil.
- Incubate at 30 °C for 7 days.
- Supernatants were acidified to pH 2.
- Final extraction done by 2:1 chloroform and methanol solvents.
- Crude biosurfactant were obtained.



Discussion

- Understanding the microbial processes which involved in bioremediation of hydrocarbons can improve the scope of field application of ecologically sustainable and nature friendly Biological techniques.
- The above experiment shows that the degradation of hydrocarbons increased from 16 to 28 % when biosurfactant producing hydrocarbon degrading bacteria were used as compared with strain incapable of producing biosurfactant. The availability of nutrients also affected the biosurfactant production.
- It is recommended that to achieve prolific bioremediation, maintaining appropriate level of nutrients is vital as it enhance biosurfactant production that further increase the rate of hydrocarbon degradation.
- After this experiment the trend of degradation by bacterial strains portray clearly that all biosurfactant producing strains performed higher biodegradation compared to non- biosurfactant producer.



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MICROBIAL FUEL CELL

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Microbial Fuel Cell (MFC) are bio-electrochemical system that harnesses the natural metabolisms of microbes to produce electrical power in the form of devices that use bacteria as catalyst to oxidize organic and inorganic matter and generate a current. MFC'S are the fuel cells in which chemical reaction is replaced by microbial reaction and the organic load of waste is used as fuel. Within the MFC'S, microbes consume the nutrients in their surrounding environment and release a portion of the energy contained in the food in the form of electricity. Activated Sludge is capable of producing electrons and protons ions. In an MFC, organic material is oxidized on the anode, and the product of oxidation is CO₂ and electrons. In Air-Cathode, electrons flow to the cathode and react with oxygen in the air to become water. MFC'S generate electricity by decomposing organic matter using exoelectrogens (exoelectrogens are the microorganisms that have ability to transfer electrons extracellularly) as the catalyst, the bacteria adhere directly to the anode, to which they transfer the electrons generated as a result of the decomposition. Many exoelectrogenic bacterial species, such as those of *Geobacter* genus are known. Exoelectrogens are widely present in various anaerobic environments such as soil, lake beds, sea beds, digestive tracts and excreta of animals, activated by sludge& compost.

Various forms of MFC'S have been developed for installation in outside the environment. There are various types of MFC'S such as Sediment MFC'S, Soil MFC'S, Plant MFC'S, Floating MFC'S etc., among these Sediment MFC'S and Soil



MFC'S are considered to generate electricity by decomposing organic matter contained in sediment soil, such as rotten leaves and dead creatures. This MFC'S are installed by inserting the anode in pond beds, rice-field beds, in the soil of a forest or in grasslands and placing the cathode in an aerobic environment. After approximately 3 days, the exoelectrogens that were originally present around the anode decompose the organic matter and the MFC naturally starts to generate power. Bacterial Inoculation of a pure culture of exoelectrogens and sterilization of the fuel are not necessary, hence the use of an MFC for power generation is uncomplicated.

Plant MFC'S can also generate electricity by using organic matter secreted from plant roots. A range of organic substrates can be used for anaerobic digestion by the microbes in bioelectricity production. Domestic wastewater can be used for continuous electricity production. Production of maximum power density using swine wastewater as a substrate in single-chambered MFC. Oil wastewater can also be used for bioelectricity production. Waste sludge also has been demonstrated to be an effective substrate in bioelectricity generation coupled with hydrogen production. Fruit & vegetable wastes were employed as a substrate for microbes isolated from high Andean region in a single-chambered MFC.

Choi and Ahn (2015) reported use of food waste leachate obtained from bio-hydrogen fermentation as a potential substrate toward enhanced electricity generation. In a study, simple substrates such as glucose, acetate, propionate and butyrate have been used as substrates in MFCs toward power generation. The power density measured in this study for the different substrates was in the order of acetate > butyrate > propionate. This is of particular importance because acidogenic degradation of organic wastes produce a range of volatile fatty acids which depending upon their affinity toward the microbes influence the electricity generation.

There are different designs for the construction of a MFC depending upon the number of chambers, the mode of operation, etc. These types primarily include:

i) Two-chamber MFC

This design is the classical design of a MFC consisting of dual chambers separated by an ion exchange membrane. These typically run-in batch modes but can function in continuous mode as well & are currently only used in laboratories.



ii) Single-chamber MFC

A single-chamber MFC involves only the anodic chamber coupled with an air cathode to which the protons and electrons are transferred. Various designs have been proposed for the construction of a single-chamber MFC.

iii) Stacked MFC

A stacked microbial fuel is a collection of MFCs connected with each other in series or in parallel connection. MFC can be stacked by achieving different configurations of both electrode as well as hydraulic flow. These can be of four types such as

- a) Series electrode connections in parallel flow mode.
- b) Parallel electrode connections in parallel flow mode.
- c) Series electrode connections in series flow mode.
- d) Parallel electrode connections in series flow mode.

Aelterman *et al.* (2006) reported a six times higher voltage & current output when connected in parallel as compared to series thereby implying an overall higher biochemical reaction rate. Thus, these studies imply a possible innovative modification in MFC technology which could assist in increasing the power output thereby contributing toward one of the applications of MFCs. Choi and Ahn (2013) obtained an overall increase in Chemical Oxygen Demand (COD) removal, Columbic efficiencies and maximal power densities in parallel electrode connection (series flow mode) while treating wastewater which was attributed to a higher stability of the oxidation-reduction potentials in overall cells.

iv) Proton Exchange Membrane

In MFC technology, electro-neutrality between the two chambers is a very important pre-requisite for its efficient operation achieved by the PEM on account of the transfer of protons across the membrane. PEM are a very important component of the MFC assembly assisting in separation of the anode and cathode chambers as well as facilitating transfer of protons to the cathode to sustain the electric current. The ideal characteristics of a PEM include the following criteria: Cost effective increased proton conductivity, Good segregationally properties, Increased mechanical strength, Electronically resistive and Endurance against heat and chemicals. The salt bridge is the simplest form of PEM that could be used in an MFC. The salt bridge consists of an ionic salt such as KCl or NaOH which is melted with agar & poured in a cylindrical cast & allowed to solidify. Upon solidification the bridge is placed between the two chambers of the MFC thereby acting as a PEM and facilitating the transfer of protons. Five per cent concentration of salt in the salt



bridge produces maximum power density of 84.99 mW/m^3 . The concentration of agarose in a salt-bridge MFC to find out that 10 % of agarose concentration acts as optimum for current and voltage generation. The electrons produced by the micro-organisms on account of anaerobic degradation of the substrate require a mediator to be transferred to the electrode. A mediator is a compound having low redox potential which is added to the growth media at specific concentration extracts the electrons from the metabolic reactions of the microbes in and supplies those electrons to the anode electrode, they should form a reversible redox couple at the electrode, should be link to NADH and have a high negative E_0 – value, should be stable in both oxidized and reduced form and soluble in aqueous systems. Exogenous chemical compounds used as mediators in MFC have certain demerits such as toxicity and their expensive nature. Mediator - less MFCs have also been developed and consequently applied in power generation using wastewater sewage. Another alternative to the mediators is the utilization of a salt bridge, the power generation capacity of a dual chambered MFC using sewage wastewater as a substrate with varying concentrations of agarose in the construction of salt bridge. The optimal concentration of agarose was found out to be 10 % as it showed maximum power density of 78.21 mW/m^2 . The production of bioelectricity technology is being used for its various applications both at industrial and research levels. MFC technology utilizing microbes for electricity generation involve degradation of various substrates which can be applied at an industrial level coupled with biofuel production.

The idea of using MFC'S for producing electricity dates back to 1911. Creations of MFC'S occurred sporadically throughout the rest of the 20th century. Recently the need of renewable and clean forms of energy and the need of wastewater treatment have triggered wide research interest in developing the MFC technology to address both of these human needs. For example, Scientific American had a popular article introducing the MFC technology. While renewable energy production & wastewater treatment are two long-term goals of developing the MFC technology, real-world applications of MFC'S are yet limited because of their low power density level of several thousand mW/m^2 . By extracting bioenergy from environments, the MFC technology exhibits a promising potential of powering sensors in remote locations where it is difficult to replace batteries. This experimental study investigates the performance of MFCs and provides ways of increasing the efficiency for such an application. Efforts are being made to improve the performance and reduce the construction and operating cost of MFC'S.



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Biodegradability of Macro Plastics

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Synthetic plastics are emerging environmental contaminants that have been found to be accumulating in a very large quantity. They are highly recalcitrant and not highly susceptible to get degraded. Burning or burning of this pollutant would cause serious bad impact on to the environment. They release harmful toxic materials which contributes to increased level of harmful gases in the environment. Currently certain enzymes realised by the microorganisms used to degrade this recalcitrant contaminant. Bacteria which are capable of producing these exo and endoenzymes has a unique mechanism to degrade large and complex hydrocarbons into simpler inorganic forms. Bacteria could be Gram positive or Gram negative (classification of bacteria based on their cell wall composition) and also few species of fungi are found to have capability to degrade plastic under favourable condition over a course of time. Most often involved common species are *Streptococcus*, *Pseudomonas*, *Micrococcus*, *Aspergillus glaucus* and *Aspergillus niger*. These are commonly occurring microorganisms in soil contributes majorly towards degradation of polymer like plastic, which converts the long chain polymer into simple monomers under set of favourable conditions which are no longer found to be toxic.



Factors affecting Biodegradation of Plastic

Temperature, oxygen, essential elements, salinity, pressure, pH and various biotic factors. Biodegradation of plastic is a technique and its advantages should be well known because plastic is one of the most life threatening contaminant which occurs in several forms and are highly recalcitrant which are not really prone to get degraded. Plastics are used for various purposes and we have arrived to a time where finding a substitute for it is very challenging. In agricultural industry alone 2 - 3 million tons of plastic are used per year. In other industries it's 10 - 30 times more than that or even more.

Photodegradation

Plastics which are extensively subjected to high intensity of solar radiation for a long period of time undergoes degradation by the Ultra-violet component present in the solar radiation that ranges the wavelength 0.295 to 0.400 μm . This radiation is absorbed by the plastic and breakage of bonds in the polymers is achieved. This leads to photo-oxidation. This makes the plastic from loss of transmissivity, discolouration and embrittlement of plastics. Loss of transmissivity will result in a lowering of the efficiency of a collector or drier and crazing or embrittlement will render the plastic more prone to damage by wind and rain.

Thermal Degradation

It generally causes change in molecular weight of polymer and properties of plastic such as ductility and embrittlement, chalking, colour changes, cracking, general reduction in their physical properties. It involves three mechanisms

- a) **Initiation:** Here there is loss of hydrogen atoms from the long chain polymer as a result of energy input from heat or light.
- b) **Propagation:** It involves variety of reactions and the most important one is where free radicle reacts with an oxygen molecule to form a peroxy radical, which enables removal of hydrogen atom from polymer chain to form a Hydrogen peroxide and this regenerates free radicle again. This hydrogen peroxide can then spilt into two new free radicals which will continue to propagate the reaction which will attack other polymer.
- c) **Termination:** It is achieved when the free radicals tend to produce inert products which cannot future be affected by any free radicals. This happens naturally when the free radicals combines or it can be made difficult by addition of stabilizers in the plastic.



Biodegradation

It is the capacity of one or more strains of microorganisms to utilize a synthetic polymer as the sole source of carbon and energy. The degree of polymer biodegradation in natural ecosystem is affected by several factors. Biodegradation is of two types:

- a) **Aerobic biodegradation** - Aerobic are mostly involved in scavenging of contaminants. They use oxygen as an electron acceptor and mineralization takes place. Carbon dioxide and water are obtained as main products.
- b) **Anaerobic biodegradation** - Anaerobic biodegradation occurs in the absence of oxygen or in oxygen free zone. They use nitrate, sulphate, Iron, Manganese and Carbon dioxide as their electron acceptors and mineralization takes place.

Factors Affecting the Biodegradation of Plastics by Microbes

- Physical and chemical properties of plastics mainly influence the biodegradation capacity.
- Physiochemical nature like surface area, hydrophilic and hydrophobicity, molecular weight, chemical structure, melting temperature, crystallinity etc plays major role.
- Molecular weight also plays major role because it identifies the polymer present in the plastic.
- Greater the molecular weight of polymer lower will be the degree of crystallinity. Since the enzymes mainly attack the amorphous domains of a polymer.
- Lower the Rate of crystalline part of polymer increases the rate of biodegradation.

Several steps involved in plastic biodegradation is explained using simple terms as follows

- Bio-deterioration defines the action of microbial population and others decomposers that are responsible for the physical and chemical factors that result in the successive degradation of plastics using its mechanical, physical and chemical properties.
- Biofragmentation refers to the catalytic action of the microbial enzyme that will cleave the polymer of the plastic into simpler oligomer, dimers or monomers. Usually ecto-enzymes or free radicals are usually secreted to perform the desired action.



- Mineralization is a prior step where complete degradation takes place where complex polymers are gradually broken down into simple monomers, dimers and oligomers and complete oxidised metabolites are released such as carbon dioxide, water and methane.

Biodegradation mode

Biodegrading bacteria are highly adaptive in nature to the environment and secretes both endoenzymes and exoenzymes that attack the substrate to cleave the molecular chains into simple monomers in eco-friendly way. Enzymes are proteins made up of -COOH, -OH, -NH₂ which helps the enzyme to cleave the polymer. Certain factors such as temperature, water availability, pH, oxygen supply, redox potential, and carbon and energy source influence the growth of microorganisms. These biodegrading bacteria doesn't produce any toxic substances to the environment.

- The initial breakdown is due to several physical and biological forces that could be wetting, heating, cooling can cause polymeric cracking.
- Synthetic polymers can also be degraded by microbial enzymes after which the monomers are absorbed by the microbial cells and biodegraded.
- Two types of enzymes are majorly involved they are extracellular and intracellular depolymerases.
- During this biodegradation, exoenzymes break down the complex polymers yielding smaller molecules of short chains that is dimers, oligomers and monomers that are small enough to pass the semi-permeable outer bacterial membrane.
- These small monomers are utilized as carbon and energy sources. This is called depolymerization. The end products are Carbon dioxide, water or Methane the degradation is called mineralization.

Conclusion

Natural plastics are synthesised from renewable sources that are completely biodegradable in their native form and they are found to be nothing but the components of plants, animals, and algae. Main advantage of these natural plastic over normal plastics is that the natural plastic is found to be biodegradable. There are many archaea and bacteria synthesize biodegradable plastics which are also polymers but they are found to be biodegradable, eco-friendly and biocompatible. Polyhydroxyalkanoates (PHA) are the best alternative over petrochemical plastics among the various biodegradable polymers. The properties of PHA are also just like polyethene and polypropylene. Many microorganisms accumulate PHA as



intracellular energy and store carbon inclusions. There are many other nutrient elements that are present such as Nitrogen, sulphur, Oxygen and phosphorous. It is observed that there are different types of PHA. And the classification is based on the number of repeating units in the polymers. Short-chain-length PHA (scl-PHA) is the polymer that contains monomers of C3 and C5 hydroxyl fatty acids Eg: Polyhydroxybutyrate (PHB) and Hydroxyvalerate (PHV). In the same way the polymers made up of C6 and C16 hydroxyl fatty acids or aliphatic carbon sources are called as medium-chain-length PHA (mcl-PHA).



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MONOCLONAL ANTIBODIES

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What are Monoclonal Antibodies?

The Serum contains totally Different kinds of Antibodies (Polyclonal) that are specific for several different antigens. In the animal, antibodies are synthesized primarily by Plasma cells, a type of B lymphocyte. Since, Plasma cells cannot be grown in tissue culture, they are not used in *In vitro* source of Antibodies. Kohler and Milstein (1975) developed a method that permits the Growth of clonal populations of cells releasing homogeneous antibodies with a specificity. In this, an antibody-releasing cell is isolated from an immunized animal, then fused with a Myeloma cell, a type of B-cell tumor. These hybrid cells during *in vitro* condition, Continue to secrete antibodies with a specificity. Antibodies that are produced by Hybridomas/ hybrid cells are known as monoclonal antibodies. The monoclonal antibodies have mainly three characteristics – (i) their specificity of binding, (ii) their homogeneity, and (iii) their ability to be produced in unlimited quantities.

Applications

Monoclonal antibodies are very important tools for several molecular immunology investigations. In particular, once utilized in combination with techniques equivalent to mapping and molecular modelling, monoclonal antibodies modify to antigenic Profiling and visualization of macromolecular surfaces. In addition, monoclonal Antibodies became key components in clinical laboratory diagnostic tests. Their wide application in Detecting and identifying serum partials, Cell markers, and pathogenic agents has Largely arisen. Ultimately, monoclonal antibodies are Only manufactured when necessary because Their production is time consuming and Frustrating. This is especially apparent when a monoclonal Antibody



can be used successfully in a pathology laboratory or resource in the medical prognosis and remedy of Patients. Given almost any substance, it's possible to provide monoclonal antibodies that specifically bind thereto substance; they will then serve to detect or purify that substance. This has become a crucial tool in biochemistry, biological science, and medicine. Methodologically these areas may contain biophysical, biochemical approaches as well as informatics and database development, genomic, and database resources required for analyses. Within the realm of diagnosis and therapy, preclinical and clinical assessments of antibodies, and compounds that induce or activate antibodies are included. The production of monoclonal antibodies has been expensive however the benefits we have from it are infinite and it has been considered a revolutionary discovery in the medical field.



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RHIZOREMEDIALION OF PESTICIDE CONTAMINATED SOIL BY PGPR

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Soil contamination is a widespread problem which has been causing deleterious changes in the biology, structure and its productivity. Growth in industrialization, urbanization, modern agricultural development and energy generation has resulted indiscriminate exploitation of natural resources for fulfilling the human desires and need, which have contributed in disturbing the ecological balance on which the quality of our environment depends. Contamination of soils, ground water, sediments, surface water and air with hazardous and toxic chemicals is one of the major problems which the industrialized world is facing today. Various recalcitrant and xenobiotic compounds, due to rapid pace of anthropogenic activities have accumulated in soil resulting in its degradation and infertility. In addition to this, persistent nature of these pollutants allows them to enter into the food chain posing serious threats to living beings. Over the years intentional, unintentional and indiscriminate use of the synthetic chemicals has released several such hazardous organic contaminants in the environment. Hazardous waste in particular pesticide is a massive environmental and health problem.



Pesticides are recalcitrant and non-biodegradable that have great property of bioaccumulation and biomagnifications leading to more serious impacts on flora and fauna. These toxic substances are persistent and harmful to non-target organisms but their usage is continuously rising year by year. They are also carcinogenic in nature and are banned in many countries due to the risk posed by their presence in the environment. Some pesticides banned several decades ago but their residues are still found in the environment. Pesticides are the chemical that are used to kill or arrest the growth of pests, insects, rodents and weeds. The low biodegradability has made these chemicals persistent in nature. These synthetic chemicals are being considered as toxic, persistent and stable and are often not used in proper manner, hence can easily contaminate air, water and soil ecosystem at alarming rate.

Among the different pesticides (organochlorine, organophosphorus and carbamates), organochlorine accumulates in the food chain causing serious problems like sterility, kidney disease, endocrine disruption and neurological disorders in the living organisms. Farmers are also at the risk of exposure to pesticides due to inappropriate use of personal protection and spraying equipment. Misuse of pesticide can also effect human health as can easily get transferred through food chain and also leads to degradation of air, soil and water . Furthermore, pesticides applied to soil can also reach to human body by inhalation (during spraying), ingestion (contaminated food material) and by body contact. Agricultural residues of pesticides can have detrimental effects as these can be easily found in food grains. All the food material gets contaminated with different harmful pesticide residues, which can further degrade the health of living organisms. Indiscriminate use of pesticides affects human health, beneficial microorganisms, plants and animals residing in different ecosystems. Therefore, a holistic and sustainable approach which is eco-friendly, cost-effective, and organic in nature, is the need of the hour.

Rhizosphere is nutrient rich area or zone surrounding the plant roots. It is rich in micro-organisms mainly characterized as plant growth promoting organisms which enhance plant growth directly or indirectly. These rhizobial microbes show immense interactions with the plants and are also involved in the remediating of the harmful pesticide pollutants found in the soil. This clean-up of the hazardous wastes and chemicals brought about by rhizo-microflora is known as rhizoremediation. Rhizoremediation is also known as rhizodegradation, microbe-assisted phytoremediation, or rhizosphere degradation and is a specific subset of phytoremediation. The first-ever study for the degradation of a compound using this method was conducted on pesticides. Important parameters for rhizoremediation are (i) Nature of the pollutants, (ii) Soil structure and hydrogeology (movement of



pollutants through soil and groundwater) and (iii) Nutritional state and microbial composition of the site. It can also be optimized using compatible plant-microbe interactions. These can be combination of plants and PGPR or may be plant- and contaminant-degrading microbes. The microbes which take part in the degradation process in the rhizosphere belong to bacteria, actinomycetes, and fungi including Arbuscular Mycorrhizal Fungi (AMF). Root exudates are the key players for the successful colonization of rhizospheric bacteria in the root vicinity and which would result in successful degradation of soil pollutants. The root exudates consists of organic acids, amino acids, sugars, proteins, alcohols, nucleotides, flavanones, enzymes and phenolic compounds for example, linoleic acid secreted by plant roots acts as surfactant to increase the bioavailability of PAH (pyrene) by forming a layer on soil particles. This results in enhanced attachment of bacteria on the pollutant. The PGPR bacteria present in the rhizosphere of plants in such contaminated sites use these exudates as their carbon and energy source and synthesize various metabolites like siderophores (iron scavenging compounds), exopolysaccharides (metal chelator and biosorption), biosurfactants (solubilization of metals and hydrophobic compounds), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (lowers ethylene, a stress hormone), organic acids (mineralize metals and solubilize organic compounds), and many other enzymes, e.g. oxidoreductases, which bring about the degradation of contaminants.

The rhizoremediation technique is advantageous over bioremediation and phytoremediation processes because of its use of indigenous rhizospheric microbes. Various contaminants and their rhizoremediation has now been done successfully in order to minimize or nullify these harmful pesticides, thereby enhancing the quality of soil and increase its productivity. However, nature harbors huge microbial diversity, and a lot has to be discovered and exploited about the plant-microbe interactions, which is largely unexplored. Unraveling these interactions and accordingly manipulating the efficacy of rhizoremediation method would further help this sustainable technology to achieve its due success and reliability.



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***Spirulina* - A RICH SOURCE OF VEGETARIAN PROTEIN FOR BOOSTING IMMUNITY AGAINST COVID-19**

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The outbreak of the Coronavirus disease of 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that has created huge trepidation worldwide, has a mortality rate of 0.5% to 1% and is growing incessantly. There are currently no therapies that may help abate this viral disease, but the use of masks and social distancing can limit the spread. Boosting immunity has been a simple way to resist viral infection and limit fatalities. The ability of algae-based nutraceuticals, mainly *Spirulina*, to boost immunity against viral diseases has already been reported clinically. *Spirulina*-based nutraceuticals boost adaptive and innate immunity, and bioactive compounds, such as Angiotensin-Converting Enzyme (ACE) inhibitor peptides, Phycobiliproteins, Sulfated polysaccharides, and Calcium-Spirulan, can serve as antiviral agents. The presence of these molecules indicates its potential role in resisting infection and COVID-19 disease progression. This review focuses on the potential role of algae (*Spirulina*) as an immune booster to combat the human Coronavirus and other Viral diseases.

Spirulina supplements or extracts are believed to potentiate the immune system, which may help fight and suppress different viral infections. Soluble extracts of *Spirulina* have been found to enhance Natural killer (NK) cell function, macrophage phagocytic activity, and red blood cell antibody response *in vitro* studies



and trials on different animals and humans. Interferon-gamma (IFN γ) cytokine plays an important role in innate and adaptive immunity in humans and is a primary activator of macrophages as well as a stimulator of NK cells and neutrophils. The administration of *Spirulina* could enhance nonspecific preventive administration measures, such as the activation of CD¹ cells, which further enhance the production of IFN γ in humans, for the prevention of viral infections.

When the algae extract was included in optimum quantities, there was a 70 % reduction in the release of TNF- α proteins, which is very encouraging. This indicates that the algae extract may be used to prevent cytokine storms if given to patients soon after diagnosis. A single tablespoon (7 gm) of dried *Spirulina* Powder contains, Protein: 4 grams (60 % - 65 %) Highest in any plant food. The quality of the protein in *Spirulina* is excellent comparable to eggs. It gives all the essential amino acids that you need Calories: 24 %, Carbohydrates: 1 %, Fat: 0.78 %, Vitamin B1 (Thiamine): 11 % of the RDA, Vitamin B2 (Riboflavin): 15 % of the RDA, Vitamin B3 (Niacin): 4 % of the RDA, Copper: 21 % of the RDA, Iron: 11 % of the RDA, Calcium, Phosphorous, Magnesium, Potassium and Manganese.

Spirulina contains 60 % protein with all essential amino acids, and it is rich in antioxidant carotenoids beta-carotene and zeaxanthin, B vitamins, vitamin K, and dietary minerals. *Spirulina* contains a blue pigment, Phycocyanin which is water-soluble, found only in *Spirulina* and other species of blue-green microalgae which has been shown to protect both the liver and kidneys from toxins. The nutrient profile of *Spirulina* versus other foods: 180 % more calcium than whole milk, 670 % more protein than tofu, 3100 % more beta-carotene than carrots, 5100 % more iron than spinach, also more antioxidant and anti-inflammatory activity in 3 g of *Spirulina* than in 5 servings of fruits and vegetables. This wide array of nutritional components makes it a valuable "Superfood" for use in human foods and health supplements as well as for feed extender for livestock. *Spirulina* increases the nutritional value of any food and valuable tool in the fight against multiple diseases. Adding a teaspoon of *Spirulina* to your diet can boost your nutrient intake and increase vitality. "For WHO - World Health Organization, *Spirulina* represents an interesting food for multiple reasons, rich in iron and protein and it is very suitable food". In 1974, the UN declares *Spirulina* algae as the answer to the World's Hunger.

Ayurvedic Properties of spirulina include light, dry quality, and easy to digest. It has a salty, bitter taste and pacifies Kapha, which may increase Vata and Pitta as three Dosha of Ayurveda. The tonic of spirulina boosts strength, proper digestion, blood purifier, and anti-inflammatory agent. According to NASA: *Spirulina* is the



most nutrient - dense food in the world. One gm of algae has 1,000 times more nutrition than any other fruit and vegetable.

The current trend toward the commercialization and cultivation of algae is targeted toward nutritional products such as *Spirulina*, *Chlorella*, *Dunaliella* and *Haematococcus* in several dozen small to medium scale production systems around the world. Algal species that are used for the development of pharmaceutical products such as lectin have high production costs. This could prove to be a challenge as further research and development of algae might be unfavorable due to its costly expenses in harvesting. Newer and cheaper methods of harvesting different algal species and strains ought to be developed to allow for an increase in commercialized application for pharmaceutical purposes. Today *Spirulina* has become a wonderful anti-oxidant. It is available in capsule, tablet, powder form, or as flakes as well. One to Three gram of *Spirulina* to a single individual (Adult) is sufficient but pediatrics consumption should be avoided.

Conclusion

There have been some good studies on *Spirulina's* COVID-19 effects, but further research is needed before any clear findings can be drawn. Finally, no high-level proof trials on *Spirulina's* role in chronic fatigue and antiviral uses exist. At this time, the literature suggests that *Spirulina* is a safe food supplement with few negative effects, but its potential as a medication is unknown.



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BIOFERTILIZERS FOR SUSTAINABLE AGRICULTURE

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Fertilizers are natural or man-made chemicals that, when applied on the plant or to soil or by fertigation, can supplement natural soil nutrients and augment crop growth and soil fertility. These make available important macronutrients such as nitrogen, phosphorus, potassium, calcium, sulfur, and magnesium along with numerous micronutrients like zinc, copper, iron, boron, and molybdenum to plants. A high production demand of standard fertilizers is observed for NPK fertilizers that provide nitrogen (ammonia, urea, ammonium sulfate, ammonium nitrate, calcium ammonium nitrate), phosphorus (di-ammonium phosphate, superphosphates, ground rock phosphates, and potassium (potash or potassium chloride, sulfate of potash or potassium sulfate, sulfate of potash magnesia, potassium nitrate, kieserite, Epsom salt). Micro-enriched fertilization, involving the addition of micronutrients to these standard fertilizers, has encouraged agronomic bio-fortification to alleviate malnutrition and micronutrient deficiencies of copper, iron, zinc, iodine, selenium, and fluorine in crop plant. Since there is constraint to plant growth as there is non-availability of nutrients especially nitrogen and phosphorus to plants despite their ample occurrence in soil, as most nitrogen is present in soil organic matter and plants have to compete with soil microbes to obtain it, while phosphorus forms precipitates with iron and aluminum (in acidic soils) or with calcium (in alkaline soils). Increased dependence of modern agriculture on an excessive, imbalanced, and steady synthetic input of chemical fertilizers has caused deterioration of soil quality and surface and ground water, and it has further reduced biodiversity and stifled



ecosystem functioning. The production and transport of chemical fertilizers, which require the use and combustion of fossil fuels, result in airborne carbon dioxide and nitrogen pollution that get deposited into terrestrial ecosystems. Because of such drawbacks of chemical fertilizers, it is essential to reduce the consumption of chemical fertilizers and pesticides in agriculture without having any adverse effect on crop production by the incorporation and usage of harmless, renewable inputs of fertilizers.

The most suitable alternatives for chemical fertilizers are Biofertilizers that include organic waste, dead organisms, as well as living organisms. Bio-fertilizers can be composed of efficient microbial strains that, by their interactions in rhizosphere, benefit crop plants by the uptake of nutrients. A bio-fertilizer of selected efficient living microbial cultures, when applied to plant surfaces, seed or soil, can colonize the rhizosphere or the interior of the host plant and then promote plant growth by increasing the availability, supply, or uptake of primary nutrients to the host. Moreover, in contrast to chemical fertilizers, bio-fertilizers are more accessible to marginal and small farmers. Bio-fertilizers containing microorganisms like bacteria, fungi, and algae have been suggested as viable solutions for large-scale agricultural practices which not only are natural, eco-friendly, and economical but also maintain soil structure as well as biodiversity of agricultural land. Besides providing nutrient enrichment to the soil, microbial bio-fertilizers promote plant growth by increasing efficient uptake or availability of nutrients for the plants and by suppressing soil borne diseases. Bio-fertilizers supplement nutrients mainly by fixation of atmospheric nitrogen, by phosphorus solubilization, and by synthesizing plant growth-promoting substances. The nitrogen-fixing bacteria of the *Rhizobia* and other groups are used for growth promotion of legumes and additional crops. In addition, Blue-green algae (BGA) as well as *Azolla* subsidize in the nitrogen budget of practicable agriculture. Arbuscular mycorrhizal fungi are important for the uptake of phosphorus and several other minerals in many plants. Phosphorus-solubilizing bacteria like *Azotobacter* and *Azospirillum* that fix atmospheric nitrogen can increase the solubility and availability of phosphorus to plants and, thus, crop yield. Further, *Azospirillum* provides additional benefits such as the production of growth-promoting substances, disease resistance, and drought tolerance. Thus, application of microbial bio-fertilizers is an effective approach in increasing and maintaining the nutrient economy of soil, thereby reducing the use of chemical fertilizers, for a proficient and sustainable agriculture.



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Bifidobacterium AND THEIR ROLE IN HUMAN GUT MICROBIOTA

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Bifidobacteria are a group of bacteria called probiotics that normally live in your intestines and stomach. They help your body perform essential functions such as digestion and staving off harmful bacteria. *Bifidobacterium* was first isolated in 1899 from the faces of breast-fed infants. The mole percentage of G+C bases of the DNA of the genus *Bifidobacterium* is 58 for the human-type strains, and the rest of the species have a mole percentage of G + C ranging between 55 and 66. The genus includes 32 described species. In the manufacture of fermented milks, *Bifidobacterium bifidum* is the species most commonly used, followed by *Bifidobacterium longum* and *Bifidobacterium breve*.

Bifidobacterium infantis is the predominant species in the stools of breast-fed infants. *Bifidobacteria* belong to a group of bacteria called lactic acid bacteria. Lactic acid bacteria are found in fermented foods like yogurt and cheese. *Bifidobacteria* are used in treatment as so-called "probiotics," the opposite of antibiotics. They are considered "friendly" bacteria and are taken to grow and multiply in areas of the body where they normally would occur.

Some early research shows that taking a specific *Bifidobacterium breve* product can reduce constipation in children 3-16 years of age. Also, most research shows that mixing *Bifidobacterium longum* BB536 with milk or yogurt and taking the mixture daily for 2 weeks increases the number of bowel movements in adults



prone to constipation. However, taking this same strain of *Bifidobacterium* for 16 weeks does not seem to reduce constipation in elderly adults receiving nutrition with a feeding tube.

Taking *Bifidobacteria* along with other probiotic bacteria strains seems to reduce side effects of treatment for the Ulcer causing bacterium *Helicobacter pylori*. Taking *Bifidobacterium infantis* 35624 (Align or Bifantis, Proctor & Gamble) for 8 weeks seems to reduce symptoms of IBS. However, it does not seem to increase bowel movements. Taking a specific product containing species of *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* seems to decrease bloating in people with IBS. Taking *Bifidobacterium infantis* along with another bacterium called *Lactobacillus acidophilus* seems to help prevent NEC in critically ill infants.

Some research suggests that taking a specific combination product containing *Lactobacillus acidophilus* and *Bifidobacterium* (HOWARU Protect) with milk helps reduce symptoms of fever, cough, runny nose, and decreases the amount of antibiotics needed in children. It may also shorten how long children have symptoms and decrease the number of days missed from day care. Also, eating food containing *Bifidobacterium longum* for 3 weeks before getting a flu. Early research suggests that taking milk containing *Lactobacillus acidophilus* and *Bifidobacterium longum* reduces “bad” Low-density lipoprotein (LDL) cholesterol in people with high cholesterol. However, it also seems to reduce “good” High-density lipoprotein (HDL) cholesterol shot and for 14 weeks thereafter seems to help prevent the flu in elderly people.

Antibiotics are used to reduce harmful bacteria in the body. Antibiotics can also reduce friendly bacteria in the body. *Bifidobacteria* are a type of friendly bacteria. Taking antibiotics along with *Bifidobacteria* might reduce the effectiveness of *Bifidobacteria*. To avoid this interaction, take *Bifidobacteria* products at least two hours before or after antibiotics. For irritable bowel syndrome: 1 billion cells of *Bifidobacterium infantis* daily in a malted milk drink.

For Lung infections in children, 120 ml of milk twice daily containing 5 billion colony forming units each of *Lactobacillus acidophilus* and *Bifidobacterium* contained in a specific product.

For Ulcerative colitis, 100 ml per day of a specific fermented milk product (Yakult Co., Japan) containing at least 10 billion live *Bifidobacterium breve*, *Bifidobacterium bifidum* and *Lactobacillus acidophilus* strains per dose has been used. Three grams of a specific combination probiotic containing living freeze-dried



bacteria species including *Lactobacillus*, *Bifidobacteria*, and *Streptococcus* twice daily has also been used.

Digesting fiber

Certain bacteria digest fiber, producing short-chain fatty acids, which are important for gut health. Fiber may help prevent weight gain, diabetes, heart disease and the risk of cancer. Eat a diverse range of foods: This can lead to a diverse microbiome, which is an indicator of good gut health. In particular, legumes, beans and fruit contain lots of fiber and can promote the growth of healthy *Bifidobacteria*.

Limit your intake of Artificial sweeteners

Some evidence has shown that artificial sweeteners like aspartame increase blood sugar by stimulating the growth of unhealthy bacteria like Enterobacteriaceae and *Bifidobacterium* in the gut microbiome. “Instead of killing all bacteria, including the beneficial ones, we should focus on shifting the balance toward a healthy microbiota by targeting harmful bacteria or enriching beneficial bacteria”.



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INHIBITION OF BACTERIAL DNA REPAIR: AN EFFECTIVE DRUG TARGET

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Introduction

Horizontal gene transfer and efficient adaptation mechanisms have resulted in increased antibiotic resistance of pathogenic bacteria, a problem faced by medical sciences globally. This has led to the increasing demand of developing new targets in drug designing. The host immune system mediates DNA damage in pathogenic bacteria. However, specialized DNA repair mechanisms constantly evolving in bacteria render this attack inefficient. These mechanisms also make bacterial cells resistant to antibiotics such as quinolone that target bacterial DNA damage. Therefore, there is a need to sensitize bacteria to these immune approaches such that immune mediated DNA damage does not repair efficiently. This can be achieved by developing drugs that target DNA repair mechanisms of bacteria. Bacterial DNA repair mechanisms serve as very specific targets to develop unique and relatively more efficient drugs. The host DNA remains intact, as the repair mechanisms are specific to pathogenic bacteria, and the target compounds such as, enzymes DNA glycosylase and prokaryotic DNA polymerase are distinct from those found in the human host, which mainly uses poly ADP-ribose polymerases. This approach might provide a way to tackle multidrug resistant pathogenic bacterial species.



Host Mediated Damage to Bacterial DNA

The innate immune system of the human host strives to hamper the pathogen entry and colonization on tissues by various mechanisms; one of the key manifestations is the generation of genotoxic compounds like 'Reactive Oxygen Species' (ROS) and 'Reactive Nitrogen Species' (RNS) by phagocytic cells like neutrophils and macrophages. Pathogens are phagocytosed through a respiratory or oxidative burst. The increased concentrations of oxygen in activated phagocytic cells is reduced to superoxide anions (O_2^-). These superoxide anions are subsequently reduced to other destructive species that mediate damage of bacterial macromolecules like DNA, proteins, and lipids. Some of the most commonly observed genotoxic compounds in phagosomes are hydrogen peroxide, hydroxyl radicals and peroxynitrites. These may induce certain fatal changes in bacterial DNA sequences such as single stranded breaks, double stranded breaks, faulty pairing or crosslinking of DNA molecules, depending on the type of ROS and RNS produced by the host cell.

Bacterial DNA Repair pathways

One of the most conserved features of bacterial DNA repair is mediated by the helicase nuclease complex composed of Add AB and Rec BCD genes. The gene products initiate the process of homologous recombination to repair double stranded breaks in the DNA molecule. This ATP dependent mechanism involves unwinding of DNA, identification of crossover hotspots and initiation of recombination and repair process. A slight modification of this process results in the SOS repair pathway. Although seen in many bacteria, it was first described in *Escherichia coli*. The SOS response is governed by a repressor protein, LexA and an inducer protein, RecA. It also involves a regulon, encompassing about 50 genes and a 20-base pair palindromic sequence, called the SOS box to which the LexA. Dimer binds when no DNA damage is reported. This represses the transcription of the regulon. RecA binds to a single stranded DNA region when DNA damage is detected and induces cleavage of LexA, leading to induction of transcription of SOS genes. DNA polymerases prone to error repair such as Pol III, Pol IV and Pol V are expressed when extreme levels of DNA damage are reported. These perform proofreading functions and replace faulty base pairs, fixing DNA damage efficiently.

Interestingly, the 'Base Excision Repair' pathway, although seen in prokaryotes and eukaryotes (albeit, with differences in enzyme composition) has evolved effectively in pathogenic bacteria like *Mycobacterium tuberculosis* and *Helicobacter pylori*. Pathogenic bacteria use this pathway to repair DNA damage caused by oxidation or due to base modifications such as deamination or alkylation.



Enzyme DNA glycosylase, first breaks the N-glycosidic bond between the defective base and the DNA backbone, creating an apurinic or apyrimidinic site. DNA polymerase I then fills single nucleotide gaps which are sealed by ligase. These DNA repair pathways in bacteria efficiently counter the fatal changes caused by DNA damage mediated by the host, ensuring the successful colonization of pathogens in the human host.

Need for New Drug Targets

As discussed above, mere oxidative damages are not fatal to pathogenic bacteria due to highly effective DNA repair mechanisms. Studies have revealed that DNA damage repair pathways which were once found to be highly conserved in bacteria, are now evolving at faster rates. This imparts protection against innate immune mechanisms of the host and establishes multidrug resistance. In the past few years, it has been realized that there is an immediate need to develop drugs that target specific bacterial enzymes and proteins that hinder the repair mechanisms, ensuring that the pathogen is sensitized to DNA damage mediated by the host innate immunity or antibiotics.

Drugs that Target DNA Repair Mechanisms

A new therapeutic approach that has taken precedence over the last few years for treating diseases caused by multidrug resistant bacteria is, the administration of DNA repair inhibition drugs. ML328 was one of the first small molecule inhibitors of bacterial DNA repair developed. However, due to its moderate potency, new drugs like IMP-1700 that are more efficient have been developed.

Conclusion

DNA repair mechanisms are an integral aspect of survival for intracellular bacterial pathogens. Thus, using this as molecular targets not only helps to develop novel and efficient therapeutic approaches but also sensitizes bacteria to DNA damage caused by existing drugs. The efficiency of this approach is highlighted in the fact that even a single break in DNA can prove fatal to the pathogen if not repaired. Future prospects include the development of a Gam-protein based therapeutic in which a protein that is a competitive inhibitor of DNA repair pathways is isolated from bacteriophage lambda. This newer therapeutic approach is considered a promising field of study in clinical sciences as the emergence of drug resistant bacteria is a global health concern.



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EFFECT OF INTEGRATED PLANT NUTRIENT SYSTEM ON SELECTED MEDICINAL PLANT- *Coleus aromaticus*

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Introduction

Coleus aromaticus is well known for its antimicrobial and pharmacological activities. Antioxidant activity is reported to be mainly due to rosmarinic acid, chlorogenic acid and caffeic acid. The essential oil of *Coleus aromaticus* has great anti-microbial activity on Gram negative as well as Gram positive bacteria, drug resistant microorganisms, phytopathogenic microorganisms and fungi. This study is to investigate the effect of integrated plant nutrition system on Coleus. The growth parameters analyzed were height of the plant, number of leaves, number of branches, leaf area, root length and girth of stem. The results are significant by having the best performing treatments in (T8-Combined use of Organic, Inorganic and Biofertilizer) has the best treatments and (T1-Control) has the poorest performing treatments. Treatments comprising of sole application of organic, inorganic and biofertilizer resulted in an average growth response whereas the combined application resulted in maximum growth.

Materials and Methods

The field experiment was conducted at the Nursery, College Of Agricultural Technology, Theni. Geographically, it is located in $11^{\circ} 05'$ N latitude and $77^{\circ} 5'$ E longitude. Organic, Inorganic and Biofertilizer were applied at 30th, 45th and 60th day



after transplanting. Plant samples were taken at peak vegetative stages for different morphological analysis, at regular periodical intervals. Pest monitoring is done. Manual collection and destruction of green larvae and aphids was done.

Treatment Details

Treatments	Fertilizers	Composition
T1	Control	None
T2	Organic Manure	Farm yard manure, Vermicompost
T3	Inorganic Manure	Urea, SSP, MOP
T4	Biofertilizer	<i>Azospirillum</i> , <i>Azotobacter</i> , VAM
T5	Organic + Inorganic	FYM , Vermicompost + Urea, SSP, MOP
T6	Inorganic + Biofertilizer	Urea, SSP, MOP + <i>Azospirillum</i> , <i>Azotobacter</i> , VAM
T7	Organic + Biofertilizer	FYM, Vermicompost + <i>Azospirillum</i> , <i>Azotobacter</i> , VAM
T8	Organic + Inorganic + Biofertilizer	FYM, Vermicompost + Urea, SSP, MOP + <i>Azospirillum</i> , <i>Azotobacter</i> , VAM

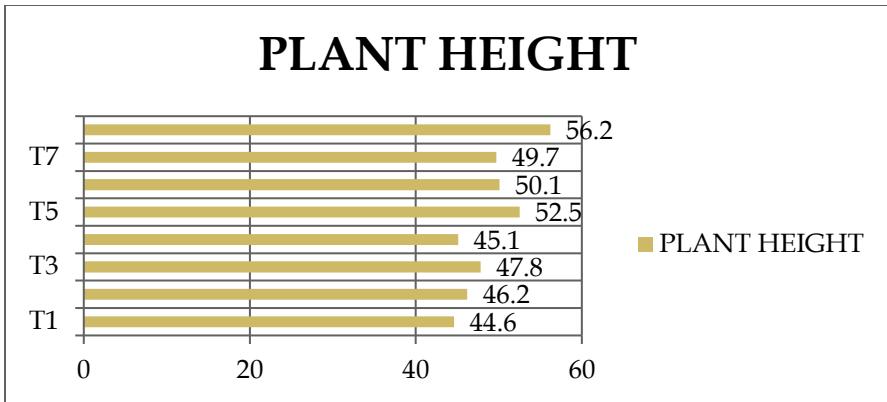
Statistical Analysis

The data generated from various experiments were subjected to statistical analysis in a Completely Randomized Block Design (CRD). The critical difference was worked for 0.005 per cent probability and the results were interpreted. The statistical analysis was carried out with AGRES software package and MS Excel worksheet.

Results and Discussion

The integrated application of inorganic, biofertilizer and organic fertilizers produced significantly taller plants in Coleus with the height of 56.2 cm and it was on par with organic + inorganic 52.5 cm and 49.7 cm organic + biofertilizer. Shortest plants were observed in control plot of 44.6 cm. Mechanism behind this is, initial application of biofertilizer increased the microbial population and enhances the uptake of nutrients in organic fertilizer, the third application of inorganic fertilizer which boost up the plants to grow well and proliferate into many branches. The increase of N in the root zone and the synergistic effect of these microorganisms on the physiological and metabolic activities of the plant and also the application of organic manures and inorganic fertilizers lead to have good growth in root length and girth of the stem





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Conclusion

The better performing treatment is T8 which increased the growth parameters of the plant such as plant height, number of leaves, number of branches, leaf area, root length and girth of stem on observation in Coleus. The reason for higher growth under integrated nutrient management may be due to sustainable or continuous availability of nutrients especially nitrogen throughout the growth period coupled with lesser leaching losses of nutrients. Thus it indicates that the treatments comprising of sole application of organic, inorganic and biofertilizer resulted in an average growth response whereas the combined application resulted in maximum growth.



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Paragonimus westermani - JAPANESE LUNG FLUKE

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Paragonimus is derived from the combination of two Greek words "Para" (on the side of) and gonimos (gonads or genitalia). A fluke invading the lungs especially either of two old forms of the genus *Paragonimus* (*P. westermani* and *P. kellicotti*) [Genus name - *Paragonimus*; Species name - *P. westermani*; Binomial name - *Paragonimus westermani*]. *Paragonimus* is the only genus in the monotypic family Paragonimidae, *Paragonimus* class under Rhabditophora and then phylum under Platyhelminthes. *P. westermani* was discovered in the Ringer in 1879 and eggs in the sputum were discovered independently by Manson and Erwinvon Beelz in 1880. Manson proposed the snail as an intermediate host and various Japanese scientists detailed the whole life cycle in the snail between 1916 and 1922. The species name *P. westermani* was named after Pieter Westerman (1859 - 1925) a Zookeeper who noted the trematode in a Bengal tiger in Amsterdamzoo. *Paragonimus westermani* is called as "Oriental lung fluke" [Parasite worm is called as fluke; worm commonly infects the lungs is called as lung fluke]. *Paragonimus westermani*, especially in Japan, Korea and China so it called as "Japanese lung fluke" and also reported from Nepal, South America and Assam. *Paragonimus* is a gene of fluke trematode (flatworm with an incomplete digestive tract) and more than 30 species of trematodes (flatworm) of the genus *Paragonimus* have been reported to infect animal and human, Among them more than 10 species are reported to infect human the most common is *Paragonimus westermani*.



Paragonimus westermani is the major species of lung fluke that infects humans causing paragonimiasis or lung fluke disease. Paragonimiasis is a food-borne parasitic infection. Paragonimiasis is an infection with parasite worm. It is caused by eating undercooked crab or crayfish and infecting agent metacercaria or adolescaria inside cyst; portal of entry through digestive tract; site of localization infection is lungs. Paragonimiasis can cause illness resembling pneumonia or stomach flu. The infection can last for years, but more serious cases of paragonimiasis occur when the worm travels to the central nervous system. Morphology: Adult worms are thick, fleshy and egg-shaped. It measures 8 to 12 mm in length by 4 to 6 mm in breadth and 3 to 5 mm in thickness. The ventral Sucker is situated near the middle of the body. The excretory vessel is large and extends from the posterior extremity to the anterior region, dividing into two equal halves. The two blind intestinal caeca (lower right part of abdomen) are unbranched and extend to the caudal region. The genital apparatus follows the same general pattern of trematodes. Lifespan of the adult worm is about 6 to 7 years. Eggs are golden brown in colour with flattened opercula. They measure 80 μm by 50 μm and each egg contains an unsegmented ovum surrounded by yolk cells.

P. westermani passes through the lifecycle in three hosts - definitive host and intermediate host. Definitive host (parasite reaches its mature form and parasite worms make use of human and animal as its definitive host; intermediate host (a freshwater snail of the genus *Melania*); second host (A freshwater crayfish or a crab). The adult worms live in the respiratory tract of definitive hosts. The eggs generally escape in the sputum and some are eliminated in the faeces. In water, ciliated embryo (miracidium) develops inside 2 to 7 week time. On attaining maturity, the miracidium escape into water and swims about in search of its snail host, species of the genus *Melania*. Inside the soft tissue of the snail, the miracidium casts off its tail and passes through the stages of Sporocyst and two generation of rediae being finally transformed into cercariae the whole cycle taking about 10 to 12 weeks. The mature cercariae escape from snails to water and enter into its second intermediate host, a freshwater crab or crayfish. Inside the crustacean host, they become encysted in the viscera, gills and muscles.

When the raw flesh of an infected crab or crayfish is eaten by man and other susceptible host, the cyst wall is dissolved by the gastric juice and adolescaria is released in the duodenum. These young worms penetrate the wall of the small intestine and enter into the abdominal cavity. Later, they migrate upwards, piercing through the diaphragm and the two layers of the pleura, to gain entrance into the lungs where they finally settle and grow to sexual maturity. Eggs are discharged into



the bronchiole and are coughed out with sputum. The cycle is repeated. As a result, the symptoms are pain in abdomen, diarrhoea, cutaneous ulceration and enlargement of the liver and other symptoms are brain tumours develop and may even terminate fatally. The main symptom is pulmonary paragonimiasis, a chronic cough with recurring attacks of haemoptysis and simulating a case of pulmonary tuberculosis.

Indirect Hemagglutination test and Enzyme Linked Immunoassay are available. The tests become negative within 3 - 4 months after successful treatment, Chest x-ray reveals abnormal shadows (Nodular, Cystic and Infiltrative) in the middle and lower lung field similar to Pulmonary Tuberculosis. Chest x-ray also helps in diagnosis of Pulmonary lesions. Praziquantel (75 mg/kg/day in three divided doses for 2 days, orally) and niclofan (as a single oral dose of 2 mg/kg) have both been treatment for Paragonimiasis. Bithionol, a dichlorophenol derivative (30 – 50 mg/kg on alternate day for 15 days has been by Yokogawa and De Jongh (1961) to give encouraging results. The measures include avoidance of the consumption of raw and freshly salted or inadequately cooked cabs and crayfish as food and Eradication of molluscan host.



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BIOSURFACTANT AND ITS APPLICATIONS

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Biosurfactants are often described with a wide range of applications due to the active biomolecules generated by microorganisms. In recent years, these surfactant biomolecules have attracted a lot of interest to their characteristic properties such as specificity, low toxicity, and relatively easy manufacture. Biosurfactants have been used in many industries as well as in organic chemicals, petroleum, petrochemical, mining, science (mainly bioleach), agrochemicals, fertilizers, food, beverages, cosmetics, prescription drugs, and many others. De-emulsifiers, wetting agents, foaming agents, spreading agents, functional food ingredients and detergents. The physical phenomenon at the surface that reduces the capacity of biosurfactants has led them to play a necessary role in the oil recovery and biological remediation of significant crude oil were used to increase the degree of hydrophobicity. Biosurfactants used together to increase the bioavailability of hydrophobic substrates through solubilization/desorption. They also regulate the accumulation and removal of microorganisms from surfaces. Compared to chemical or artificial surfactants, biosurfactants gained many advantages as well as their biodegradability, biocompatibility and digestibility. Biosurfactants are often used in environmental remediation through biodegradation and detoxification of commercial wastewater and in the biological remediation of contaminated soils. Their specificity and manageability of the raw materials together created the most popular surfactants. In recent times, the ecological effects of composite surfactants have been given extraordinary importance. Growing natural concerns, the development of biotechnology and the advent of stricter laws have made biosurfactants an expected choice as opposed to the composite surfactants available. Some reviews have aimed



to improve the level of biosurfactant formation by changing the factors that affect the type and level of biosurfactant released by a microorganism. The most important factors are carbon and nitrogen sources, probable limit values for food supplements and other physical and material limit values. In this chapter we desire to emphasize the appearance of biosurfactants and their various applications. Biosurfactants have several advantages when placed alongside their synthesized chemical counterparts. They are, Biodegradability: microbes simply break down biological surfactants. Low toxicity: Biosurfactants are more toxic than surfactants made from chemicals. In addition, it can be reported in absolute terms that the biosurfactants had higher EC50 values (effective concentration to reduce 50% of the control population) than the artificial dispersants. Accessibility of raw materials: Biosurfactants are made from raw materials from below, which are accessible in large quantities. The carbon contribution can be returned by hydrocarbons, carbohydrates and / or lipids, which can be used individually or together. Physical factors: several biosurfactants don't seem to be suffering from environmental factors admire temperature, pH and ionic strength tolerances.

Commercial Applications of Biosurfactants

The worldwide production of surfactants amount increased to 17 million metric tons in 2000 (including soaps) expected future growth rates of 3-4% per year globally. These chemically synthesized surfactants are mainly petroleum based and are usually non biodegradable thus remain toxic to the environment they find themselves. Also these compounds may bio-accumulate and their production processes and by-products can be environmentally hazardous, due to this increasing awareness on the need to protect the ecosystem, environmental scientist have been tightening environment regulations thus necessitating an increased interest in surfactants of microbial origin as possible alternatives to chemically synthesized ones. Biosurfactants have several applications in agriculture, medicine, petroleum and industry. Use of Biosurfactants in Agriculture: One way to improve the solubility of biohazardous chemical compounds such as PAHs is to use surfactants as mobilizing agents that increase the apparent solubility of hydrophobic organic contaminants (HOC). Surfactants are also used in agriculture to make heavy soils hydrophilic in order to achieve good wet capability and even distribution of the fertilizer in the soil. They also prevent certain fertilizers from caking during storage and encourage the spread and penetration of toxins in pesticides. Application of biosurfactants in medicine - To enlighten about the wide range of applications of biosurfactants in medicine, they include: Antimicrobial activity: The diverse structures of biosurfactants give them the ability to perform in a variety of ways. Due to their structure, biosurfactants exert their toxicity on the permeability of the cell



membrane, similar to a detergent with a similar effect. Several biosurfactants have potent antibacterial, antifungal, and antiviral activities; these surfactants play the role of anti-adhesion agents to pathogens, which makes them useful for the treatment of many diseases as well as their use as therapeutic and probiotic agents. Anti-cancer activity: Some microbial extracellular glycolipids induce cell differentiation rather than cell proliferation in the human promyelocytic leukemia cell line, and exposure of PC 12 cells to MEL improved acetylcholinase esterase Activity and disrupted the cell cycle in the G1 phase with subsequent neurite outgrowth and partial cell differentiation, this suggests that MEL induces neuronal differentiation in PC 12 cells and forms the basis for the use of glycolipids. Microbial extracellular cells as new reagents for the treatment of cancer cells. Antiadhesives: Biosurfactants inhibit the adhesion of pathogenic organisms to solid surfaces or sites of infection, the precoating of the urethral catheter made of vinyl by passing the surfactin solution through it prior to inoculation with the medium resulted in a reduction in the amount of *Salmonella typhimurium*, *Salmonella enterica*, *Escherichia coli* and *Proteus mirabilis*. The pretreatment of silicone rubber with *Streptococcus thermophilus* surfactant inhibited the adhesion of *Candida albicans* and glass-adsorbed *Lactobacillus fermentum* and *Lactobacillus acidophilus* surfactants, reducing the number of adherent uropathogenic cells of *Enterococcus faecalis* by 77% has been. Bacterial lipopeptides are potent non-toxic and non-pyrogenic immunological adjuvants when mixed with conventional antigens.

Conclusion

Biosurfactants exhibit several properties that could be useful in many areas of the food industry; recently, its anti-adhesive activity has attracted attention as a new tool for inhibiting and altering biofilms on food contact surfaces. The combination of special properties such as emulsifying, anti-adhesive and antimicrobial activities that biosurfactants have, suggests possible application as multipurpose ingredients or additives. Information on toxicity combined with high production costs appears to be the main cause of the limited use of biosurfactants in the food sector, however the use of agro-industrial waste can lower biosurfactant production costs as well as waste treatment costs and also an alternative for the food and food industry, not just upgrading their waste, but also becoming a manufacturer of microbial surfactants - there is much more research going on. The prospect of new types of surfactant compounds from microorganisms may help detect different molecules in terms of structure and properties, but the toxicological aspects of new and current biosurfactants need to be highlighted in order to certify the safety of these compounds.



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CELL TO CELL ADHESION

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For different components to be exchanged in cells, communication of cells is required. Cell adhesion is essential for the communication and regulation of the cells. Cell adhesion causes tissue development. There are some important adhesive molecules for cell adhesion, they cause adhesion or attachment, which we will see in detail later.

What is Cell Adhesion?

Tissues contain some adhesive molecules that cause attachment between cells, making it easier to communicate or exchange between cells. This adhesive property of cells is called cell adhesion. Cell adhesion is found in all multicellular animals.

How Cell to Cell Adhesion occurs?

Cell interaction occurs when Cell Adhesion Molecules (CAMs) interact with a transmembrane protein present on the cell surface. Cell adhesion is a process in which cells interact and attach. Cell adhesion molecules have four families: Integrins, Immunoglobulin superfamily (IgSF), Cadherin, and Selectins. All of these adhesion molecules have different functions.

A most important class of adhesive molecules: **Integrin**

- Integrin is a transmembrane protein found in all animal cells
- There are two types of integrins: a) Alpha subunit and b) Beta subunit
- It facilitates Cell-Extracellular Matrix (ECM) adhesion.



In multicellular organisms, Cell Adhesion Molecules (CAMs) allow cells to bind to one another the resulting structure is called Junction.

According to their functions, the Cell Junctions have the following types. They are as follows: a) Anchoring Junctions, b) Occluding junctions, c) Channel-forming junctions and d) Signal-relaying junctions.

- a) **Anchoring Junctions:** It includes, Adherence junctions, Desmosomes, Hemidesmosomes. which holds cells together and strengthens them.
- b) **Occluding Junctions/Tight Junctions:** Which seal the gap between neighboring cells.
- c) **Channel-forming junctions (Gap junctions):** which allows the transport of molecules to occur between cells.
- d) **Signal-relaying junctions:** it is a neuronal junction in the nervous system.

If cell interaction is mediated by Cadherins, Cell-cell junctions occurs while Cell interaction is mediated by Integrins cell-matrix junctions occur. Cell adhesion plays an important role in regulation of fundamental cellular processes, such as cell survival, proliferation and migration. Integrin binding to matrix initiates signals that modify cellular adhesion, locomotion, and gene expression. Cell adhesion, is critical for proper embryonic development. Dynamic changes in cell adhesion allow stationary cells to become migratory and mediate morphogenesis in the proper spatio-temporal manner.



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PESTICIDE FORMULATIONS USING CHITOSAN AS A SOURCE FROM PROKARYOTIC CELL

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India has 170 million ha of arable land with average pesticide consumption of 0.5 kg/ha due to more than 2,000 species of insects and 800 fungi are known so far as agricultural pests and pathogens. In terms of the total consumption of pesticides, India is placed tenth in the world. Interestingly, there is always scope for more pesticide consumption in India in the coming decades owing to the growing population coupled with the high demand for food grains and intensification of agriculture under a fast-changing climate. The major insect pests which cause >30% loss in yield in India are *Helicoverpa armigera* on pulses, cotton, vegetables and sunflower, *Spodoptera* on vegetables, *Pyrilla* on wheat, rice, millet and sugarcane, recently introduced pest *Ceratovacuna lanigera* (woolly aphids) on sugarcane, mealy bug on cotton (*Phenacoccus gossypiphilous*) and grapes (*Maconellicoccus hirsutus*). While legumes such as groundnut, cowpea, etc. are prone to attack by soil-borne plant pathogenic fungi such as *Sclerotium rolfsii*, *Fusarium oxysporum*, *Rhizoctonia solani* and others. The development of resistance to chemical pesticides and the negative impact of pesticides on the environment and human health has fueled a hunt for safer, more ecologically friendly pest management options. Several researchers provoked to evaluate alternative methods for the control of important agricultural pests. The use of biological agents to control plant pests has a lot of potential as a chemical-free alternative.



Biological control agents are widely acknowledged to be safer, less harmful to human health and more environmentally friendly than relying on the use of high-velocity pesticides. Biological control agents like chitinolytic enzymes inhibit growth and development in insect, nematode and fungi because protective covers of both insects and fungi, respectively viz; cuticle and cell wall share common structural component chitin. The role of chitinolytic enzymes is being studied as biopesticides or chemical defense proteins in transgenic plants and microbial biocontrol agents. Entomopathogenic fungi appear to overcome the host's physical barriers by producing a variety of extracellular enzymes, including chitinolytic enzymes, which help in the penetration of the cuticle and promote infection. After cellulose, chitin is the two most common biopolymer on the planet. New bacterial enzymes can be found and exploited by metagenomic techniques through extensive cloning and screening hidden among uncultivated microorganisms. Enrichment is also another well-known method, as it allows for the selection of organisms that have evolved to feed on a particular substance.

It is also important to find alternatives to chemical pesticides and *Bacillus thuringiensis* toxin because resistance to these molecules has already developed. Despite the fact that chemical pesticides will continue to be used for many years, the need for environmentally acceptable alternatives is urgent if we are to avoid further harming the Earth's ecosystems. Novel biocontrol agents that serve as pesticide alternatives, particularly those that do not create resistance and are sustainable and environmentally friendly, have begun to come on the market and should be a top priority in the future. Chitinolytic bacteria could be a viable alternative to these poisons because they are already present in the soil and endophytic microbiome. Chitinolytic bacteria like *Bacillus subtilis*, *Bacillus popilliae*, *Bacillus thuringiensis*, *Bacillus sphaericus*, *Bacillus licheniformis*, *Pseudomonas fluorescens*, *Burkholderia cepacia* (formerly *Pseudomonas cepacia*), *Streptomyces griseoviridis*, *Serratia entomophila*, *Paenibacillus sp*, *Pantoea dispersa*, *Nocardia orientalis*, *Serratia marcescens*, *Vibrio alginolyticus*, *Streptomyces cinereoruber* etc. are important for pest and pathogen control. Because chitin synthesis is limited to insects, fungi, and some algae, many of them are plant pathogens, this molecule in the pathogen is a logical target for pest control

Combining chitinases with other bioactive peptides and lytic enzymes, such as glucanases, β protease and lipase as found in natural systems, is expected to increase their potential. As a result, the employment of combinatorial methods should be emphasized. The enormous potential of genetic engineering will allow us to combine natural plant responses with transgenes of microbial or insect chitinases. Inhibition of dangerous pests like phytopathogenic fungi and unwanted insects that is



appropriate and target specific. Depends on the availability of highly active and stable chitinase preparations at a reasonable price. In the future, sophisticated protein engineering may open up new avenues for producing chitinases that act reliably as biocontrol agents even in severe and extreme environmental circumstances. The synergistic mechanism of chitinases and glucanases in the fight against fungal biocontrol is well-known. However, serious and committed research efforts are still required to understand how to develop effective and highly stable formulations of both catalysts that should be more progressive in the treatment of fungal plant diseases.



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***Trypanosoma cruzi* – CHAGAS DISEASE**

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Chagas disease is named after the Brazilian physician Carlos Chagas, who discovered the disease in 1909. Chagas disease is caused by the Protozoan parasite *Trypanosoma cruzi*, which is transmitted to animals and people by insect vector that are found only in the America. Chagas disease (*Trypanosoma cruzi* infection) is also referred to as “American Trypanosomiasis”. The name *Trypanosoma* derived from Greek word, “Trypano” means insect; “soma” means body. *Trypanosoma cruzi* causes Chagas disease, a zoonotic disease that can transmitted to human by blood sucking Triatomine bugs (Genus name - *Trypanosoma*; Species name - *cruzi*; Binomial name - *Trypanosoma cruzi*). *Trypanosoma* comes under Family Trypanosomatidae; Phylum under Euglenozoa and then Class under Kinetoplastid. *Trypanosoma cruzi* in human blood Giemsa stain. They are typically seen as c-shape. *Trypanosoma cruzi* is transmitted by Triatomine bugs. Parasite need a host body and the hematophagous insect triatomine (Description: Kissing bug, Cone-nose bug) is the major vector in accord with a mechanism of infection. This infection usually occurs after bugs defecate on the bite site and are rubbed into the wound by the host scratching and portal of entry through skin or conjunctiva. In the skin a localized swelling “Chagoma” develops when the entry is through the conjunctive an Oedematous swelling eye lids of one eye develops.

Morphology

Three main Morphological forms are i) Trypomastigote form, ii) Amastigote form and iii) Epimastigote form.



- i) **Trypomastigote form:** The Trypomastigote forms appear in the Peripheral blood. It measures 20 μm in length, flagellated and it has a central nucleus and a large, oval kineoplast situated at the posterior wedge-shaped end. Multiplication of the parasite does not occur in the Peripheral blood. The Trypomastigote forms are either taken up by the insect host or enter tissue cells where they continue to live as Amastigote forms.
- ii) **Amastigote form:** The amastigote forms are oval bodies or round and non-flagellated, measuring 2 to 4 μm in diameter having nucleus and kinetoplast. These are particularly found inside the cells of skeletal and heart muscles. Multiplication of the parasite occurs at this stage only. A parasite of muscular and nervous tissue and also the rough endoplasmic reticulum system existing in the amastigote form.
- iii) **Epimastigote form:** This form found in the vector. It has a kinetoplast adjacent to the nucleus. An undulating membrane runs along the anterior half of the parasite. It multiplies by binary fission.

Immunology

Serum antibodies develop in *T. cruzi* infection but as the parasite continues to grow as amastigote from inside the rough endoplasmic reticulum cells and Parenchyma cells they are not exposed to the action of these Antibodies.

Life cycle

T. cruzi passes its life cycle in two hosts: One in man or the reservoir host; the other in the transmitting insect, the reduviid bug (*Panstrongylus megistus*, *Triatoma infestans* and *Rhodnius prolixus*).

- **Development in Reduviid Bug:** The trypomastigote forms are taken up by the bug during the act of biting. In the “stomach” (mid-gut) of the bug they are transformed into amastigote forms and thereafter multiply by binary fission. Later the amastigote forms are transformed into Epimastigote forms which migrate backwards to hind-gut and in turn multiply by longitude fission. In 8 to 10 days' time, metacyclic forms of Trypomastigote appear and are excreted in the faeces of the bug.
- **Development in Man:** Man is infected either by faecal matter of the bug being rubbed into the wound caused by the bite or by a possible contamination of the conjunctivae and other exposed mucous membranes with the fingers. The metacyclic trypomastigotes thus introduced, invade tissue cells and are transformed into amastigote forms. These multiply by binary fission after passing through promastigote and Epimastigote forms



again transformed into Trypomastigote forms which are liberated in the blood.

- **Pathogenic lesions:** Skeletal muscle and nervous system (thyroid gland in some region) and are all due to the invasion of the parasite inside the tissue cells. The cells of the reticulo-endothelial system are particularly involved. The parasite multiplies only in the amastigote forms. Parasitic multiplication within the cells leads finally to destruction of the cell.

Clinical Features

Incubation period is 7 to 14 days. The acute form occurs in children and infants. It is characterized by fever, conjunctivitis, unilateral oedema of the face, enlarge of the spleen and lymph nodes, anemia and lymphocytosis. It lasts for a period of 20 to 30 days, often terminating fatally with symptoms of meningoencephalitis or myocardial failure. The chronic form is seen in adults and adolescents. Its characterized by disturbance of cardiac rhythm (Heart block, Adams – Stokes syndrome) and Neurological manifestation (Spastic paralysis). It may last for a period of 12 years.

Laboratory Diagnosis

- a) Detection of parasite Deoxyribo Nucleic Acid by Polymerase Chain Reaction method is now used for detection for positive cases with very few parasites in the blood.
- b) Xenodiagnosis: By allowing a laboratory – bred parasite free reduviid bug to feed on an individual suspected to be suffering from chagas disease and 2 weeks later examining the intestinal contents for the presence of the parasite.

Treatment

Nifurtimox (lampit) in a dose of 10 mg/day in three divided doses orally after meal for 92 - 120 days is used. Alternative drug benznidazole is given orally in a dose of 5 – 10 mg/kg/day in two divided doses for 30 - 90 days.

Prophylaxis

- a) Attack on the Parasite - Treating the disease with a specific drug, if available.
- b) Attack on the Vector - Use of insecticide and use of building materials which are impermeable to the bug.
- c) Personal Prophylaxis - Avoiding the bites of the insect by using mosquito-nets.



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Biodegradation of Xenobiotic Compound Using Microbes

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Introduction

During the 1950s and 1960s many low- and middle-income countries were struggling to feed the growing need of their population which saw a boost due to the industrial revolution and rise of employment. The Green Revolution was introduced in these countries, which saw a sharp rise in the yield of the food grains production making the agriculture produce available for exports due to the use of high yielding varieties of seeds, advanced irrigation technology and chemical pesticides, fertilizers. The Indian economy which is largely based on agriculture has been immensely benefited by the advent use of pesticides, there has been a fourfold increase in production of food grains by the application of pesticides. The term pesticide encompasses a wide array of components from fungicides, insecticides, nematicides, rodenticides, molluscicides, herbicides among others. But the main players are organophosphate, and organochlorine compounds. They have completely transformed the field of agriculture by keeping a control on the population of pests and not allowing the growth of unwanted weeds and herbs near the crop of interest so that nutrient availability is not shared. The excessive use of organochlorine compound as DDT in the earlier times used to significantly reduce the mosquito borne diseases malaria but due to the extreme toxicity and bioaccumulation of such xenobiotic compound the developed countries banned its use in the developing countries.



What are these Xenobiotic compounds?

A substance foreign to the biological system is known as xenobiotic compound. Chemical fertilizers pesticides oil spills are the key sources of xenobiotic compounds entering the environment. Due to their potential toxicity to both wildlife and humans, several persistent organic pollutants (POPs) have now been totally banned from production and use in many countries around the world. No population is protected from the pesticide ill effects.

What is the effect of this Pesticide on Soil and Environment?

Due to the leaching of heavy metals from the fertilizers and related compounds not only contaminate the soil flora but also affect the water table and cause poisoning of the groundwater. The worldwide deaths and chronic diseases due to pesticide poisoning number about 1 million per year. High risk groups exposed to pesticides include production workers, formulators, sprayers, mixers, loaders and agricultural farm workers. During manufacture and formulation, the possibility of hazards may be higher because the processes involved are not risk free. Organochlorine compound is virtually found in the tissues of all life forms due to the phenomenon of Biomagnification.

i) Biomagnification

Organophosphorus compound toxicity can be classified into two broad categories namely Axons and Thions the axons include Axons which have a P=O double bond and include phosphotriesterase and phosphotulates. The organophosphate poisoning act by interfering with the neurotransmitters namely acetylcholinesterase activity, OPs covalently bond to the hydroxyl group of serine phosphorylation, inhibit the enzyme's active site and cause the accumulation of acetylcholine at synapses. Also, these Ops impair the reproductive function of humans causing infertility, premature birth to birth defects

Solution for Accumulation of Xenobiotic pesticides and its Deleterious effects

Bioremediation, means use of microorganisms to remediate/ destroy or to immobilize pollutants from the environment. The fate of pesticides in the environment is determined by both biotic and abiotic factors. Pesticides are degraded in the environment principally by the action of indigenous microorganisms. Organophosphate pesticides are generally regarded as safe for use on crops and animals due to their relatively fast degradation rates. The degradation rates vary as a function of microbial composition along with different environmental factors, such as pH, temperature, and availability of sunlight. Some studies have shown that



organophosphate pesticides degrade rapidly by hydrolysis on exposure to sunlight and air.

Degradation of Xenobiotic Compounds by Microorganisms

Most pesticides, such as organophosphate, carbamate, or pyrethroid are biodegradable compounds which can hydrolyze spontaneously at high pH and by enzymes to less toxic materials. Enzymes play a key role in degradation of these toxic pesticides and most of these enzymes are produced by bacteria which are naturally equipped with the mechanisms to deal with these toxins produced by manmade activities. The microbes by means of gene transfer can acquire the gene necessary for production of enzymes which can be helpful for the degradation, also the group of bacteria can form a consortium and can together bring about degradation of complex and toxic pesticides which can be utilized by other groups of organisms. The genera of organisms like *Flavobacterium* spp., *Micrococcus* spp., *Arthrobacter* spp. are some of the significant players in the degradation



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BIDIRECTIONAL COMMUNICATION BETWEEN THE CENTRAL AND THE ENTERIC NERVOUS SYSTEM (GUT MICROBIOME)

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The Gut Microbiome (GM) is a clear community of microorganisms that inhabit our gut. In the colon majority of the single-cell present in not less than 1 trillion GM reside in grams of intestinal. The approximately 40 trillion bacteria are present in our gut, but best guess of using to date technology. In Human body containing 30 trillion cells, so more than cells present the more bacteria. Most of our gut bacteria more belong to 30 spp., or can be up to 1,000 different spp. in all there collectively called the microbiome. The deep of understanding of the gut-brain once of the complex communication system, but an important role in the interaction between Central Nervous System (CNS) and Enteric Nervous System (ENS), metabolic pathway, prevent the many diseases, immune activation, entero-endocrine signalling. The gut has defences against pathogens but at the same growth and survival of healthy gut bacteria.

The study of GM to brain, the study testing animal is germs free animal, probiotic, antibiotic and infection. In the absence of microbial colonization is an altered expression and turnover of neurotransmitters in both nervous systems and gut



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sensory-motor functions. The Neuromuscular abnormalities resulted in a reduction in gene expression of enzyme involved in the synthesis and transport of neurotransmitters, muscular contractive protein. Also show that microbiota influences stress activity and regulate the set point for Hypothalamic Pituitary Adrenal (HPA) activity. In the age dependent manner only microbial colonization leading to gut normalization. Also, dysfunction was reported altered expression of brain derived neurotrophic factor. It's mainly involved memory and located in the hippocampus and cerebral cortex, these functions are regeneration, muscle repair, differentiation. Some studies are confirmed that affect anxiety and the HPA system by influencing brain neurochemistry by through the use of probiotics and/or antibiotics. The chronic treatment with *Lactobacillus rhamnosus* JB-1 induced region depended changes in GABA (Gamma Amino Butyric Acid) mRNA in the brain. GABA_{B1b} increased in the cortical cingulate and prelimbic region, GABA_{Aα2} reduced in the prefrontal cortex and amygdala, but increased in the hippocampus. The probiotic is reduced stress, anxiety, and depression like behaviour. Take up the oral antibiotic in specific pathogen free and increased exploratory behaviour and hippocampal expression of Brain-derived neurotrophic factor (BDNF) or Abrineurin.

The microbiota communication with the brain involves the vagus nerve, which transmits information from the luminal environment to CNS. The microbiota affects mucosal immune activation. The uptake or treatment with antimicrobials, increases substance P expression in ENS, an impact normalized by the administration of *Lactobacillus paracasei* which also attenuates antibiotic-induced visceral hypersensitivity.

The brain to gut microbiota's different way of psychological stressors modulates the composition and overall biomass of the enteric microbiota by independently from duration. Within 2 hrs significantly change the community profile because of social stressor exposure and reduce the relative proportions of the main microbiota phyla. The parallel neuroendocrine output efferent systems, both directly via host-enteric microbiota signalling and indirectly via changes in the intestinal environment. These are efferent neural pathways, associated with the pain-modulator endogenous pathways called an emotional motor system. The communication between CNS effectors and bacteria banks on the presence of neurotransmitter receptors on bacteria. That binding sites for enteric neurotransmitters produced by the host are present bacteria and may influence the function of components of the microbiota contributing to extend prediction to inflammatory and infection-induced. High affinity for the GABA system has been reported in *Pseudomonas fluorescens* with having binding properties almost like those of



a brain receptor. *Escherichia coli* possesses a receptor for host-derived epinephrine that would block specific adrenergic antagonists.

The brain also is affected by microbiota composition and function by a change of interesting permeability, allowing bacterial antigens can penetrate the epithelium and induced an immune response in the mucosa. If the stress increased in colonic paracellular permeability calls for overproduction of Interferon- γ and a decrease in mRNA expression of ZO-2. corticotropin delivering chemical receptors are engaged with colonic boundary brokenness in light of gentle pressure in neonatal maternal detachment in grown-up rodents that prompts discouragement and upgraded weakness to colitis. If possible, anxiety in the microbiota habitat make by perturbation occurs through the enhancement in the section of defensin, an antibiotic peptide, from Peneth cells.

The Gut Microbiota (GM) has been playing an important role in bidirectional interaction between the ENS and CNS or gut and nervous system. It interfaces with CNS by directing mind science and impacting neuro-endocrine frameworks related to pressure reaction, nervousness, and memory work. A considerable lot of these impacts give off an impression of being strain-explicit, recommending a possible job of certain probiotic strains as the novel adjuvant methodology for neurologic issues.



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PRODUCTION OF MILK FROM MICROBES

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As peoples are the largest consumers of the milk, but due to consuming too much milk can cause various problems to human. As lactose present in milk, it's not been easily digestible that's why it's important to produce a milk from fermentation of bacteria. Lactose itself can't be absorbed and it first needs to be broken down into its components: glucose and galactose. The milk produce from microbes can be considered as the world's cleanest milk because it has no lactose, no cholesterol and even its not from animal. This milk produces without cow.

The milk comes from cow contain lactose, cholesterol and saturated fats which cannot be easily digestible by peoples. Also people waste lots of water, money, and energy to give us milk, and in animal factories milk production means animal abuse. That's why we need to come up with a different kind of milk that is sustainable, affordable and ethical. So that for the production of milk, scientists first extract the genes from the cow that how they exactly produced a milk, and transformed it into a microbe. This genetically engineered microbes allow to grow at different level. And this microbe using a fermentation process produce a milk protein. And from this protein they created milk. It looks exactly the same, taste exactly the same as the one gets from cow. From this milk can also produce milk products like cheese, yoghurt and other milk products.



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Ideonella sakaiensis - ANTICIPATION TO ECO-FRIENDLY PLASTIC REMEDIATION

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Plastic waste is being increasing and accumulating in the environment causing pollution over years. Recycling of plastic doesn't seem to be a very promising solution because there is a limit to which plastic can be recycled. Also, plastics (like water bottles, straws, grocery bags) ultimately go into the water bodies and then accumulate into the ocean. Now, inside the ocean microplastics are ingested by marine algae as food and they enter the food chain. When these marine algae are consumed by aquatic organisms. They keep on concentrating and bioaccumulating with increasing trophic levels and ultimately reach humans via sea food consumption (bio-toxification). And it can lead to plastic poisoning.

Currently, the plastic recycling factories are the only option for plastic waste management although they are not much efficient in waste management. And recycling is not a sustainable solution. So, microbes seem to be a better option. A bacterium named *Ideonella sakaiensis* was discovered outside a plastic recycling factory in Osaka, Japan. This species of bacteria has a potency to degrade plastic (i.e. PET: polyethylene terephthalate) by breaking it down into environment friendly components. PET is the commonly used type of the plastic and it is being used widely in packaging, bottles, and containers. This bacterium releases an exogenous enzyme called MHETase when attaches to the PET surface followed by release of PETase enzyme. Both these enzymes are responsible for PET degradation and the bacteria uses PET as a source of fuel to produce energy by using it as a food and carbon source. *Ideonella sakaiensis* is a negative-gram bar-shaped bacterium. It is an aerobic bacterium that have flagellum that makes it able to motile or move quickly.



This bacterium grows optimally in temperatures of 30 – 37 °C and in acidity level of pH 7 - 7.5. The strain of Ideonella itself is positive to catalase and do cytochrome oxidation test.

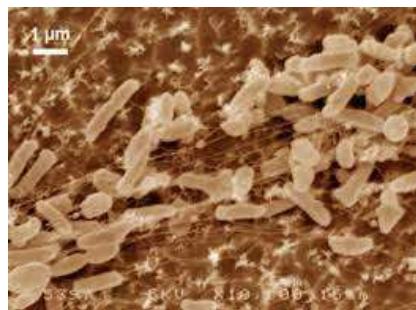


Figure – 1: *Ideonella sakaiensis* (as viewed under electron microscope)

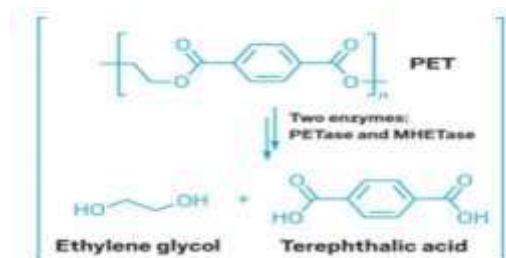


Figure – 2: Conversion of PET to eco-friendly compounds by *Ideonella*

Current research on *Ideonella* focused on transformation of this plastic eating bacteria to make it survive as a free-living bacterium in water as well as terrestrial habitats. There are free-living microbes that are found almost everywhere in soil and water, like-*Azotobacter*. So, transformation of the genes of these free-living microbes into the *Ideonella* genome can make it survive in soil and water freely. *Azotobacter* have few similar characters to *Ideonella* that like being gram negative, does not produce endospore, mobilize with flagella.

The PET producing gene of *Ideonella* was inserted into *Vibrio fischeri* which is a marine bioluminescent bacterium. Also, the molecular structure and biochemistry of the enzyme PETase has been studied to synthesize this enzyme at industrial level. The PETase enzyme have been genetically modified and combined with MHETase for faster degradation of plastics. In conclusion, rigorous research is required to manifest the implementation of *Ideonella* to practical use for a safer plastic degradation.



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FERMENTED FOOD: A PROMINENT SOURCE OF PROBIOTIC

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Fermented foods are defined as “foods or beverages produced through controlled microbial growth, and the conversion of food components through enzymatic action”. Many foods have historically undergone fermentation, including meat and fish, dairy, vegetables, soybeans, other legumes, cereals and fruits. There are several variables in the fermentation process including the microorganisms, the nutritional ingredients and the environmental conditions, giving rise to thousands of different variations of fermented foods. Historically, food fermentation was performed as a method of preservation, as the generation of antimicrobial metabolites (e.g., organic acids, ethanol and bacteriocins) reduces the risk of contamination with pathogenic microorganisms. Fermentation is also used to enhance the organoleptic properties (e.g., taste and texture), with some foods, such as olives, being inedible without fermentation that removes bitter phenolic compounds.

There are two main methods through which foods are fermented. Firstly, foods can be fermented naturally, often referred to as “wild ferments” or “spontaneous ferments”, whereby the microorganisms are present naturally in the raw food or processing environment, for example sauerkraut, kimchi, and certain fermented soy products. Secondly, foods can be fermented via the addition of starter



cultures, known as “culture-dependent ferments”, for example kefir, kombucha and natto. One method of performing a culture-dependent ferment is “backstopping”, in which a small amount of a previously fermented batch is added to the raw food, for example sourdough bread. Starters used to initiate fermentation can be either natural (e.g., backslappings), or selected commercial starters to standardize the organoleptic characteristics of the final product.

Fermented foods hold a firm place in cuisine from almost every culture in the world. In the West, there has been a surge in popularity of fermented foods in recent years, major reasons including the proposed health benefits of fermented foods and surging interest in gastrointestinal health. There are several mechanisms through which fermented foods may exert beneficial effects in health and disease. Fermented foods are foods and beverages that have undergone controlled microbial growth and fermentation. Fermentation is an anaerobic process in which microorganisms like yeast and bacteria break down food components (e.g. sugars such as glucose) into other products (e.g. organic acids, gases or alcohol). This gives fermented foods their unique and desirable taste, aroma, texture and appearance.

The most commonly defined probiotic bacteria include member of the genera *Lactobacillus*, *Bifidobacterium*, lactic acid and non-lactic acid bacteria. Different species belong to these genera are considered as probiotics. Many fermented dairy products including curd, cheese, yoghurt was supplemented with variable probiotic strains, like *Lactobacilli*, *Streptococcus* and *Bifidobacterium* strains. Fermented vegetables, including sauerkraut, olives, mustard pickles, pickles, and kimchi are important sources of probiotic bacteria namely *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Pediococcus cerevisiae*, *Leuconostoc*, *Weissella* and *Lactobacillus* spp.

Lactobacillus brevis, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Pediococcus acidilactici* and *Leuconostoc fallax* are the predominant in fermented vegetable product gundruk, sinki, and khalpi. Fermented porridges and gruels are widely consumed in many African countries. These cereals were made using pearl millet, millet, sorghum, and maize as starting grains. Bacteria commonly found in kombucha a fermented beverage made from sweetened tea include the acetic acid bacteria belonging to the genera, *Acetobacter*, *Gluconacetobacter*, and *Gluconobacter*, as well as Lactic acid bacteria. Most of the yeasts associated with kombucha are species of *Saccharomyces*, although other yeast genera may also be present.



In conclusion, Probiotic microorganisms, including bacteria and yeasts, are attracting a growing interest due to their promising physiological effects as well as the value they add to probiotic-containing food products. There is a growing body of evidence that probiotics may play a beneficial role in human health. Established effects in humans include alleviation of symptoms linked to lactose intolerance or to irritable bowel syndrome. They also include reduced diarrhea associated with antibiotic treatment, rotavirus or Traveller's diseases. It should be emphasized that the beneficial properties of probiotic microorganisms are highly dependent on the strains, which means that each strain or product requires demonstration of the specific effects *in vivo*.



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BISMUTH - A 'GREEN' ANTIMICROBIAL AGENT

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Antimicrobials are substances which are used to terminate or slow down the growth of viruses, bacteria and other microorganisms. They are essential in preventing and treating infections but they pose a global threat to public health when microorganisms develop antimicrobial resistance. Emergence of various antibiotic resistant microorganisms is occurring worldwide which endangers the efficiency of pre-existing antibiotics. With drug resistant microorganisms on the rise, it's high time we revisit metal based antimicrobials.

Metal based antimicrobials have been used to treat various infections since ancient times. Ancient Egyptians used to treat fevers and skin irritation using Antimony. Antimony based complexes were used widely during start of 20th century to treat leishmaniasis. Along with antimony, arsenic based compounds were also used to cure various infections. These heavy metals are generally used in the form of liquid suspensions. They are toxic and not meant to be exposed directly to vital organs or swallowed. But placed between lead and polonium at position 83 on the periodic table is an elemental exception. Bismuth is a heavy metal which has puissant antimicrobial activity and has been used to treat gastrointestinal infections since 18th century. Bismuth has this strange double character of being a heavy metal with this powerful antimicrobial activity, but at the same time seemingly very low toxicity within humans. Today it is used as a part of combinational therapy to treat stomach ulcers caused by drug resistant gram negative bacterium *Helicobacter pylori*.



Bismuth subsalicylate has been marketed as an OTC medicine named Pepto-Bismol since 1919 to treat gastrointestinal problems.

Organic antibiotics have been researched for almost half a century while metals have received much less attention. It is because mode of action of metal complexes is more intricate than organic antibiotics. Organic antibiotics mainly hit one specific target, whereas metals often appear to be more scattershot. This characteristic property of metal based antimicrobials exerts a pleiotropic effect on bacterial cells. As metals hit multiple targets, it's harder for the bacteria to evolve resistance. It has been observed that bimolecular proteins are the principle targets for bismuth, especially those particular proteins which possess cysteine (thiol) rich domains or metal binding sites with suitable coordination environment for bismuth. Also one more additional advantage for using bismuth (III) is that for the regulation of their metabolic activities and survival, bacteria have to import ions, and similar ions can ride the same pathway. This aspect of bacterial cells can be utilized for making transport of bismuth ions more convenient. Even if a cell decides to shut down its ion uptake because it wants to prevent a metal that mimics the ion from coming in, it is not going to survive anyway.

One more interesting aspect of bismuth lies in its hydrolytic chemistry. When any bismuth complex is reacted with water, it forms chunks of bismuth oxide. These complexes or clusters of bismuth oxide are biologically inactive, shutting down bismuth's antimicrobial activity. These clusters are composed of 9 – 38 bismuth atoms in size. We know that bacteria tend to develop resistance against a complex when it is present in less concentration than what is needed to kill the bacteria. If the complex is essentially inactive which here is bismuth, then the bacteria cannot possibly develop resistance against the complex. It is due to these factors that *Helicobacter pylori* hasn't been able to develop antimicrobial resistance against bismuth complexes even after 100 years of use.

In case of *Helicobacter pylori*, when the culture was exposed to bismuth sub citrate for over 12 hours, it was observed that bismuth sub citrate disrupts bacteria's central carbon metabolism, reducing its energy production and growth. After further detailed analysis based on metagenomics, it was found that many systems were involved in this, including the glycolysis pathway and citric acid cycle. Therefore bacterial central metabolism might be an important target that results in drugs that bacteria can't easily adapt to.



Even though bismuth drugs are quite effective in treating *Helicobacter pylori* infections and reducing the prevalence of resistant strains, their mode of action is not fully understood. Scientists from all over the world are coming up with different strategies and advances to understand the mode of action accurately. Now instead of focusing on changes during protein or gene expression, or metabolite production by bacteria, researchers are using Transcriptomics, Metabolomics and Metalloproteomics to correlate multiple cellular changes with respect to numerous intracellular and extracellular processes simultaneously.

Given the notable resurgence of scientific interest in the physiochemical and bioinorganic properties of bismuth and similar elements, it is indeed becoming clear that organometallic compounds have the potential to play new and essential roles in killing drug resistant organisms. Given its history and varied applications in medical chemistry, bismuth has earned the reputation of a ‘green’ non-toxic metal. Research papers based on use of novel Bismuth compounds are increasing day by day. Organometallic compounds have given promising results at early-stage clinical assays and can be used as integral weapons against fighting drug resistant microorganisms.



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AMINOGLYCOSIDE RESISTANCE (16S rRNA Methyltransferase) IN GRAM NEGATIVE BACTERIA

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Aminoglycosides are an important class of broad spectrum antibiotics that target 16S rRNA in bacterial ribosomes and disrupt protein synthesis. They are active against various gram positive and negative organisms. The commonly used aminoglycosides include Tobramycin, Amikacin, Neomycin, Gentamicin and Streptomycin (TANGS). Aminoglycoside play an important role in the treatment of several infections caused by Gram negative bacteria (e.g., *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and also used in combination with β lactam antibiotics. The emergence of 16S rRNA methyltransferases (16S RMTase) around the world is a growing concern due to their ability to confer high-level resistance (Minimum inhibitory concentrations (MICs) > 256 mg/L) to all clinically relevant aminoglycosides. There are several mechanisms of aminoglycoside resistance; the most common mechanism of resistance to aminoglycoside is the enzymatic inactivation, which is mediated by three classes of enzymes: acetyltransferases, nucleotidyl transferases, and phosphotransferases. Other mechanisms of resistance to aminoglycoside include decreased cellular permeability, active efflux, nucleotide substitution of the target molecule.



The 16S RMTases is an emerging aminoglycoside mechanism. It was first discovered in Enterobacteriaceae and in *Pseudomonas aeruginosa* in the late 1990's. Within 16S rRNA, two sites of methylation that lead to different aminoglycoside resistance phenotype have been identified. One group of 16S rRNA are produced by the Istamycin producer *Streptomyces tenjimariensis*, methylates residue. It confers resistance to Kanamycin and Apramycin but not Gentamicin. Another group of 16S rRNA are produced by Gentamicin producer *Micromonospora purpurea*, methylates residue and confers resistance to Kanamycin and Gentamicin but not Apramycin. RmtA was found among clinical isolates of *Pseudomonas aeruginosa* in Japan, RmtB has been detected in various species of Enterobacteriaceae including *Klebsiella pneumoniae*, *Escherichia coli*, and *Clostridium freundii*. ArmA was initially found in *Clostridium freundii* and later in *Klebsiella pneumoniae* and it has also been identified in clinical isolates of Enterobacteriaceae.

RmtD and RmtA have only been reported from *Pseudomonas aeruginosa*. Hence, these findings indicate that 16S RMTases genes are already distributed globally among pathogenic Gram negative bacilli. The global spread of Gram negative bacilli producing 16S rRNA methylase is concerning for several reasons. First concern is, high level of resistance to most clinically useful Aminoglycosides such as Gentamicin, Tobramycin and Amikacin, which cannot be overcome by dose adjustments. Second the all structural genes of known 16S rRNA methylases are associated with mobile genetic elements like Transposon. Third, these Gram negative bacilli possess a high potential for developing multidrug resistance especially in combination with various β -lactamase genes. For the detection of 16S rRNA methylase resistance Disc diffusion tests followed by PCR confirmation is recommended.



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PHENOTYPIC DETECTION OF METALLO-BETA LACTAMASE (MBL) PRODUCING GRAM NEGATIVE BACTERIA

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Antibiotic resistance produced by Metallo-β-lactamases (MBL) enzymes is particularly worrying due to activity patterns that include most carbapenems and beta-lactam medications. MBL are zinc-based enzymes that break the amide bond in the beta-lactam ring. Multidrug-resistant (MDR) Gram negative bacteria (GNB) pose the greatest hazard to public health. As a result of mobile genetic elements that can quickly spread through the population, GNB are becoming more resistant. So far, there have been five different types of acquired MBL identified. In the pharmaceutical sector, terminologies like VIM, IMP, GMP, and SIM are employed. Because their molecular structures are so distinct. IMP and VIM variants have been discovered all over the world. The Carbapenemase epidemic will not go away by itself. Such community-based outbreaks will be difficult to contain. Fast diagnosis is necessary to minimize the spread of MBL producing GNB.

MBL gene identification is mostly based on molecular methods. While PCR analysis often yields satisfactory findings in the detection of MBL, it is not cost-effective for everyday testing in clinical laboratories. Carrier discovery, on the other



hand, is based on screening in culture media. Two tests are recommended for detecting MBL production: the Modified Hodge Test (MHT) and the Combined Disc Test (CDT). The MHT can be used to identify bacteria that generate Carbapenemases quickly and easily. The MHT must be used to analyse all isolates having a moderate or sensitive zone diameter on disc diffusion for Carbapenemases production. The zone size difference in the Disc Potentiation Test (DPT) with EDTA must be monitored since the MBL gene does not always confer resistance. Chelating agent EDTA is utilized. The position of Zn(2+) is crucial for substrate binding and hydrolysis. Because these agents have been reported to block metallo-lactamases, CuCl₂, FeCl₂, EDTA, and thiol compounds including mercaptoacetic acid, 2-mercaptopropionic acid, and mercaptoethanol were utilized and assessed for IMP-1 suppression. EDTA generates a clear growth-inhibitory zone between the two discs. Because it has a poorer specificity than imipenem and meropenem, ertapenem is not recommended as an indication carbapenem.

One strategy adopted by bacteria to combat beta-lactam antibiotics is the creation of MBL that require Zn (2+) to function. Despite the fact that the number of Enterobacteriaceae that produce Carbapenemases is rather vast, MHT detection provides a feeble positive result. MHT has good sensitivity for KPC and OXA enzymes, but is insensitive to VIM, IMP, and NDM, according to numerous studies. Despite the fact that certain isolates have a small amount of indentation, they do not create Carbapenemases. The E-test is 100 percent sensitive, according to a comparison of different phenotypic assays for the identification of MBL in bacteria. Due to cost and availability limits, it is probable that not all laboratories will be able to execute the E-test.

Large investigations have indicated that the CDT has the highest rate of sensitivity, followed by the E-test. When bacterial protein extracts are incubated with carbapenems, MALDI-TOF MS detects particular carbapenem breakdown products. Because of its simplicity, CLSI recommended that Enterobacteriaceae with high carbapenem MIC's or low disc diffusion inhibition zones be tested for Carbapenemases production using the MHT. This does not apply to *Pseudomonas aeruginosa*, where acquired Carbapenemases are becoming more common. For MDR *Pseudomonas aeruginosa*, a more precise combination disc test is more possible. The DPT, is proven to be a more sensitive test, because the MHT produces a false positive result.

Unlike the qualitative Double Disc Synergy Test, the DPT and the E-test are both partly quantitative, allowing for the computation of an MBL index. The CDT and E-test MBL index is a measure of relative MBL activity that is comparable



within the approach. The MBL indexes derived by these two procedures are incomparable due to significant changes in methodology between disc potentiation and E-test. A zone size of more than 8 mm in an EDTA-incorporated disc suggests that EDTA has a negative effect on *Pseudomonas aeruginosa* in addition to its chelating activity. As a result, there are no widely used tests for detecting MBL. The E-test advantage has been that it is the only approach that permits the MIC to be determined. In conclusion Several investigations have shown that modified variants of the Carba NP test (E-test) may detect Carbapenemases-producing *Pseudomonas aeruginosa* with a sensitivity and specificity of more than 90 % in most situations. It is critical to discover MBL-producing bacteria and more precisely quantify their percentage positive in hospitals, to restrict their further spread. According to study evaluations, phenotypic tests should be evaluated and chosen based on the local context, as the most common kinds of MBL display genetic heterogeneity across regional boundaries.



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RESPIRATORY TRACT INFECTION CAUSED BY *Moraxella catarrhalis* IN CANCER PATIENTS

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Respiratory Tract Infection (RTI) refers to any of a number of infectious diseases involving the respiratory tract. An infection of this type is normally further classified as an Upper Respiratory Tract Infection (URI or URTI) or a Lower Respiratory Tract Infection (LRI or LRTI). The respiratory tract is the part of the human system that plays a vital role in breathing processes. There are a number of acute and chronic infections that can affect the lower respiratory tract. The two most common infections are bronchitis and pneumonia. RTI remain substantial causes of morbidity and mortality globally, the common bacterial pathogens being *Streptococcus pneumoniae* and *Hemophilus influenzae* followed by *M. catarrhalis*. *M. catarrhalis* is a bacterium belonging to the Neisseriaceae family. It was first described in 1896 as *Mikrokokkus catarrhalis*. It has since been named *M. catarrhalis*, *N. catarrhalis* and *B. catarrhalis* but is commonly known by *M. catarrhalis*. Its β-lactamase producing ability draws even more attention toward its varying patterns of resistance. *M. catarrhalis* is gram negative diplococcus bacterium. *M. catarrhalis* has particularly become an important pathogen in patients with immunocompromised status and in patients with chronic pulmonary diseases. It has been considered harmless commensals of the upper respiratory tract. It colonizes the respiratory tract of a small proportion of adult



and larger proportion of children. Identification of *M. catarrhalis* is done by gram staining culture and biochemical tests. A majority of strains produce beta-lactamase especially in clinical significant isolates. Many treatment failures with ampicillin or amoxicillin are due to the production of this enzyme. The genus *Moraxella* also includes the normal human microflora of the genital tract. This emergent role of *M. catarrhalis* calls for intensified efforts to understand its interaction with the human host. Respiratory tract colonization is a key element in the pathogenesis of *M. catarrhalis* infection and remains incompletely understood. Currently available data on age specific colonization rates indicate that infants are exposed to *M. catarrhalis* very early life, whereas carriage rates in older children and healthy adult are low. The latter data are difficult to explain for a number of reasons. Because it is an exclusively human pathogen.



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DOCTOR ACTINOBACTERIA

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Introduction

Actinobacteria is a Gram positive bacteria with High G +C DNA content. It exists as either Dormant spores or actively growing mycelium with filaments called hyphae. They are phenotypically diverse and exhibit wide variety of morphologies. The highly differentiated mycelium and spore production create long distance dispersal, so their ubiquitous nature, capability to survive in extreme habitat and staggering diversity of their biosynthetic capability for specialized metabolites made actinobacteria as a doctor for treating and recovering varies problems associated with abiotic and biotic components.

Actinobacteria treating the Soil

Bioremediation is needed to reduce and remove the pollution we produce and to provide healthy soils for future generations. Actinobacteria can bioremediate the soil by degrading complex polymers and recycle the organic carbon. It can degrade high doses of pesticides, chemical complexes, and heavy metals. Actinomycetes utilise toxins as carbon sources and in turn synthesise commercially viable antibiotics, enzymes and proteins. Especially Genus *Streptomyces* can resist several metal ions and can utilize harmful chemical residue of pesticide as carbon source and convert them to non-toxic form, streptomyces also produce wide range of siderophore (metal ion chelator) to protect from negative effect of heavy metals and produce Extracellular polymeric substances (EPS) to remove heavy metals.



Actinobacteria treating the Humans

Actinobacteria produce variety of drugs that are extremely important to human health and nutrition. They are found in the human body, from the skin to mucosal surfaces. Their presence and abundance in these specific sites correlate with the individual's health status. *Corynebacterium*, *Propionibacterium*, *Rothia*, *Actinomyces*, and *Bifidobacterium* are the five most important genera of Actinobacteria that live in healthy individuals. As new microbial diseases, diseases due to multidrug-resistant pathogenic bacteria are sturdily increasing thus search for new antibiotics is must, chemically synthesized antibiotics are highly toxic so antibiotics derived from microorganisms are preferred as they are non-toxic. Almost 80% of the world's antibiotics are derived from Actinobacteria, mostly from the genera *Streptomyces* and *Micromonospora*. The Genus *Streptomyces* produce antibiotic compounds that are used as Antitumor (Piericidins), Antibacterial (Trindamycins), Anticancer (Chinkomycin), Antiparasitic (Avermectin), Antifungal (Daryamides), Immunosuppressive (Hygromycin), Antioxidative, Cancer chemoprevention, Diabetogenic (Streptozotocin) and Food preservative (2-Allyloxyphenol).

Actinobacteria treating the Plants

Actinobacteria can treat the plant and stimulate plant growth generally by two ways, either directly or indirectly. Indirect growth promotion by preventing the harmful effects of one or more deleterious microorganisms through biocontrol, antagonism of soil plant pathogens, colonization or by biosynthesis of antibiotics can prevent pathogen invasion and establishment. Direct promotion of plant growth through synthesis of phytohormone by actinobacteria and facilitating the plant uptake of soil nutrients. *Streptomyces olivaceoviridis* had a pronounced effect on yield components (spikelet number, spike length, and fresh and dry mass of the developing grain) of wheat plants. The *Streptomyces rimosus*, *Streptomyces rochei* and *Streptomyces olivaceoviridis* produce substantial amounts of exogenous auxins (IAA), as well as gibberellins and Cytokinins.

Actinobacteria also control various plant diseases, about 60 % of the new insecticides and herbicides reported recent years originate from *Streptomyces*. Actinobacteria had the ability to reduce damping off severity in tomato plants, Polyoxin D control rice sheath blight caused by *Rhizoctonia solani*, Kasugamycin is a bactericidal and fungicidal metabolite discovered in *Streptomyces kasugaen*, which acts as an inhibitor of protein biosynthesis in microorganisms but not in mammals, kasugamycin control rice blast *Pyricularia oryzae* and bacterial Pseudomonas diseases in several crops.



Actinobacteria also control the nematode, The production of avermectins by a species of *Streptomyces* is a highly nematicidal compounds. *Streptomyces avermitilis* produces ivermectin, which has an excellent activity against *Wuchereria bancroftii*. Actinobacteria synthesis secondary metabolite that act as Bioherbicides, *Streptomyces saganonensis* produce herbicidines and herbimycins that controls monocotyledonous and dicotyledonous weeds. Anisomycin, growth inhibitor for annual grassy weeds such as barnyardness and common crabgrass and broad-leaved weeds by destroying the ability of the plants to synthesize chlorophyll.

Actinobacteria treating the Invertebrates

Some *Streptomyces* and other actinobacteria live in symbiosis with invertebrates where they mostly play a protective role, producing antibiotics that are used to defend the host's larvae or food source against infections by pathogens, Bacteria variously serve as a food source for marine sponges or as a disease-causing agent, but actinobacteria and other antibiotic-producing bacteria associated with marine sponges also offer protection against disease, *Acromyrmex octospinosus* leaf-cutter ants grow the fungus *Leucoagaricus gongylophorus* as the sole food source for their colony and use antibiotics made by symbiotic actinobacteria to suppress the growth of other microorganisms. Members of the genus *Streptomyces* have been identified in the guts of various arthropod species, including termites, beetles, millipedes, wood lice and earthworms, *Streptomyces spp.* have been isolated from termite guts play a role in cellulose or lignin degradation.



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PEPTIDE VACCINE DEVELOPMENT AGAINST HIV

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Human Immunodeficiency Virus (HIV) continues to impose a significant burden of disease worldwide. HIV belongs to the family of lentiviruses, which means being acting slowly. Lentiviruses cause diseases that progress over a long period disturbing the immune system in humans. HIV provides the basis to grow Acquired Immune Deficiency Syndrome (AIDS). AIDS is characterized by the selective targeting of the CD4+/CD8+ T cells by HIV which fatally impairs the immune system. The window period for this retrovirus may last from several weeks to few months altogether before the seroconversion (detection of earliest antibodies in blood serum raised against HIV). However, a person is said to be diagnosed with AIDS, only when the CD4+ T cell count drops below 200 cells/microlitre, time period for which is indefinite and may last from few months to years. This makes the immune system highly vulnerable to opportunistic infections.

For a person infected from HIV it can take 10 - 15 years to develop AIDS. On the medical frontier there have been many advances, but still there is no effective cure or vaccine available for HIV. There have been many attempts for making a successful HIV vaccine using attenuated form or mRNA approach but the mutative nature of the virus makes the usage of vaccine deficient. There are two closely related human lentiviruses causing AIDS, referred to as HIV-1 and HIV-2. Although HIV-1 is more common and prevalent species of HIV, HIV-2 is relatively new. HIV-1 and 2 shares various biological and genetic properties such as genome structures, mechanism of trans-activation and manner of CD4+ T cell depletion. The virulence



properties of HIV-2 vary appreciably from HIV-1 and may range from comparative attenuation in certain individuals to elevated pathogenicity in others.

After infected by the virus our immune system acts upon the pathogen but it can be rarely identified because it implements its genetic material into the host's DNA. And throughout the time of infection the virus keeps mutating itself by varying its spike proteins. Different strains of the virus immerge and the host body makes broadly neutralizing antibodies (bNAbs). BNAbs can elicit the virus in an earlier stage but it requires a long time to be matured and mutated until then the virus has already been mutated.

The broadness of neutralizing antibody reaction against HIV-2 is divergent from that of HIV-1. Cervicovaginal secretions of infected women demonstrated that one-third of them generated IgA response to HIV-2 envelope antigens confirming lesser viral replication as compared to HIV-1. HIV-2 infected individuals also show prominent cross-reactivity by IgG and IgA against heterologous envelope antigens in contrast to HIV-1 for this compartment.

Vaccine development is a complex and challenging process. Peptide vaccines provide several advantages in comparison to conventional vaccines. Peptide vaccines are a safe and economical technology compared to traditional vaccines made of dead or attenuated pathogens, inactivated toxins, and recombinant subunits. Peptide vaccine production is relatively inexpensive due to the ease of production and simplistic composition. Additionally, peptide vaccines avoid the inclusion of unnecessary components possessing high reactogenicity to the host, such as lipopolysaccharides, lipids, and toxins. In this case, fusion peptides of surface glycoproteins are used in vaccines. There is widespread agreement that the development of a successful HIV vaccine will be dependent on the ability to induce potent protective antibodies capable of neutralizing diverse HIV isolates. These antibodies, termed broadly neutralizing antibodies (bnAbs), are found in nearly 50% of HIV infected individuals, but only after multiple rounds of immune selection and viral escape. BnAbs commonly target a few sites of vulnerability on the HIV envelope glycoprotein (or Env trimer): the CD4 binding site, the gp120-gp41 interface, the fusion peptide, and the membrane-proximal external region. The isolation of bnAbs from HIV infected patients and identification of their target epitopes on the conserved regions of HIV Env trimer have provided possible pathways for vaccine design. However, many bnAbs recognize complex conformational epitopes or have undergone extensive affinity maturation from their germline state. These factors have complicated schemes for devising effective vaccine regimens for eliciting bnAb responses.



To overcome this issue, peptide vaccines can be developed to trigger the bnAbs inside the host body. Using the recombinant DNA technology, multiple copies of variable fusion peptides would be made which would trigger the bnAbs formation. Several doses at different time intervals can provide enough mutation and maturation period for bnAbs, which would elicit the HIV later on.

The development of an effective AIDS vaccine is the most daunting task because of the variable and divergent characteristic of the virus along with extremely long and time taking protocols for vaccine development which calls for a minimum duration of 6-10 years. Approach of recombinant peptides can be a possible strategy to overcome the aforementioned obstacles and challenges. It offers a promising vaccine development approach starting with acknowledgement of fusion peptides and recombination techniques. Synthetic peptides could be made knowing the gene sequences for it, which could resemble the immunogenic or conserved domains of hyper variable HIV strains.

HIV-1 and 2 both have varieties of fusion peptides which triggers the broadly neutralizing antibody response. The mRNA sequences coding for fusion peptides can be used in vaccines. But these approaches are not entirely capable of dealing with various complications that arise during the development of new vaccines. Therefore, directly using varieties of fusion peptides with the help of recombination for vaccine can be more specific and it would be triggering bnAbs efficiently, which would then elicit the virus more effectively.



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BIOREMEDIATION BY *Pseudomonas putida*

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Bioremediation is the use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms. Various microorganism involves in the process of Bioremediation, *Pseudomonas putida* is one of them. Professor Ananda Mohan Chakrabarty was an Indian American Microbiologist, Scientist and Researcher. Professor Chakrabarty genetically engineered a new species of pseudomonas bacteria that is *Pseudomonas putida* in 1971. *Pseudomonas putida* is a Gram negative, rod shaped, saprophytic soil bacterium, belongs to family Psudomonadaceae, Genus *Pseudomonas*, species *Pseudomonas putida*. In 1981, Dr. Chakrabarty received a patent on a Genetically modified *Pseudomonas* bacterium that would eat up oil spills, the first patent of its kind. He was the first person to win a patent on a living organism. A variety of *Pseudomonas putida*, called multiplasmid hydsrocarbon degrading pseudomonas, is the first patented organism in the world.

Pseudomonas putida demonstrates a very diverse aerobic metabolism, including the ability to degrade organic solvents such as tolune and also convert styrene oil to biodegradable plastic Polyhydroxyalkanoates (PHA). This helps degrade the polystyrene foam which was thought to be non-biodegradable. This ability has been put to use in bioremediation, or the use of microorganism to degrade environment pollutant.



Bioremediation of P-Nitrophenol (PNP) by Pseudomonas putida 1274 strain

PNP is a chemical compound and is an important intermediate in the manufacturing of Azo dyes and a number of pesticides. PNP occurs as contaminants of industrial effluents and it is the most important environmental pollutant and causes health and environmental risks. It is toxic to many living organism and it may accumulate in the food chain. Therefore, rapid removal and detoxification of PNP is necessary.

Pseudomonas putida 1274 shows good growth, can tolerate high concentrations of organic molecules and degradation at 37 °C in neutral pH. Acidic and alkali pH retarded the growth of *Pseudomonas putida* as well as the PNP degradation. On the basis of specialized techniques, hydroquinone was identified as major degraded product. The removal of the nitrate group and formation of Hydroquinone as one of the intermediate. Research suggest that *Pseudomonas putida* 1274 strain would suitable aspirant for bioremediation of nitro -aromatic compounds contaminated sites in the environment.

Biodegradation of Arsenic by Pseudomonas putida KT2440

Accumulation of Arsenic has potential health risks through consumption of food. By inserting the Arsenite [As(III)] Sadenosylmethionine methyltrasferase (ArsM) gene into the chromosome of *Pseudomonas putida* KT2440. Recombinant bacteria methylate inorganic arsenic into less toxic orgnoarsenicals. This has the potential for bioremediation of environmental arsenic and reducing arsenic contamination in food. *Pseudomonas putida* KT2440, a strain that colonizes the plant roots in which there is a mutual relationship between plant and bacteria. *Pseudomonas putida* induces plant growth and protects the plants from pathogens.

Biodegradation of Styrene by Pseudomonas putida CA-3

Styrene is less toxic than benzene and PAH, proven carcinogens. However, it is classified as a mutagen and thus potentially carcinogenic. It is main use in the production of the polymer polystyrene and in the production of plastics, rubber, resins, and insulators. Styrene has toxicological effects on humans, animals, and plasts. *Pseudomonas putida* CA-3 is capable of converting the aromatic hydrocarbon styrene, its metabolite phenylacetic acid, and glucose into Polyhydroxy alkanoate (PHA). When growing on the toxic pollutant styrene as the sole source of carbon and energy, acetyl- coenzyme A (CoA) was identified as the end product of styrene metabolism in *Pseudomonas putida* CA-3.



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AGAROSE GEL ELECTROPHORESIS

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Agarose

Agarose is a linear carbohydrate polymer extracted from seaweed, agarobiose.

Gel Electrophoresis

Gel Electrophoresis is a laboratory method used to separate mixtures of DNA, RNA, or proteins according to molecular size. In Gel Electrophoresis, the molecules to be separated are pushed by an electrical field through a gel that contains small pores. The molecules travel through the pores in the gel at a speed that is inversely related to their lengths. This means that a small DNA molecule will travel a greater distance through the gel than will a larger DNA molecule. As previously mentioned, gel electrophoresis involves an electrical field; in particular, this field is applied such that one end of the gel has a positive charge and the other end has a negative charge. Because DNA and RNA are negatively charged molecules, they will be pulled toward the positively charged end of the gel.

Gel Concentration

Gel Concentration the percentage of agarose used depends on the size of fragments to be resolved. In general if the aim to separate large DNA fragments , a low concentration of agarose should be used, and if the aim is to separate small DNA fragments, a high concentration of agarose is recommended.



Making an Agarose Gel

An agarose gel is prepared by combining agarose powder and a buffer solution.

Place the Gel in the Electrophoresis Chamber

Add enough electrophoresis buffer to cover the gel. Make sure each well is filled with buffer.

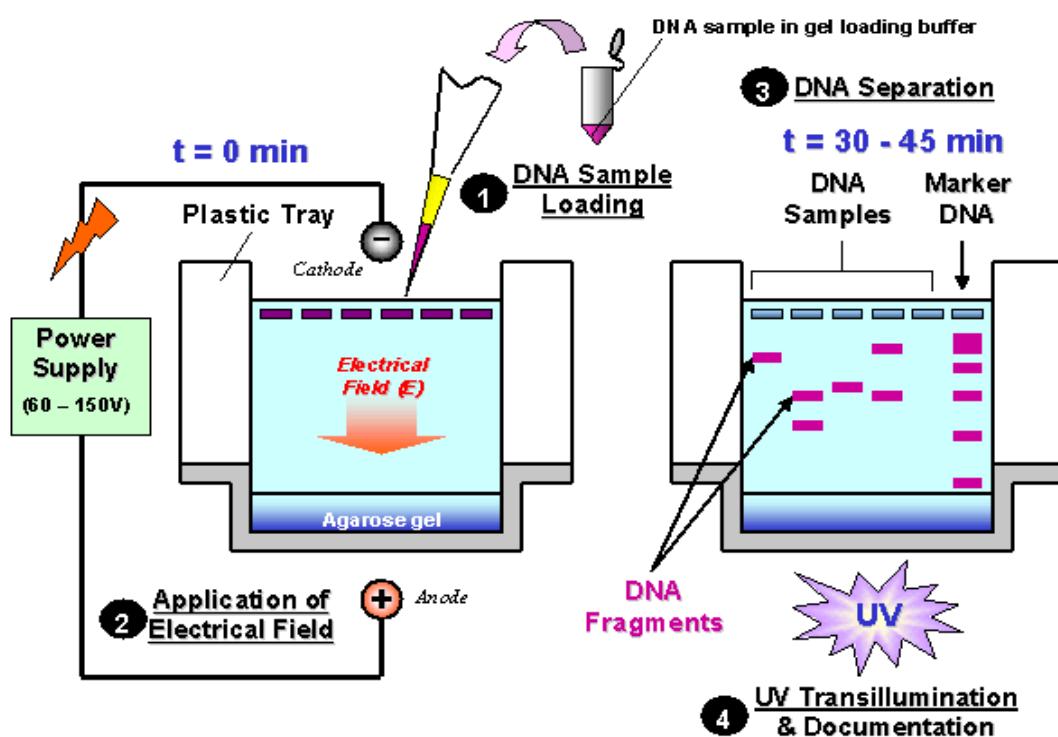
Loading the Gel

Carefully place the pipette tip over a well and gently expel the sample. The sample should sink into well. Be careful not to puncture the gel with pipette.

Running the Gel

Place the cover on the gel electrophoresis chamber. Connecting the electrical leads to power supply. Be sure the leads are connected correctly. DNA migrate towards the anode. When power is turned on bubbles should form on the electrode in the electrophoresis chamber.

Steps Involved in Agarose Gel Electrophoresis



Staining the Gel

Ethidium bromide binds to DNA and fluorescent under UV light allowing the visualization of DNA on a gel.

Application

Estimate of the size of DNA molecules. Analysis of products of a PCR. Separation of DNA fragments for extraction and purification.

ADVANTAGES	DISADVANTAGES
Non toxic gel medium	High cost of agarose
Gels are quick and easy to cast	Fuzzy bands
Good for separating large DNA molecules	Poor separation of low molecular weight samples.



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Escherichia coli IN FRESHWATER AQUACULTURED FISH IN WEST BENGAL

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Aquaculture is growing more rapidly than any other animal production sector in many developed and developing countries because it is an increasingly important source of protein available for human consumption. Its relative contribution to the total amount of fish produced for human consumption changed from 5 % in 1962 to 44.1 % in 2014. According to Food and Agriculture Organization in 2016 total of 580 species and species groups were farmed around the world. The main aquaculture products are finfish, crustaceans, molluscs and aquatic plants. A large proportion of fish products come from small-scale producers in developing countries or low income-deficit countries. More than 80 % of global aquaculture products are produced in freshwater. Aquaculture consists of a broad spectrum of systems, from small ponds to large-scale, highly intensified commercial systems. Indian aquaculture has demonstrated a six and half fold growth over the last two decades. According to Food and Agriculture Organization and Marine Products Export Development Authority, more than 75 % of the aquaculture production is being contributed by three Indian Major Carps (IMCs) namely Catla (*Catla catla*), Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*) followed by exotic carps comprising silver carp, grass carp and common carp contributing to the balance 25 %. National freshwater fish productivity has registered marked rise from 0.6 tonnes/hectare/yr in 1974 to 3 tonnes/hectare/yr in 2018. Many farmers have also demonstrated productivity levels as high as 8 - 12 tonnes/hectare/yr.



West Bengal has occupied 2nd position in producing fish over 16.5 % of India's total fishery production and over 23 % of India's inland fishery production during the yr 2014 - 2016. West Bengal contributed approximately 21-23 % of inland fish production of the country. The domestic demand for fish in West Bengal is high because almost all the people of is state consume fish. Food safety is a prerequisite for protecting consumer health. It also serves the interest of producers and those involved in culture and marketing of fresh fish. Consumption of fish and fishery products is associated with a variety of human health hazards, and broadly the same hazards are present in farmed fish as they are present in corresponding fish varieties caught in the unhygienic condition. Food safety hazards in aquaculture include fish disease agents and hygienic aspects (microbiological agents), and contaminants such as environmental pollutants.

Escherichia coli, the predominant faecal indicator bacteria, have been traditionally used as indicators of faecal contamination and sanitary status of water. The poor unhygienic conditions of the pond area, water source, landing centers, storage and domestic retail markets exacerbate the problem of poor hygiene and consumer safety of fish. Most strains of *E. coli* or faecal coliform are harmless, but some may cause diarrhoea. According to International Comission on Microbiological Specifications for Foods, strains of *Escherichia coli* carry typically virulent properties which emerged as a serious health hazard in human, nevertheless consumption of even low numbers of organisms bears the risk for life-threatening illness. Among the enteric bacteria, *E. coli* is always considered to be of faecal origin, and exists only transiently in other environments. It is also found in abundance in almost any moist environment, notably soil, water and the domestic environment. Human pathogens have been reported to be associated with different types of fish. The fish that live in water polluted with human and the animal faecal matter may carry considerable numbers of bacteria such as *E. coli* and other coliforms. Human infections and intoxications caused by pathogens transmitted from fish are quite common and the following bacteria have been recorded: *Salmonella* spp., *E. coli*, *Vibrio parahaemolyticus*, *V. cholerae*, *Staphylococcus aureus* and *Listeria monocytogenes*. Outbreaks usually occur due to the consumption of raw or insufficiently heat treated fish, which may be contaminated with bacteria from water environment (*E. coli*) or terrestrial sources [coliforms]. It is worth noticing that this microorganism have pathogenic strains standing out as emerging zoonotic potential, as well as shigatoxigenic (STEC) and enteropathogenic (EPEC). At present there are several classes of entero-virulent *E. coli*, namely entero-toxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), entero-haemorrhagic *E. coli* (EHEC), entero-invasive *E. coli* (EIEC), entero-aggregative *E. coli* (EAggEC), diarrhoea-associated haemolytic *E.*



coli and cytolethal distending toxin (CLDT)-producing *E. coli*. *Escherichia coli* is not a natural inhabitant of the fish microbiota, nevertheless, it can be isolated from the animals gut due to its presence in contaminated aquatic environments. In most cases, these microorganisms are part of normal microbiota from fish, when colonizing human sites, they can cause some diseases, like urinary tract infection. The presence of *E. coli* in fish farming may, therefore, lead to a serious health public risk.

In addition, since some *E. coli* strains and serotypes can cause human diseases, understanding the ecology of this bacterium is important to prevent infection and spread of this pathogen to food, soil, and water. The FIOs are assessed to minimize the risk to the public, and this is achieved through monitoring and management practices set by regulatory agencies. International Commission on Microbiological Specifications for Foods, acceptable levels of an indicator organism in fish and fishery products.

	N	C	M	M
Coliforms (CFU/g)	5	2	10	100
<i>Escherichia coli</i> (CFU/g)	5	1	10	10

- N- Number of representative sample units
- C- Maximum number of acceptable sample units with bacterial counts between m and M
- m- Maximum recommended bacterial counts for good quality fish and fishery products
- M - Maximum recommended bacterial counts for marginally acceptable fish and fishery products

Significance of *E. coli* seem to be highest in the food animal industry, acting as reservoir for intra- and interspecific exchange and a source for spread of *E. coli* determinants through contaminated food to humans. Thus, public health potential of commensal *E. coli* in food animals can be a concern. Good public health depends on regular monitoring of water quality as faecal contamination is a serious problem due to the potential for contracting a disease.



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ANTIBIOTIC RESISTANCE IN COAGULASE - NEGATIVE *Staphylococci*

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The term nosocomial infection is applied to infections developing in the hospitalized patients, not present or in incubation at the time of their admission. The hospital environment is highly laden with a wide variety of pathogens. Patients shed them from their bodies; hospital personal spread them through hands, clothes, hospital dust and air. Hospital infections are in a sense; diseases of medical progress. Advances in treatment of cancer, organ transplantation, implanted prostheses and other sophisticated medical technologies enhance the risk of infection to patients.

Staphylococcus is the most important pathogenic bacteria responsible for a variety of diseases in humans and other animals. They are the most common cause of hospital acquired infections and antibiotic resistant strains have become endemic in hospitals in most countries causing major public health issues. *Staphylococcus* are widespread in nature although they are mainly found living on the skin, skin glands and mucous membranes of mammals and birds. *Staphylococcus* are usually divided into two groups based on the ability to produce coagulase, an enzyme that clots blood plasma. The coagulase positive group that includes is *S. aureus* which is an important human pathogen. Coagulase negative group is a large and heterogenous group with a diverse natural habitat that includes humans, birds, fishes and other animals. The species most frequently isolated from human material is *S. epidermidis*. Coagulase -Negative *Staphylococci* (CoNS) formerly regarded as harmless inhabitants of the skin are often found as contaminants in clinical specimens. They are now



regarded as major cause of significant clinical infections and are also involved in animal diseases. Two main reasons for increasing rate of CoNS infections are the spreading antibiotic resistance among CoNS and increasing use of medical devices in recent years. It is however difficult to distinguish between pathogenic CoNS and non-pathogenic resident flora, since their virulence factors have not yet been well defined. The virulence factors of coagulase negative Staphylococci include surface proteins that promote colonization of host tissues, invasions that promote bacterial spread in tissues, surface factors that inhibit phagocytic engulfment (capsule), biochemical properties that enhance their survival in phagocytes (catalase production) and membrane damaging toxins that lyse eukaryotic cell membranes. Coagulase-negative *Staphylococci* also produce several enzymes that could contribute to virulence such as lipase, esterase, DNase and TNase. However, the role of these enzymes on pathogenesis of CoNS remains unclear.

In recent decades CoNS have emerged as important nosocomial pathogens due to the progress that has been made in the field of medicine. CoNS are associated with bacteremia, wound related infections, intravascular catheter-related infections and a variety of postoperative infections. They have the ability to develop resistance to all described antibiotics and cause diseases and show wide variability in their pattern of resistance to antimicrobial agents. Multidrug-resistant CoNS are currently a common finding among hospitalized patients. The emergence of multidrug resistant CoNS has limited the therapeutic arsenal, thus increasing the risk of treatment failure and costs. Antibiotic resistance in CoNS strains may be unstable. Penicillin resistance is developed by the production of beta lactamase (penicillinase) which inactivates penicillin by splitting the beta lactam ring. β -lactamase is encoded in staphylococci by the blaZ gene, which is located on a large plasmid. Methicillin resistant *S. epidermidis* (MRSE) is recognized as pathogens that can cause serious nosocomial infection that contribute to mortality and morbidity in hospitals around the world. Resistance to methicillin in *Staphylococci* is primarily mediated by penicillin binding protein (PBP-2a) which is a unique cell wall synthesizing enzyme. With the increase in methicillin resistance in Staphylococcal species, other antibiotics have been used in the treatment of serious infections caused by this group of bacteria. Aminoglycosides are a group of antibiotics with a complex mechanism of activity. Tobramycin and Gentamicin are possible to use for treatment of Staphylococcal infections. To avoid development of resistance to aminoglycosides, combination therapy has been suggested; combining aminoglycoside with a β -lactam antibiotic, vancomycin, orrifampicin. Over the past several years, resistance of the CoNS to many classes of antimicrobial agents has emerged. The glycopeptide vancomycin has been regarded as one of the last resorts for treatment of infections due to MRSA and





MR-CoNS. Increasing use of vancomycin has led to the emergence of CoNS with decreased susceptibility to vancomycin. The major CNS is generally more resistant to antimicrobial agents than is *Staphylococcus aureus*.

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Rhizobium sp.

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Introduction

Rhizobia are symbiotic diazotrophs (prokaryotic organisms that carry out nitrogen fixation) that form a symbiotic association with legumes. This association is symbiotic in that both the plant and rhizobia benefit. The plant supplies the rhizobia with energy in the form of amino acids and the rhizobium fixes nitrogen from the atmosphere for plant uptake. The reduction of atmospheric nitrogen into ammonia is the second most important biological process on earth after photosynthesis. The actual process of dinitrogen fixation can only be carried out by diazotrophs that contain the enzyme dinitrogenase. Nitrogen is the most critical nutrient needed to support plant growth. Unfortunately, atmospheric dinitrogen (78 % of air we breathe) is extremely stable due to triple bonds which can only be broken by energy intensive ways. These include electrical N₂ fixation by lightning where oxides of N come to ground with rain, the Haber-Bosch process in industrial fertilizer production, and biological N₂ fixation in legumes by bacterial symbionts such as *Rhizobium etli*. Biological fixation of nitrogen was the leading form of annual nitrogen input until the last decade of the 20th century. It is gaining attention once again as sustainability becomes a central focus to feed a world population of over 7 billion people.



What is Rhizobium?

Rhizobium is a genus of Gram negative soil bacteria that fix nitrogen. *Rhizobium* is the bacteria that live in symbiotic association with the root nodules of the leguminous plants. Fixation of nitrogen cannot be done independently. That is why rhizobium requires a plant host. *Rhizobium* is a vital source of nitrogen to agricultural soils including those in arid regions. They convert nitrogen into ammonia. Ammonia, being toxic in nature. is rapidly absorbed into organic compounds. Nitrogen fixation helps in increasing soil productivity and soil fertility. The various behavioral factors such as drought stress, nutrient deficiency, salt stress, fertilizers, pesticides of nitrogen-fixing systems are reviewed.

Morphology

i) Cellular

- **Staining** – Gram negative.
- **Morphology** - Rods 0.5 to 0.9 x 1.2 to 3.0 um. Commonly pleomorphic under adverse growth.
- **Motility** - Motile by one polar or subpolar flagella or two to six peritrichous flagella.
- **Specialized structures** - Fimbriae have been described on a few strains Usually containing granules of Poly- β -Hydroxybutyrate (PHB) which are refractile by Phase Contrast Microscopy. Non-spore forming bacteria.

ii) Colonial

- **Solid surface** - Colonies are circular, convex, semi-translucent, raised and mucilaginous, usually 2 - 4 mm in diameter within 3 - 5 days on Yeast Mannitol - Mineral Salts Agar.
- **Liquid** - Pronounced turbidity develops after 2 or 3 days in agitated broth.

Scientific Classification

Kingdom	Bacteria
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Hyphomicrobiaceae
Family	Rhizobiaceae
Genus	<i>Rhizobium</i>



Physiological Growth Parameters

- **Tropism:** Chemoorganotrophic, utilizing a wide range of carbohydrates and salts of organic acids as carbon sources, without gas formation.
- **Oxygen:** Aerobic, possessing a respiratory type of metabolism with oxygen as the terminal electron acceptor. Often able to grow well under oxygen tensions less than 1.0 kPa.
- **pH:** Optimum pH, 6 – 7.
- **Temperature:** Optimum temperature, 25 – 30 °C.
- **Media:** In Yeast Extract Mannitol Agar (YEMA) medium with Congo Red, *Rhizobium* sp. produces Pink slimy colonies to distinguish itself from other bacterial contamination.

Environmental Habitat

The organisms are characteristically able to invade the root hairs of temperate - zone and some tropical-zone leguminous plants (Family Leguminosae) and incite production of root nodules wherein the bacteria occur as intracellular symbionts. All strains exhibit host range affinities (host "specificity"). The bacteria are present in root nodules as pleomorphic forms (bacteroides) which are normally involved in fixing atmospheric nitrogen into a combined form (ammonia) utilizable by the host plant.



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ANTIOXIDATIVE SYSTEM OF *Deinococcus radiodurans*

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Deinococcus radiodurans is an extremophilic bacterium and one of the most radiation resistant organisms. It can survive cold, dehydration, vacuum, acid and it is known as a polyextremophile. It is extremely resistant to radiation and has the ability to repair double strand breaks in its DNA. The antioxidative system is not only equipped with the common Reactive Oxygen Species (ROS) scavenging enzymes (e.g.: catalase, superoxide dismutate) but also armed with a variety of non-enzyme antioxidants (e.g.: carotenoids and manganese species). The small manganese complexes play an important role in the antioxidative system of *Deinococcus radiodurans*.

Oxidative stress induced by ROS is deleterious for all organisms. In humans, oxidative modifications of cellular macromolecules by ROS underlie various degenerative diseases, cancer, and aging. The extremophilic bacterium *Deinococcus radiodurans* is well known for its extreme resistance to various stresses including Ionizing radiation (IR), Oxidative stress and Desiccation. The mechanisms responsible for the extreme resistance of this robust bacterium remain unclear. At high doses of IR, *Deinococcus radiodurans* can rapidly reassemble DNA fragments into complete Chromosomes.



Deinococcus radiodurans has been considered an ideal model bacterium to study the stress resistance against environment fluctuations. Numerous thermo stable catalases have already been found in extremely thermophilic bacteria e.g.: *Thermus thermophilus*, *Geobacillus* sp., *Thermopakistaniensis* and *Deinococcus radiodurans* but in fungi they are still rather rare. It has some applications for the treatment of mixed oxidative wastes containing ionic mercury. It can be used for the treatment of nuclear energy waste. It has been genetically engineered to consume and digest solvents and heavy metals in these radioactive environments. The mercuric reductase gene has been cloned from *Escherichia coli* into *Deinococcus* to detoxify the ionic mercury residue frequently found in radioactive waste generated from nuclear weapons manufacture. It is used by engineering for uranium precipitation in radioactive sites.

I conclude that the proteome is well protected by the antioxidative system in *Deinococcus radiodurans*, than other ionizing radiation sensitive bacteria after exposure to ionizing radiation, the undamaged proteins exert their function to repair the injured cellular macromolecules such as DNA, lipid and protein restore the cell to normal physiological state. It is used as a means of information storage that might survive a nuclear catastrophe. It could perform a major role in biomedical research and in nanotechnology.



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"BLACK FUNGUS" IN COVID-19 PATIENTS, ITS INFECTION, SYMPTOMS AND PREVENTION

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"Mucormycosis" is also known as Black Fungus or Black Fungal Disease. If the disease is not treated at the right time, then it can be more fatal. During Covid - 19 pandemic this infection was seen more in patients suffering from Covid-19 with diseases like HIV /AIDS, uncontrolled diabetes, Mellitus cancer. This simple fungus infection is seen in the initial stages, but its fatal results can be seen in a short time. People with black fungus disease symptoms, should immediately contact an experienced doctor. They need to be careful to avoid this disease. Covid -19 affected persons can suffer from this disease due to exposure to fungal spores in the air. Due to not being cautious at the right time, these fungus spores can go inside the body and damage the lungs. The numbers of patients reporting this disease in the hospitals were higher than those who suffered from corona in about 10 - 15 days ago.

Black Fungus Infection Symptoms

- Black Fungus Symptoms includes excessive runny nose.
- Persons can feel swelling and pain in eyes when they come in contact with the infection.
- In some black fungal infection eyelid loss and blurred vision have also been seen.



- Dark spots can also be seen around the nose of the infected patient.
- Some patients have complete vision loss.

In some cases, due to the spread of eye infarction, the eyes of some patients had also been removed by surgery because the infection could spread to the brain through the eye.

Black Fungal Infection Causes

Black Fungus is present almost everywhere. It is usually produced automatically on rotting soil, plants, manure, and fruits and vegetables. This infection is ubiquitous as it can be commonly seen in air and soil. Generally, patients who are prone to Black Fungus Infection are those who have recently got rid of the corona virus. These infections seen in most of the patients aged around 35 years of age in the normal age group. A weak immune system, over use of steroids and uncontrolled diabetes put people at risk for this black fungus infection.

Black Fungal Disease Prevention

Black Fungus Disease can be prevented by checking their sugar level on time and keep it limited. Patients recovering from corona should be tested to keep their sugar right. Intravenous Amphotericin –B is an antifungal medication used to treat black fungus infection.



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BIOLOGY OF *Bipolaris*

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Fungi are important yield reducers in crop plants. These eukaryotic microorganisms use enzymatic and/or toxic potential for successful establishment in the host. Extensive diversity has been documented in plant pathogenic fungi at inter- and intra-species level. Variations in pathogenicity require proper understanding of organism biology. One of the significant pathogens of food crops is *Bipolaris* belonging to Ascomycetes group. Different species/strains of this pathogen are known to infect various crops like barley, maize, oat, rice, sorghum and wheat. They can also infect other wild grasses. This pathogen can incite different symptoms in plants like necrosis, leaf spots, leaf blights, root rots, foot rot and so on. This pathogen is majorly considered as necrotroph owing to its capacity to produce toxic metabolites and kill the host tissues. In some of the plants *Bipolaris* is known to survive as endophytes and in absence of the hosts as saprophytes. So far, more than 52 species have been recorded. The species of *Bipolaris* are responsible for major epidemics like Southern corn leaf blight in USA and brown spot of rice in India. Various related genera are *Drechslera*, *Exserohilum*, *Helminthosporium* and *Curvularia*. The genus *Bipolaris* is characterised by having conidia which can germinate on both sides where terminal cells produce germ tubes. Asexual stage of the fungus referred as anamorph named as *Bipolaris* and its sexual stage the teleomorph is *Cochliobolus*.

Hyphae are dark, septate and extensively branched. During asexual reproduction mycelium produces conidiophores bearing conidia. The arrangement of conidia on conidiophore varies in different species. Conidia are dark coloured, multi-



septate, cylindrical or curved, disproportionately swollen. During sexual reproduction black perithecia containing ascospores are produced.

Variability exists in this pathogen with respect to morphology, pathogenicity, toxin producing ability, enzymatic potential and other biochemical parameters. This resulted in identification of different races of the pathogen. The fungus produces necrotic symptoms on host plants and the type of lesions depend upon the fungal species and the host on which it infects. Numerous spots coalesce to cover the entire leaf and the infection may occur in other parts of the plant also. In many crops this fungus is seed-borne and seed-transmitted. Infected kernels cause the seedlings to blight and die within few weeks of planting.

Interestingly, several toxic metabolites causing necrosis in the host plants have been identified in *Bipolaris*. Phytotoxins are the metabolites showing toxicity to plants and produced by microorganisms. *Bipolaris maydis* Race T is known to produce T-toxin a polyketol showing toxicity to maize and responsible for Southern corn leaf blight. The other toxin produced is HC-toxin acting as specific pathogenicity factor in Northern leaf spot and ear rot of maize. Victorin toxin is responsible for blight disease in oat. Most of these *Bipolaris* metabolites have been characterised and known to interfere with host metabolism resulting in various symptoms. These toxins have established their role as pathogenicity or virulence factors. Different classes of metabolites are produced by *Bipolaris* spp. These are polyketides- bipolarinone, cochliomycin, cochliobolic acid, radicinol, radicinin, lunatoic acid A involved in antibacterial, phytotoxic, antifungal activity. Quinones like mitorubrinic acid A and B; and terpenes like cochlioquinone A and B, ophiobolin A show leishmanicidal and cytotoxic activities. Another metabolite maydispenoid acts as an immunosuppressive compound.

Some species of *Bipolaris* are pathogens of human beings and the most predominant are *B. spicifera*, *B. hawaiiensis*, *B. cynodontis*, *B. micropus*, *B. australiensis*, *B. setariae* from nasal region, skin, lungs and eyes.

Significance of *Bipolaris* as plant and human pathogen is documented. The plant diseases caused by *Bipolaris* spp. need to be controlled by integrated disease management practices using resistant species, chemical fungicides like organophosphates and biocontrol agents apart from physical methods. Human pathogenic *Bipolaris* spp. requires to be challenged by antifungal compounds like fluconazole.



Considering the pathogenic potential of *Bipolaris* spp. it is important to understand variability. Detection of toxigenic potential could prove valuable in identification of races of the pathogen and effective disease management practices. Quarantine measures will be able to prevent the introduction of new species/strains to new areas. Understanding of metabolite profile may lead to the development of mycoherbicide formulations from *Bipolaris*. Other biological activities shown by such metabolites may offer useful biological compounds.



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ACTINOMYCETES

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Actinomycetes are useful as producing organisms of antibiotics, enzymes and other bioactive compounds. They are also important resources for the development of industrial products. The compounds they produce are valuable for industrial and pharmaceutical purposes and the enzymes are valuable. Actinomycetes are the most widely distributed groups of microorganisms in nature. They are attractive and charming filamentous Gram positive bacteria. They make up in many cases, especially under dry alkaline conditions, a large part of the microbial population of the soil. Based on several studies among bacteria the actinomycetes are noteworthy as antibiotic producers. Actinomycetes are a group of morphologically diverse, gram positive bacteria having high hexosemine in the cell wall to the extent of 2-18% and identification of aerobic actinomycetes is based on analysis of whole cell diamino pimelic acid.

The actinomycetes are also a group of physiologically diverse bacteria. This diversity is seen both in the production of extra cellular enzymes and in the thousands of kinds of metabolic products which they synthesis and excrete. Actinomycetes are wide spread in nature, occurring typically in soil, composts, aquatic habitats and colonizing plants.

The majority of actinomycetes are free living. These microorganisms are found in a wide range of aquatic and terrestrial environment, but some form symbiotic associations and others are pathogenic in man, animals and plants. The organisms are chemoorganotrophs; collectively they can degrade a wide range of



substances which includes agar, cellulose, chitin, keratin, paraffin and rubber. In recent years, there has been an increasing interest in discovering new agricultural antibiotics for the protection of our living environments. These actinomycetes are prolific producers of antibiotics and other industrially useful secondary metabolites.

Actinomycetes population forms an important component of the soil microflora. According to the estimate of Alexander, 70-80% of the actinomycetes in virgin and cultivated soils are *Streptomyces* species. *Streptomyces* species are also found to occur in fresh water and marine environments. Actinomycetes hold a prominent position as targets in screening programmed due to their non antibiotic bioactive molecules of pharmaceutical interest. Since, the discovery of actinomycin, the first antibiotic from an actinomycete, many commercially important bioactive compounds and antitumour agents have been produced using actinomycetes.

The actinomycetes are a group of bacteria which possess many important and interesting features. They are of considerable value as producers of antibiotics and of other therapeutically useful compounds, they exhibit a range of life cycles which are unique among the prokaryotes, and they appear to play a major role in the cycling of organic matter in the soil ecosystem. Actinomycetes have been shown to be a promising source of wide range of enzyme inhibitors, immunomodifiers and have an ecological advantage over bacteria in desert soils which tend to be dry and alkaline.

The ecological niche of most actinomycetes is probably the aerobic zone of the soil, where they live saprophytically at the expense of a wide variety of organic substrates. Saprophytic actinomycetes are important primary colonizers of soil organic material, the bulk of which is in form of insoluble polymers. Actinomycetes are able to penetrate and solubilize the polymers. Most of actinomycetes are isolated from fresh and sea water sediments. Many bioactive actinomycetes were isolated from marine sediments. Actinomycetes have some unique properties that may be related to their ability to survive and grow in soils. They are prolific producers of extra cellular enzymes that degrade the complex macromolecules substrates commonly found in soils.

Most ecological studies of actinomycetes have been carried out with the dilution plate procedure, which does not differentiate between forms of growth in the natural habitats nor provide a direct assessment of activity.

The actinomycetes are microscopic soil microorganisms known to play a very supporting role in the degradation of organic matter in coffee habitats. Ecological significance of actinomycetes are degradation of lignin, degradation of



organic matter, formation and stabilization of compost piles, formation of stable humus, production of antibiotics, combine with other soil microorganisms in breaking down tough plant and animal residues. Major groups of Actinomycetes are Streptomycetaceae, Nocardiaceae, Micromonosporaceae, Actinoplanaceae, Dermatophilaceae, Frankiaceae and Actinomycetaceae. Some of the ecological parameters influences on growth and development of actinomycetes. Which includes, alkaline and neutral soils are more favourable for the development of actinomycetes is in the range of 6.5 to 8.0. they cannot survive in acidic pH. In soils, with pH less than 5.0 they are almost absent, water logged soils with 80 – 90 % moisture is detrimental for the survival of actinomycetes, the percentage of actinomycetes in the total microbial population increases with the depth of soil. However, they are also found in surface soils. The ideal temperature for the growth of actinomycetes in the range of 25 - 30 °C. As such most actinomycetes are mesophilic. However, thermophilic actinomycetes play an important role in the transformation of various organic residues inside, Nocardia and Micromonospora. compost pits. The most common genera of actinomycetes inhabiting the soil are the *Streptomyces*, *Nocardia* and *Micromonospora*.

Most of the soil actinomycetes Can able to produce enzymes like Dextranases and Xylanase. Enzymes have been used directly and indirectly by mankind for thousands of years. Their initial discovery and use were led to several observations, adaptations and continued adaptations. In general, the enzymes themselves were expressed through the use of live microorganisms. Today, enzymes have many applications in a wide variety of areas of which the general consumers is unaware. Food application microorganisms are now becoming the dominant source for a wide variety of types. This trend will increase in future due to ease in genetic manipulation and also wide variety of enzymes have been found and developed to replace existing enzymes of animal and plant origin.

The biochemical diversity of microorganisms makes them logical sources of wide variety of enzymes for use in food and other biotechnological system. Several microbial enzymes are employed in fruit juice processing, most important are Pectinases. These processing aids are often produced by Solid State Fermentation (SSF) method. Fruits and berries contain varying amounts of pectin, which acts as a binding layer between plant cells to hold adjacent cell walls together. In plant juice production, some of this pectin is extracted during pressing; it causes increases in juice viscosity, leading to difficulties in obtaining optimal juice yields and in juice clarification and filtration. These problems can be overcome by adding pectinase



preparations to the fruit pulp before pressing. Similar enzyme treatment is used to increase the yield of oils from olive pulp, palm fruit and coconut flesh.

In wine making, commercial enzymes are used for several purposes, as well as for juice extraction. Enzymes are being increasingly used in textile processing for the finishing of fabrics and garments especially in desizing, biopolishing and denim washing. Paper manufacture is a major world industry. Microbial enzymes can be used in several stages of pulp and paper processing. To enhance pulp digestion and bleaching, to improve drainage rates in water removal during paper formation, to increase fiber flexibility, to remove xylan, resins and contaminants. Among these microbial enzymes, dextranase and xylanase enzymes are most important. Dextranase are high molecular weight polysaccharides. The few dextranases detected in higher organisms are expected to have novel functions. Purified and specific enzymes can be used in basic research for the analysis of more complicated structures of carbohydrates as well as in biotechnological applications to produce and modify glycosylated residues in receptor proteins. The most interesting dextran related applications may be to create hydrolysis of microbial dextran.

Xylan, it is an important constituent of renewable resource and it is one of the most abundant hemi cellulose in plants. Xylanases involve the conversion of xylan, which is present in wastes from agricultural and food industry, into xylose and used for the clarification of juices, for the extraction of coffee, plant oils and starch and for the production of fuel and chemical feed stocks and in Paper and Pulp industry. Actinomycetes xylanases have attracted considerable sugars from hemicelluloses, bioleaching of pulp and paper industry and to other industrial applications.

Actinomycetes are considered as saprophytes in soil, composts and aquatic habitats. Among bacteria, filamentous fungi, actinomycetes and a small number of yeasts have been shown to produce dextran and xylan hydrolyzing enzymes. But fungal enzymes show the highest reaction rate at 50°C and contamination most commonly occurred in fungi compared to actinomycetes. Bacteria are very well producers in submerged cultivation, but some of the reference shown as fungal enzymes production efficiency by Solid State Fermentation means. As actinomycetes adopted to perform well in SSF we intend screen the enzymes (dextranases and xylanases) from soil isolates of Actinomycetes. In Actinomycetes, *Streptomyces* and *Nocardia* are the major and most important producers of enzymes are dextranases and xylanases. Those are industrial important and significant enzymes. Hence, have chosen this topic for our research work.



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MICROBIAL PLASTIC DEGRADATION

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A growing accumulation of plastic wastes has become a severe environmental and social issue. The current methods for disposing of plastic wastes mainly include landfilling, incineration, and mechanical and chemical recycling. In most countries, especially the developing countries, landfilling is the major method for plastic wastes disposal due to its operability and low cost. However the accumulated plastic wastes have occupied a great amount of land. Incineration of plastic wastes can reduce the demand of landfills and recover heat energy, but we also need to reduce the environmental effects of secondary pollutants generated from the incinerating process, such as dioxins, carbon monoxide, nitrogen oxide, and so on.

In recent years a number of studies have reported that several microorganisms and enzymes are capable of degrading synthetic plastics, such as Polyethylene (PE), Polystyrene (PS), Polypropylene (PP), Polyvinyl chloride (PVC), Polyurethane (PUR), and Polyethylene terephthalate (PET). *Ideonella sakaiensis* is a bacterium from the genus Ideonella and family Comamonadaceae capable of breaking down Polyethylene terephthalate (PET) plastic which was isolated from outside a plastic bottle recycling facility. *Ideonella sakaiensis* was identified in 2016 by a team of researchers from Kyoto Institute of Technology and Keio university, after collecting samples of PET debris in a search of bacteria which relied on the plastic for carbon growth. The bacterium was observed to utilize two distinct enzymes that reacted with water to breakdown PET plastics into terephthalic acid and ethylene glycol, substances which on their own pose no threat to the environment.



The discovery of *Ideonella sakaiensis* has potential importance for the recycling process of PET plastics. Prior to discovery of the bacterium, the only known consumers of PET were a small number of fungi including *Pestalotiopsis microspora*, and knowledge of the new species has spurred discussion about biodegradation as a method of recycling. The bacterium can currently break down a thin film of PET in a little over six weeks, so it is thought that any prospective applications in mass recycling programs will have to be preceded by enhancement of its abilities through genetic modification.

Compare with chemical process PET degradation using microorganisms and enzymes consumes less energy and environmentally friendly option. We believe that the present research results bring us closer to achieving an ideal model for PET recycling so long as we are able to enhance the activity level and stability of these newly discovered microbial enzymes.



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VIROIDS: AN OVERVIEW

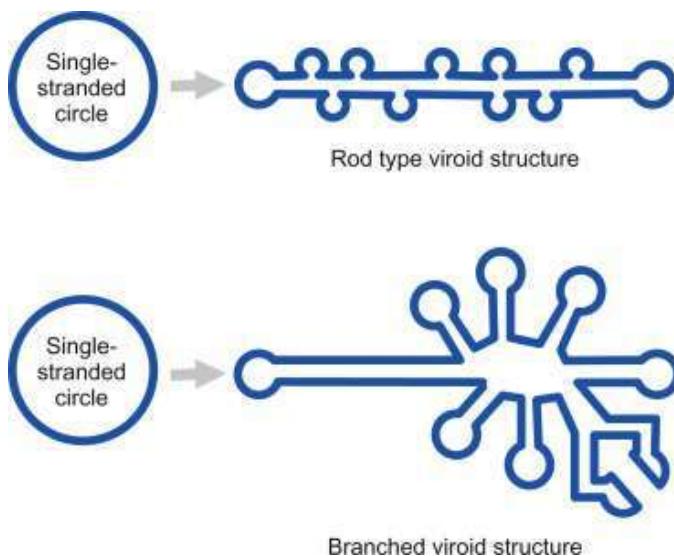
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A class of extremely simple infectious agents termed VIROIDS was discovered in 1967. Viroids are small, circular RNA molecules that do not encode any protein and that are infectious as naked RNA molecules. Potato spindle tuber viroid (PSTVd) was the first pathogen of a eukaryotic host for which the sequence was established. Sequenced viroids range from 246 to 375 nucleotides and possess extensive internal base pairing that results in the RNA being rodlike and about 15nm long. Viroids infect plants (but no other forms of life) and are replicated at the expense of the host cell. All evidence indicates that viroids do not code for any proteins. Consequently, they must rely completely on normal plant processes for their replication and spread throughout the plant. There are 27 viroids characterized so far out of which 25 infect dicotyledonous plant and the other two infect monocotyledonous plants. Coconut Cadang Cadang Viroid (CCCV) and Coconut Tinangaja Viroid (CTiVd) infect Monocotyledons. Some viroids have devastating effects, for example, CCCVd has killed millions of coconut palms in the Philippines, while others cause epinasty, rugosity, chlorosis and necrosis on leaves, internode shortening of stems leading to stunted plants, bark cracking, deformation and color alterations of fruits and storage organs, and delays in foliation, flowering and ripening. Many viroids are important agricultural pathogens, whereas others replicate without causing symptoms. Viroids are often transmitted through vegetative propagation of plants but can also be transmitted during agricultural or horticultural practices in which contaminated instruments are used.



Because viroids have no protein coat, they lack attachment proteins and cannot recognize and penetrate healthy cells as can a true virus. Viroids can infiltrate a plant cell only when its surrounding membrane is already damaged. They often take advantage of damage done to plant tissue by insects. Once inside, viroids may be passed from one plant cell to another via cellular junctions. Viroids replicate by a rolling circle mechanism. Most viroids replicate in the plant cell nucleus and rely on RNA polymerase II for RNA synthesis. A smaller group of viroids (e.g., chrysanthemum chlorotic mottle viroid) have a highly branched structure, rather than a rod with bulges, and replicate in the chloroplast.



How viroids damage plants are still rather mysterious. However, it appears that the viroid and/or its replicative intermediates trigger RNA interference. This results in the production of short single-stranded RNA molecules (known as vsRNA—viroid short RNA) of the same size as the microRNAs widely used by plant cells to regulate genes. This decreases expression of multiple plant genes, hence causing disease.

Viroids have properties that make them candidates for survivors of the RNA world: small genome size (to avoid error catastrophe caused by error-prone replication), high G+C content (for greater thermodynamic stability), circular genomes (to avoid the need for mechanisms to prevent loss of information at the ends of linear genomes), no protein content, and the presence of a ribozyme, a fingerprint of the RNA world.



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RESEARCH ARTICLE ON MIRACLE OF GLOMALIN

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Introduction

India is an agricultural country. Agriculture and its allied activities act as main source of livelihood for more than 80 % population of rural India. It provides employment to approximately 52 % of labor. Its contribution to Gross Domestic Product (GDP) is between 14 to 15 %. It is world's largest producer of milk, pulses, and spices. Today's condition we see that population goes on increasing, requirement of food and employment is also important need for them. For this reason, challenges are that rising agricultural productivity. For this we have to overcome the problems that interrupt in rising in agricultural productivity.

Here, I am discussing about one of them soil erosion. Soil erosion is a gradual process that occurs when the impact of water or wind detaches and removes soil particles, causing the soil to deteriorate. Soil deterioration and low water quality due to erosion and surface runoff have become severe problem worldwide. The problem may become so severe that the land can no longer be cultivated and must be abandoned. Many agricultural civilizations have declined due to land and natural resource mismanagement, and history of such civilization is good reminder to protect our natural resources. Erosion is serious problem for productive agricultural land for water quality concerns. Many studies indicate that soil erosion results in large decreases in soil productivity. Impact of soil erosion on soil productivity was largely determined by subsoil properties because they effect root growth, soil water availability, and plow layer fertility. Thus, the loss of the topsoil can have



considerable impact on yield, where nutrient availability, root growth environment, and soil water availability are essential for plant development. To reduce this problem one of the important beneficial protein helps called glomalin. What is glomalin?

Glomalin is a glycoprotein discovered in 1996 by Sara F. Wright, a scientist at the USDA Agricultural Research Service. The name comes from Glomaleres, an order of fungi. Glomalin is produced by abundantly on hyphae and spores of Arbuscular Mycorrhizal (AM) fungi in soil and in roots. A specific protein glomalin has not yet been isolated and described. However, Glomalin- Related Soil Proteins (GRSP) have been identified using a Monoclonal antibody (Mab32B110) raised against crushed AM fungi spore. It is defined by its extraction conditions and reaction with antibody Mab32B11. Glomalin molecule is a clump of small glycoproteins with iron and other ions attached glomalin contains from 1 to 9 % tightly bound iron.

Production of Glomalin

Glomalin occurs in very large amounts in soil, typically in the range of several to 10 mg g⁻¹ soil. It is produced by only AMF. Glomalin have N-linked oligosaccharide side chains, and the extract also contains Fe in variable percentages. It is not known in what way Fe is bound in the molecule. In its native state, glomalin is assumed to be insoluble and hydrophobic. Evidence (e.g., from NMR studies) is mounting that glomalin is a compound distinct from humic acid. The pathway of release of glomalin into soil is not clear. Glomalin is actively secreted at the hyphal tip, or it could be sloughed off hyphae as they grow through soil. It is not known what factors control glomalin production.

AMF are obligate biotrophs living in symbiosis with the roots of ca. two third of higher plants. They are ubiquitous in soils of terrestrial ecosystems. Micorrhizae are symbiotic relationships that form between fungi and plants. The fungi colonize the roots system of a host plant, providing increased water and nutrient absorption capabilities while the plant provides the Glomalin is an important molecule in aggregate stabilization. When aggregates are not stabilized, they break apart with rainfall. Organic matter and nutrients within disrupted aggregates may be lost to rain and wind erosion. The chemistry of glomalin makes it an ideal stabilizing coat. Glomalin is a sticky substance to create stable soil aggregates. The gluing of soil particles together into aggregates help in maintain pores and channels in the soil for air and water to enter and move through it. Glomalin Related Soil Protein (GRSP) contribution to soil carbon and nutrients at vertical soil profiles and underlying



mechanism were not well defined yet. Glomalin ultimately increases soil fertility, reduces soil erosion. AMF helps in plant growth development, improves nutrient cycle, and increases water infiltration. By these increases in crop production and economic condition of the country also stable and developed.



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PLANT GROWTH-PROMOTING BACTERIA THAT CAN WITHSTAND HIGH SALINITY FOR INCREASED CROP YIELD

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Soil is the upper layer of the earth's crust. It is composed of air, water, minerals and other living organisms. The thin layer of soil surrounding plant roots is known as the Rhizosphere. Beneficial bacteria are present in the Rhizosphere which play an important role in plant growth. *Rhizobacteria* (PGPR) act as growth promoters as well as disease suppression in many crop plants. The rhizosphere contains all essential nutrients for plant growth. Rhizospheric soil is resourceful in microorganisms and bacteria, which release certain enzymes that protect the plant from the pathogen, exist in the soil.

Nitrogen content, nodulation in leguminous plant and reduction yield has been reported due to soil salinity. Soya bean nodules sensitive to NaCl. Photosynthetic capacity is also decreased due to soil salinity. Plant cells produce antioxidant enzymes and non-enzymatic oxidants to protect them from oxidative stress. Rhizobacteria improve plant growth and yield directly and indirectly. Indirect mechanisms have been observed with PGPR strains and direct mechanisms may act on plant growth regulator mineral solubility and atmospheric nitrogen fixation. PGPR also prevents the damaging effect of the phytopathogenic organism. *Bacillus subtilis* can induce plant resistance to stress and produce plant growth hormones.



Soya bean nodulation could be enhanced by *Serratia proteamaculans*. Bacterial exo-polysaccharides are produced by PGPR to bind cation i.e. Na^+ . It is reported that the increasing population of exo-polysaccharides producing bacteria in root decrease the content of Na^+ in the soil, thus decreasing soil salinity.

Soil salinity can be prevented by inoculation of *Bacillus sp.* *Serration sp.* and PGPR. exo-polysaccharides producing bacteria play an important role of PGPR in reducing salinity stress in soybean seedlings grown under soil salinity conditions in the greenhouse. The root system is attached to the soil environment in which some bacteria improve growth and increase the yield of crop plants called plant growth-promoting.

Rhizobacteria (PGPR) play an important role in the nutrient cycle and maintains the productivity of the plant. But in some cases when PGPR is applied in the field, it fails to induce the desired effect. The saline condition of the soil can affect the nature and strength of plant microbial interaction. It also affects the productivity of crop plants and soil biota systems. Several studies have shown that *Rhizobial* interaction with leguminous plant inhibits due to salinity, because of this nodulation N^2 fixation is also decreased. Absorption of Ca^{++} is also decreased which reduces the growth of the roots and root hairs. Several molecules play an important role in the interaction between plants and microorganisms. In Rhizosphere soil microorganisms can detect plant hosts and start their colonization. As colonies occur on the roots, their number is high in the Rhizosphere from those found in the soil. The colonization efficiency of microorganism can be decreased due to salinity of the soil.

Plant nodulation and nitrogen fixation have been increased, when rhizosphere (PGPR) and rhizobium co-inoculate under normal growth conditions. Soybean nodulation and nitrogen fixation can be increased due to co-inoculation of *B. japonicum* with PGPR strain at optimal and suboptimal root zone temperature. PGPR can be used instead of pesticide and fertilizer. PGPR rapidly grows in rhizospheric soil and increases the nutrient absorption capacity of the plant. It is also helpful for disease suppression by multiple mechanism activity. PGPR carried out various biotic activities of the soil to increase yield and sustainable crop reduction. PGPR colonize plant improve plant growth by phosphate solubilization, nitrogen fixation, production of indole-acetic acid, siderophores cyclopropane carboxylase and degradation of environmental of pollutant and production of hormones antibiotics. Some strains of PGPR detoxify heavy metal and also biological control activity of phytopathogens and insects.



Beneficial microbes occur in root nodules of leguminous plants in the rhizosphere of fix atmospheric N². It has been studied that plants release certain chemicals that stimulate beneficial microbes to move towards the plant root. These microbes form nodules for N² fixation. Iron is essential for growth in all kinds of organisms. Siderophores are produced by PGPR compete to attain Fe³⁺ (ferric ions) from the surrounding. Siderophores mean “Iron Carrier”.

Bradyrhizobium japonicum, *Bacillus subtilis* and *S. proteamclans* improve growth, photosynthetic rate and total dry weight. Mineral uptakes of plants like N, P, K, Calcium and Na in soil salinity treatment were decreased as compared to non-salinity treatment. In this way, there is no interaction found between salinity and strain.

In conclusion, general nutrient imbalance membrane destabilization also shows salinity in the soil. Exo-polysaccharides producing bacteria play an important role of PGPR in reducing salinity stress. It is found that the concentration of cation was an increase in non-salinity treatment with PGPR but K⁺ and Na⁺ uptake were decreased by 1.2 - 11.8 % and 0.4 - 4.3 % under soil salinity. The negative effect can be caused by salinity on soil micro-organisms. In the future, this research will play an important role on rhizosphere microbes. Modern tools and techniques for the enhancement of PGPR can serve as a key in sustainable agriculture by improving soil fertility, plant tolerance, crop productivity, and maintaining balanced nutrient cycling.



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BIOSURFACTANTS: EVOLVING TRENDS AND POTENTIAL APPLICATION

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Biosurfactants are known as surface-active molecules as it reduces the surface tension of the hydrophobic substrates thereby increasing its bioavailability. Biosurfactants are polymers, totally or partially extracellular, amphipathic molecules containing polar and non-polar moieties which allow them to form micelles that accumulate at interphase between liquids of different polarities such as water and oil thereby reducing surface tension and interfacial tensions thus facilitating hydrocarbon uptake and emulsification. It consists of hydrophilic and hydrophobic moieties which provide them the ability to accumulate between two immiscible fluids. It is produced by the broad range of microbes including bacteria, fungi, and yeast. They are composed by sugars with long chain of aliphatic or hydroxyaliphatic acids. It divided in three major groups such as Rhamnolipids, Trehalolipids and Sophorolipids. There are numerous microbes which produce biosurfactants but the exploration of potent biosurfactant producer is a need of hours to effectuate and accelerate its commercial production for industrial use. In recent times, due to their unique properties like specificity, low toxicity and relative ease of preparation, these surface-active biomolecules have attracted wide interest. These compounds are majorly categorized according to their molecular weight, physico-chemical properties and mode of action. They are eco-friendly, easily biodegradable and non-toxic materials. Due to their unique functional properties, biosurfactants are used in several industries including organic chemicals, petroleum, petrochemicals, mining, metallurgy (mainly bioleaching), agrochemicals, fertilizers, foods, beverages,



cosmetics, pharmaceuticals and many others. They can be used as emulsifiers as well as demulsifiers, wetting agents, foaming agents, spreading agents, functional food ingredients and detergents. The interfacial surface tension reducing ability of biosurfactants made them to play important role also in the oil recovery and bioremediation of heavy crude oil.

When compared to chemical or synthetic surfactants, biosurfactants gained several advantages including their biodegradability, biocompatibility and digestibility. Other advantages include selectivity and specific activity at extreme temperatures, pH and salinity. It can be produced by the use of cheaper, renewable substrates as potential carbon sources obtained from various industries such as agricultural wastes (sugars, molasses, plant oils, oil wastes, starchy substances and lactic whey), dairy industry whey, distillery wastes, animal fat and oil industries. Biosurfactant production is basically growth associated as the substrate will be utilized and cells will grow, and simultaneously biosurfactant will be produced. It has been reported that yeast shows higher production of biosurfactant than bacteria this is due to presence of a rigid cell wall.

It can be used in Paint as protective coating agent and employed for the modification of rheological properties of oil and gas wells as well as for demulsifying crude petroleum. In agriculture it can be used as fertilizers to prevent caking during storage. It can also be used in electrolytic plating of metals. In pharmaceutics, biosurfactants can be used for gene delivery and recovery of intracellular products as well as they can be served as antimicrobial substances. While, in bakery and ice-cream formulations, biosurfactants are impotent to control the consistency, slowing staling and solubilizing the flavour oils. Biosurfactants also show an advantage over synthetic surfactants such as low irritancy or anti-irritating effects, better moisturizing properties and compatibility with skin and hence it is much demanded in beauty industries. Several biosurfactants have also shown antibacterial, anti-fungal, antiviral, anti-adhesive, and anti-cancer activities.

Biosurfactants extracted from Generally Regarded As Safe (GRAS) microorganisms like lactobacilli and yeasts may contribute to the various health beneficial properties. Due to their specific attributes, such as emulsifying, anti-adhesive and antimicrobial activities they are recommended as multipurpose fixings or added substances. Biosurfactants have attracted great scientific attention due to their high surface activity, reduced toxicity, easy biodegradability, better environmental compatibility, higher foaming and higher selectivity. Due to their structures and properties, biosurfactants have applications in different industrial processes as well as possible novel uses in the future. Indeed, biosurfactants are





expected to become known as the “multifunctional materials” of this modern century. Thus, Biosurfactants can be served as green alternatives in a variety of applications including bioremediation, pharmaceuticals, agricultural disease control and cosmetics.

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MICROBIAL FUEL CELL

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Introduction

Microbial Fuel Cell (MFC), a bioelectrochemical process which transforms chemical energy of organic compounds into electrical energy which is catalyzed by microorganisms. MFC is used as an alternative source for energy generation, which is reliable, clean and efficient process. MFC is a technology in which microbes convert chemical energy produced by the oxidation of organic /inorganic compounds into ATP by sequential reactions in which electrons are transferred to a terminal electron acceptor to generate an electrical current. Bacteria used in MFCs are known as Exoelectrogens. They are electrochemically active and can transfer electrons outside their cells. MFCs help in get rid of wastewater streams and bioremediation.

Working

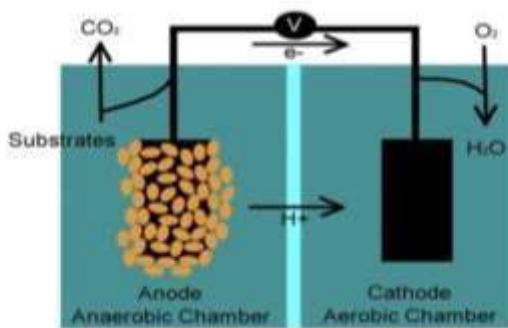


Figure - Schematic of a Simple Two Chambered MFC



- An MFC has two electrodes held in separate compartments. The anode and cathode compartments connected by an external circuit and are separated by a semi permeable cationic membrane.
- Microbes resides in the anode compartment which is anaerobic in nature, where they metabolize organic compounds present in waste water, acts as electron donor and generates electrons and protons.
- The cathode chamber is aerobic in nature, acts as electron acceptor, which has the advantage of the oxidation.
- Bacteria breaks the electron bonds present in the waste water and release protons and electrons
- The electrons move to cathode through the electrical circuit connected within anode, while the protons (H^+ ions) migrate through the semi permeable cationic membrane.
- The protons (H^+ ions) combine with oxygen present in the cathode compartment and forms contaminants free H_2O (water).
- The free electrons produced by bacteria, attached to the anode are extracted using an external circuit and used accordingly.

Applications

a) Power Generation

Microbes can perform dual duty of degrading effluents and generating power. MFC's are used to produce electrical power in the course treatment of industrial, agricultural and municipal wastewater. Microbes oxidized organic compounds present in these waters and produce a huge amount of electricity which is also used for the further treatment of waste water.

b) Underwater Monitoring Devices

MFC's can be used to collect the data, understanding and modelling ecosystem responses through sensors. These sensors require power for operation particularly for the sensors present in rivers, lakes and deep waters. Sediment fuel cells has been developed to monitor environmental systems such as creeks, rivers and oceans

c) BOD Sensing

Another potential application for the MFC technology is to use it as a sensor for pollutant analysis, process, monitoring and control. Biological Oxygen Demand (BOD) is the amount of dissolved oxygen required to meet the metabolic needs of



aerobic organism in sewage. An MFC-type BOD sensor can be kept operational for over 5 years without extra maintenance.

d) Wastewater Treatment

MFC's are used in water treatment to harvest energy utilizing anaerobic digestion. This process can also reduce pathogens as it requires temperature above 30 °C and requires an extra step in order to convert biogas to electricity.



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EFFECT OF FERTILIZERS ON EARTHWORM AND SOIL ORGANISMS

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Earthworms are presumably the main individuals from the soil biota. Despite the fact that they are not numerically prevailing in soils, their huge size makes them one of the significant supporters of all out biomass, and their exercises are to such an extent that they are critical in keeping up with soil fruitfulness in an assortment of ways. Aristotle was quick to cause to notice their job in turning over the dirt and he suitably called them "the intestines of the earth". However, it was not until the last part of the 1800s that Charles Darwin (1881), in his conclusive work, "The Formation of Vegetable Mould Through the Action of Worms", characterized the outrageous significance of earthworms in breakdown of dead plant and creature matter that arrives at soil and in the proceeded with upkeep of soil structure, air circulation, waste, and richness. Other than the presence of pesticides in the soil, miniature living beings liable for some, serious illnesses may likewise pollute the soil through contaminated excreta of creatures and people and along these lines, be in earthworm's environmental factors. The natural connections in soil between human microorganism miniature organic entities and earthworms are in a roundabout way explained by surveying the antibacterial properties of earthworms' coelomic liquid or its concentrate.



Earthworms generally participate in various cooperation, soil living beings have with each other. Better information on the earthworms/miniature creatures' associations is helpful in natural insurance (for example utilization of earthworms in organic control of vermin and in sewage treatment). *Eisenia fetida* is a most loved worm animal types for fertilizing the soil and is habitually utilized as an organic screen for testing the impacts of toxins on soil biota. The structure of securing earthworms that are significant individuals from soil biodiversity and misusing their biological system administrations for the ecological assurance and suppression of phytopathogenic miniature living beings. Its goal is to give an outline of stresses identified with pesticide details and miniature organic entities, that earthworms are looking in their living climate. In particular, the allowed appraisal of the harmfulness of six pesticides definitions (Gather together GT Additionally, Stratos Ultra Jardin, Capiscol, Polyvalent, Polyflor and KB Limace) and six miniature creatures (*Geotrichum candidum* ATCC 203407, *Escherichia coli* UCMA 10579, *Enterobacter cloacae* UCMA 10580, *Listeria monocytogenes* UCMA 6115, *Salmonella typhi* UCMA 10598 and *Staphylococcus aureus* UCMA 6834) on *Eisenia fetida*.

Essentially all-natural manures advantage to earthworms. The expansion of creature fertilizer, sewage squanders, and spent malt from breweries, paper mash, or potato handling waste all showed a beneficial outcome on earthworm numbers. Increments of natural material can twofold or significantly increase earthworm numbers in a solitary year. The ammonia and salt content of some liquid manure can have an adverse effect on earthworms, but populations usually recover quickly and henceforth increase. Normally, the use of inorganic fertilizers also has a positive impact on earthworm numbers. This is probably an indirect effect of the increased crop biomass production and consequent increases in organic residues. Earthworm numbers in meadows receiving inorganic fertilizer averaged nearly twice the earthworms in unfertilized meadows on the Georgia piedmont. Ammonia and ammonia-based fertilizers can adversely affect earthworms. Annual use of ammonium sulphate, anhydrous ammonia, and sulfur-coated urea has been shown to decrease earthworm populations. Research at Park Grass showed that after extremely long exposure to several levels of ammonium sulphate (0, 48, 97, and 145 kg/ha), the populations of earthworms were inversely proportional to the dose of nitrogen applied. This is probably due to the effect these fertilizers have on lowering soil pH. Direct exposure to anhydrous ammonia during application will kill up to 10 % of the population. However, farmers report increased numbers in the long run due to higher yields and more food for earthworms to feed upon. Still, some farmers have switched from anhydrous ammonia to 28 % nitrogen to avoid killing earthworms during nitrogen application. Others have converted to using manures in order to



protect and increase earthworms. Lime seems to benefit earthworm populations in otherwise acid soils because most species of earthworms favour neutral pH levels and require calcium for growth. Lime may indirectly benefit earthworms by increasing plant growth and therefore plant residues.

In general, most herbicides are harmless to earthworms. The triazine class of herbicides has a moderate impact on earthworm numbers. Herbicides used prior to World War II, including lead arsenate and copper sulphate, are moderately toxic to earthworms. The main threat of toxicity to earthworms is from long-term build-up of these compounds in the soil. The majority of the carbamate class of insecticides are toxic to earthworms. The toxic effects of carbofuran (Furadan) have been studied extensively. Other insecticides in the carbamate class that have proved highly toxic to earthworms are aldicarb (Temik), aminocarb, bufencarb, carbaryl (Sevin), methiocarb (Measural), methomyl (Lannate), oxamyl (Vydate), promecarb, propoxur (Baygon), and thiofanox. Generally, insecticides in the organophosphate class are less toxic to earthworms. However, organophosphate insecticides that are extremely or highly toxic are phorate (Thimet), chloropyrifos (Dursban, Equity, Tenure, etc.), ethoprophos (Mocap), ethyl-parathion, and isazophos. Aromatic organochlorine insecticides (used predominantly in the 1950s – 1970s) are generally not very toxic. Exceptions are chlordane, endrin, heptachlor, and izobenzan. Carbamate fungicides (carbendazim and benomyl) have shown toxic effects to earthworms. Other broad-spectrum fumigants (fungicides and nematicides) are very toxic to earthworms.

As conclusion, it may be concluded that the acute toxicity of inorganic fertilizers and pesticides on *E. fetida* using a simple paper contact method was actually significant in confirming the toxic impending. The application of environmentally level-headed doses of urea exposed the possible harmful effects on earthworms when comes in contact directly. Thus, in future, this method will be necessary to find an approach to establish the sensitivity of the earthworm's acute toxicity before going for the evaluation in soil (i.e., acute and chronic toxicity tests in artificial soil sample). Soil being a very complex system, it is often difficult to compare toxicity information directly. The necessary precautions and regulations should be implemented for the usage of the chemical fertilizers like urea on the agricultural lands. The social awareness is most needed for this serious issue about the soil health.



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ROLE OF PALLIATIVE CARE IN CANCER

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Cancer is an abnormal and uncontrolled growth of cells in the body and it's termed as malignancy. It can occur on any organ or part of the body. Cancer growth destroys the normal cells and tissues. Tumor is caused by abnormal proliferation of cell. There are two types of tumors. Benign tumor is non-cancerous. It grows only in one place; it cannot spread to other parts of the body. Malignant tumors are cancerous tumor. It can easily spread through other parts of the body. Cancer is caused by many factors. Environmental or genetic factors. Exact causes are not known, likely causes – Benzene and other chemicals pollutants like asbestos, environmental toxins, such as certain poisonous mushrooms and poison that can grow on peanut plants (aflatoxins). Food habits, infections – hepatitis B and C. Drinking excess of alcohol and smoking, chewing pan. It is also caused by chemicals which are used in our day to day life like perfumes, soaps etc.., Radiation may cause cancer of bone marrow, skin lymph system, bone. Different types of diagnosis, treatments are given to the patients, but it causes side effects. Method of diagnosis, Radiological, histological, hematological, cytological, Tumor markers, immunohistochemistry and molecular. Cancer treatment will be given by the depending upon the diagnosis. There are 4 main different types of treatment - surgery, Radiotherapy, Chemotherapy and Palliative care. Depending on the organs, affected stage of the disease, types of cells - the treatment will be given to the patients. Surgery is the most common form of treatment - Biopsy, curative, palliative (Useful in breast cancer stomach and gut cancer). Radiotherapy can be given as curative intent. Enables reduction of mass so as to control the progress of disease and



as a palliative measure. Like X-ray Measured dose focused on the tumor. External irradiation, or internal irradiation (Brachytherapy) Ex; Uterus cancer prostate cancer. Chemotherapy is the use of drugs to destroy cancer cells. Some cancers are well known to be quite resistant to chemotherapy variety of chemotherapeutic agents like alkylating agents, anti-metabolic agents, plant alkaloids, hormonal agents, and interferon. Palliative care is a supportive care helps patient and family to cope throughout the illness. Side effects affects adjoin area\organs, normal cells to get damaged, diarrhea, vomiting, nausea, skin discoloration, hair loss, fatigue, anemia, drop of immunity mucositis (inflammation of the mucous membrane).

Palliative Care movement is founded by Dame Cicely Saunders, of UK, in 1948 and regarded as the founder of Modern Hospice Movement. In India, Dr. Rajagopal, introduced it. Palliative care “Improves the Quality Of Life” of patients and that of their families who are facing challenges associated with life threatening illness, physical, psychological, social or spiritual distress. Palliative care is a specialty medicine given by the team of doctors, nurses, psychological therapists, social workers, clergy and volunteers. Each year an estimate of 40 million people is in need of palliative care. Only 14 % of people currently receive palliative care in the world. Aspects of Palliative Care “Pain Is The Worst of Evils”. Total Pain is a term proposed by Dame Cicely Sainders. A multi-dimensional need of families and patients requires a whole person approach (body, mind, soul, physical, emotional, social and spiritual). In focusing on the management of pain in palliative care, we are dealing primarily with cancer pain. Palliative care approach is to Treat not only the disease; but the person as a whole with the disease. For patients with curable, non-curable disease, suffering begins not just at the terminal stage, it even occurs during diagnosis, during treatment and being supportive throughout the disease. Psychological pain is most common reactions and concerns (anxiety, fears, anger, sadness, guilt). Anxiety occurs in most phases. Shock, emotional numbness, disbelief are commonly expressed. Fear, sadness and guilt may continue to the advanced stage. Regret, Guilt – a sense of hopelessness, may be felt in terminal and in the advanced stage. Social distress– self isolation, loss of job, disgust due to malodorous and disfiguring wound on face, loss of respect, insecurity due to loss of position and neglect by family. Spiritual is a personalized belief through which one understands the purpose of life, this is often expressed as religion but it can refer to other concepts like belief in GOD, relationships, disappointments, remorse, death anxiety, nature, energy and force etc.



Barriers include issues related to use and availability of Morphine. Physician's underestimation of pain due to deficiency in understanding the basic principles of pain management. Hesitation to report pain due to lack of patients and care givers knowledge of pain (Poverty, lack of family support, access to health care, drug non availability and lack of professional awareness). Poverty – Alcoholism and loss of job due to present health status will make him/her depend on government resources for treatment. Lack of Family Support –Conflicts in the family due to his/her health emotionally distances from their family. Difficult Access to Health Care –The time taken and the difficulty to travel due to loss of daily wages. Drug non availability – Pain not responding to Tramadol (mild opioid) and demanded use of powerful opioids like Morphine, not being available in the hospitals. Lack of Professional Awareness – Doctors unawareness on palliative approach will compromise accessing him/her from total Pain and the scope of relief.

Cancer is a serious illness. Early diagnosis increases the chances for cure. The causes of cancer are not known. But there are certain risk factors increases the cancer. Palliative care is the integral part of total cancer care. Palliative care can be started at the time of diagnosis, during treatment when treatment cannot be done are at the end stage. palliative care can be given in the hospital, at home or hospice. Palliative care can be given not only for cancer but any long term illness. Palliative care is improves the quality of life of patients and care givers.



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MUCORMYCOSIS: A LIFE THREATENING POST-COVID INFECTION

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Introduction

Coronavirus disease 2019 (Covid-19), caused by severe acute respiratory syndrome coronavirus is now a worldwide pandemic. Some most common symptoms of COVID-19 are fever, dry cough, shortness of breathing and myalgia. Management of a patient infected with the virus is a challenging task. Despite this, the case fatality rate has improved with time because of increased awareness and knowledge about the disease.

Background

Mucormycosis, known as "Black fungus" in INDIA, is a fungal infection caused by the family of molds Mucoromycetes. The Mucoromycetes is a fungus with at least 20 pathogenic species divided into 12 genera. *Rhizopus* is the genus that has been linked to the most Mucormycosis cases in the literature. Mucormycosis may be a rare, opportunistic, aggressive, fulminant, and often fatal infection. In India, Mucormycosis (Black Fungus) is one the most commonly observed secondary infection in COVID-19 infected patients in post-COVID-19 stage.



Forms of Mucormycosis

Clinically, five major types of Mucormycosis are recognized which include the rhino cerebral (44 % – 49 %), which is the most frequent form, followed by cutaneous (10 % – 16 %), pulmonary (10 % – 11 %), disseminated (6 % – 11.6 %), and gastrointestinal (2 % – 11 %) forms.

- **Rhinocerebral Mucormycosis** - Most typical form in patients with diabetes and renal transplants.
- **Cutaneous Mucormycosis** - Often seen in patients with burns or other sort of local skin trauma, also occur in patients who don't seem to be immunosuppressed.
- **Pulmonary Mucormycosis** - Generally found in patients with hematologic malignancy or profound neutropenia.
- **Disseminated Mucormycosis** - Usually seen in neutropenic patients with pulmonary infection.

Threat to Life

A new hurdle stands in front of medical science as variety of cases have been seen. These infections are more dangerous than black fungus due to its acute effect on the lungs and other body parts including nails, skin, stomach, kidney, brain, and mouth. Therefore, we must be vigilant about the secondary fungal infections in COVID-19 patients. Early diagnosis of Mucormycosis becomes mandatory because it can become life-threatening due to the invasive ability of the fungi into blood vessels, embolizing to distant organs, including the brain.

Causes

COVID-19 infection was a risk factor but the surge in Mucormycosis cases is also because of indiscriminate use of steroids and uncontrolled diabetes. Following risk factors can also be a cause of Mucormycosis:

- Long term corticosteroid use
- Skin trauma (burns, surgery ,or injuries)
- Excess iron in body

Symptoms

Mucormycosis can commonly affect areas like nose, eyes, brain, and sinuses. Therefore, recovering COVID-19 patients should seek medical help as and once they experience the symptoms given below.



- Swelling within the face
- Pain and numbness
- Nasal or sinus congestion.

Conclusion

The cases of Mucormycosis are not new however; a spike in such cases has been reported during COVID era. The rise in cases of Mucormycosis is due to COVID-19 remain associated with impaired immune system of infected patient. The central research institution across the globe including CDC emphasizes rise in Mucormycosis cases after COVID-19 disease. The poor and or impaired immune functioning is major cause of rise in Mucormycosis cases and clinical findings further confirmed. The cell mediated immunity like Th1 and IFN- γ are primarily involved in giving protection during viral infections. However, novel SARS-CoV-2 infection remains related to impaired functioning of not only cellular but also humoral immunity triggers higher risk of mycosis.



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POST- COVID HEALTH MANAGEMENT: A HOLISTIC APPROACH TOWARDS AYURVEDA AND YOGA FOR POST- COVID19 RECOVERY

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Introduction

The Ayurvedic perspective on the pathophysiology of novel corona virus disease can be explained by considering the disease as *Aupasargika roga* (infectious) in nature. Being infectious in nature, the COVID-19 disease has spread globally causing vast number of life loss. Despite worldwide efforts to contain it, the pandemic is continuing to spread for want of clinically-proven prophylaxis and therapeutic strategy. The dimensions of pandemic require an urgent harnessing of all knowledge systems available globally.

Utilization of Traditional Chinese Medicine (TCM) in Wuhan to treat COVID-19 cases sets the example demonstrating that traditional health care can attribute to treatment of post-covid management successfully. So, looking forwards to Ayurveda, an age-old science, to deal with post-covid recovery is need of an hour to add on.



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Management

The standard protocol for management of the Covid-19 infection is performing Panchavidha Shodhanam (Panchakarma), administration of Rasayana (immunomodulatory supplements), specific therapies and remedies as well. Even adopting Sadvritta (ethical code of conducts), wholesome food habits with proper lifestyle and administration of other supportive psychological therapeutic measures, will help in managing Covid-19 infection.

The ancient traditional principles of management in pandemic diseases can be precisely understood through the integrative methods. Ayurveda physicians have described performing the Panchakarma in the preliminary phase that may help fight the viral entry into the host body or may reduce the viral load in an infected individual. The administration of Rasayana may delay the pathogenesis process by increasing the immunity and eliminating the toxic effects of virus on the individual.

General Ayurvedic Recommendations for Building Immunity

Keep your Agni (digestive fire) strong. Follow *dincharya*, the first important thing which Ayurveda recommends. To maintain a proper dincharya, wake up in the morning, scrape your tongue and then brush your teeth. Drink a glass of warm water and practice some pranayama. Pranayama strengthens immune system including pranavaha srotas, respiratory system. Rasa vaha srotas, lymphatic system will also be cleansed to boost energy. This will maintain the balance of ojas (immunity, strength), tejas (digestion), and prana (vital life force).

How Ayurveda helps in Post-COVID Management?

According to this age-old science called Ayurveda, any abnormalities in the body are caused due to the imbalance of Vata, Pitta and Kapha doshas. Not to forget the accumulations of Ama (toxins) in body. So, the focus is to bring the doshas in balance and detoxify the body to make it ready for overall healing. The impact of any disease depends upon severity of disease, prakriti, satwa, satmya and age of an individual. Following are few ayurvedic integrations that one can take and make a part of life style

- ***Amrit Kaslah*** - Boosts immunity and strengthens all the organs of body, helps in detoxification and heals the body within.
- ***Chyawanprash*** - Strengthens immune system as well as respiratory system.
- ***Triphala*** - Helps to regulate excess pitta in the body and eliminates toxins as well.



- **Ashwagandha** - Acts as an adaptogen that helps the body adapt to various types of stress, calms and strengthens the nervous system, and promotes stamina and general well-being.
- **Shatavari** - Helps to increase the strength of musculoskeletal system.
- **Guduchi** - Effective against viral fevers, used as an anti-inflammatory, antipyretic and immunomodulatory interventions.
- **Turmeric** - Used as antioxidants.
- **Ayurvedic Panchakarma Therapy** - Helps in cleansing of body to reduce toxins. Abhyanga (whole body massage) with lukewarm sesame oil followed by Swedan (whole body steaming) are recommended as detoxifying therapies.
- **Yoga and Pranayama** - Removes lactic acid from the body and helps to eliminate fatigue, improves lung functions.
- **Steam Inhalation** - Provides relief in nasal and throat congestion, bronchoconstriction, headache and sinusitis.
- **Herbal Tea** - Helps to improve metabolism.
- **Gargles (Gandusha)** - Induces additional immunomodulatory, antioxidant and antimicrobial benefits in oral cavity.
- **Rasayana Therapy** - Effective in immunomodulation and restoration of immune Haemostasis.

Conclusion

It is very important to follow a strict post-covid regimen along with maintaining a positive state of mind to recover completely. It has proved to provide a long term relief to patients and boosts immunity. Ayurvedic treatment can help heal the body organs and are free from any side effects (exceptions, medication without prescription and that too in excess amount, and any pre-existing illness).



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CURRENT TRENDS OF FERMENTATION IN FERMENTED FOODS

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Fermentation is growing demand, supported by consumer perceptions of it as a natural and 'healthy' food preservation method. Fermentation refers to an anaerobic process involving the application of natural bacteria feeding on the starch and sugar present in the food to produce lactic acid. This helps preserve food and extend shelf life. Fermented foods sit at the intersection of two meg-trends that are coming to define consumption patterns in Europe: the demand for natural products that deliver added health benefits. Because fermentation is a natural process it delivers on consumer expectations around clean labelling and a desire to avoid synthetic additives and preservatives. According to a survey commissioned by specialist public relations group ingredient communications last year. Averagely, 73 % of consumer said they are willing to pay a higher retail price for products made with ingredients they recognize and trust.

Fermentation also carries health-related benefits. Fermented foods contain necessary enzymes. Omega-3 fatty acids, probiotics and vitamin-B. Across various categories – from ambient vegetables to health drinks and dairy products -fermented foods have come to be associated with positive digestive health. Fermentation also appeals to millennial consumers, who are seeking out novel taste and texture experiences and international cuisines. A survey from the research group found the 49 % of millennial and gen Z consumers like to experiment with new and unusual flavours. Fermentation is a popular food preparation method in African and Asian



cultures. And increasingly European consumers are experimenting with exotic flavours found in Korean Kimchi or cultured drinks like Kombucha and Lassi. Which have been long-popular with consumers in China and India.

Fermentation is most commonly used in the dairy aisle in Europe. But, with growing demand for plant-based options and an increased uptake of flexitarian diets, Carcano suggested that the greatest potential for future growth in fermented products lies in less developed areas. Future growth lies first with alternatives to Yogurt and to fermented milk drink. One can also expect increase demand for a variety of fruit juices, protein and cereals based juices. In order to help food makers meet this need, Dupoint is launching Danisco Vege cultures, a new portfolio of cultures specially formulated for the plant based fermented products market. Fermentation also offers fish and meat producers a method for attaining clean label certification for their products without using harmful synthetic preservatives. The growing popularity of fermented foods implies the need for innovative and creative packaging formats that preserve the integrity of the live probiotic culture in the foodstuff, without sacrificing the convenience and aesthetic aspects.



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FOOD ADDITIVES

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Food Additives are substance added for food to maintain or improve its safety, freshness, taste, texture, or appearance. Food additives need to be checked for potential harmful effects on human health before they can be used. Some food additives have been in use for centuries for preservation such as salt, sugar or sulfur dioxide. Food additives can be derived from plant, animal or they can be synthetic. Many modern products, such as low-calorie, snack, and ready-to-eat convenience foods, would not be possible without food additives. There are four general categories of food additives: Nutritional additives, processing agents, Preservatives and Sensory agents.

Nutritional additives are used for the purpose of restoring nutrients lost or degraded during production, fortifying or enriching certain foods in order to correct nutrients to food substitute. The fortification of food began in 1924 when iodine was added to table salt for the prevention of goiter. Vitamins are commonly added to many foods in order to enrich their nutritional value. For example , vitamins A and D are added for to dairy and cereal products, several of the B vitamins are added to flour , cereal, baked foods , and pasta, and vitamins C is added to fruit beverages cereals , dairy products, and confectioneries.



Functions

Improve the taste or appearance of a processed food. (Ex) Beeswax-glazing agent is used to coat apples. Improve the keeping quality or stability of a food. (Ex) sorbitol added to mixed dried fruits to maintain moisture level and softness of the fruit. Ensure nutritional value. Maintain uniform quality and to enhance quality parameter like flavor, ECT. in large scale production.

Preservatives

Preservatives prevent spoilage of the food due to fungi, bacteria, and other microorganisms. (eg.) sodium benzoate, sodium nitrate, benzoic acid, BHA .

- **Sodium Benzoate:** In this preservatives used to preserve carbonated drinks, pickles and sauces. **Side effects:** Asthma, and suspected to be a neurotoxin and carcinogen, may cause fetal abnormalities.
- **Sodium nitrite and Nitrate:** Used to preserve sausages, smoked fish, canned meats. **Side effects:** nitrite is a carcinogen, nitrate risk of miscarriages, fetal death.
- **Benzoic acid:** Used to preserve drinks, low sugar products, cereals, meat products. **Side effects:** may temporarily inhibit digestive enzyme function.
- **BHA (Butylated hydroxyanisole)/BHT (Butylated hydroxytoluene):** Used to preserve potato chips, vegetable oils. **Side effects:** may be carcinogenic to humans toxic to central nervous system and liver. Color additives make the food look colorful and so taste. These are described as one of the most dangerous additives. Color additives used to color beverages, powder, ice creams, and custard. It will cause allergies, asthma and hyperactivity. (eg.) , Erythrosine and brilliant blue.
- **Erythrosine:** Cherries in fruit cocktail and canned fruits baked foods, dairy products, and snack foods. **Side effects:** Cancer.
- **Brilliant blue:** Used to dairy products and sweets. **Side effects:** Skin rashes and Carcinogen.



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DETECTION AND PROLIFERATION OF VIRUSES IN EMBRYONATED HEN'S EGG

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Viruses can replicate only in living cells. For studying the growth of viruses and production of virus components it is necessary to have access to cells cultivated in the laboratory. Certain viruses which are pathogenic for liver and intestinal epithelial cells can only replicate in these highly differentiated cells and therefore have not yet been efficiently cultivated *in vitro*. In the early phase of research on viruses their properties could have only be studied by the transmission of virus infected to non-infected plants and animals. During the 1930, several methods were developed for the cultivation of poxviruses, herpes viruses and certain myxovirus in embryonated hen's egg. The introduction of this technique was a major step forward and it still being used for the identification of virus infectivity, for vaccine production and for diagnostic purposes. Proliferation of viruses in both live animals and embryonated hen's egg are included in the concept of *in vivo* systems. The introduction of chick embryo in 1931 as a useful medium for the growth of viruses opened the door to the systematic study of viruses. The most known viruses were adapted to grow in the chick embryo. With refinement in technique, accurate determination of virus identity, growth cycles and antigenic structure became possible. Prior to the advent of cell culture, animal viruses could be propagated only on whole animals or embryonated hen's eggs. Good Pasture in 1931 first used the embryonated hen's egg for the cultivation of viruses. The procedure of cultivation of



viruses in embryonated eggs depend on the type of egg which is used. The egg used for the cultivation must be sterile and shell should be integral and healthy. A hole is drilled in the shell of embryonated egg and viral suspension or suspected viral containing tissue is injected into the fluid of egg. Viral growth and multiplication in the egg embryo is indicated by death of embryo, by embryo cell damage or formation of typical pocks, lesions on egg membranes. An embryonated egg offers various sites for the cultivation of viruses. Different kinds of viruses are propagated in either of the embryonal membranes surrounding the Amnion, Chorioallantoic membrane, Yolk sac, Allantoic etc. The chorioallantoic membrane (CAM) is highly susceptible to infection with poxviruses and it is used for their isolation and quantification. During infection newly synthesized virus spreads from the primarily infected cells to neighbouring cells to form the localized pock like structure. Virus growth and replication in the CAM is indicated by visible lesions (pocks); grey white area in transparent CAM. Each pock is derived from single virion. The morphology of the pocks may vary depending on the nature of the virus. Under optimal conditions, each infectious virus particles can form one pock. Thus this method is suitable for plaque studies. Herpes simplex virus can also be inoculated *via* CAM. The area of the membrane available for virus growth can be increased by opening a small window in the shell and shell membrane moving the air space from the end to directly below the window. The virus is then introduced through this window directly on to the CAM. The window can be sealed with paraffin wax. After 2-3 days of incubation many of the poxvirus, herpes virus, forms pocks of varying size and consistency the CAM. After 2-6 days of incubation virus is shield into the fluid which can easily harvested. This is an excellent source of virus for morphological and serological studies and production of vaccines. Allantoic cavity is the most popular and simple method for viral inoculation. Allantoic inoculation is employed for the growth and replication of the influenza virus for vaccine production. This will provide a rich yield of influenza and some paramyxoviruses. Other allantoic vaccines include yellow fever and rabies vaccine. Duck eggs provide a better yield of rabies virus and were used for the preparation of the inactivated non-neuronal rabies vaccines. But they need a longer incubation period than embryonated hen's egg. Most of avian viruses can be isolated using this method. Amniotic sac is employed inoculated for primary isolation of influenza virus and mumps virus. Injection of virus into the amniotic cavity is difficult and necessitates the cutting of a window for accuracy. Several types of cells are in contact with the amniotic fluid. These are the epithelial cells of the amnion, the epidermal epithelium and the cells lining the respiratory and alimentary tracts of the embryo. With the most of the myxoviruses, inoculation performed on 13-14 day old eggs followed by 2-4 days of incubation, is the most proficient method of isolating and propagating of new strains. New virus is shed into



the amniotic fluid which has a maximum volume of 3-6ml in the 13-14 old eggs. Much training is mandatory to successfully harvest pure amniotic fluid. Growth and replication of virus in egg embryo can be detected by haemagglutination assay. The yolk sac inoculation is also a simplest method for growth and multiplication of virus. The yolk sac acts as a blood forming organ and as a means of transporting nutrient material to the embryo. Inoculation requires much practice but harvesting the yolk sac is much easier than the amniotic fluid. The new virus is present in the actual sac and this is washed clean before emulsifying. Vaccines against typhus, Rocky Mountain spotted fever, Q-fever, and rabies are all produced by from yolk sac material. Mostly mammalian viruses are isolated by this technique. Immune interference mechanism can be detected in most of avian viruses. This method is also used for the cultivation of some bacteria. Embryo inoculation, although unnecessary for the propagation of virus, various technologies have been devised for direct inoculation of the embryo. Intra cerebral inoculation has been employed in the study of herpes simplex virus and rabies virus with success. Intra venous inoculation is also used. However, these techniques are difficult, and while useful to gain specific information they have never become routine. In the case CAM inoculation the pocks illustrate the growth. By staining the yolk sac emulsion the elementary bodies or virus particles can be seen. The Myxoviruses, Arboviruses can also be seen in allantoic or amniotic fluids. Myxoviruses adsorb the erythrocytes, hence these erythrocytes are clumped or agglutinated and this property is called as haemagglutination. The presence of viruses in allantoic or amniotic fluid can be detected by adding an aliquot of fluid to a suspension of susceptible erythrocytes. If virus is present the erythrocytes are agglutinated.



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TURMERIC THE THERAPEUTIC CRUDE

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Curcuma longa, a flowering plant of the ginger family, Zingiberaceae, the roots of which are used in cooking. The plant is a perennial, rhizomatous, herbaceous plant native to the Indian subcontinent and Southeast Asia, that requires temperatures between 20 °C and 30 °C and a considerable amount of annual rainfall to thrive. Plants are gathered each year for their rhizomes, some for propagation in the following season and some for consumption.

The rhizomes are used fresh or boiled in water and dried, after which they are ground into a deep orange-yellow powder commonly used as a coloring and flavoring agent. Turmeric powder has a warm, bitter, black pepper - like flavor and earthy, mustard-like aroma. Although long used in Ayurvedic medicine, where it is also known as haridra (golden colour). Chemical composition of turmeric includes curcuminoids, vitamins, minerals and Phytochemicals like carotenoids, carotenes and xanthophylls etc. Turmeric contains 3 – 6 % polyphenolic compounds, collectively known as curcuminoids, which is a mixture of curcumin, demethoxycurcumin and bisdemethoxycurcumin. Curcuminoids are major components responsible for various biological actions. One tablespoon of ground turmeric powder contains 29 calories, 6 grams of carbohydrate, 2 grams of fiber, 1 gram of fat, and little protein. It contains about 16 % of your daily iron needs (2.8 mg) and 26 % of your daily manganese needs (0.5 mg). It also contains vitamins B3, C, E and K. Other components of turmeric include minerals such as calcium, copper, iron, magnesium, potassium, and zinc.



Health Benefits of Turmeric

Applying turmeric to a wound can help sanitize the site and enhance tissue formation, collagen deposition, tissue remodeling, and wound contraction. Turmeric was also found useful in speeding up skin healing after a Caesarean section without causing any adverse effects. More research is needed to determine the ideal curcumin formulation for aiding the repair of skin wounds. Weight management: Curcumin interacts with white adipose tissue, often thought to be the unhealthy fat store in the body, and suppresses inflammation caused by obesity. A study found that curcumin helped with the overall weight loss, loss of body fat, and reduction of weight circumference in those that had already successfully lost weight from following a healthy diet and exercising. Arthritis relief: Free radicals damage body cells and induce inflammation, which lies at the crux of degenerative disorders such as osteoarthritis and rheumatoid arthritis. Diabetes control: Some studies found that turmeric may help moderate insulin levels and improve glucose control in patients with diabetes or pre-diabetes when taken in appropriate amounts. It may also help improve insulin resistance, which is a precursor for type 2 diabetes. Proper digestion: Turmeric can stimulate your gallbladder to produce more bile to improve fat metabolism and overall digestion. This digestive aid helps reduce flatulence, bloating, and other discomforting symptoms of inflammatory bowel disease. Other Health Benefits of Turmeric are cholesterol reduction, prevention of Alzheimer's disease, liver disease prevention, anti-aging, anti-venom and oral care.



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ANTIBACTERIAL PROPERTY AND PHYTOCHEMICAL SCREENING OF *Drynaria quercifolia*

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Medicinal plants have been used as traditional treatments for numerous human diseases in many parts of the world. Most of the medicinal plants are said to have various properties like anti-inflammatory, anti-diabetic, anti-fungal, anti-oxidant and various other properties. *Drynaria quercifolia* belongs to the family Polypodiaceae. It consists of a densely scaled rhizome. This article shows the anti-bacterial property in the roots of *Drynaria quercifolia* against various bacteria like *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Escherichia coli*.

The roots of *Drynaria quercifolia* were cut into small pieces and shade dried and the components in the roots were extracted by Soxhlet apparatus. This method uses various solvents like water, acetone, chloroform, ethanol, methanol, etc. In this study, two solvents ethanol and methanol were used to extract the contents in the roots. Here the dried root is soaked in respective solvents in the Soxhlet apparatus and around 10 to 15 cycles were repeated for each of the solvents, for the efficient extraction of all the constituents in the root. The extract solution obtained, was heated. This causes the evaporation of the solvents and the dried extract is obtained. The prepared extracts were further used for the analysis of phytochemicals and anti-bacterial activity.



Phytochemicals occurs naturally in the medicinal plants, leaves, roots, fruits that show defence mechanism against various diseases. These are various compounds present in plants that exhibit various properties. The phytochemical analysis is done to screen the plant extracts for various compounds like alkaloids, tannins, carbohydrates, proteins, flavonoids, etc. This analysis is done by series of reactions that involves a series of reagents and methods. The plant extracts were exposed to various reagents like Fehling's, Benedict's reagents, ferric chloride, sodium hydroxide, ammonium hydroxide, etc., based on the compound to be screened. For example, for the test to detect flavonoids (Alkaline reagent Test), both the extracts were treated with 10% of sodium hydroxide, so the formation of an intense yellow colour indicates the presence of flavonoids. Similarly, various tests performed to find the presence of phytochemicals.

The ethanolic and methanolic root extracts of *Drynaria quercifolia* were screened for phytochemicals. The ethanolic root extract showed the presence of flavonoids and reducing sugars and also showed the absence of saponins, alkaloids, tannins, steroids, carbohydrates and cardiac glycosides. The methanolic root extracts showed the presence of flavonoids, saponins, alkaloids, reducing sugars, steroids and carbohydrates and they also showed the absence of tannins, cardiac glycosides.

The evaluation of the anti-bacterial activity is done to screen the biological activity of the plant extracts against bacteria. The principle behind this assay is that the agar plate with wells containing different concentrations of the plant extracts whose activity against the test organisms inoculated on the surface of the agar by spread plate method has to be screened, the extract diffuses radially outwards through the agar producing a concentration gradient. The extract will be present in higher concentration near the well and as the distance from the well increases the concentration of the extract decreases. The region with greater concentration of the plant extract inhibits most of the bacterial growth. The extracts with concentration of 500 µg/ml and 1000 µg/ml were loaded into the wells. The zone of inhibition was measured after 24 hours of incubation. The clear zone of inhibition (clear ring) is observed. Wider the zone surrounding the well, the more susceptible is the test organism. The zone width is a function of initial concentration of the extract, its solubility, its diffusion rate through the agar and the growth rate of the test organism. Muller Hinton Agar (MHA) is the most commonly used growth medium for antibiotic susceptibility testing.

The anti-bacterial activity of the root extracts of *Drynaria quercifolia* were observed by the formation of the zone of inhibition around the extracts, in the well. The zone of inhibition showed that the ethanolic and methanolic extracts of *Drynaria*



quercifolia showed significant anti-bacterial effect against *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Escherichia coli*, *Serratia* species. Simultaneously, disc method was followed by dipping the disc in the extract and placing them on the inoculated media. For example, In *Staphylococcus*, the zone of inhibition measured in methanolic and ethanolic extracts of *Drynaria quercifolia* in well was found to be 19mm and 16mm respectively, and in disc it was found to be 13mm and 19mm respectively.

In conclusion, the root extracts of *Drynaria quercifolia* in the solvents methanol and ethanol shows various important phytochemicals and proves that it possesses a very high anti-bacterial property. Therefore, it is necessary to study the maximum potential of these medicinal plants in the field of medicine and pharmaceutical sciences for novel and fruitful application of their anti-bacterial activity.



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IMPORTANCE OF DRAGON FRUITS RICH NUTRIENTS AGAINST COVID -19

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Due to upcoming pandemic diseases, it is important to study different medicinal property of plant. The present status of coronavirus infection world wide variety creates a savior health problem. Due to less immunity these viruses may infect easily. So, we require strong immunity against to fight these infections we are searching that medicinal plant and fruit that boost our immunity and make strong to fight against various Air borne disease.

Red dragon fruit which is widely used as herbal medicine plant. Dragon fruit is rich in Vitamin C. These dragon fruit rich in Vitamin C having the antioxidant properties to boost the immunity and to fight against corona virus infection. Dragon fruit belong to family of Cactaceas and of genus *Hylocerous undatus*. Another name 'pitaya' call in Central Americans. It is rich in vitamin and helps the digestive process due to its fiber and it prevent from colon cancer and diabetes, also neutralizes toxic substance such as heavy metal and high blood pressure. Lycopene is present in red fleshed varieties. It acts as natural antioxidant which is important to fight cancer, heart disease and low blood pressure.

Asthma and Cough can be cured by regular consumption of dragon fruits. Dragon fruit also contain fiber, Vit C and minerals, calcium and magnesium iron. Dragon fruit is rich source of phytoalbumins which is very important for antioxidant property. When cell use oxygen to generate energy, free radicals are formed. This



byproduct is Reactive Oxygen Species (ROS). ROS is helpful for cellular response and immune system, but these radical and oxidant generate oxidative stress at high level. These antioxidants prevent free radical and repair damage caused by ROS. In this way, it improves the immune system and help to fight against Cancer.

Percentage wise data of Dragon fruit

- 2.9 g of Fibers (11 % DV)
- 2.5 mg of Vitamin C (4.16 % DV)
- 18 mg of Calcium (1.8 % DV)
- 59 IU of Vitamin A (1.18 % DV)
- 40 mg of Magnesium (10 % DV)
- 0.74 mg of Iron (4.1 % DV)

Note: DV- daily value, IU- international unit

Conclusion

The present investigation shows that the antioxidant and antimicrobial activity property of dragon fruit against Covid-19 has proven that it boosts immunity because of rich in Vit C, Vit A, calcium, iron, fiber and magnesium. These all nutrients are useful to improve immunity to fight against Corona virus situation.



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EXPRESSION VECTOR - AN OVERVIEW

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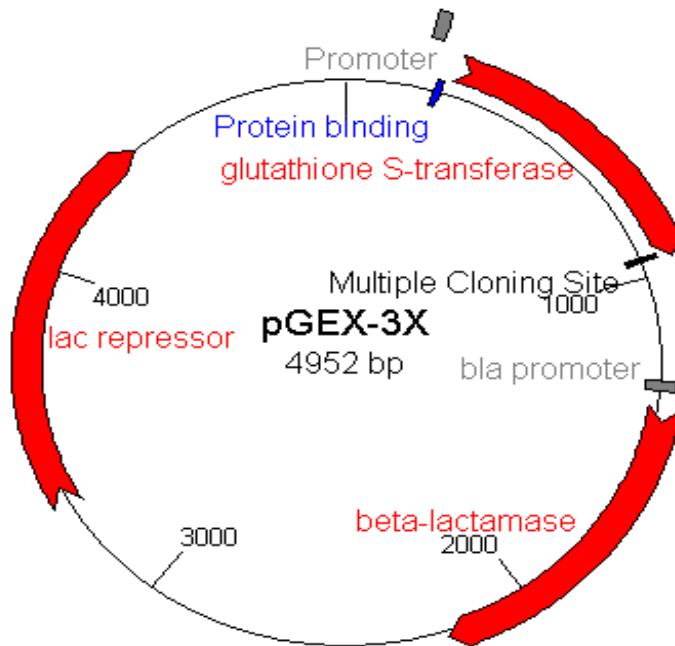
Expression vector is a very important topic now a days because of it's huge application in research and medical field. Before we discuss about the definition of expression vector, we should know what is expression and what is vector? In central dogma, DNA produce from DNA is called replication, RNA produce from DNA is called transcription and protein produce from RNA is known as translation. This transcription and translation portion of central dogma is responsible expression of gene. A vector is a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell where it can be replicated and/or expressed. e.g- plasmid, cosmid, lambda phage etc.

We use cloning vector for transferring our gene of interest to the host cell and replicating it to increase the copy number but expression vector is used to express the gene. Cloning vector is consist of origin of replication, selectable marker gene and multiple cloning site whereas in addition to these elements expression vector is made up of inducible promoter, transcription translation initiation sequence, elements of transcription, purification tag (His tag) and fusion protein tag.

The origin of replication (also called the replication origin) is a particular sequence in a genome at which replication is initiated. A selectable marker is a gene introduced into a cell, especially a bacterium or to cells in culture, that confers a trait suitable for artificial selection. A multiple cloning site (MCS), also called a polylinker, is a short segment of DNA which contains many (up to ~20) restriction sites. A promoter which is induced by an inducer. Start codon and stop codon for translation and termination sequence for transcription is required for expression. To



express a vector inside the bacterial cell, it must carry Shine-Dalgarno sequence. Six histidine tag is required for purification. Fusion protein tag is not a very important element. It may be present or may not be. The fusion protein usually act as a reporter, glutathione transfer, proper and better solubility in the water.



The expression host of choice for the expression of many proteins is *Escherichia coli* as the production of heterologous protein in *E. coli* is relatively simple and convenient, as well as being rapid and cheap. A large number of *E. coli* expression plasmids are also available for a wide variety of needs. Other bacteria used for protein production include *Bacillus subtilis*. A yeast commonly used for protein production is *Pichia pastoris*. Examples of yeast expression vector in *Pichia* are the pPIC series of vectors, and these vectors use the AOX1 promoter which is inducible with methanol. Baculovirus, a rod-shaped virus which infects insect cells, is used as the expression vector in this system. Many plant expression vectors are based on the Ti plasmid of *Agrobacterium tumefaciens*. In these expression vectors, DNA to be inserted into plant is cloned into the T-DNA, a stretch of DNA flanked by a 25-bp direct repeat sequence at either end, and which can integrate into the plant genome. SV40 are commonly used in mammalian expression vectors to drive gene expression. *Escherichia coli* cell lysate containing the cellular components required for transcription and translation are used in this *in vitro* method of protein production. The advantage of such system is that protein may be produced much faster than those produced *in vivo* since it does not require time to culture the cells, but it is also more expensive.



Expression vector in an expression host is now the usual method used in laboratories to produce proteins for research. Most protein pharmaceuticals are now produced through recombinant DNA technology using expression vectors. These peptide and protein pharmaceuticals may be hormones, vaccines, antibiotics, antibodies, and enzymes. The first human recombinant protein used for disease management, insulin, was introduced in 1982. In recent years, expression vectors have been used to introduce specific genes into plants and animals to produce transgenic organisms, for example in agriculture it is used to produce transgenic plants. Gene therapy is a promising treatment for a number of diseases where a "normal" gene carried by the vector is inserted into the genome, to replace an "abnormal" gene or supplement the expression of particular gene. We hope in future more research will conduct on expression vector.



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FERMENTED CHEESE

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Fermented Cheese

Fermented cheese is a food found all around the world, Cheeses are fermented by adding different types of bacteria to dairy products and allowing the bacteria to eat the lactose.

Examples of Foreign Fermented Cheese

- **Ambra Di Talamello** – Ambra di Talamello is an Italian cheese that originated in the Marche region of Italy and is produced in Talamello, Italy. This cheese is aged for a few months, Ambra di Talamello is a registered trademark and is designated as “the official commercial name of foss a cheese”.
- **Limburger** – Limburger is a cheese that originated in the Herve area of the historical Suchy of Limburg in Belgium. The Cheese is especially known for its strong smell caused by the bacterium *Brevibacterium linens*. The texture of this cheese is semi soft. Aging time of these cheese is 2-3 months. One of the most traditional ways of eating limburger is the limburger sandwich.
- **Shanklish** - Shanklish is a type of cow's milk or sheep milk cheese in Levantine cuisine. Shanklish cheese is found in the region Syria, Egypt, Lebanon and Turkey. Shanklish varies greatly in its texture and flavor. Shanklish is generally eaten with finely- chopped tomato, onion and olive oil; and often accompanied by Arag. Shanklish is also mashed up with eggs or crushed in a pita with cucumbers, olive oil and mint for breakfast.



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ARBITRIUM: VIRAL COMMUNICATION SYSTEM

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Viruses communicate with each other in a molecular language .Through this system, they can decide whether to stay in a dormant state in the host cell or to replicate and attack the host. When a bacteriophage infects a cell, a short peptide is released which serves as a messaging molecule to other phages. This viral protein is called arbitrium. When more cells are attacked by phages, more protein is released which lets the viruses know that the availability of hosts are decreasing. They then stay in lysogenic state. This decision is made by the virus and do not depend on the bacterial cues. It is now clear that viruses interact with each other, to co-infect a cell, bringing down the cell's immune defences.

Arbitrium is a six-amino acids long peptide that is released when *Bacillus* phage infects a cell. Arbitrium system is encoded by three genes: aimP, aimR, and aimX. The hexapeptide is produced by aimP and aimR produces the intracellular peptide receptor. AimP (hexapeptide - arbitrium) can reduce the expression of AimX which is the negative regulator of lysogeny or genetic inhibitor of dormancy, produced by aimX. This reduction in expression occurs when AimP binds to AimR. When this happens, it leads to lysogeny. The version of this communication peptide varies in different bacteriophages. If it is found that the viruses that infect humans and other organisms communicate in a similar manner, learning and intercepting this communication system can be helpful in preventing viral diseases.



The receptor for arbitrium in phage can interact with unrelated stretches of DNA of the bacterium. This could mean that the peptide has other functions and not limited to the decision making on whether to stay in lysogenic or lytic phase. For example, it could alter the gene of the host. Different phages communicate in different languages and can only understand their own. They can also listen to the communication happening between bacteria –quorum sensing- and use the QS signalling molecules to their own benefits. If a phage is made to infect a disease causing bacteria, they can sense the QS signals and destroy them.

Altruistic behaviour is seen in viruses. Phages cooperate selflessly, sacrificing themselves, to overcome the host. When bacteria were bombarded with viruses that had specialized anti-CRISPR protein, the initial attack killed the viruses but it also weakened the cell's CRISPR –based immune defence. This made it possible for the other phages, even the ones that do not possess any anti-CRISPR proteins, to kill the host. The viral particles are more infectious when they are in these cooperating clusters. Disrupting this altruistic behaviour and clustering can help ward off viral diseases. Taking advantage of this same behaviour of viruses can help treat bacterial infections.



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INHIBITORY EFFECT OF GINGER AGAINST FISH BACTERIAL PATHOGENS

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Ginger *Zingiber officinale* is an erect, slim herbaceous perennial plant that possess a fleshy and thick underground rhizome and having one or more aerial leafy stems, that grows up to 1.25 m tall. Ginger is grown in the tropical weathers of Australia, West Africa, India, Jamaica Brazil, China, and some parts of the United States. In the first year of growth, it produces a green, straight stalk like stem about 60 cm high growing from the rhizome. Its leaves grow and measures about 12-30 cm long which dies off each year. The crop grows preferably in warm, Sunny conditions and may profit from shade during hot days, especially when young. Shading is however generally may profit from shade during hot days, especially when young. considered redundant. The optimum rainfall is 2500 – 3000 mm, well-distributed over the year. The odour and taste of ginger plant are aromatic, characteristic and pungent.

Ginger is a member of the family Zingiberaceae; a small family with more than 45 genera, and 800 species; its scientific name is *Zingiber officinale* (*Z. officinale*). Ginger is truly a world domestic remedy. It is also used in India and other place like the ancient Chinese where the fresh and dried roots were considered distinct medicinal products. Fresh ginger has been used for cold-induced diseases, nausea asthma, cough, colic heart palpitation, swelling, dyspepsia, less of appetite, and rheumatism, in short for the same purposes as in ancient china. In nineteenth century ginger serves as a popular remedy for cough and asthma when the juice of fresh



ginger was mixed with a little juice of fresh garlic and honey. A paste of powdered dried ginger was applied to the temples to relieve headache and fresh ginger was mixed with a little honey, tapped off with a pinch of burnt peacock feathers to alleviate nausea.

Ginger (*Zingiber officinale*) is used as a spice in many Asian foods, especially in Indian cuisine along with garlic. A number of researches have investigated the antimicrobial activity of ginger. Ginger is thick scaly rhizomes which are aromatic, thick lobed, branched, have a scaly structure and they possess a spicy lemon like scent. The rhizomes contain both aromatic and pungent components. For centuries, it has been an important ingredient in Chinese, Ayurveda and Tibb-Unani herbal medicines for the treatment of catarrh, rheumatism, disease, gingivitis, toothache, asthma, stroke, constipation and diabetes. Several reviews have appeared in the literature about this may reflect the popularity of the subject and its common use as a spice and a medicinal plant.

Ginger has several ethnomedicinal and nutritional values as spice and flavouring agents in Ethiopia and elsewhere. In last few decades, ginger is extensively studied for its medicinal properties by advanced scientific techniques and a variety of bioactive compounds such as tannins, flavonoid, glycosides, essential oils, furostanol, spirostanol, saponins, phytosterols, amides, alkaloids have been isolated from the different parts of the plant which were analysed pharmacologically.

Antibacterial activity of extracts of *Allium sativum* (garlic) and *Zingiber officinale* (ginger) has been evaluated against four different bacteria namely *Escherichia coli*, *Salmonella Typhi*, *Staphylococcus aureus* and *Bacillus cereus*. Two methods were used to determine the antimicrobial activity of garlic and ginger extracts namely disk diffusion method and agar well diffusion method. Garlic extract exhibited excellent antibacterial activity against all four test organisms while ginger extract showed antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus* only. In addition, agar well diffusion method showed higher zone of inhibition when compared with the zone of inhibition produced by the spice of same concentration against the test microorganism by disk diffusion method. The Whatman Number 1 (6 mm diameter) were sterilized in a dry heat sterilizer and kept in the refrigerator for further use. A lawn of each bacterial isolate was prepared on MHA plates using a sterile cotton swab from the inoculum showing growth. MHA plates were dried for 15 minutes in the laminar air flow cabinet. Three filter paper discs were placed one on top of other on dried MHA plates and ginger (20 µl) were added on each disc separately.



The present study was designed to evaluate the antimicrobial activity of fresh ginger extract. These have been traditionally used in folk medicine, and are still used in the alternative system of health care. The antibacterial activity of these commonly used Indian spices was tested against bacterial fish pathogens, namely *Escherichia coli*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Bacillus cereus*, which are responsible for many health-related problem. These were tested using disc diffusion method bacterial fish pathogens. Fresh ginger affected inhibitory growth of *Staphylococcus aureus*.



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BIPOTENTIAL EFFECT OF GARLIC AGAINST BACTERIAL PATHOGENS FROM INFECTED FISH

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Garlic belongs to family of Alliaceae and its scientific name is *Allium sativum*. Other members of the family include onion, leek, shallot and leek. Garlic is widely used in culinary and medicine. It has a pungent not flavour but mellows and improves with cooking. It has been utilization to fight infection such as cold, cough, asthma, diarrhoea, flu, headache, sore throat, abdominal discomfort and respiratory tract infection. The antibacterial properties of crushed garlic have been known for a long time. Various garlic preparation been shown to exhibit a wide spectrum of antibacterial activity against Gram -negative and Gram -positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, and *Clostridium*. Even acid-fast bacteria such as *Mycobacterium tuberculosis* are sensitive to garlic. Analysis of steam distillation of crushed garlic cloves performed over a century ago showed a variety of allyl Sulfide which are isolated and identified as the component responsible for the remarkable antibacterial activity of sulfide which are isolated and identified as the component responsible for the remarkable antibacterial activity of crushed garlic cloves. The compound turned out to be an oxygenated sulfur compound which they termed allicin from the Latin name of the garlic plant, *Allium sativum*.



Garlic (*Allium sativum*) belongs to the family Alliaceae. Its close relatives include the onion, shallot, and leek. It has been used throughout recorded history for both culinary and medicinal purposes. It has a characteristic pungent, hot, flavour that mellows and sweetens considerably with cooking. leaves and stems are sometimes eaten, particularly while immature and tender. Garlic has been used as medicine in many cultures for thousands of years, dating as far back as the time that the Egyptian pyramids. It is also claimed to help prevent heart diseases including atherosclerosis, high cholesterol, high blood pressure, and to improve the immune system as well as protection against cancer. A daily dose of 1 mL/kg body weight of garlic extract for six months can result in significant reduction in oxidant (free radical) stress in the blood of patients with atherosclerosis and cholesterol circulating in the bloodstream. Garlic's ability to prevent these oxidation reactions may explain some of its beneficial effects in atherosclerotic cardiovascular diseases.

Allium sativum is a perennial bulbous plant that initially came from middle Asia, and is at present grown globally. Garlic can grow up to feet in height or more. The bulb is the main part of the plant which is used for medicine. Each garlic bulb is made up of 4 to 20 clove. Each garlic clove may weigh about 1 gram in weight. Fresh, aged, dried or garlic can be used garlic supplements. Each of the supplements may have different effects to the body. It commonly used as seasoning. It helps prevent certain heart diseases including atherosclerosis, high cholesterol, high blood pressure and boost the immune system as well as protect against cancer. The medicinal potency of garlic is due to glycoside, vitamins B, C and D allisation II and I. It also contains volatile sulfur oil, which has a vermicidal action.

Filter paper discs (6 mm) were prepared using a punch machine. Filter paper discs were sterilized in a dry heat sterilization and kept in the refrigerator for further use. A lawn of each bacterial isolate was prepared on MHA plates using a sterile cotton swab. MHA plates were dried for 15 minutes in the Laminar air flow cabinet. Three filter discs were placed one on top of other on dried MHA plates and garlic extract (20 µl) were added on each disc separately. All plates were incubated at 37°C for 18-24 hours and the zone of inhibition (diameter in mm) were measured on the agar surface. The present study conducted to see the antimicrobial activity of ginger extract of garlic (*Allium sativum*) against infected fish pathogens *Escherichia coli* and *Staphylococcus* bacterial species using Disc diffusion method.



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VEGETABLE MARKET WASTE MANAGEMENT: SUSTAINABLE APPROACH

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Fruits and vegetables have a crucial role in human diet and nutrition. They are considered as the main source of nutrients such as dietary fibers, vitamins, mineral and photochemical compounds, therefore the demand for such important food commodities has increased very significantly as a result of the growing population and the changing dietary habits.

India is the second largest producer of fruits and vegetables in the world. By 2025 India will have a population of 1403 million from the current population 1391 million (2020 - 2021) so, constantly growing population and the changing dietary habits demand for fruits and vegetables increased very significantly in past few years this trend is likely to continue in the future. The total fruit and vegetable production of India in year 2020 - 2021 was 103.23 million tones and 193.61 million tons respectively. But from this production, about 40 to 50 percent of the total output worth Rs. 13,300 crore is wasted every year.

Fruits and vegetables waste can be generated in different steps of the food supply chain, from farm gate, wholesale, cold storage, processing and retail market. The huge amount of this waste is dumped on land to rot in open air without any treatment. It emits a foul odour, is a source of greenhouse gases, and creates a big nuisance by attracting birds, rats and pigs, vectors of various diseases. The rotting vegetables and fruits waste thus is an environmental threat and causes pollution. Thus, the sustainable waste management is necessary.



Why Sustainable Vegetable Waste Management is needed?

Sustainable waste management is the collection, transportation, valorization and disposal of the various types of waste, in a manner that does not risk the environment, human health or future generations. The goal of sustainable waste management is to reduce the amounts of natural resources consumed, reusing and recycling the materials taken from nature to maximum extent and creating minimal waste.

Fruits and vegetables waste are nutritionally very rich and are excellent sources of minerals, dietary fibers, pigments, Phenolic compounds, sugar derivatives, organic acids etc. Recycling of these nutritionally rich wastes in to value added by products recycles essential nutrients back to soil and in long run may also reduce dependency on fertilizers. Value added product derived from processing of vegetables and fruits waste include: Bio-Fertilizer, Enzymes, Organic acid, Bioactive compounds, Bio-pigments and colorants, Bio-degradable plastic, Biofuel, Compost, etc.

The zero-waste concept is an appealing solution to all issues. It forms basis of the sustainable approach, which aims at designing of processes to convert the raw material and waste into value added products without generation of any additional waste. The zero waste concepts have two strategies to eliminate the waste.

- a) Minimization of waste
- b) Reusability of generated waste.

There are many methods for managing the wet/organic waste such as landfill, dumping, composting, feed in digester of biogas. The common methods used for management of vegetable market waste are composting of waste to produce organic fertilizer, use of vegetable waste as feed to livestock, Processing of fruit and vegetable culls to separate juice from pulp.

Proposed way to for sustainable management of Vegetable Market waste are:

- a) Large amount of generated waste can be pulverized to reduce waste volume.
- b) Compressing of waste and separation of liquid and solid component.
- c) As vegetable and fruits contain natural indigenous microbes that can be used as biofertilizer.
- d) Nutrient rich solid component can be processed via composting and resulting product can be used as soil conditioner.
- e) Conversion of solid waste to animal feed *via* processing.
- f) Waste can be used as substrate for the production of important biopolymers degrading enzymes.



- g) Development of technology for the extraction of value-added products such as Organic acids, Bioactive compounds from the waste
- h) Anaerobic digestion of organic rich vegetable waste to produce Biofuels.

Above approaches aims for the development of technology for vegetable market waste management by transforming solid waste into organic fertilizer for agricultural system; recovery and utilization of methane from vegetable waste and minimizes greenhouse gas emissions through application of zero waste concepts and sustainable waste management.



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CLINICAL SIGNIFICANCE OF SUPEROXIDE DISMUTASE

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Superoxide dismutase (SOD) a common antioxidative enzyme found in all aerobic organisms. Superoxide dismutase brings about the dismutation or the breakdown of Superoxide (O_2^-) radical into Molecular oxygen (O_2) and Hydrogen peroxide (H_2O_2). Superoxide anion is produced as a by-product of aerobic metabolism and can be detrimental if left unchecked. Hydrogen peroxide is also damaging to the cell and is further catalysed by catalase enzyme. Hence SOD is an important antioxidant and gives defence to all aerobically respiring living cells. There are many types of SOD, and it has been classified based on the type of cofactor used for activation as - Cu/Zn SOD (copper and zinc), Iron (Fe) and manganese (Mn) SOD and Nickel (Ni) SOD.

Almost all types of living cells make use of antioxidants to deal with oxidative stress and Reactive Oxygen Species (ROS). In higher organisms SOD, protects cellular components from ROS. SOD outcompetes superoxide and hence protects it from superoxide toxicity. In this study we aim to do the spectrophotometric assay of antioxidative enzymes produced by *Aspergillus* sp. and *Cladosporium* sp. As mentioned earlier SOD performs the same functions of cell survival and oxidative stress tolerance and helps against host invasion in fungi just like in human beings and plants. The focus of our study is to isolate antioxidative enzymes from fungi rather than higher plants as extraction and isolation will be much easier in fungi as



compared to plants. Fungi being a simplest eukaryote organism can be easy to purify and the purity of SOD obtained will be much higher and it is also economical and commercially feasible.

Many studies have shown that SOD is necessary for survival in all aerobically metabolizing cells. Superoxide radicles (O_2^-) is formed when a single electron is passes through auto-oxidation from reduced haemoproteins and other enzymes. Free radicles such as singlet oxygen (O^{\cdot}) and its derivatives can cause oxygen toxicity. All aerobic organisms synthesize SOD which can protect from oxygen toxicity. Mammalian mitochondria produces singlet oxygen during electron transfer chain (ETC). Singlet oxygen damage is seen both in prokaryotes as well as eukaryotes. ROS which mainly includes O^{\cdot} and H_2O_2 readily react with biomolecules and on reaching excessive concentration can react and oxidize nucleic acids, proteins, and singlet oxygen. Singlet oxygen can act in response with DNA and cause G to T transversions and cause mutations. It can also cause tissue damage and inflammation. Singlet oxygen is also formed by photo oxidation in Prokaryotes and induces genotoxicity and mutagenesis. Not that ROS are all bad, in fact they are involved in the body's natural defence system and healing mechanism. Regular exposure to low levels of singlet oxygen leads to optimal neural function, faster healing, increased longevity etc. but prolonged exposure or heightened concentrations of ROS can cause tissue damage, cancer, cardiovascular and neurological diseases, hypertension, aging and so on.

SODs are powerful anti-inflammatory enzymes also has other beneficial properties such as anti-ageing, helps boost immunity and flush out toxic substances from the body. As people grow older the natural synthesis of antioxidants and SOD is reduced so antioxidants supplements can be taken to fulfil the need of the body and protect it against mutagenesis and various other fatal degenerative diseases. Hence the main aim of this study is to spectrophotometrically analyse antioxidants from fungal species commonly found in soil (*Aspergillus* sp. and *Cladosporium* sp.) and to quantify the isolated SOD.

In the present study, two common genera of soil fungi namely, *Aspergillus* sp. and *Cladosporium* sp. are cultivated by submerged cultivation for 5 days and pellets are separated by filtration. Pellets are disrupted by detergent method (0.5 % Triton X 100, 1 % CTAB, 1.5 % Tween 80, 5 % SDS). The pellets of both the fungal species were suspended in detergent solution for 40 mins under shaker incubation at 30 °C. The disrupted pellets were centrifuged (REMI™_sKBM 80 plus) at 12,000 rpm for 15 mins at 4 °C. Culture media and cellular extracts are analysed for intracellular and extracellular total proteins and their panel of antioxidative enzymes.



Superoxide dismutase activity was analysed by Beauchamp and Fridovich (1971) method. It is a photochemical assay consisting of methionine, riboflavin, and *p*-nitro blue tetrazolium. Crude extracts of the sample were analysed for SOD isoenzyme activity at 550 nm. It includes both secretory (extracellular) enzyme as well as intracellular enzyme assays. Secretory SOD, intracellular SOD and total SOD activity of *Aspergillus* sp. and *Cladosporium* sp. are summarized in the below given table.

S. No	Sample	Intracellular SOD activity (U/mg protein)	Extra cellular SOD activity (U/mg protein)	Total SOD isoenzyme activity (U/mg protein)
1	<i>Aspergillus</i> sp.	1.33	0.350 mg/ml	1.68 mg/ml
2	<i>Cladosporium</i> sp.	1.38	0.792 mg/ml	2.167 mg/ml

SOD isoenzyme activity profile of *Aspergillus* sp. and *Cladosporium* sp. crude extracts. SOD being the first line of defence against oxygen toxicity, higher SOD isoenzyme activity was expected. Previous research revealed, SOD protect cells from exogenous as well as endogenous reactive oxygen radicals. Hence all aerobic organisms have been observed to produce extracellular and intracellular SOD. In the present study, both secretory and cellular SOD isoenzyme activity was analysed. Interestingly, *Aspergillus* sp. and *Cladosporium* sp. have showed almost equal extracellular SOD activity. Whereas the intracellular SOD isoenzyme activity is higher in *Cladosporium* sp. compared to *Aspergillus* sp. Indicating that, *Cladosporium* sp. has a higher potential of super oxide scavenging activity. These results indicate that commercial production of microbial SOD is feasible and economical for the pharmaceutical grade SOD against many fatal and degenerative diseases.



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CATALASE AND PEROXIDASE ACTIVITIES IN *Aspergillus* sp. AND *Cladosporium* sp.

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Catalase is an important antioxidative enzyme, evolved to eliminate Reactive Oxygen Species (ROS). Catalase can be peroxisomal and cytosolic in origin and localization. In filamentous fungi, catalase functioning in conidia and mycelia are expressed by two different genes. Catalase (CAT) is a common aerobic enzyme, found in all aerobically respiring organisms, which catalyze the breakdown of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2). Catalase is a very potent enzyme and even very minute quantities can convert millions of H_2O_2 molecules into water and oxygen. Catalase is a tetramer and contains 4 iron-containing heme groups as prosthetic group, just like peroxidase enzyme. The heme group allow it to react with hydrogen peroxide. Catalase has a very high specific activity, which can be seen without the help of an instrument, the formation of bubbles (effervescence) seen by the addition of H_2O_2 to any microbial or tissue sample shows the potency of the enzyme and hence even minute quantities can breakdown many H_2O_2 molecules.



Hydrogen peroxide is a very harmful by-product of many metabolic aerobic pathways. H_2O_2 is a commonly used bleaching agent and even small quantities can be detrimental to the cell. To prevent damage from H_2O_2 to cells and tissues, it should be converted to other less harmful substances such as gaseous oxygen and water. Catalase production is seen in all those cell organelles where H_2O_2 production is high quantities, such as cytosol, mitochondria, peroxisomes and chloroplast. Catalase is also an isozyme which shows the versatile role of the enzyme. Different catalase isoenzymes such as CAT-1, CAT-2 and CAT-3. Catalase deficiency can cause the organisms to develop many anomalies such as chlorosis, head sterility and sensitivity to photorespiration in plants and can cause oxidative stress and H_2O_2 toxicity etc. in humans.

Peroxidases (PRX) also called as peroxide reductases, as the name suggests is a big group of enzymes that commonly breakdown peroxides. Peroxidases perform redox reaction (oxidation-reduction reactions) with the help of free radicals. Peroxidase are produced in both plants and fungi, but the fungi peroxidase is considered more efficient. The class II heme-comprising peroxidases, belonging to the peroxidase-catalase superfamily, are enzyme which have the property to breakdown lignin also called as delignification by using H_2O_2 as the substrate, this activity involves donating electrons to ascorbic acid (Vitamin C) and ferricyanides to break them down to harmless components. Both peroxidase (PRX) and catalase (CAT) are heme containing enzymes that catalyze hydrogen peroxide. Both react or work in a similar mechanism. A large number of peroxidase have been identified in fungal species and so for our study we have chosen fungal organisms (*Aspergillus* sp. and *Cladosporium* sp.) for the spectrophotometrically analyse their Catalase and Peroxidase production potential.

In the present study, two common genera of soil fungi namely, *Aspergillus* Sp. and *Cladosporium* sp. are cultivated by submerged cultivation for 5 days and pellets are separated by filtration. Pellets are disrupted by detergent method (0.5 % Triton X 100, 1 % CTAB, 1.5 % Tween 80, 5 % SDS). The pellets of both the fungal species were suspended in detergent solution for 40 mins under shaker incubation at 30 °C. The disrupted pellets were centrifuged (REMI™ KBM 80 plus) at 12,000 rpm for 15 mins at 4 °C. Culture media and cellular extracts are analysed for intracellular and extracellular total proteins and their panel of antioxidative enzymes. Catalase and Peroxidase activity are determined by simple spectrophotometric method.

The catalase test was performed by simple UV spectrophotometric method using 20 mM hydrogen peroxide. The reaction mixture contained 30 µl of crude cellular extract and 2970 µl of 20 mM hydrogen peroxide prepared in pH 7.4 sodium



phosphate buffer. The absorbance was read at 240 nm at 20 seconds time interval for 3 minutes. Total CAT activity was determined spectrophotometrically by following the decline in absorbance with H_2O_2 ($E = 36 \text{ M}^{-1} \text{ cm}^{-1}$) catabolized, according to the method of Beers and Sizer (1952) at 240 nm.

Catalase activity

Table – 1: Estimation of extracellular Catalase activity in *Aspergillus* sp. and *Cladosporium* sp.

Sl. No.	Sample	Catalase activity (U/mg protein)	Time interval (20 S/3 min)
1	<i>Aspergillus</i> sp.	0.115	20 sec
2	<i>Cladosporium</i> sp.	0.146	20 sec

Catalase being an intracellular enzyme, the cellular extract was analysed for catalase activity. *Aspergillus* sp. and *Cladosporium* sp. have shown considerably good amount of catalase activity in the crude extract. Results indicated, *Cladosporium* sp. could produce slightly higher amounts of Catalase compared to *Aspergillus* sp. against H_2O_2 toxicity.

Peroxidase activity

Extracellular (Mn Peroxidase) activity was monitored with phenol red at room temperature. Reaction mixture was prepared using 25 mM Lactate, 0.1 mM Manganese sulphate (MnSO_4), 1 mg Bovine Serum Albumin (BSA), 0.1 mg Phenol red, d 0.5 ml of culture filtrate in 20 mM sodium succinate buffer (pH 4.5). The reaction was started by the addition of 0.1 ml of 0.1 mM H_2O_2 to the reaction mixture. The absorbance was read at 610 nm at time intervals of 1 minutes up to 3 mins. The reaction was stopped by adding 50 μl of 10 % NaOH. Extracellular Mn peroxidase was analysed Spectrophotometrically at 240 nm. Peroxidase activity was determined using the below formula: Enzyme activity (Units/L) = $(\Delta\text{Abs} \times \text{Total assay volume}) / (\Delta t \times \epsilon \times 1 \times \text{Enzyme sample volume})$. Where, ϵ (extinction coefficient) for peroxidase is $26.6 \text{ mM}^{-1} \text{ Cm}^{-1}$.



Table – 2: Estimation of extracellular Peroxidase activity in *Aspergillus* sp. and *Cladosporium* sp.

S1. No	Sample	Extracellular Peroxidase activity (Units/L)	Time of exposure	Total time
1	<i>Aspergillus</i> sp.	0.00097	1 minutes	3 minutes
2	<i>Cladosporium</i> sp.	0.00150	1 minutes	3 minutes

Results have indicated that, both the test organisms produce extracellular peroxidase isoenzyme. Extracellular peroxidase activity is higher in *Cladosporium* sp. compared to *Aspergillus* sp. The enzyme activity is considerably low in the test fungal species. However, with modified culture media and altered physical factors peroxidase synthesis can be enhanced.



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MICROBIAL ENDOPHYTES AS A NOVEL SOURCE FOR TREATING UROLITHIASIS

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Urolithiasis, one of the major lifestyle disease caused due to deposition of Calcium Oxalate in Urinary tract, may results in Kidney failure. The present study illustrates the application of various endophytic micro-organisms including Fungi, Bacteria and Actinomycetes for the treatment of Urolithiasis. It is based on the fact that endophytic micro-organisms may have the ability to produce Oxalate Oxidase enzyme associated with the degradation of Kidney stone. Hence, microbial endophytes might be utilized as a novel therapeutic agent to treat Urolithiasis.

Globalization with modernization has given rise to fast changing life style. As a result of which people are suffering from many lifestyle disorders such as Cancer, Diabetes, Heart diseases, Kidney Disorders. Urolithiasis is one of the major and common health problems affecting a large population all over the world. Urolithiasis is characterized by complex pathophysiology, high recurrence rate as well as multifactorial etiology. Among the different types, Calcium Oxalate (CaOx) based kidney stones are predominant and near about 85 % of the kidney stones are formed due to deposition of Calcium Oxalate compounds. Use of plants as an antiurolithiatic agent have wide acceptance. But in recent years a new trend in treatment is introduced, as it is discovered that many vital activities of the host plant



are known to be influenced by the presence of microorganisms. Compounds isolated from plants are largely the products of plant metabolism. However, microorganisms living in symbiosis with plants also produce bioactive compound. These are referred as Endophytes.

Endophytes are mainly classified into three types i.e Bacterial Endophytes, Fungal Endophytes and Actinomycetal endophytes. These endophytes are found to possess bioactive potential. According to recent studies the bioactive compounds produced by endophytes includes oxalate oxidase, one of the enzyme needed for the degradation of urine stone. Oxalate oxidase is produced by different species of Bacteria, Fungi, Mosses and Higher plants which can be used for the degradation of Calcium Oxalate, but their endophytic nature is not discussed. Hence, more research is needed regarding the same. While many studies suggests that Bacterial endophytes produce Oxalate Oxidase *viz.*, *Ochrobactrum intermedium*, *Pseudomonas*, *Lactobacillus*, *Oxalobacter*. Hence, Considering the above reviews microbial Endophytes could be utilized as a novel source of antilithiatic compound for treatment of Urolithiasis.



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PLANT MICROBIOMES

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Microbes are essential for the maintenance of life on earth. Microbes are vital in the cycling of nutrients in the soil and play an important role in making nutrients available to plants. It is also essential for compost and fertilizer production in agriculture. Thus, microorganisms are necessary for plant growth and development. Microbiome refers to the community of microorganisms that inhabit a particular area. The plant-soil microbiome refers to the dynamic community of microorganisms that are found in association with plants and the soil. This community comprises of the bacteria, archaea and the fungi which has both positive and adverse effects on plant growth. Their important roles include nutrient uptake, protection from pathogens and regulation of host immunity. Like the plant microbiome, soil microbiota is also important for the proper establishment of plants in soil, nutrient cycling, and nutrient mobilization. The rhizosphere is a region of rich microbial diversity influenced by deposition of plant mucilage and root exudates. Microbes that can exist on the surface plant tissues are termed epiphytes and endophytes are those microbial genomes located inside plant tissues. Thus, enabling them to exist as facultative epiphytes and endophytes. The microbial component of the plant holobiont, also called the plant microbiota which comprises all microorganisms in the rhizosphere (root zone), Phyllosphere (near or on plant tissues) and Endosphere (within the plant tissues) has important functions of supporting plant growth and health. Microbes in these niches can establish constructive, neutral or detrimental associations of varying degree with their host plants.



The Plant microbiome is a key determinant of plant health and its productivity. Plants live in association with diverse microbial consortia. These microbes are referred to as the plant's microbiota, live both inside (Endosphere) and outside (Episphere) the plant tissues, and play vital roles in the ecology and physiology of plants. However, in the plant microbiota, the microbial diversity varies with varied forms of agricultural practices. Such practices impact the plant microbiome composition and functions with positive and negative consequences on the growth of plants. Cropping systems, like intercropping and no-tilling farming, have increased the microbial community's diversity, crop yield, and organic carbon levels in the soil. Whereas, monoculture or short rotations of crops more rapidly deplete soil nutrients and increase the population of plant species-specific soil pathogens and root herbivores, thereby resulting in poor crop yield.

Core and Satellite Plant Microbiomes

Some microorganisms are found to be closely associated with certain plant species independent of soil and environmental conditions are defined as the core plant microbiome. For example, a core microbiome of potato (*Solanum tuberosum*) comprises of *Bradyrhizobium*, *Sphingobium*, and *Microvirga*. Similarly, a core microbiome of rice particularly comprising of Deltaproteobacteria, Alphaproteobacteria, and Actinobacteria was found. The core plant microbiome is important for plant fitness and enrichment of microbial taxa containing essential functions genes for the fitness of the plant halobiont. Some microbes that occur in low abundance in a reduced number of sites are called satellite plant microbiomes. The importance of satellite taxa is increasingly being recognized as drivers of key functions for the ecosystem. A study demonstrated that taxa occurring in low abundance are critical for reducing unwanted microbial invasions into soil communities. Similarly, low abundance bacterial species largely contributed to the production of antifungal volatile compounds that protect the plant against soil-borne pathogens.

Factors affecting Plant Microbiome

The microbial composition in and around the plant organ is influenced by a variety of biotic and abiotic factors. Factors such as pH, salinity, soil type, structure, moisture, organic matter, etc. and external environmental conditions along with the human practices influence the microbiota of the rhizosphere along with the host-related factors such as the age of the plant, its development stage, pathogen presence and the composition of root exudates determine the presence of microbial community.



Functions on Plant Microbiota

The plant microbiome comprises of beneficial, neutral or pathogenic microorganisms. Plant growth-promoting bacteria (PGPB) can promote plant growth by either producing phytohormones like auxin, cytokinin and gibberellin to promote plant growth directly or strains of *Pseudomonas* spp., *Arthrobacter* spp. and *Bacillus* spp. secrete enzymes such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase that helps to reduce the level of ethylene, a stress hormone thereby promoting stress tolerance and plant growth. The functional importance of the plant microbiome includes mobilization / acquisition of nutrients, out-competition and antagonism of pathogens, plant stress alleviation, plant growth promotion, co-metabolism and degradation of toxins present in and around the plant. The severity of plant disease depends on multiple factors like pathogen population size, host susceptibility, environmental condition and biotic factors like the plant microbiota that determine the outcome of plant-pathogen interaction. The continuous use of agricultural soils can build pathogen pressure and can also develop disease-suppressive soils containing microorganisms mediating disease suppression. Hence, the manipulation of the plant microbiome has the potential to reduce the risk of pests and diseases in the plant community.



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METHODS OF ISOLATION AND ADVANTAGES OF CELLULOLYTIC SOIL MICROBES

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Cellulose is one of the most abundant organic molecules in environment. The structure of the cellulose differentiates from the other molecules by its unique linkages. Cellulase is a proteinaceous enzyme which has an ability to degrade complex cellulose to simple sugars. Cellulase are an inducible bioactive molecule which can be produced by the microorganisms upon supplementation with cellulose material. Both bacteria and fungi could produce cellulase enzyme. The growth rate of bacteria (doubling time of bacteria is approximately 20 minutes) is faster than the fungal growth. But the maintenance of the bacterial culture is far most difficult than the fungal culture. Therefore, there is a need to properly find the source for a particular experiment. Some of the bacterial species such as *Cellulomonas* species, *Pseudomonas* species, *Micrococcus* and *Bacillus* species have a cellulolytic property. Though many microbes have cellulolytic activity only few microbes have met an industrial utility. In general, there are 3 types of cellulase which is involved in degradation of cellulose such as

- ***Endoglucanases (Endo-beta-1,4-glucanases)*** - Cuts at random internal amorphous sites in cellulose polysaccharide molecule generating an oligosaccharide of various lengths. which are also called as Carboxymethyl cellulases. These



enzymes are Glycoproteins with an approximate molecular weight of 5.3 k - 14.5 k daltons.

- **Exoglucanase (Exo-beta-1,4-glucanase)** - Cuts on non-reducing and reducing sugar ends of cellulose polysaccharide chain liberating either cellobiose (cellobiohydrolase) or glucose (glucanohydrolases) as main products.
- **Cellobiases (Beta-glucosidase)** - Hydrolyse soluble cellobiose and cellodextrins to simple glucose molecule.

As the cellulolytic soil microbe is present in very minimal count in any soil sample, composting the soil with the cellulose rich organic matter could increase the cellulolytic soil microbial content. To compost the soil, we should make sure to include equal amount of green and brown leaves, soil with cellulose rich components (could be an artificially synthesized cellulose powder or any easily available cellulose rich agricultural wastes such as fruit peels like orange peels etc).

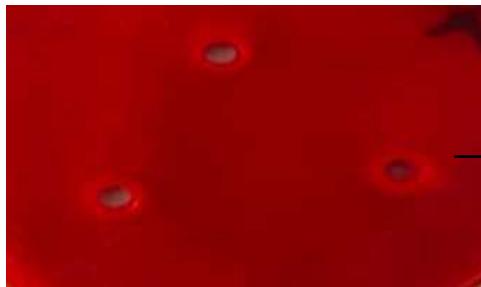
Depending upon the source and availability of cellulose molecule the duration of composting varies. With good ventilation, sunlight and moisture maximum of 3-5 months can give a good yield of microbes.

Isolating microbes from soil is one of the most cost-effective way to isolate cellulolytic soil microbes. Upon serial dilution of the soil sample (expected to contain cellulolytic soil microbes with other microbes) is then spread plated on the agar plate. The agar used in this process are of 2 types- CMC Agar (carboxymethylcellulose) agar (composed of carboxymethylcellulose, MgSO₄, KCl, NH₄H₂PO₄, Agar, Yeast extract), Cellulose Agar(composed of cellulose, Potassium phosphate, ammonium sulfate, Calcium chloride, L-asparagine, Yeast extract, Magnesium extract, Agar).Though both have different composition both have the same carbon source that is cellulose which allows only cellulolytic soil microbes to survive whereas other microbes which don't have cellulase enzyme die due to lack of energy. After incubation at 37° C for 24 hours the plates are visualized by flooding the plate with 0.1% aqueous solution of Congo red for 15minutes and washed with 1M NaCl.

Screening of desired cellulolytic bacteria-the colonies would be taken and the parameters was investigated for colony morphology, gram staining, catalase production, VP reaction, indole production, starch hydrolysis and citrate utilization. The results were compared with the Bergey's manual of determinative bacteria. To estimate the cellulolytic activity of the organism- The cellulase activity of each culture was measured as the amount of reducing sugars liberated was determined by using a DNS (dinitro salicylic acid) method. The highest activity having bacteria was isolated and selected. Cellulase activity can be measured by



- CMC Method where the enzyme extract reacts with CMC broth with a particular buffer to give glucose. DNS method is used in the detection of the glucose.
- Filter Paper Assay - Whatman filter paper strip used as carbon substrate, Beta-Glucosidase Assay where p-nitrophenyl-beta-D-glucopyranoside (PNPG)used as carbon source.
- Zone of Hydrolysis Test by Agar Well Diffusion Method.



Zone of hydrolysis

Figure – 1: Extracted enzyme in the well has the capability to hydrolyze cellulose in the agar

Advantages of cellulolytic soil microbes-recently the research is more on using cheap unwanted substrates and soil microbes in producing more economical products such as

- Bioethanol-where we can use cellulolytic soil microbes to act on agro waste to make biofuel which is nothing but second generation of biofuel production.
- Bioplastic production using cellulolytic soil microbes.
- Landfill clearance - Many agro waste specifically cellulose rich wastes like orange peel from the juice factory which creates a landfill as they can't be degraded easily. Therefore, could be utilized to make economically valuable product.



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BIOTERRORISM

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Bioterrorism is the use of infectious agents or other harmful biological or biochemical substances as weapons of terrorisms. A biological attack, or bioterrorism, is the intentional release of viruses, bacteria, or other germs that can sicken or kill people, livestock, or crops. The five most common examples of bioterrorism attacks or the diseases that shaped human history include:

- Anthrax (*Bacillus anthracis*).
- Smallpox (*Variola major*).
- Plague (*Yersinia pestis*).
- Q fever (*Coxiella burnetii*).
- Ebolavirus.
- Coronavirus.

Anthrax disease

Bacillus anthracis, the bacteria that causes Anthrax, is one of the most likely agents to be used in a biological attack. Anthrax is a serious infectious disease caused by gram-positive, rod-shaped bacteria known as *Bacillus anthracis*. Anthrax can be found naturally in soil and commonly affects domestic and wild animals around the world. Although it is rare in the United States, people can get sick with anthrax if they come in contact with infected animals or contaminated animal products. Anthrax can cause severe illness in both humans and animals. Antibiotics are usually used to treat anthrax. Antibiotics that may be prescribed include penicillin, doxycycline, and ciprofloxacin. Inhalation anthrax is treated with a combination of



antibiotics such as ciprofloxacin plus another medicine. They are given by IV (Intravenously).

Small Pox

The origin of Small pox is unknown. The finding of smallpox-like rashes on Egyptian mummies suggests that smallpox has existed for at least 3,000 years. Smallpox is caused by infection with the variola virus. The virus can be transmitted: Directly from person to person. There is no cure for smallpox, but vaccination can be used very effectively to prevent infection from developing if given during a period of up to four days after a person has been exposed to the virus. This is the strategy that was used to eradicate the disease during the 20th century.

Plague

Plague is a disease that affects humans and other mammals. It is caused by the bacterium, *Yersinia pestis*. Humans usually get plague after being bitten by a rodent flea that is carrying the plague bacterium or by handling an animal infected with plague. Bubonic plague: Patients develop sudden onset of fever, headache, chills, and weakness and one or more swollen, tender and painful lymph nodes (called buboes). This form usually results from the bite of an infected flea. The bacteria multiply in the lymph node closest to where the bacteria entered the human body. Unlike Europe's disastrous bubonic plague epidemic, the plague is now curable in most cases. It can successfully be treated with antibiotics, and according to the CDC, treatment has lowered mortality rates to approximately 11 percent.

Q fever

Q fever is a disease caused by the bacteria *Coxiella burnetii*. This bacterium naturally infects some animals, such as goats, sheep, and cattle. *C. burnetii* bacteria are found in the birth products (i.e., placenta, amniotic fluid), urine, faeces, and milk of infected animals. People get infected by breathing in dust that has been contaminated by infected animal faeces, urine, milk, and birth products that contain *Coxiella burnetii*. Direct contact (e.g., touching, being licked) with an animal is not required to become sick with Q fever. Chronic Q fever is serious and can be deadly if not treated correctly. Chronic Q fever infection requires months of antibiotic treatment. Chronic Q fever is more likely to occur in people with heart valve disease, blood vessel abnormalities, or in people with weakened immune systems.



Ebola Virus Disease (EVD)

Ebola virus disease is a rare and deadly disease in people and nonhuman primates. The viruses that cause EVD are located mainly in Sub-Saharan Africa. People can get EVD through direct contact with an infected animal (bat or nonhuman primate) or a sick or dead person infected with Ebola virus.

Ebola virus was first discovered in 1976 near the Ebola River in what is now the Democratic Republic of Congo. Since then, the virus has been infecting people from time to time, leading to outbreaks in several African countries. Recovery from EVD depends on good supportive care and the patient's immune response. Investigational treatments are also increasing overall survival. Those who do recover develop antibodies that can last 10 years, possibly longer.

Coronaviruses (CoV)

Coronaviruses are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). A Novel Coronavirus (nCoV) is a new strain that has not been previously identified in humans. On 31st December 2019, WHO was informed of cases of Pneumonia of unknown cause in Wuhan city, China. A Novel Coronavirus was identified as the cause by Chinese authorities on 7th January 2020 and was temporarily named “2019-nCoV”. Most Common Symptoms: Fever, Dry cough and Tiredness. Vaccines invented for the disease are Covaxin Vaccine, Covishield Vaccine, Moderna Vaccine and Sputnik V Vaccine.

Though biological weapons are unique in their invisibility and their delayed effects. We can assure following certain steps to prevent ourselves becoming affected by any disease by following simple procedures like wearing a mask to reduce inhaling or spreading germs be it any microorganisms, washing frequently with soap and water and wearing clean clothes.



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BIOLOGICAL ACTIVITIES AND SOURCES OF HEPTACOSANE

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Heptacosane is a higher n-alkane containing 27 carbon atoms. Being a hydrophobic molecule, it is practically insoluble in water. This is an aliphatic hydrocarbon lipid molecule that plays a significant role in chemical signalling molecular transport. Moreover, this is a plant metabolite produced in flowering plants, conifers, and other gymnosperms.

Biological activities of Heptacosane

- It exhibits pheromonal activity in many insects thus allowing organisms to identify mating partners of the opposite gender.
- The attraction of insects can be elicited by mimicking the synthetic mixtures of alkanes compounds may provide clues to sustainable pest management programs such as baited traps.
- This has important economic consequences including production outcomes, environmental and societal effects.
- Besides, Heptacosane is also produced by *Fusarium* species when grown in the presence of elicitors. Few *Streptomyces* species also produce Heptacosane as a Secondary metabolite which is confirmed by the GC-MS technique.



- This compound possesses good antibacterial activity especially in gram-positive organisms but has no antifungal activity. This is a good antioxidant agent too.
- In the urgent need for new antibiotics, Heptacosane would be a promising drug against the challenging Gram positive bacterial infections.

Molecular Formula ($C_{27}H_{56}$)



Figure - 1: Chemical structure depiction of Heptacosane

Few Sources of Heptacosane

- Heptacosane is the major component of the contact sex pheromone of an insect *Neoclytus acuminatus* (commonly known as Red-headed ash borer).
- *Labellum* extracts of an Orchid flower - *Ophrys sphegodes* Miller.
- Surface waxes and leaves of Spiny bitter guard - *Momordica cochinchinensis*.
- Waxy skin of apples - *Malus domestica*.
- Roots of pointed guard - *Trichosanthes dioica*.
- Essential oil of soul vine - *Banisteriopsis* sp.
- Leaves of silver buffalo berry - *Shepherdia argentea*



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PLANT METABOLITES: A PROMISING SOURCE AGAINST COVID-19

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In the modern epoch, the majority of public health problems are the account of viral infections globally. As per the report of WHO, contagious diseases hit above 17 million people each year. The unforeseen explosion of the deadly COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has damaged the worldwide healthcare, social, and economic system. COVID-19 was initially spotted in the Chinese city Wuhan in December 2019. Since there, it has covered the globe nearly. In the present situation, COVID-19 has evolved as the biggest health hazard globally to *Homo sapiens*. In spite of the accessibility of antiviral drugs and vaccines for such viruses, there are various drawbacks in the medications involved, which hold several side effects and active mutation causing drug resistance. By seeing the present worldwide situation, scientists and researchers have discovered medicinal plants and their metabolites as a potent source against COVID-19. For instance, scutellarein is a natural flavonoid obtained from the roots of the plant *Scutellaria baicalensis* (Lamiaceae) is found to be effective against SARS-CoV-2 since this flavonoid is able to inhibit the nsP13 helicase of SARS-CoV-2 via changing its ATPase activity. The pharmacological investigation of two more flavonoids baicalin and baicalein isolated from the roots of *Scutellaria baicalensis* has reported their potency towards the COVID-19. However, the accurate medicinal impresses of this plant are even now required to be discovered in clinical trials. Another flavonoid named Quercetin from leek (*Allium porrum*) has proved its remarkable capacity by inhibiting Human ACE2 (angiotensin-converting enzyme II) enzymes while in-silico



study for SARS-CoV-2, hence could possess the potency to inhibit the link of the SARS-CoV-2 to the cells. In a molecular docking study for the protease domain, receptor-binding domain of spike glycoprotein and the receptor-binding domain of the ACE2 (angiotensin-converting enzyme II), hesperidin, a flavone glycoside richly present in several citrus plants revealed its significant binding affinity toward the COVID-19 virus.

Some other plant flavonoids such as curcumin from *Curcuma longa*, brazilin from *Caesalpinia sappan* and galangin from *Alpinia galanga* too displayed their activity against COVID-19. Phyto-terpenoids constitute a broad spectrum of secondary metabolites that include a wide range of structures, results and properties like antiviral. A triterpenoid saponin glycyrrhetic acid also known as glycyrrhizin obtained from the roots of *Glycyrrhiza glabra* is a vital compound by inhibiting the replication of clinical isolates of the SARS-associated virus. Moreover, this Phyto-compound prevents the absorption and penetration of the virus in early-stage. *Cannabis sativa* is a central Asian plant which possess a Phytocannabinoid and Cannabidiol (CBD). Since, the compound is a modulator of Angiotensin-converting enzyme II (ACE2) expression in COVID-19 target tissues, by turning down ACE2 which is connected to the Receptor-mediated entry into human lung epithelial cells may give the right approach for minimizing COVID-19 severity. One more class of bioactive compounds that holds potential activity towards coronaviruses are alkaloids. Such as homoharringtonine isolated from the plant *Cephalotaxus fortunei*, not only possess anticancer activity but is also found to be effective against SARS-CoV-2 by preventing the replication of the virus. In one more study, it is reported that phthalideisoquinoline alkaloids such as noscapine isolated from the species of Papaveraceae family for example *Papaver somniferum*, apart from having antitussive and nonnarcotic effects, also hold strong and stable interaction with the Mpro enzyme of SARS-CoV-2, hence, they may be propitious possibility against COVID-19. Medicinal plants essential oils are generally used in elective medicine and its medication to cure various health illnesses. In some research studies, essential oils derived from plants are reported to have antiviral bioactivities against SARS-CoV-2. As proof, in a molecular docking study eucalyptol an essential oil obtained from *Eucalyptus* spp. have shown its potential against the virus. Medicinal plants and their bioactive compounds are the pool of unique antiviral drugs. In the present scenario, COVID-19 is one of the greatest public health threats that demand to be controlled quickly. Hardly any nation is left which is untouched by the pandemic. Acknowledging this fact, the plant bioactive compounds that have antiviral properties must be investigated in such a way that could discover new possibilities.



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Pseudomonas aeruginosa – AN IRRESISTIBLE CLINICAL PATHOGEN

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In 1882, Carle Gessard discovered a ubiquitous free-living bacterium *Pseudomonas aeruginosa* through an experiment. Later in 1894, Migula named these bacteria for the resemblance of the cell 'Monas' in both size and active motility (Figure - 1).

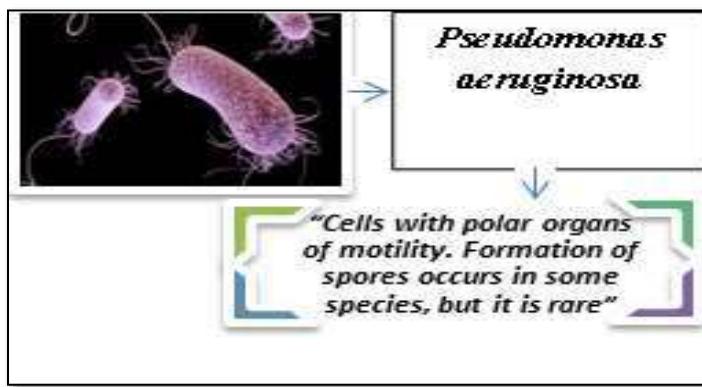


Figure - 1: Microscopic appearance and literal explanation of *Pseudomonas aeruginosa*



They are gram-negative, obligate aerobes possessing a low degree of pathogenicity and invasiveness. Being a clinically significant opportunistic pathogen, it often targets the host with a weakened immune system. It causes a variety of both complicated and uncomplicated infections depicted in fig 2.

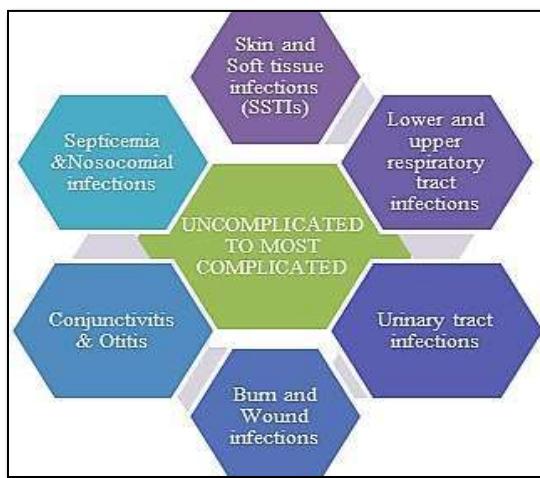


Figure - 2: Types of infections caused by *Pseudomonas aeruginosa*

What makes *Pseudomonas* an Irresistible one?

The high frequency in the emergence of antibiotic resistance to most of the available antibiotics makes these bacteria an irresistible clinical pathogen. Although they don't have intrinsic resistance to the drugs, still they develop drug resistance during the administration of antibiotics. The few contributing factors behind are given below.

- a) Multi-faceted bacteria which could survive in multifarious hosts.
- b) Emergence of integrins.
- c) Exclusion of antibiotics from the cell due to efflux by MexAB - OprM (Impermeability mediated resistance).
- d) Accumulation of resistance after exposure to various antibiotics.
- e) Limitations of therapeutic options leading to increased mortality and morbidity.
- f) Mechanisms of resistance carried by the plasmids

It is very difficult to treat because of the limited susceptibility to currently available antimicrobial agents like carbapenem and third- generation cephalosporins. Yet, *Pseudomonas* serves as a challenge to the field of medicine in its virulence, pathogenicity and resistance to antibiotics, since the time of discovery.



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COLoSTRUM - "THE LIQUID GOLD"

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Colostrum is the nature's gift to the immune system. The colostrum is the pre-milk fluid produced by female mammals in the mammary glands just before they give birth. While it is technically not milk at all, colostrum is often called "first milk" as it is obtained in the first milking after birth. Birth is the triggering event that ceases colostrum production in the mother and signals the body for the milk to come in or for the mammal "to freshen". Colostrum, the first milk produce when starting breast feeding, is the ideal nourishment for a new born. It's a highly concentrated, full of protein and nutrient dense-so a little goes a long way in baby's tiny tummy. It's also low in fat, easy to digest, and brimming with components that start baby's development in the best possible way. And perhaps even more importantly, it plays a crucial role in building the immune system. Colostrum looks thicker and more yellow than mature milk. Its composition is different too, because it's tailored to new born specific needs. After the first milking, the fluid beings to change into the milk, containing less colostrum and more milk as time passes. This transitional period lasts for 2 - 3 days. This fluid is referred to as transitional milk. In all other mammals other than humans, colostrum is crucial to the survival of newborn. This is because of the high concentration of immuno factors that are transferred through the colostrum. In humans only, some immuno factors are transferred through the placenta. The colostrum is still very important, but if the newborn baby does not receive the colostrum, death is eminent, as it is in all other mammals. Colostrum and lactoferrin supplements can be used on a diary basis to enhance the immune system as a preventative against illness. Colostrum might look clear, but it's often a golden-



yellow or light orange colour because it contains high levels of beta-carotene. Colostrum also tends to be thicker than transitional and mature breast milk. Occasionally, blood from inside the milk ducts can make its way into the colostrum. Colostrum mixed with blood can look red, pink, brown, or rust coloured. While potentially alarming to see, a bit of blood in the colostrum is not harmful or cause for concern. Colostrum production can start as early as the beginning of the second trimester of pregnancy. Colostrum may only come in small amount, but it is packed full of concentrated nutrition. Hence, it is referred as "Liquid Gold" because it contains everything that the body needs in the first few days of life. The colostrum is made up of components that protect the newborn and help them fight off infection, illness and diseases. Colostrum is higher in protein and lower in fat and sugar, compared to transitional and mature breast milk, making it easier to digest and nutrients packed. Colostrum is full of antibodies, white blood cells, and other immune properties, it functions like child's first immunization. The high levels of Secretory Immunoglobulin A (SIgA) found in colostrum protect your baby's gastro intestinal tract and help to kill off viruses and bacteria. Colostrum is a natural laxative. It helps the infant move their bowels and get rid of meconium - the tar-like poop that collects in the bowels before baby is born. Since meconium contains bilirubin, the laxative effect of colostrum helps to prevent new born jaundice. Carotenoids and Vitamin A is important for baby's vision (Vitamin A deficiency is a major cause of blindness worldwide), as well as keeping skin and immune system healthy. Babies are usually born with low reserves of vitamin A, so colostrum helps make up the deficit. Colostrum is rich in minerals too, such as magnesium, which supports the baby's heart and bones; and copper and zinc which help develop the immune system. Zinc also aids brain development, and there's nearly four times more zinc in colostrum than in mature milk to support the newborn brain development. Colostrum has a similar make up of amniotic fluid (which baby has been swallowing and excreting in womb) hence ideal easing for baby's transition to the outside world. Studies report colostrum helps manage infants with chronic diarrhea. In eight children's with diarrhea, ranging from nine months to three yrs of age, *E.coli* was present in all eight cases, *Ascaris lumbricoides* in four and *Giardia lamblia* in one. All eight children were given 20 ml of fresh human colostrum daily for seven days. In addition those who had giardiasis received metronidazole treatment, while cases with ascariasis were given antihelminthic therapy. The result indicates that colostrum provided effective antidiarrheal action in some patients with chronic diarrhea of infective origin. Studies show the activity of human colostrum in stimulating DNA synthesis was 20 times greater than that of bovine serum. Only human colostrum contains two different growth factors: CAGF, an epidermal growth factor, and CBGF, a platelet differentiation growth factor. Colostrum is life's perfect first food for enhanced



immunity and healthy longevity. Hundreds of scientific studies show the amazing health benefits of supplemental colostrum for virtually all aspects of health. The numerous immune enhancing component in colostrum have been proven to help fight off allergies, diarrhea, asthma, cancer, lupus, diabetes, fibromyalgia, heart diseases, chronic fatigue, crohns and colitis, peridontal diseases, multiple sclerosis, intestinal disorders etc. The powerful growth factors in colostrum have been shown to build muscle and lose fat, improve athletic performance, increase energy and vitality, reduce wrinkles, improve skin elasticity, quicken wound healing. It seems unimaginable now, but in just one year the baby could be walking and on the verge of talking. Colostrum may produce for short time, but it makes an invaluable contribution to those first 12 months, and to rest of life. Hence “COLOSTRUM – THE ORIGINAL LIQUID GOLD OF LIFE”



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AMBIGUOUS NATURE OF VIRUSES: TWO SIDES OF A COIN

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Viruses are a type of microorganisms that are incapable of self-replication. After invading a host cell (either animal or plant), viruses use host's DNA or RNA to create numerous copies. They are capable of causing wide range of diseases that affect the host body, have fatal effects and higher mortality rate. In many years history these are evident but this article is against that opinion and highlighted the unseen good side.

The kind that makes someone Sick

One of the most relevant at the moment is Corona Virus. Globally, it has claimed the lives of a large number of people. A person-to-person transmission occurs when we breathe it in and out; and after entering the body, it attacks and destroys the lung cells of our bodies. These in turn cause long-term damage to a person's heart, lungs and brain as well as respiratory problems. The causative agent of AIDS is HIV, also known as the human immunodeficiency virus. Once an individual is infected with the HIV virus, their immune system gradually deteriorates and become immunocompromised, which allows other opportunistic infections. Though symptoms are related to that of the common flu or fever appear early but the consequences are severe. It is to take note that once someone is infected with HIV,



he/she will never be able to fully recover as there is no absolute cure available. Another very common virus is the influenza virus, a respiratory virus. It is extremely dangerous for children, the elderly, pregnant women, and those who are immunocompromised. Chills, muscle pain, headaches, exhaustion, cough, congestion, runny nose and fatigue are the general symptoms of an infected person.

The Good Sides

There are numerous other types of viruses that can harm us, or so do we think. But if we think from an evolutionary perspective, we can see every living thing attempts to survive, so is the virus. Along with all the negative impacts of the viruses, there are some positive aspects as well.

Viruses such as retroviruses, adenoviruses, adeno-associated viruses (AAV), and herpes simplex viruses have been modified in the lab and used as a vector for gene therapy. In the laboratory, viruses can be modified to act as vectors for delivering corrected, therapeutic DNA into cells, where it can be integrated into the genome to correct genetic disease and alter abnormal gene expression. The virus then infects the human. Following that, the virus inserts that gene into the host cell's genes. Then there's a homologous pair recombination in which the person's abnormal gene is replaced with the one that was inserted into the virus. As a result, the virus can be easily used to treat a person's genetic disease via gene therapy.

Although the corona virus pandemic is taking the global attention right now. But antibiotic resistance is another upcoming threat and challenge for science that is yet to answer. The abuse of excessive and unregulated use of antibiotics has made many bacteria resistant, as a result antibiotic resistance. For this global concern bacteriophage therapy may be used as an alternative to antibiotics. Bacteriophages are viruses that kills bacteria while causing no harm to humans. So, this is how the bacteriophage virus can be used to treat bacterial diseases in humans in place of antibiotics.

In addition, an attenuated viral vaccine can be produced as a preventive measure for viral diseases. As viruses are weakened, they have a difficult time reproducing once inside the body. By this method vaccines for measles, mumps, German measles (rubella), rotavirus, oral polio, chickenpox (varicella), and influenza are made. Viruses typically cause disease by replicating multiple times within the body. Vaccine viruses do not cause disease because they do not reproduce very much, but they do replicate well enough to induce "memory B cells" that protect against infection in the future.





From the above description we can deduce that the viruses have both negative and positive aspects, like two sides of a coin. Now it's up to us to decide whether the virus is truly bad or good.

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THALASSEMIA: THE FAULT IN OUR GENES

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Thalassemia is an inherited haemoglobin blood disorder that results from inadequate production of normal haemoglobin, leading to severe anaemia. Decreased production of alpha globin gene and beta globin gene leads to defective haemoglobin leading to alpha thalassemia and beta thalassemia, respectively. These two types of thalassemia are again found in two stages- Heterozygous, which called alpha and beta thalassemia minor and Homozygous, which called alpha and beta thalassemia major. Heterozygous or minor phase are usually asymptomatic but Homozygous or major phase produces acute haemoglobin decaying.

Genetic description

In normal case scenario chromosome 16 and chromosome 11 contains two alpha polypeptide-forming alpha globin genes and two beta polypeptide-forming beta globin genes respectively. In homozygous condition both the genes in homologous positions are lost, which is called major case and in the case of heterozygous condition only one gene of the two is lost, also known as minor case. Thus, giving rise to 4 categories- Alpha thalassemia major, Alpha thalassemia minor, Beta thalassemia major, Beta thalassemia minor.

Associated problems

The beta thalassemia trait has the ability to prevent malignant malaria. The main symptoms of thalassemia are anaemia, especially haemolytic anaemia. In



addition, as the amount of haemoglobin decrease, the size of red blood cells becomes smaller, the bones become weak and deformed and the liver spleen grows.

Available therapies used to treat Thalassemia

i) Blood transfusion

Individuals with thalassemia major and many with intermedia are treated primarily with blood transfusions. Transfusions have a dual purpose: to improve anaemia and to suppress ineffective erythropoiesis, respectively. Thalassemia major has serious growth, skeletal and neurological complications that are prevented by chronic transfusions. Transfusion-related complications become a major source of morbidity once they are initiated. Also, regular blood transfusion overload iron in the body, and has a detrimental effect on the heart and liver. For this reason, iron chelation therapy is required for remove extra iron from body.

ii) Uses of Drug

Various pharmaceutical drugs are used to increase haemoglobin levels, among which hydroxyurea is most effective. It normalizes the function of different signalling pathways, consequently increases the level of haemoglobin and eliminates the need for frequent blood transfusions.

iii) Hematopoietic Stem Cell Transplantation (HSCT)

HSCT is a method of transplanting multipotent hematopoietic stem cells, that generally derived from umbilical cord blood, or peripheral blood and bone marrow. It can be autologous, allogeneic or syngeneic. This method is used to treat hemoglobinopathy which is an inherited blood disorder found in thalassemia patient. For normal haematopoiesis after conditioning to overcome the immunological barrier allogeneic stem cells are used as vectors to correct genetic defects with normal genes.

iv) Gene Therapy

Lentiglobin gene therapy is a method of treating thalassemia by overcoming the problem of death and any clonal dominance. In this case of beta thalassemia, blood stem cells are collected from the patient's body and edited *ex-vivo* to remove mutations in the BCL11A gene. There are some limitations in using gene therapy for thalassemia. The genes are inserted into the body through a vector, which is usually a virus. As a result, there is a possibility of tumor formation through viral poisoning. Another emerging treatment methods for thalassemia using CRISPR / CAS9 method. Homology-directed repair is used to correct the haemoglobin E mutation





using a single-stranded DNA template containing the normal HBB gene sequence. To ensure that the correct sequence was present in the extracted iPSCs, a Sanger sequence was used.

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BIOACTIVE BACTERIAL METABOLITES

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In recent times, microorganisms as a source of new biologically active substances have attracted much research attention due to the enormous diversity of living conditions of microorganisms and insufficient knowledge about them. Despite significant advances in medicine, diagnosis, and treatment of infectious diseases, pathogenic microorganisms continue to pose a serious threat to the world's human population. Their effects are significant in both developing countries. Due to their limited access to medicines and in developed countries where the uncontrolled administration of antibiotics has led to the widespread prevalence of multi-drug resistant bacteria. The strategy of creating new synthetic antibiotics by modifying existing natural antibiotics has not proven to be sufficiently effective, pathogenic microorganisms become resistant to new drugs after their first appearance. The global community, represented by the World Health Organization, is justifiably concerned about the future of mankind and is promoting the search for new antimicrobial agents that can become an alternative to modern antibiotics. Mankind has long learned to use the resources of the world's ocean, which covers more than 70 % of the earth's surface. Nevertheless, marine bacteria did not arouse the interest of researchers until the middle of the 20th century, although some studies on the biological activity of the metabolic products of these microorganisms were published as early as the end of the 19th century including the bottom sediments, it represents a huge reservoir of microbial biodiversity, comprising around 3.67×10^{30} microorganisms. The few studies of the last few decades have shown that the marine



ecosystem, with its unique diversity of habitats and rich biota, is an inexhaustible resource of naturally occurring biologically active chemicals. In the last decades numerous compounds with outstanding pharmaceutical effectiveness have been described from marine organisms, which can become sources for new therapeutics. In particular, antibacterial substances, which are secondary metabolites of marine bacteria, are attracting the researchers' attention. Due to its high antibacterial potential, these substances are the subject of extensive research in the marine microbiology and chemistry of marine natural substances, which are currently being intensively developed and, due to their unique properties, have become one of the focal points of modern marine biotechnology.

Overview of bacterial metabolites and their biotechnological potential, approximately 70% of the identified secondary metabolites of marine bacteria are represented by Non-Ribosomal Peptides (NRP) or a mixed family of polyketide-ribosomal peptides with varying biological activity. Molecules those are extremely diverse in their structures and functions, with a registered number of over 25,000, which is less than 2% of the total number of natural metabolites of microorganisms that are not yet available for research. Secondary metabolites are organic compounds with a complex chemical structure and a multitude of physiological functions that are required to implement the survival strategies of bacteria under adverse conditions and to act as mediators with the external environment and intercellular communication media. Secondary microbial metabolites such as antibiotics, pigments, growth hormones, anti-tumor agents and others are not essential for the growth and development of microorganisms, but have shown great potential for human and animal health. Among the microorganisms that produce the above compounds, bacteria, including actinobacteria and fungi, produce a variety of small bioactive molecules with significant potential for use in medicine. These bioactive compounds are mainly made by activating cryptic gene clusters which are not active under normal conditions and therefore expression of these clusters would be useful. Secondary metabolites that are isolated from microbes and that have antimicrobial (antibacterial, antifungal, antiprotozoal), antitumoral and antiviral activities, formerly known as antibiotics. Today its original definition should be extended to all those secondary (microbial) metabolites that regulate growth and replication processes and show some kind of response (regulatory, inhibitory, stimulatory) to the (life cycle of) prokaryotes or eukaryotic cells on a biochemical level, in minimal concentration. In addition to the so-called "classic antibiotics" (microbial compounds that have antimicrobial and antitumoral and antiviral activities), this broader definition should include practically all bioactive compounds that are obtained from microbes or others. This group of metabolites is an essential



component that supports the life processes of marine bacteria, fungi, archaea and others.

Microorganisms that are rich sources of these compounds. Among these complex biomolecules, substances with various biological properties have been found, including antibacterial, antifungal, antiviral and antiproliferative agents, exotoxins, metal carriers, hormones, immunomodulators, pigments and enzyme inhibitors. Have activity play an important role in the vital functions of bacteria and are widely used in pharmacology, cosmetics, the food industry and agriculture. However, some bacteria (such as *Clostridium botulinum*, *Vibrio cholerae*, *Escherichia coli*, and *Yersinia sp.*) Synthesize exotoxins, which are secondary metabolites and cause disease in humans. In particular, the secondary metabolites of marine bacteria have proven to be a rich source of natural substances with a new structure and excellent antibiotic activity; For example, among the actinomycetes, which are the primary source of biologically active natural products for clinical or pharmaceutical applications, more than 5000 antibiotics have been identified that are associated with secondary metabolites. Modern science regards secondary metabolites as a group of low molecular weight, structurally diverse and complex bioactive compounds. It has been found that the active synthesis phase of these molecules in microorganisms occurs at the end of the exponential phase and at the beginning of the stationary phases of their growth. Its production is induced by the depletion of nutrients and adverse habitat conditions.

The genes responsible for the biosynthesis of secondary metabolites are divided into a small number of groups. In contrast to the primary metabolites, the biosynthetic pathways used to produce these molecules are numerous and not yet fully understood. For biosynthesis, bacteria use multi-stage biosynthetic pathways in which specific enzymes or multi-enzyme complexes are involved, which are intermediate or end products of intracellular metabolism. Biosynthesis comprises cascade regulation, the mechanisms of which have been investigated at the transcription level. Among the most important biosynthetic pathways for secondary metabolites with antibacterial activity are the best characterized non-ribosomal pathways (with peptide synthetase as the key enzyme), β -lactam, polyketide (types I-III, with polyketide synthase as the key enzyme), ribosomal polyketides, oligosaccharide and Shikimate ways. Considerably increased interest in obtaining new antibiotics from secondary metabolites of bacteria is linked to the advances in biotechnology in recent decades and is based on the revealed mechanism of synthesis of the main classes of microbial metabolites by polyketide synthase and non-ribosomal peptide synthetase, which are widely used biosynthetic pathways by



marine microorganisms to produce antimicrobial substances. The total number of the microbial metabolites recognized until now, including both bioactive and inactive compounds, is around 50,000 and the number of all known natural products is around one million. Regarding the possible number of bioactive natural products do not forget that natural product chemists in the past rarely investigated the isolated compounds, especially not for a wide range of bioactivity. Also remember that in the past countless of compounds, including microbial metabolites formerly believed to be inactive, proved to be active in later investigations, or were rediscovered with screening of different stock of microbes or with specific screening methods. It is unpredictable how many “new” bioactive metabolites will be discovered in this way.



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METHODS OF MICROALGAE CULTIVATION

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Microalgae are unicellular photosynthetic, prokaryotic or eukaryotic organisms with tremendous potential as an energy crop. Microalgae are a promising feedstock for next-generation biofuel production due to the fact that they are probably 10–20 times more productive than any other biofuel crop and can be grown on a big scale without competing for arable land or precious biodiverse landscapes. Importantly, they are also able to grow in saline and even wastewater. Microalgal oil is a valuable commodity and used as a substitute for omega-3-rich fish oil. Microalgae accumulate large quantities of lipid bodies containing triacylglycerides during adverse conditions, such as under nutrient deprivation. Under these circumstances, microalgae stop dividing but are still able to perform photosynthesis and the accumulation of triacylglycerides is considered a survival approach to undergo adverse conditions. Cultivation of microalgae for biodiesel production, however, aims at maximizing lipid productivity which takes both growth and lipid content into attention. Several methods are available for microalgae cultivation and some of the important ones are:

- a) Open pond method
- b) Closed pond method
- c) Photobioreactors
- d) Hybrid method.



a) Open pond systems

Open pond systems are the most common system of algae cultivation. It consists of a series of closed loop channel around 30 cm deep and paddle wheel which enable recirculation of microalgae biomass to ensure equal distribution of nutrients and prevent sedimentation of microalgae biomass and the algae are exposed to natural solar radiation. Typically the ponds are called raceway ponds because their shape resembles a race track. Feeding the pond with nutrients is done in front of the paddle and harvesting is done behind the wheels. It has relatively cheaper construction, maintenance and operation cost but faces constraints like dependence on weather and contamination with undesirable species or organisms.

b) Closed pond systems

Closed pond systems are usually constructed with in the glasshouses and better suited for smaller systems. This system will take care of many problems associated with open raceway ponds like better control over the environment and less chances of contamination and the only constraint is a higher establishment cost.

c) Photobioreactor

Photobioreactor is a bioreactor system that is used to culture microalgae in an enclosed system which does not allow direct exchange of material between the culture and environment. Photobioreactor is able to conquer numerous constraints confronted by open pond culture design. It provides more efficient land usage, contamination free, single strain microalgae culture with higher nutrient, metabolic efficiency and higher biomass production per unit of substrate. Based on the shape it may be tubular, vertical column or flat-plate photobioreactor. The highly controlled growth condition of photobioreactor elevates the capital and operating costs.

d) Hybrid system

Hybrid system of algae cultivation is a combination of both open and closed system. This system will improve the algal production and yield. In this system, first the selected strain of algae is grown in a closed bioreactor and then the algal broth is transferred to an open pond. It helps in reducing the risk of contamination and produces higher algal yield.



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ANTIBIOTIC RESISTANT BACTERIA IN COW DUNG

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Cow dung is being used in agriculture as well as for the household and religious purpose from the ancient time. Cow dung is traditionally used as organic fertilizer in Indian sub-continental farming for centuries. Cow dung is rich in nutrients specially Nitrogen, Potassium, Phosphorous which is use as a fertilizer. Cow dung increases the mineral states of soil, enhance the resistance of plants against pests and diseases, also stimulate plant growth and other beneficial activities such as sulphur oxidation and phosphorous solubilisation. Normally composition of cow dung is about 80% water and supports a matrix of undigested plant material that is rich in nutrients, microorganisms and their growth.

Antibiotics are group of reagents that can inhibit the growth of bacteria or destroy them and have been widely directed to keep humans and animals healthy. In the past decades, worldwide utilization of Antibiotics for both human and animal are on the climb. Veterinary antibiotics are used on regular basis for treatment and prevention of disease in animal in commercial livestock farming. Globally the consumption of antibiotics in animal feeds was increased by 8 % from 2009 to 2011. But animals are not able to effectively metabolize antibiotics. Depends on antibiotics, they may be either completely metabolised or a portion may be excreted via urine or feces in either its original form or a portion its original state or as active/non active metabolites.



Antibiotics Resistance is the ability of microorganisms to avoid the harmful effects of an antibiotic by destroying it, transporting it out of the cell or undergoing changes that block its effects. The overuse of antibiotics clearly drive the evolution of resistance.

Indiscriminate use of antibiotics in animal feeds is one of the prime breeding grounds for tough, drug- resistance bacteria. Antibiotics resistance development by overuse of antibiotics, particularly sub therapeutic use of antibiotics in food – producing animals in animal feed has been noted down since 1972.

Bacteria develop resistance mechanisms by using instruction provided by their DNA. In bacteria, genes can be inherited from relatives or can be acquired from non - relatives on mobile genetic elements such as plasmids. This horizontal gene transfer can allow antibiotics resistance to be transferred among different species of bacteria. Resistance can also occur spontaneously through mutation. Antibiotic remove drug-sensitive bacteria competitors, leaving resistance bacteria behind to reproduce as a result of natural selection.

Enterococcus faecalis and *Escherichia coli* have presented serious challenges clinically, as both are leading cause of nosocomial infections, gastrointestinal infections, urinary tract infections and are becoming increasingly resistant to treatment with antimicrobials.

Antibiotic resistance bacteria in livestock manure used as fertilizer and spread over agriculture land, may pose a threat to the health of humans. Antibiotic resistance bacteria of cow dung spread in environment through contaminated crops or groundwater and have potential consequences for human health if transferred to human pathogen.

India is now slowly realizing the adverse effects of using antibiotics in livestock causing antibiotic resistance. As animal health can not be ignored, judicious use of antibiotics is required to maintain the health of animals, but only when they are ill. But by that time, often the animals would have been treated by antibiotics and other drugs by an unskilled person. Therefore there is need that livestock owners are made aware about antimicrobials resistance and other harmful effects of the antibiotics.



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MEDICINAL USES OF *Psoralea corylifolia* SEEDS

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The name *Psoralea corylifolia* is derived from the Greek word Psoraleos, which means “affected with itching or leprosy”. *Psoralea corylifolia* is a medicinally significant plant in the Fabaceae family that has been endangered. Bakuchi, babchi, bavachi, Bu Ku Zhi, Ku Tzu, and buguzhi are some of the common names for it. *Psoralea corylifolia* is used in Ayurveda and Chinese medicine to treat a variety of ailments. The plant includes antibacterial and cytotoxic effects and is cardioactive. *P. corylifolia* is also known as 'Kusthanashini,' or leprosy destroyer. It is a pigment manufacturer. Because of its potential to heal leprosy, *Psoralea corylifolia* seeds are utilized in indigenous medicine systems to treat various ailments. Diuretic, aphrodisiac, laxative, anti-helminthic, and utilized in febrile circumstances, the seeds are used. These seeds are sweet, bitter, astringent, and caustic. They provide strength and vitality, as well as improved digestion and mental receptivity. The seeds are used in Ayurveda to treat alopecia, inflammation, leukoderma, leprosy, psoriasis, and eczema as a paste and as an ointment for external and internal usage.

In India, the powder of seeds is mixed with Haratalabhasma (yellow arsenic) and converted to paste with the urine of the cow. Leukoderma lesions are treated using this paste. A mixture of powdered seeds and buttermilk has been used externally to treat ringworm and scabies in another formulation. For the treatment of leprosy, the seed oil is administered orally with betel nut leaves. Dermatosis has been treated with bakuchi (*Psoralea corylifolia*) adjuvant therapy in combination with other



native plants such as Amalaki and Khadira. Bakuchi and Karanja oils are also combined with Vaseline to treat chronic skin disorders. The plant's seeds are also used to treat bilious disorders, snakebites, and scorpion stings. The seeds are also utilized in scent making.

The seeds of Bakuchi in powder form mixed with the decoction of Bibhitaka (*Terminalia bellirica* bark) and Kaakodumbara (*Ficus hispida*) were effective to treat vitiligo. When applied externally, a combination of bakuchi and Haratalabhasma provides treatment from leukoderma. It has a wide range of applications because it is an essential component of both the allopathic and traditional medical systems in different regions of the world. Psoriasis, leucoderma, and vitiligo are all treated with it in traditional Chinese medicine and Indian systems of medicine such as Ayurveda, Siddha, and Unani. Babchi seeds are taken orally to treat a variety of ailments, including intestinal worm infestation. Overdosing on babchi seeds, on the other hand, can cause headaches, nausea, diarrhea, and vomiting, among other things.

Seed and extract powder are used as a diuretic, anthelmintic, laxative, and for healing wounds. Seeds are antipyretic and alexiteric. Seeds are used as stomachic, stimulant, aphrodisiac, and diaphoretic. It is an effective invigorant against impotence, menstruation disorder, and uterine hemorrhage. It shows coronary vasodilatory activity. It is a cure for gynecologic bleeding. It is also useful to treat spermatorrhea and premature ejaculation. The seeds act as deobstruent and heal the ulcer, heart troubles, and cure blood disorders and elephantiasis. Seeds are given in scorpion-sting and snake bite. Seeds are useful in bilious disorders. The crude drug has been used for the treatment of enuresis, pollakiuria, painful feeling of cold in the waist and knees, and weak kidney. It is used in the treatment of debility and other problems related to kidney inefficiencies, such as febrile disorders, low back pains, frequent urination, incontinence, and bedwetting. Most of the bioactive compounds of medicinal interest are present in seeds. So, the seeds of *Psoralea corylifolia* have been the main focus. There is a variety of diverse bioactive compounds such as Psoralen, Isopsoralen, Bakuchiol, Bakuchicin, Bavachin, Isobavachin, Bavachinin, Bavachalcone, Isobavachalcone, Neobavachalcone, Corylifol A, B, C, D, E, Corylifolin, Corylifolinin, and others.



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BIOREMEDIATION: AN EFFECTIVE TECHNIQUE OF ENVIRONMENTAL CLEANUP

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Environmental pollution has been a rising problem in the past few decades. Industrial activities, human-generated hazardous waste, leakage during the transport of toxic chemicals are being released and accumulated within the ecological components. Toxic materials mainly include hydrocarbons, xenobiotics, industrial wastes such as toxins produced in dairies, tanneries, distilleries. The effluent from the textile industry contains carcinogenic amines, toxic heavy metals, pentachlorophenol etc. Dairy waste consists of cleaning chemicals, detergents used in saponification. Increase of pollutants in the ecosystem has induced the need to develop cost-effective methods for their removal. Degrading the pollutants to neutral chemicals and removing these pollutants from contaminated environments is today's utmost need. Microorganisms have the ability to break down a huge range of organic compounds and absorb inorganic substances. Microorganisms metabolise contaminants like oil, pesticides from soil, water bodies and other environments. Microorganisms are used to clean up the toxic environment with the technique known as Bioremediation.

The objective of bioremediation is to modify the contaminants into a less toxic, neutral or nontoxic chemical species. It is a unique approach towards earth repair. Remediation of polluted sites uses organisms like plants, fungi, algae and



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bacteria. That's the reason why it is sustainable. Bioremediation aims to encourage organisms, to work by supplying optimum levels of nutrients essential for their metabolism and manipulation of environmental parameters. Bioremediation techniques are decided on the basis of the location and the extremity of the contamination. These are broadly classified into in-situ and ex-situ methods. Effective methods of in-situ (Remediation carried out at the site of contamination) are Bioventing and Biosparging. This approach avoids the excavation and transport of the heavily contaminated site. Ex-situ method involves excavation, involving methods such as landfarming, composting, biopiles and use of Bioreactors. There is a high risk of exposure to a toxin free environment to the pollutants. To make a microorganism-friendly environment in the vicinity of the contaminated site is a productive approach.

Bioremediation is a promising approach for degradation of numerous pollutants. These pollutants serve as a carbon, energy source for microbial growth and as an electron acceptor. Complex mixtures of hydrocarbons commonly occur in crude oil and gasoline. Aerobic degradation occurs with molecular oxygen (O_2). Bioventing and biosparging increase the availability of oxygen (Injection and Circulation). Other strategies of bioremediation involve enzyme systems, Carboxylation and fumarate addition. The enzyme groups used are oxidoreductases, hydrolases, lyases, transferases and isomerases etc. These enzymes have a strong degradation capacity according to their substrate affinity. 'Cytochrome P450 alkane hydroxylases' reduces harmful hydrocarbons. Yeast species *Candida maltosa*, *Candida tropicalis*, and *Candida apicola* have this enzyme system. Decomposition or immobilization of dyes and suspended solids is achieved by using metabolic strength of microorganisms like *Pseudomonas* spp., *Xenophilus* spp. etc. Algae and fungi also efficiently take part in remediation processes. Algae such as *Microcystis* spp. can decolorize bleaching effluents. Adsorbable organic halides (AOX), a group of bioaccumulative chemicals are removed using mixed culture of alga *Chlorella*, *Chlamydomonas* and *Microcystis* spp. AOXs are produced when the halogens react with organic matter. Mycoremediation is also one of the approaches to bioremediation. *Phanerochaete chrysosporium*, *Pleurotus ostreatus* are ligninolytic fungal species. These fungi reduce Polycyclic Aromatic Hydrocarbons (PAHs). *Mycorrhizae* are fungi which colonize with root tissues, increase the absorption area and enhance heavy metal uptake. Xenobiotics are degraded by Methanotrophic, Methanogenic bacteria and *Sphingomonads* as well.



Rate of bioremediation is affected by various factors- pH, temperature, carbon source, concentration of pollutants. Acceleration of bioremediation is achieved by creating an organism-friendly, nutritive environment. Bacteria which are able to degrade chemical species naturally are developed using strain improvement methods to enhance the quality of the technique. The characters mainly include specific enzyme production, respiratory abilities in presence of the contaminant. Bioaugmentation is a standard way to improve performance of indigenous organisms by adding microorganisms of specific catabolic activities. Effective steps in bioaugmentation are- Immobilization or Entrapment of cells, Quorum sensing (QS) etc. The removal of heterocyclic compounds from refineries is improved using immobilized naphthalene-cultivated *Arthrobacter spp.* Quorum sensing ensures colonisation of efficient bacteria. Manipulation of the genome (Development of Genetically Modified Organisms - GMOs) can be a strong approach towards enhancing degrading capabilities. *Pseudomonas putida* is a GMO. It is genetically engineered with plasmids encoding for catalytic degradation of toluene, xylene etc. *Flavobacterium spp.* and some *Pseudomonas spp.* have a *pod* gene. This gene is involved in degradation of pesticides such as parathion, methyl parathion.

Bioremediation does not cause a major disturbance of normal activities. Recent study suggests its efficiency and advances. Biosorption, biostimulation are emerging methods in the pollutant degradation techniques. It uses natural components to improve the state of contamination. It is economically profitable as well. Use of naturally occurring microorganisms makes the method sustainable and fulfils the requirement of biodegradation. It prevents accumulation of toxic chemicals, reduces the possibility of bioaccumulation and thus future possibilities of biomagnification. Bioremediation techniques can be considered as an effective cleanup strategy towards a healthy environment.



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BIOLUMINESCENCE IN MICROBIAL WORLD

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The phenomenon of light emission as a result of an enzyme-catalyzed oxidation reaction in living organisms is called Bioluminescence. Bioluminescent bacteria are widely distributed light-emitting organisms and the majority of them are found in marine water. The enzyme that catalyzes bioluminescence in bacteria is luciferase. However, the catalytic machinery involved in the emission of light also includes enzymes that supply & regenerate substrates of luciferase.

Mechanism of Bioluminescence

Lux genes are the DNA sequences coding for the proteins in luminescent microbes. Luciferase is the heterodimer composed of α and β polypeptide subunits encoded by luxA & luxB genes respectively. reduced flavin mononucleotide (FMNH_2), molecular oxygen, and long-chain fatty aldehyde are the substrates of the luciferase enzyme. The excess of energy which is liberated by oxidation of FMNH_2 & associated fatty aldehyde with reduction of molecular Oxygen is released as blue/green light emission. ($\lambda_{\text{max}} \sim 490 \text{ nm}$).



Applications of Bacterial Bioluminescence

Bioluminescent bacteria are capable of acting as biosensors. The feature of light emission with help of luciferase enzyme can be successfully produced in other non-luminescent bacteria along with the generation of aldehyde substrate. This can be done by the insertion of luxCDABE genes. The non-luminescent bacteria modified with this method provide an easy alternative for the detection of growth & living conditions of bacteria. This feature can also be used in detecting pathogens present in food. By culturing the food sample in presence of a genetically modified bacteriophage inserted with the luxCDABE gene, bacterial contamination in food resources can be detected. The presence of toxic waste in the environment can be monitored by the expression of lux genes. The genetically modified bacteria carrying lux gene insert can also be used to study the effect of antibiotics against bacterial infections in mammals. The intensity of luminescence will decrease in infected tissue as bacteria are killed by antibiotics. This screening method helps determine the proper dosage of the respective antibiotic.

Examples of Bioluminescent Bacteria

- a) *Allivibrio fischeri* – It is found in symbiotic association with Hawaiian bobtail squid. The squid use bacteria's luminescence to attract prey & to communicate with other squids.
- b) *Photobacterium phosphorium* – It lives in symbiosis with marine organisms usually in their gut. Marine organisms get benefited by the luminescence of bacteria as camouflage, communication signals and escaping predators.
- c) *Photorhabdus luminescens* – It is a nematode-symbiotic bacteria belonging to the family Enterobacteriaceae.
- d) *Photobacterium profundum* - It is a deep-sea Gamma Proteobacterium, belonging to the family Vibrionaceae.



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TOWARDS EFFECTIVE RISK FACTORS FOR ANTI-SARS-COV-2 VACCINE EFFICACY

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Global immune weakness is a risk factor for anti-COVID-19 vaccination effectiveness, especially among elderly people who have been exposed to many causes contributing to immune system weakening, as already explained. These factors also lead to diseases like obesity/obesity: e.g. type II diabetes, metabolic syndrome, and immunological cancer. Mechanical explanations for this disease include antigen recognition weakness, decreased immune cell number and functionality, increased level/length and time of humoral component immunological modifications, reduced cell response initiation, and cell memory disturbances. Other immunodeficiency correlations include age-related humoral and immunological cell disorders; immunosenescence; malnutrition; protein-energy-micronutrient deficiencies and telomeres. In addition, prior or present therapies affect the scaled inefficacy of vaccinations, especially in immunocompromised adults and children.

Exposure to immunosuppressive medicines or residence in low socioeconomic level developing nations is associated in comparison with resident in developed countries with increased viral mortality. Current prevalence of global obesity in adults and children is also a risk factor for ineffectiveness of anti-SARS-CoV-2 vaccine due to higher IL-6 levels and lower IgG concentrations. Parasitic



illnesses, such as severe pneumonia in respiratory tract infections, may also influence subsequent immune responses to the SARS-CoV-2 vaccine.

The vaccination efficacy is affected by the additional purity and safety, by the knowledge gaps of the relative contribution of the inborn and adaptive reactions to protection against individual pathogens and by the precise mode of action for individual adjuvants. Anti-SARS-CoV-2 vaccinations will be provided by vaccine market participants in the context of the present legislation on orphan medicines and vaccines to combat SARS-CoV-2 pandemics. This indicates that vaccine efficacy can also be affected by a design error, failure to warn and carelessness of testing. Under those settings, there will be no long-term safety testing of human vaccines. Whether anti-SARS-CoV-2 vaccines will be first licensed in developed countries or if all facilities of vaccine production will be adequate to secure a reliable timely supply of new anti-SSARS-CoV-2 vaccines compatible with the requirements and improved WHO design strategy for adult vaccines or Immunobiography and Immune response in elderly subgroups are compatible.

In laboratory experiments with mouse or rabbits, the safety of the vaccination is initially tested. If after receiving the vaccine the animals do not show signs of sickness, then human testing starts, and the number of subjects gradually grows. The following is the typical duration of the clinical trial for the classical vaccination (after preclinical phase – *in vitro* and *in vivo*): In phase I, also called the first human test, a small group of healthy volunteers are vaccinated (10 to 100). The goal is not to assess if the vaccination protects against the disease, but if it is safe or if it causes serious side effects.

A large number of individuals (100 - 1,000) get the proposed vaccine in Phase II and an even bigger cohort in Phase III (1,000 - 100,000). Separate investigations in adults, children and the elderly may be necessary. Sometimes, when given to a small number of people, immunizations that appear safe exhibit secondary effects when given to a large number of people. This is because there is less chance of any uncommon issue when the group of subjects is limited. Continuous monitoring is crucial in the event of delayed-effect problems. In a pandemic, the successive trials may be shortened and partially overlapped, but it is crucial that the general purpose vaccine be followed up by thousands of vaccinated persons for several months. If more severe inflammatory indications occur or if the vaccine creates a malfunction in the immune system in other ways, the proposed vaccine may not be acceptable. Delays in the production process necessarily arise when such detrimental impacts are detected. At present, there are only two potential SARS-CoV-2 vaccines at the first stage by way of a waiver from the rule of skipping animal research.



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FORTUITOUSLY A NEW ELUSIVE OF METAL EATING BACTERIA

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A new elusive of metal eating Bacteria that feeds on manganese and use the metal as their source of calories have been discovered by Caltech microbiologists. None of such bacteria had been described or found until now and such microbes were predicted to exist over a century ago.

Professor of environmental microbiology at Caltech, Jared Leadbetter says, "These are the first bacteria found to use manganese as their source of fuel". In the journal Nature, Jared Leadbetter in collaboration with Hang Yu, a postdoctoral scholar, described the findings in the July 16 issue of the journal nature. The study also reveals that a process called chemosynthesis, where carbon di oxide is converted into biomass using manganese can be carried out by the bacteria. This led to "A wonderful aspect of microbes in nature is that they can metabolize seemingly unlikely materials, like metals, yielding energy useful to the cell."

Previously, researchers knew of bacteria and fungi that could oxidize manganese, or strip it of electrons, but they had only speculated that yield-to-be-identified microbes might be able to harness the process to drive growth. But, after performing unrelated experiments using light, a chalk-like form of manganese, Leadbetter found the bacteria serendipitously, Before departing for several months to work off-campus, in his Caltech office, he had left a jar glass soiled with the substance soak in tap water. The glass jar was found to be coated with dark material



when he returned. "I thought, 'What is that?'" he explains. I started to wonder if long-sought-after microbes might be responsible, so we systematically performed tests to figure that out". The jar's contents had been replaced with a new substance, dark and crusty. Two new species of bacteria were responsible, according to a study the researchers published in *Nature*. The bacteria, tentatively christened *Candidatus Manganitrophus nodulliformans* and *Ramlicbacter lithotrophicus*, that can borrow electrons from metals like manganese and use them as fuel for growth. When metals lose electrons, they oxidize; in the case of the dirty jar, this caused the contents to transform from Manganese carbonate into Manganese oxide. This is because both the species likely came from tap water, the findings could help control manganese oxide pollution in drinking water the researchers say. He found that a new bacterium that had likely come from the tap water itself had generated that black coting, which was in fact oxidized manganese. Manganese oxides are common in nature and take the form of a dark, clump substance.

They can also form in water-distribution systems and have been found in subsurface deposits. He said "There is evidence that relatives of these creatures reside in groundwater, and a portion of Pasadena's drinking water is pumped from local aquifers". Leadbetter says, "There is an entire set of environmental engineering literature on Manganese is one of the most abundant elements on the surface of the earth, Manganese oxides take the form of a dark, clumpy substance and are common in nature; they have been found in substance deposits and can also form in water-distribution system getting clogging by manganese in the drinking water distribution systems. However, the reason why such material is generated there such and how it is generated is not known. Many scientists considered that this was due to bacteria using manganese for energy, but until now, there was no evidence supporting this idea."



Manganese oxide nodule generated by the bacteria discovered by the Caltech team. These nodules are generally about 0.1 to 0.5 millimeters in diameter. Image is a scanning electron micrograph with false colorization.

Credit: Hang Yu/Caltech



Geochemistry of groundwater can be easily understood by researchers with the help of the findings. The process of bioremediation is known, a process where bacteria can degrade pollutants in groundwater. In this similar manner to how humans use oxygen in the air, various key organisms will “reduce” manganese oxide while doing this, which means they donate electrons to it. Where the manganese oxide comes from in the first place? For so long this question wondered by scientists. Leadbetter also states “The bacteria we have discovered can produce it, thus they enjoy a lifestyle that also serves to supply the other microbes with what they need to perform reactions that we consider to be beneficial and desirable”.

The very possible relevance to understanding manganese nodules that dot much of the seafloor is also in the research findings. As early as the cruises of the HMS Challenger in the 1870s, marine researchers knew these round metallic balls, which can be as large as grapefruit. At the bottom of many of Earth's oceans, such nodules have been found, since then. Within these nodules, rare metals are often found in concentrated forms, and mining companies have been making plans to harvest and exploit these nodules in recent years. However, information on how the nodules form in the first place was not known quite well. Yu and Leadbetter plan to further investigate the mystery as they are now wondering if the microbes similar to what was found in freshwater might play a role in the formation of these nodules. Yu said, "This underscores the need to better understand marine manganese nodules before they are decimated by mining."

"A major intellectual gap in our understanding of Earth's elemental cycles are filled by this discovery from Jared and Hang, he also said that the diverse ways in which the evolution of life on our planet has been shaped by manganese, an abstruse but common transition metal." Woodward Fischer (Professor of geobiology) at Caltech was not involved with the study, he said.



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VIRUSES AS BIOMATERIALS

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Viruses have long been studied as deadly pathogens to cause disease in all living forms. By the 1950s, researchers had begun thinking of viruses as tools in addition of pathogens. Bacteriophage genomes and components of the protein expression machinery have been widely utilized as tools for understanding the fundamental cellular process. On the basis of these studies, several viruses have been exploited as expression systems in biotechnology.

Advances in synthetic biology and chemistry have influenced many areas of research, industry, and medicine by allowing for the fabrication of nanoscale devices with increasingly controllable structures. Even so, the large-scale manufacturing of such materials remains challenging, and it is difficult to prepare structurally homogeneous populations of particles. In contrast, bionanomaterials based on viruses allow for the templated assembly of millions of identical nanoparticles and their production in living cells. Viruses are ubiquitous in the environment, and those that infect bacteria, mammals, or plants have all been used to manufacture Virus-Based Nanoparticles (VNPs). Viruses are an ideal starting point because they have evolved naturally to deliver nucleic acids and can therefore be subverted for the delivery of other molecules, such as drugs and imaging reagents.

Bacteriophages and plant viruses are nucleoprotein assemblies in which the nucleic acids are tightly enclosed in a capsid comprising multiple copies of identical coat proteins. The natural function of the virus capsid is to protect the viral genome from nucleases and physical hazards. Virus coat proteins are therefore stable and



resistant to chemical and physical degradation, which is an advantage for the development of VNPs because it means they have a long shelf life and can withstand the chemical treatments necessary for conjugation with targeting ligands or loading with payloads such as drugs, fluorophores, or contrast agents. . Finally, viruses replicate prodigiously, allowing the inexpensive manufacture of VNPs on an industrial scale.

VNPs comprise regular arrays of virus coat proteins and have highly defined three-dimensional structure, providing an engineering scaffold that is superior to synthetic particles. The structure of VNPs can be altered by modifying the nucleic acid template that codes for viral proteins prior to synthesis, and by chemically decorating the particles by adding conjugates to specific amino acid side chains. VNPs are composed primarily of protein and are therefore known for their biocompatibility, biodegradability, ability to cross biological barriers, and efficient delivery of cargo to target cells. Viruses have evolved to interact with specific cellular proteins, deliver their nucleic acid cargo, and hijack the intracellular machinery to produce the components of progeny viruses. These properties have led to the development of VNPs based on mammalian viruses for use in gene therapy, but it is difficult to rule out pathogenic effects resulting from natural virus-host interactions. In contrast, VNPs based on bacteriophages and plant viruses are regarded as safe because even the fully functional viruses cannot infect humans.

A battery of techniques can be used to tailor and modify virus-based materials, including genetic engineering, encapsulation, biomaterialization, infusion, and bioconjugation. Genetic engineering allows the basic structure of the coat protein to be changed by inserting, removing, or substituting particular amino acid residues. Such changes include terminal extensions (adding sequences to the N terminus or C terminus of each coat protein), the insertion of sequences that form surface loops, or the insertion or exchange of individual amino acids to introduce side chains that allow functionalization or to alter the overall physicochemical properties of the VNP. Prominent examples of such modifications include the introduction of purification/immunodetection tags, the introduction of epitope sequences so that the VNP functions as a vaccine, and the introduction of targeting sequences that allow the VNP to target specific receptors. Conjugation involves the selective covalent addition of payload molecules to particular amino acid residues of the coat protein. Infusion is achieved by incubating the intact VNP in a solution containing the cargo, and encapsulation requires the carrier to be assembled around the payload. Biomaterialization is the accumulation of minerals in and around the cells and tissues of living organisms, but in the context of VNPs it refers to the ability



of virus coat proteins to assemble around a mineral core or nucleate mineralization. For example, an electrostatically engineered cowpea chlorotic mottle virus (CCMV) was used to facilitate the nucleation and oxidation of an Fe (II) cargo, leading to the formation of spatially constrained iron oxide nanocrystals suitable for magnetic resonance imaging (MRI) or hyperthermia treatment applications.

Virus - Based Nanoparticles in Therapeutic Interventions

The ability of bacteriophages and plant viruses to enter mammalian cells without further replication makes them suitable as tools for therapeutic interventions. Virus-based nanomaterials can be tailored to target particular cells, including cancer cells and specific cells of the immune system. They can present antigens to the immune system, meaning they can also be used as vaccines. VNP interactions with the immune system are advantageous for immunotherapy and immuno/chemo combination therapies, but often not for imaging applications or drug delivery. Therefore, several strategies have been developed to shield VNPs from the immune system while directing them to specific target cells. The clearance of VNPs by the mononuclear phagocyte system can be overcome by tailoring the surface chemistry or shape of the particles. For example, surface PEGylation can minimize nonspecific interactions between VNPs and macrophages, thus prolonging their circulation time. Targeting can be achieved by the genetic or chemical addition of ligands that bind to receptors overexpressed on particular cell types, such as cancer cell

Virus - Based Materials as Imaging Probes

Imaging molecules can be added to the external surface or internal cavity of VNPs by Genetic modification (Bioluminescent proteins), infusion, encapsulation, and/or bioconjugation. The precise spacing and controlled loading of imaging molecules can be used to tweak the signal-to-noise ratio depending on the dye or contrast agent used. The genetic addition of GFP or the red fluorescent protein m Cherry to the Potato virus X (PVX) coat protein allowed for *in vivo* imaging of Human Tumor Xenographs in Mice.



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OVERVIEW OF ZIKA VIRUS

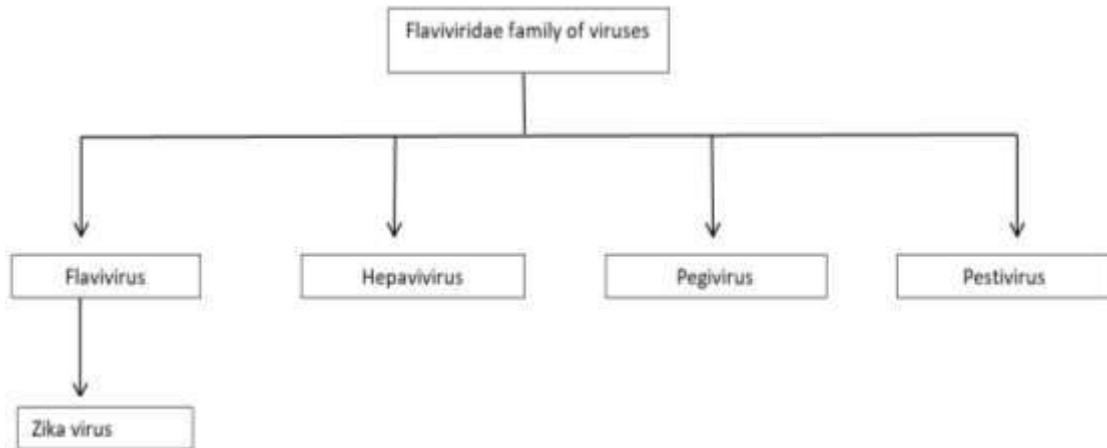
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The first cases of the zika virus were found in 1947 in Uganda and only minor outbreaks and few cases were reported in Asia and Africa during the periods ranging between 1960 to the early 21st century. An outbreak was diagnosed in the year 2007 on a place called Yap Island and similarly in 2013 epidemic was reported in French Polynesia. In Brazil, the zika virus was recognized in April 2015 and was reported to be the etiological agent of breakouts of an acute exanthematous illness that began in the later period of 2014 in numerous cities of the Northeastern region of the country. After Brazil, the cases spread through South and Central America. In Continental US, the Maiden cases of Zika via local transmission were reported in the week of 24 July 2016. In the United States of America, on 19 April 2017 223 cases of suspected local transmission were reported.

Zika virus has emerged as a virus belonging to the family *Flaviviridae* that spreads *via* mosquito as the vector of the disease. The virus has an antigenic and phylogenetic identity that is connected to the Spondwenivirus. The diseases caused by the Zika virus are primarily arboviral. The Zika Virus structurally resembles an icosahedron. It is an enveloped RNA virus that is made up of a single strand. A covering of dense projections that constitute membrane and envelop glycoproteins surrounds the lipid envelope.





Etiology

The virus may spread through person-to-person contact, transfusion of blood, transplantation of organs, and perinatally and primarily are communicated via the bite of a female *Aedes, Aegypti* in addition to *Aedes albopictus* mosquitoes. Zika has symptoms that are connected to dengue, West Nile fever, and Yellow fever along with Japanese encephalitis viruses genetically.

Prognosis

Maximum cases of infection are mild and they normally get cured on their own but critical diseases of neurological have been reported that also comprise Guillain-Barre syndrome. There is also colossal concern that congenital disabilities like microcephaly, brain and eye abnormalities can be induced by the virus if it is acquired during the period of pregnancy. There have also been cases of acute myelitis and meningoencephalitis reported subsequently Zika virus infection. Hence a complete Neurologic examination is an advice when zika is suspected.

Physical effects

The maximum number of patients with acute Zika virus infections are either asymptomatic which ranges between 60-80% for they possess only mild symptoms estimated incubation phase between bites and symptoms is 2-14 days for mosquitoes bite. In asymptomatic infection, the most common symptoms include rashes, conjunctivitis, fever, and headache. Normally the rashes maculopapular with low-intensity fever and is a short period. Other frequent symptoms and signs include arthralgia, myalgia, and retro-orbital pain.



Treatment

The non-availability of a vaccine or a clinically tested drug to treat the infection coupled with the probability that another breakout might occur in the future defines an unachieved medical necessity. A large number of drug molecules are being under examination through the employment of repurposing study, screening through the compound library, and other new techniques during a short span of few years.



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INTERACTION BETWEEN RHIZOSPHERIC MICROBIOME AND ROOT EXUDATES UNDER DROUGHT CONDITIONS

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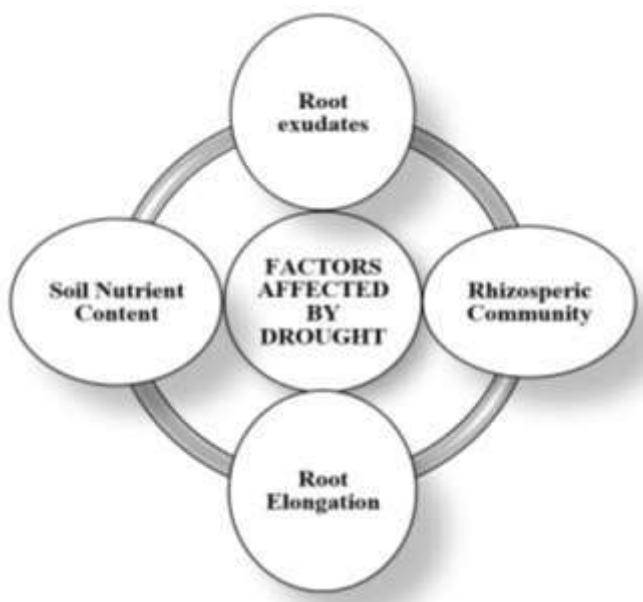
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Soil has a diverse array of microorganisms precisely known as Rhizospheric microbiome. They provide soil their colour, texture and other characteristics. One of the examples is Actinomycetes giving the peculiar smell of soil. Root exudates are simply the secretions of plant root which are low molecular weight compounds. They provide nutrition to soil microbes and play an important role in their interaction with both biological and chemical reaction taking place inside the soil. The compounds present in root exudates (preferably amino acids and organic acids) are found to mimic as bacterial signals required for quorum sensing. They recruit beneficial microorganism for nitrogen fixation, plant growth promoting factors and mycorrhizal fungi association. Additionally, their role is found to be extremely useful in biodegradation of pollutants and during plant stress like salinity, water-logging, temperature and drought, etc. Studies have found that root exudates increase soil nitrogen which leads to delay in flowering. Similarly, under high CO₂ concentration plant has been observed to exude high carbon leading to enhancement of organic soil matter. Such examples clearly exhibit how different environmental condition alter root exudates which in turn affects rhizospheric microbiome and can also impact our future positively.



Recent research focus has been on root exudates under drought condition in order to increase the existence of flora in arid and semi-arid regions. It is found to be one of the major strategies to survive in drought conditions. An interesting study says that slow growing plants invest less time in acclimatizing to drought stress than those which are fast growing. Scientists hypothesized that fast growing plants do not show rapid response to reduce water stress due to their intimacy with rhizospheric bacteria while slow growing plants depend on their interaction with mycorrhiza fungi which continue to function even in drought conditions.

Another important adaptation by plants to cope drought stress is Biological Nitrification Inhibition (BNI) as it reduces the nitrogen expenditure. Research proves that exposure of roots under drought results in high BNI activity. It was observed that such a response was initiated owing to gene specificity found in drought tolerant plant like pearl millet. Drought sensitive plants were observed to induce root elongation under the action of root exudates in order to reach deep layer of soil for water and nutrient uptake. Citrate, Vitamin B₂ were also observed to increase under drought stress in both sensitive and tolerant species which result in antioxidant production in plant.



All the above fact state that there is a strong association between root exudates, plant microbes and the way plants react to different stimulus under their influence. Studies in similar area can help us to find mechanism to conserve plants in both dry and wet lands which is the need of the hour.



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Stevia rebaudiana AS AN ANTIMICROBIAL AND ANTI-QUORUM SENSING AGENT

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Natural substances having therapeutic capabilities have been used for a long time; plant, animal, and mineral products were the primary sources of medicines. New antimicrobial chemicals have been discovered from a variety of sources, including bacteria, animals, and plants. Knowledge of these agents might lead to the discovery of new antibacterial agents that are both effective and safe. To protect themselves from microbial infection and degeneration, plants can produce antimicrobial natural compounds. Natural antibacterial chemicals, particularly those produced from plants, are becoming increasingly popular for food preservation. Many plant phytoconstituents having biological activity, such as alkaloids, flavonoids, tannins, and phenolic compounds, have been shown to have antioxidant properties. Many of these biologically active compounds have been found to be useful in the treatment of chronic disorders such as cancer, cardiovascular disease, and neurodegenerative disease. Plant-based medications are currently used by 66 percent to 75 percent of the world's population. *Stevia* is a nutrient-dense plant belonging to the Asteraceae family, it is considered as a natural sweetner. *Stevia*'s sweetness comes from the rebaudiosides A-F, diterpene glycosides stevioside, dulcoside and steviolbioside, which are found in the leaves. The dry extract of *Stevia*



rebaudiana leaves are enriched with enormous phytoconstituents making it a wonder drug as it is effective as vasodilators, cardiotonic, anaesthetic, and anti-inflammatory.

Stevia leaf extracts have been shown to inhibit microbes in studies, however their antibacterial activity is lower than that of a conventional antibiotic like ciprofloxacin. The extracts could be a good candidate for more research into their potential use in food preservation, pharmaceuticals, and natural plant-based goods. The primary goal of medicinal plant research is to determine plants with pharmacological action, and thus to explore new antimicrobial compounds or molecules that might be turned into drugs through various chemical processes and utilized to control or prevent illnesses. Furthermore, the pure extracts produced from *Stevia rebaudiana* leaves showed no harmful or genotoxic action. Antimicrobial activity has been discovered in herbal extracts containing secondary metabolites such as alkaloids, terpenes, sterols, flavonoids, carotenoids, and other secondary metabolites. *Stevia rebaudiana* inhibitory activity is aided by the presence of substances such as rutin, quercitrin, and dihydrodeoxy Streptomycin. Because the plant extract is chemically complex in nature, all of its components may be jointly responsible for its antibacterial activity, bacteria cannot build resistance to it. *Stevia* extracts have been demonstrated to reduce biofilm development at greater doses and pH levels. The Extracellular Polymeric Substance (EPS) matrix protects biofilm, which is a structural form of a microbial group. The biofilm is thought to be the primary cause of infection and plays a key role in the development of antibiotic resistance. Terpenoids, steroids, saponins, carotenoids, phenolics, furanones, alkaloids, peptides, tannins, and lactones are some of the natural compounds found in plants that have antibiofilm potential. The capacity of metabolites to permeate the EPS layer or the mucous layer that encases the bacteria is connected to their ability to degrade biofilms. Many antimicrobials are unable to enter biofilms because EPS acts as a barrier, protecting bacterial cells within. The secretion of quorum-sensing (QS) signals the production of EPS. The QS is a bacterial cell mechanism that determines the state of their extracellular environment and plays a key role in biofilm development. To inhibit biofilm formation, interrupting QS release and preventing the production of EPS is the primary concern. Stevioside, Reb A, Reb C, and Dulc A are the most prevalent glycosides in *Stevia* leaves by dry weight. Stevioside and Reb A, the two most frequent steviol glycosides, differ only by one glucose moiety, with stevioside containing two glucose molecules and Reb A containing three. Reb A, according to research, disrupts gut microbiota balance and lowers dopamine transporter mRNA and nucleus accumbens tyrosine hydroxylase levels when compared to a control group. Although some studies have found that steviol glycosides have no effect on gut microbial makeup and growth, there is little question



that *Stevia* glycosides change gut microbial equilibrium. However, the method by which *Stevia* extract affects the gut microbiota is unknown. Autoinducers (AIs) are the signalling molecules that are found in QS. AIs are either unmodified peptides or post-translationally modified peptides in Gram-positive bacteria, having variations in structure and sequence. N-acyl homoserine lactones (AHLs) are the major AIs in Gram-negative bacteria. The most frequent family of AIs, these signalling molecules are permeable via the membrane and adhere to their cognate receptor LuxR homologs in the cytoplasm, where they perform regulatory role. AHLs created by various bacterial species range in length and substitution of the acyl chain but possess the identical homoserine lactone moiety. AHLs are created proportionally to bacterial density by the LuxI protein family until they reach a concentration level, or thresholds, at which they form a LuxR–AHL complex, which subsequently serves as a transcriptional factor to control a specific gene expression. Reb A, a purified glycoside from the *Stevia* plant, has potential bacteriostatic properties. Pure *Stevia* extracts have been proven to limit light production in bioluminescent bacteria as well as inhibit production in bacteria that create biofilms. This could mean that consuming too much *Stevia* as a substitute could upset the gut's microbial equilibrium. The elements Steviol and Reb A are thought to allosterically modulate the pathway by binding to the cytoplasmic receptor of the QS machinery in a non-competitive manner. Exposure to steviol glycosides or their derived aglycon steviol in unknown amounts over time may have a deleterious impact on gut microbial balance by interfering with AHL-mediated bacterial networking and causing health issues. More studies may be undertaken to help further explain the effects of these sweeteners and to change the maximum daily consumption limits based on the effects observed in other studies and the increased consumption of *Stevia*. Further studies would also allow us to understand the synergistic actions of *Stevia rebaudiana* extracts with antibiotics to fight antibiotic resistance.



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BIOTRANSFORMATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN MARINE POLYCHAETES

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Polycyclic Aromatic Hydrocarbons (PAHs) are the compounds that are composed of few or more aromatic benzene rings. These compounds are the organic pollutants that are present in the marine ecosystem and are ubiquitous. They are heavily carcinogenic and have acute toxicity. PAHs are produced when naturally occurring elements such as coal, crude, gasoline and oil are burned. These particles combine and bind to the particles present in the air. There are many other sources through which PAHs are thrown into the environment. Some of them are naphthalene (produced commercially to make other chemicals), cigarettes, high-temperature cooking which also leads to the formation of PAHs in meat and other food and nourishments. PAHs are also formed from petrochemical products and the combustion of fossil fuels. Anthropogenic pollutants that are present in the environment mainly consist of PAHs. Hence exposure of people to PAHs is very common because of the air contaminated with these harmful elements.

Polychaetes

The dominant macrofauna of the marine environment mainly constitutes of deposit-feeding polychaetes. The PAHs are accumulated by the polychaetes and the biotransformation of these Polycyclic aromatic compounds (PAHs) in marine



polychaetes occurs through a two-phase process i.e. Phase I and phase II that contains enzymes that help in the biotransformation of PAHs to marine polychaetes. Cytochrome P450 (CYP) enzyme family includes the enzymes present in phase I in marine polychaetes. Bulk sediments are ingested by deposit-feeders like marine polychaetes in large amounts. They are also exposed through body surfaces so that when the body surface comes in contact with the PAHs, biotransformation takes place. A common example is lugworm *Arenicola marina*. This lugworm ingests the sediment and the organic matter and microorganisms present in the sediment are absorbed for its benefit. Because of this, they have a major role in the food web as they are the food source for many other birds, fishes, etc. Biotransformation of PAHs makes the hydrophobic PAHs hydrophilic.

Phase I Biotransformation of PAHs in Marine Polychaetes

Monooxygenase activities are present in Polychaetes. The biotransformation of PAHs can be estimated by measuring the production of metabolites. Fluoranthene that is accumulated from porewater and sediment can be biotransformed by *Capitella species I*. PAHs such as benzo(a)pyrene (B(a)P) and benzo(a)anthracene (B(a)A) can be biotransformed by *Nereis virens*. These PAHs are biotransformed *via* enzyme-catalyzed reactions. The CYP activity in *Nereis virens* is formed in microsome fraction of the gut tissue. B(a)P can be biotransformed in six or more marine polychaete species. This biotransformation efficiency can be determined by the enzyme activity.

Phase II Biotransformation in Marine Polychaetes

Phase II biotransformation also contains enzymes such as glucuronosyl transferases and sulfotransferases that play a role in biotransformation in marine polychaetes. The most essential phase II conjugation pathway is glucosidation in insects and other invertebrates because studies say that this particular pathway is restricted in invertebrates. Sulfotransferase found in marine polychaetes that contain sulfate metabolites are generally deficient as compared to another phase II metabolites. According to the study, the vital phase II metabolite is pyrene-1-sulfate. The most essential and necessary phase II conjugate present in tissue is pyrene-1-glucuronide in the marine polychaetes such as *N.virens* and *N.diversicolor*. And the other conjugates present were pyrene-1-sulfate and pyrene-1-glucoside. The most prominent metabolites present in *Capitella capitata* and *A.marina* are pyrene-1-sulfate and pyrene-1-glucoside.

The efficient biotransformer among all is *Nereis virens* because pyrene which is present as the phase II metabolite was extracted from the gut tissue after the exposure period of 5 days. And the amount of pyrene extracted was 80 %. Amount of pyrene



extracted from whole worms after 5 days of exposure is 75 % in *Nereis diversicolor* and they were present in the form of pyrene-1-glucuronide. The amount of fluoranthene that is present as aqueous metabolites is 45 % in *Capitella species* after 10 days of exposure.

Excretion

Excretion of xenobiotics takes place through gut in polychaete species. Chloragogen tissue is the tissue by which the gut of the polychaete is lined. The function of the tissue is it resembeles with that of vertebrate liver. In *Nereis diversicolor*, the conjugates of pyrene were present in fluid as well as in defecation water. Hence this indicates that after the biotransformation of phase I and II, the pyrene metabolites are eliminated. This elimination takes place via gut.

CYP enzymes play a crucial role in biotransformation of PAHs. CYP genes from the marine polychaetes have been identified. Two CYP genes identified in *Nereis virens* are CYP4BB1 and CYP342A1. Metabolites that are potentially carcinogenic are formed when CYP enzyme mediated phase I biotransformation of PAHs occurs in marine polychaetes. The metabolites formed are important prey for fishes. Few data suggests that PAH phase I metabolites resembles vertebrate phase II biotransformation and the phase II conjugation pathway differs even among closely related species.



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ROLE OF ACTINOBACTERIA IN TEA ECOSYSTEM

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Tea (*Camellia sinensis* (L.) O. Kuntze.) is an economically important perennial crop plant mostly cultivated in India. Tea is prone to attack by several pests and pathogens. It requires relatively higher quantum of nutrients like N, P, K. Constant uptake of nutrients will leach in the soil and the residues that remain in plant or soil limits the crop productivity that ultimately result in extensive annual crop loss. Agrochemicals are commonly used in agricultural production to control or prevent diseases, pests and weeds in order to maintain high quality of agricultural products and eliminate or reduce yield losses. With this industrialized system, tea is produced at reduced costs and farmers therefore get higher profits from their farm but serious concerns were being raised about health risks, reiterated that heavy doses of chemical fertilizer, although developmental resistance and environmental contamination. To retain sustainable agriculture, it is essential to limit the use of these harmful chemicals and replace them with eco-friendly agricultural practices. Plant growth promoting actinobacteria, also known as biofertilizers, being environment friendly and renewable, offer superior alternative to these hazardous and non-renewable fertilizers and fungicides.

Nitrogen (N) is an essential macronutrient in plant metabolism. Being a foliage crop, the requirement of N in tea soil is quite high, as N plays an important role in increasing tea productivity. N is usually met by chemical fertilizers such as urea, and sulphate of ammonia. However, high doses of chemical fertilizers have an adverse effect on crop sustainability, as well on soil health and productivity. Nitrogen



fixation *via* biological means entails the role of microorganisms. the potential of some special actinobacteria to fix atmospheric nitrogen plays a significant role to supply nutrients for the growth of plants.

Phosphorus (P) is a vital nutrient requirement in all types of plants, including tea. It plays an indispensable role in photosynthesis, respiration, energy storage and transfer, cell division and enlargement, and several other biochemical reactions in the living plant. It affects the environment, lowers soil fertility and reduces microbial diversity and function. Actinobacteria are prime microorganisms in the soil they have recently been reported to solubilize complexes of phosphate and making P available to the plant's uptake. The ability of actinobacteria to solubilize P makes them a better candidate to use as natural fertilizers.

Potassium (K) is a vital component of plant nutrition and performs a multitude of important biological functions in order to maintain quality plant growth and development and additionally helps crops to develop resistance to stress as well as insect pests and diseases. The consumption of all K fertilizers is imported, which leads to a huge amount of expenditure. Actinomycetes have the ability to solubilize the unavailable form of K-bearing minerals to release K in an available form. The production of organic acids secreted during K transformation and nutrient cycling which could create opportunities for maximum crop yield with low-cost technology.

The maximum loss of crop in tea plant is attributed to pathogens. Among the various tea diseases, leaf disease considered as important one. Such as Blister blight disease caused by *Exobasidium vexans* and Grey blight caused by *Pestalotiopsis theae*. Fungicides also gave satisfactory protection against these diseases. However, the extensive use of chemical fungicides has a harmful effect on soil health. production of phytohormone by Actinomycetes such as Indole-acetic acid (IAA), utilization of 1- aminocyclopropane-1-carboxylate (ACC), production of Siderophores, Hydrogen cyanide (HCN), Lytic enzymes and Antibiotics. These mechanisms are engaged in suppress the deleterious pathogens.

Actinomycetes have an important role in growth promoting activities and antifungal activities. All these qualities of this special group of bacteria make them inevitable tools in increasing tea productivity and quality. Moreover, there are great numbers of evidences that prove Actinobacteria as potential biocontrol agents in various crops. Considering all these aspects, it is high time that we focus on Actinomycetes as alternative tool for reducing harmful chemical usage to promote eco-friendly and sustainable farming practices.



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ANTAGONISTIC ACTIVITY OF SYNBiotics

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The human gut comprises a variety of beneficial microorganisms called the gut microbiota. These organisms help in the support of normal physiological functions of the gut. Probiotics have been selected from members of the normal healthy intestinal microflora, most of them belonging to the species *Lactobacillus* or *Bifidobacterium*. Upon ingestion, these probiotics aid in microbial modification leading to multiple health boosting properties which include the maintenance of the gut barrier and the regulation of the host immune system. It has been observed that these probiotics undergo antagonistic relationships with many bacterial pathogens and thus can be used to control their effect on our health. The use of probiotics either alone or in combination with prebiotics, has led to novel therapeutic and preventive approaches.

Probiotics and synbiotics are used to treat chronic diseases, mainly due to their role in immune system modulation and the anti-inflammatory response. A prebiotic is a high-fiber food component that provides the host with a health advantage linked with the modification of the gut microbiota. Synbiotics refers to the use of prebiotics and probiotics in tandem, but only if the net health benefit is synergistic. For gastrointestinal illnesses and other pathologies, both probiotics and synbiotics are used as therapeutic agents. They are consumed in multiple forms like cheese, yogurt and fermented food. Prebiotics are thought to aid in the treatment of



osteoporosis, obesity, and diabetes. In addition to this, it also regulates mineral absorption, energy expenditure, lipid metabolism, and glucose levels alongside preventing excessive weight gain.

Multiple studies have shown that many species of probiotics have antimicrobial efficacy against infections. According to one study, *Lactobacillus* species produce hydrogen peroxide, which inhibits *Pseudomonas* species. *Lactobacilli* with large genomes, such as *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus rhamnosus* may utilize a wider spectrum of complex carbohydrates as substrates and hence colonize more effectively than species with smaller genomes, which can only utilize simple saccharides. *Streptococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa* are all inhibited by neutralized supernatant fluid from an *Lactobacillus casei* strain.

In a study conducted, *in-vitro* growth inhibition of *Escherichia coli*, *Shigella sonnei*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Clostridioides difficile* was observed in the presence of the probiotic *Lactobacillus rhamnosus* and *Lactobacillus reuteri* and the prebiotic fructooligosaccharide. Fructooligosaccharide alone has no inhibitory effect on *Escherichia coli*. It also has no inhibitory effect on the growth of the other pathogens studied. Based on results of similar studies, multi strain probiotics should be used if a high *in-vitro* growth suppression of a broad spectrum of pathogens is desired. *Lactobacillus casei* is the most active group of strains in the presence of other alternative prebiotics, galactose-erythritol and galactose-xylitol. Another study suggested that a mannanoligosaccharide or fructooligosaccharide containing diet in mice stimulated the growth of *Lactobacillus acidophilus* and its sustenance. Fructooligosaccharide and inulin in dietary treatment showed similar effects on *Bifidobacterium lactis*. Such effects have also been observed in humans, as the administration of probiotic *Lactobacillus rhamnosus GG* with galactooligosaccharides was seen to significantly increase the amount of *Lactobacillus rhamnosus* in feces.

Probiotics appear to have a promising impact in minimising complications in critical illness. This non antibiotic approach to infectious ICU problems is of special importance, given the restricted supply of new antibiotics in the near future. A deeper understanding of the mode of action of probiotic and prebiotic components is required to improve its application to support human health without any adverse side effects. Biopreservation of blood with native Lactic acid bacteria appears as a promising tool to reduce the growth of undesirable bacteria in blood. A deeper understanding of the relationship between antagonistic metabolite development,



microbial interactions, production, and performance is necessary in food preservation success based on in situ antimicrobial metabolite formation. Newer claims suggest that they may help to prevent osteoporosis, obesity, and possibly type 2 diabetes. Probiotic efficacy has been significantly demonstrated in the prevention and treatment of antibiotic-associated diarrhoea, rotavirus diarrhoea, and allergy prevention.



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THE EXPENSIVE FUNGUS OF THE HIMALAYAS

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'Kira jari' is an Indian term for the caterpillar fungus which is now coming to light as the 'Himalayan gold'. The caterpillar fungus is mainly endemic to Tibetan alpine and subalpine meadows and is called 'Yartsa gunbu'- summer grass, winter worm. It is a parasitic fungus named '*Ophiocordyceps sinensis*' that hosts on the larvae of ghost moth (*Hepilus fabricius*) by infecting the whole body of the moth through mummification and eventually killing it. It is only found at high altitudes of 3000-5200 meters of Tibetan and Himalayan alpine meadows.

The life cycle starts when the larvae of the ghost moth get infected by the spores of the fungus. The ingested spores enter the caterpillar's torso and start consuming it from inside by spreading their hyphae forming an endoparasitic complex (Dead caterpillar + Fungus). Ultimately taking control over the brain, the caterpillar migrates towards the soil surface and settles just under the surface and gets mummified, that is, it gets converted to sclerotium which is covered by the intact exoskeleton of the caterpillar to thrive during winter. During the spring and early summer season, the fruiting body (stroma) erupts out from the shoot region of the insect and emerges to the soil surface to disperse spores and continue the life cycle. They have various traditional medicinal and pharmacological benefits among the Chinese and Bhutanese indigenous medicine. People believe that it can cure diseases like cough, anaemia, back and knee pain, rheumatism, asthma, pulmonary diseases, tuberculosis, cancer-related diseases, renal and liver diseases, sexual dysfunction. As a matter of fact, in 1964, the species was classified as a drug in the Chinese



pharmacopoeia as an aphrodisiac. Hence also called as the ‘Herbal Himalayan Viagra’. In India, they are found in the western part of the Himalayas like in Nanda Devi biosphere reserve and its alpine meadows of Dhauliganga valley, Gori mandakini, Pindar and Rishiganga valley; Askot Wildlife Sanctuary in Uttarakhand; Kanchendzonga biosphere reserve in Sikkim; Dehan-Debang biosphere reserve in Arunachal Pradesh. The highest production of caterpillar fungus is from the Tibetan region, followed by China, Nepal, India, Bhutan. The estimated annual harvest of the fungus in the Tibetan region was 45 tons and in the Himalayan region of India was 2.2 tons.

The local rural communities living in this area have got a good source of income aside from tourism. The collection of this fungus starts from early May till the end of June depending on the weather conditions and snow-cover in the collection region. The collectors set up a base camp near the collection area and stay there for 20 - 30 days depending on the availability of food and shelter. They then carefully scan the alpine meadows looking for the fruiting body above the surface of the soil and hand-pick them such that the fungus should not break and come out as a whole. The cleaning process is also done carefully with a toothbrush to remove the soil, air-dried to remove moisture and kept in a dry place until marketed. The superior quality is golden yellow, unbroken, dried completely and have short stroma than larvae and is priced individually. The damaged quality is broken caterpillar, broken stroma and is sold at lower prices. China has the highest economic gain by trading caterpillar fungus, estimated to be \$140000/kg in 2011. The economic contribution of trading this fungus in Tibet was estimated to be \$225 million/50000 kg in 2004 and in India it was estimated to be \$13571428.6 in 2012. This increase in demand and prices for the fungus cause excessive collection of the fungus which posed a threat to sustainability and resources. In India, very few efforts have been taken in the Indian laboratory and very little research has been conducted regarding caterpillar fungus cultivation, habitat and other aspects for an alternative solution. Hence there should be an increase in scientific research and investigation regarding various aspects of caterpillar fungus like the economical and medicinal importance, conservation of the species and the habitat in which it is grown and on how to reduce overexploitation of the species. Appropriate attention should be given to the rural communities that are dependent on this livelihood.



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SURVEILLANCE AND OXA 48 OF CARBAPENEM RESISTANT MDR GRAM NEGATIVE BACILLI IN HOSPITAL

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Antibiotics are major role in treating many infections. Bacterial resistant to antibiotics may possess major concern, as Carbapenem (Meropenem, Imipenem, Ertapenem, Doripenem) is reliable β -lactum antibiotics used for risk infection like Blood stream infection, Bacteremia, Septicemia. In recent year, the prevalence of OXA 48 Carbapenem Resistant MDR (CRMDR) strain are increasing hospital care setting there by increase in a causing mortality as well as treatment cost in many countries .The surveillance is necessary to curb the bacterial resistance which provides time to develop novel strategy to cope with this problem. The prospection and consecutive surveillance analysis which included 1791 gram negative bacteria were recovered from 3600 Clinical specimen from specimen from 2015-2019.The standard protocol was used for all clinical specimen and analysis. The antibiotics sensitivity pattern and Minimum inhibitory concentration for each isolated evaluated by Viteck 2 compact system.

The result of 780 (43.5 %; 780 of 1791) of CRMDR strain bacteria. In this 780 CRMDR, *Klebsiella pneumoniae* (44.4 %; 347 of 780) exhibited higher resistant strain followed by *Acinetobacter baumannii* (26.7 %; 209 of 780), *Escherichia coli* (11.1 %; 87 of 780), *Pseudomonas aeruginosa* (9.1 %; 87 of 870), *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp. (1.7 %, 1.0 %, 1.0 %) (Table - 1). These total of 780 CRMDR were



isolated from period of 5 years. One of important findings, rate of CRMDR is increased in the period of (2015 - 2019) (24.2 % - 73.3 %) (Table - 2).

Table - 1. Organism wise Surveillance of Carbapenem Resistant MDR Gram Negative Bacteria (CRMDR)

Name of the GNB isolates	No. of CRMDR isolates recovered (n = 780)	Year wise surveillance of CRMDR (2015 - 2019)				
		2015	2016	2017	2018	2019
<i>Klebsiella pneumoniae</i>	347	50	52	70	83	92
<i>Acinetobacter baumannii</i>	209	32	40	32	55	60
<i>Escherichia coli</i>	87	6	8	20	25	38
<i>Pseudomonas aeruginosa</i>	71	6	8	18	25	30
<i>Proteus spp.</i>	14	-	6	4	4	-
<i>Citrobacter spp.</i>	23	1	-	4	3	-
<i>Enterobacter spp.</i>	14	2	-	2	4	-
Total	1791	97	114	150	199	220

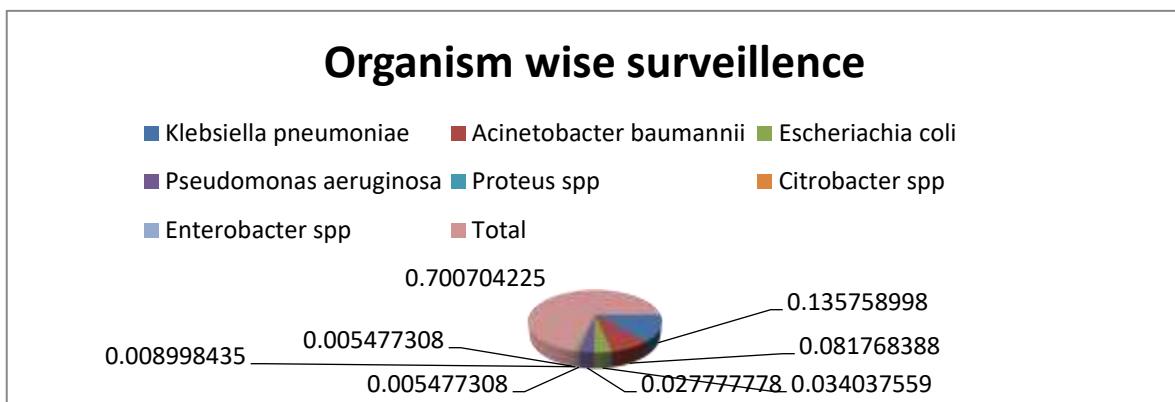
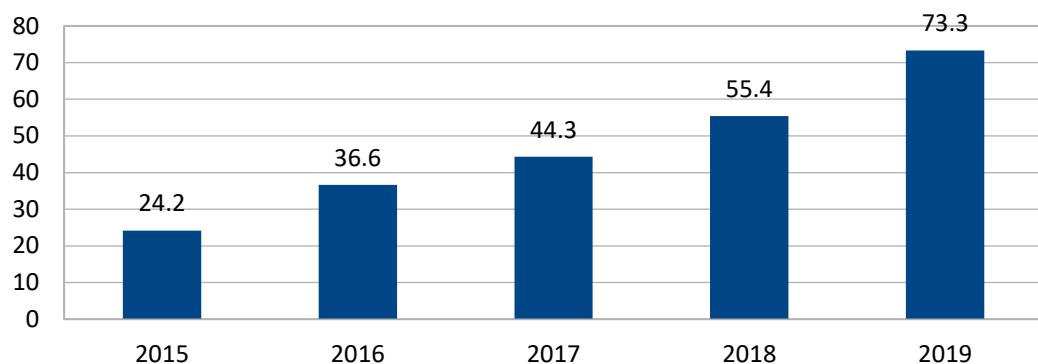


Table – 2: Year wise surveillance of Carbapenem Resistant MDR Gram negative bacteria (CRMDR)

Year wise of surveillance	2015	2016	2017	2018	2019
%	24.2	36.6	44.3	55.4	73.3



Year wise of surveillance of CRMDR Strain



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MICROBES AND HUMANS

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The surfaces of the human body inside and out, for example the skin, mouth and the intestines, are covered in millions of individual microorganisms that don't do us any harm. In fact, they help to protect us from becoming infected with harmful microbes. They are known as the normal body flora.

"Hilaire Belloc", a great poet wrote a humorous poem in the title "The Microbe" in 1912.

"We inherit every one of our genes, but we leave the womb without a single microbe" - Michael Specter

Friend or Foe?

The main microorganisms in and on our bodies are

- Protozoa
- Algae
- Fungi
- Bacteria
- Viruses

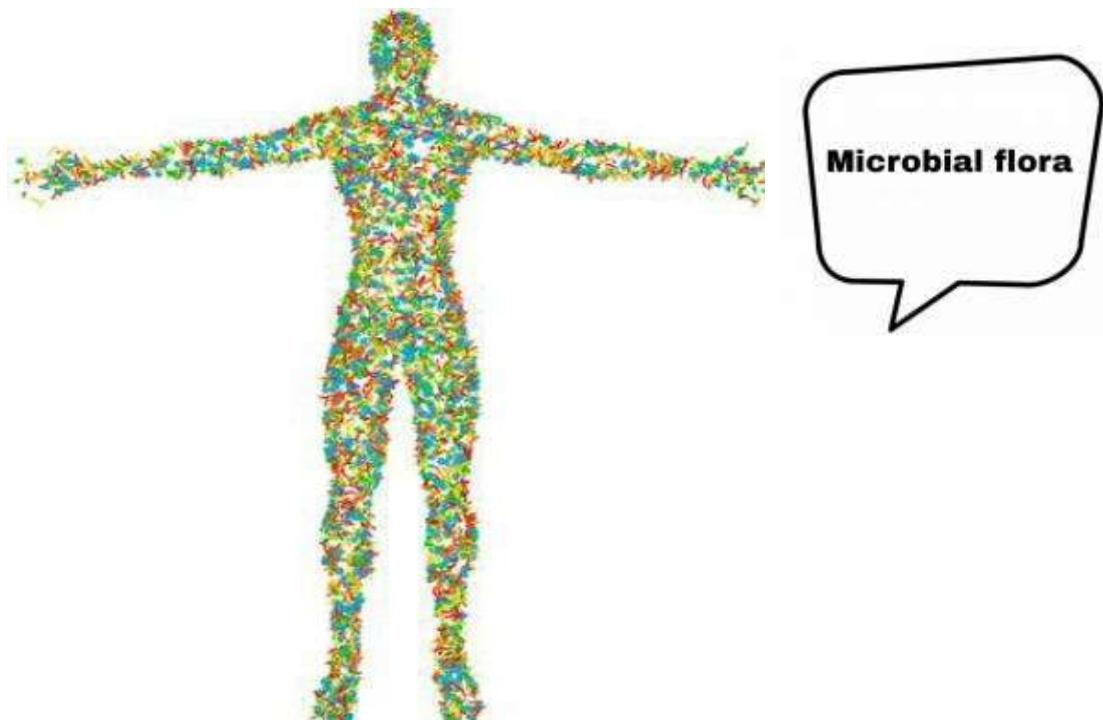
Many people think of bacteria, viruses and other microorganisms as our enemy. But, microbes are found almost everywhere and are both helpful and harmful. Microbes are essential to make many foods we enjoy, such as bread,



cheese, wine and other diary products and also microbes cause infectious diseases such as flu and measles that are the harmful.

- a) **Making Curd from Milk:** The bacterium called *Lactobacillus* reproduces in milk and helps to convert milk into curd.
- b) **Making Bread:** The fungus called yeast reproduces in flour dough and produces carbon-di-oxide during respiration which makes the dough soft and helps in making bread, cakes, biscuits, pastries, etc.

We can't see them, we can't hear them and we can't feel them, but they are with us. There are 10 trillion cells in the average body. For every cell, we have 10 times as many microorganisms – that's 100 trillion microorganisms on and in our bodies! We are a walking, talking ecosystem. But, don't worry – without microorganisms, we wouldn't survive. So, microorganisms are both Friend and Foe.



Human Interactions with Microbes

Microbial interactions are crucial for a successful establishment and maintenance of a microbial population. This include both practical and symbolic uses of microbes and negative interactions in the form of human, domestic animal and crop diseases.



Host – Microbe Relationships

- ***Pathogen:*** A parasite capable of causing disease in a host.
- ***Host:*** Any organism that harbors another organism.
- ***Symbiosis:*** An association between two or more species. Symbiosis includes
 - a) **Mutualism** - Both members of the association living together benefit from the relationship.
 - b) **Commensalism** - Two species live together in a relationship such that one benefits and the other one neither benefits nor is harmed.
 - c) **Parasitism** - One organism, the parasite, benefits from the relationship, whereas the other organism, the host is harmed by it (E.g. Bacteria, Viruses, Protozoa, Fungi and Helminths).



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ORAL MICROBIOME AND ITS DETECTION METHODS

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Microbiome is a term used to describe the bacteria that live in our bodies. Joshua Lederberg, a Nobel Laureate, coined the word "microbiome" to describe the existence of an interacting group of commensals, symbiotic, and pathogenic microorganisms which are literally present everywhere in human body. Genome of all microorganisms that reside in the oral cavity is called oral microbiome. Oral cavity contains diverse microbiota comprising over 700 unique bacterial species as mentioned in Table - 1. Various surfaces in the oral cavity are coated with a wide variety of bacteria forming a bacterial biofilm. Some of the bacteria among them are implicated in diseases like periodontitis and caries, which are the most common bacterial infections seen in the human mouth.

Microscopy and Culture

The detection of bacterial taxa was previously done using culture-dependent methods. Microscopy, biochemical and other phenotypic analyses, sugar utilization, growth conditions, and antibiotic sensitivity were among the tests performed. Culture-based approaches were unable to fully demonstrate the true diversity of the oral microbiome. Presently efforts of numerous researchers have resulted in isolation, cultivation, identification, characterization and classification of nearly 50 % bacterial species. They have found over 700 bacterial species in the oral cavity. The problem



with traditional culture methods and culture-based analytical technologies is that many bacterial species found in biological samples cannot be cultured, rendering these methods unsuitable for analysis.

Table – 1: Different Sites in oral cavity and oropharyngeal region harbouring bacterial species

Different Sites in Oral Cavity and oropharyngeal Region	Major bacterial species
Tongue	<i>Capno cytophaga, Porphyromonas gingivalis, atypica, Selenomonas sp., Enterococcus faecalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia and Eikenella corrodens</i>
Oropharynx	<i>Streptococcus pyogenes, Streptococcus mutans, Streptococcus pneumoniae, Haemophilus influenzae, Streptococcus mutans, Streptococcus anginosus, Haemophilus parainfluenzae and Streptococcus salivarius</i>
Tooth Surface	<i>Actinomyces, Streptococcus mutans, Eubacterium and Peptostreptococcus.</i>
Tonsil	<i>Staphylococcus, Streptococcus viridans, Haemophilus influenzae and Neisseria sp.</i>
Gingival crevice	<i>Porphyromonas, Fusobacterium, Prevotella, Veillonella parvula, Streptococcus mitis, Streptococcus sanguinis, Propionibacterium acnes, Leptotrichia buccalis and Actinomyces odontolyticus.</i>
Dental Plaque	<i>Rothia, Actinomyces, Microbacterium, Corynebacterium, Mycobacterium, Propionibacterium and Bifidobacterium.</i>

Molecular Methods

Due to availability of several culture-independent techniques and Gel-based techniques, it is possible to do microbial community review at a high throughput by using Denaturing Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE), and Restriction Fragment Length Polymorphism (RELP) techniques. Various polymerase chain reaction-based methods available for the identification of microbes are PCR-DGGE, random amplified polymorphic DNA, arbitrarily primed PCR, multi-locus sequence typing, repetitive element-based PCR, PCR RELP, and terminal RELP are all terms for polymerase chain reaction (PCR).



Metagenomic Analysis of Oral Microbial Diversity

In recent times, the greatest advance in the development of culture-independent methods are probably “Omics” methods. This involve researching the entire microbial community's DNA, RNA, proteins, or metabolites. Microbiomics and Metagenomics are two emerging research areas that seek to detect and classify the role of microbes in the body as well as the existence of microbiome activity in health and disease . Metagenomics not only gives information on the kind of organisms present but also on their potential function through, analysis of metabolic pathway genes. It can also provide information on the use of protein-coding sequence databases and sequence the entire DNA of a given sample. The oral microbiome is undoubtedly, well-studied human microbiome till date due to easily obtainable samples. Human microbiomes have co evolved over the course of millennia, and under homeostasis, coexist in a symbiotic relationship. Perturbations to the human microbiome, however, can result in imbalance, potential dysbiosis, and disease.



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MIDDLE EAST RESPIRATORY SYNDROME (MERS)

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Middle East Respiratory Syndrome Corona Virus (MERS) are highly transmissible and pathogenic virus that emerged in humans at the beginning of 21st century. This virus originated in bats and genetically diverse corona viruses that are related to SARS and MERS were discovered in bats worldwide. MERS were transmitted directly to humans from market, civets and dromedary camels. Corona virus are members of sub-family Coronavirinae in the family Coronaviridae and the order Nidovirales. MERS is a novel Corona virus discovered in 2012 and is responsible for Acute Respiratory Syndrome in humans. The disease is heavily endemic in dromedary camel population of east Africa and the middle East through bats and alpacas can serve as potential reservoir for MERS virus. Dromedary camels seems to be the only animal host responsible for the spill over human infection.

MERS virus is a lineage C beta corona virus with a genotype that is very closely related to bat coronaviruses from the same lineage such as Btcov-HKU4 and Btcov - HKU5 through its evolutionary pathway is still unclear. In Saudi Arabia bat fecal samples collected in October of 2012 were tested for MERS RNA. Nearly 4 years after the first report of the emergence of Middle East Respiratory Syndrome Corona virus in humans and more than 1800 cases later. A number of studies on wild birds and swine in Hong Kong fecal camels in Australia and bats in several countries have not identified MERS in these species. Putative precursors of MERS



have been detected in species of African bats. MERS virus has high homology with bat coronavirus HKU4 or HKU5 and its polymerase Gene (RdRP) has 90 - 92 percent amino acid homology with the bat corona virus HKU4 or HKU5. MERS virus was isolated secretions obtained from the nose of a camel and those who were infected with to this virus we're closely contact with camels suggesting that camels served as an intermediate host for the transmission of MERS virus. The MERS virus receptor is an exopeptidase dipeptidyl peptidase IV (DPPIV) also known as D26 which is a multifunction glycoproteins. DPP IV us highly expressed in liver, kidney, intestinal epithelial cells and prostate. MERS were more visible than SARS in pleural effusion and the pneumothorax.

Since, the discovery in 2012 Middle East Respiratory Syndrome Corona virus (MERS virus) has infected over 2,000 people and has mortality rate of approximately 35 percent. Adaptation of MERS spike has been a challenge in the field as DPP4 from most non- permissive species contains glycosylation that completely abrogates the interaction as and block infection. Without viral replication it has not been possible to experimentally demonstrate evolution of the MERS virus spike DDP4 was only semipermissive viral infection and therefore served as a tool to test MERS virus spike adaptation *in vitro*. Now, it is widely accepted that many viruses have existed in their natural reservoir for a long time. The constant spillover of viruses from natural hosts to humans and other animals is largely due to humans activities including modern agricultural practices and urbanization therefore the most effective way to prevent viral zoonosis is to maintain barriers between natural reservoir and human society in mind of the one health concept.



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LANTIBIOTIC NISIN FROM *Lactococcus lactis*

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Certain strains of *Lactococcus lactis* subspecies, *lactis* produce bacteriocin Nisin (or group N inhibitory substance) discovered by Rogers and Whittier in 1928 in England. Nisin, a potent bacteriocin of length 35 amino acids belonging to the group of lantibiotics, has been commercially used worldwide in food and dairy industries. It has minimized sulphur dioxide in the food industry and prevents the spoilage of certain foods. Being a reducing agent maintains the antioxidant benefits of wine polyphenols. Nisin biosynthesis occurs during the bacterium's exponential growth phase and is completely inhibited when the cells enter the stationary phase. It has cationic and hydrophobic characteristics. The molar mass of Nisin is 3.5 kDa. The functional properties of Nisin might be because of the presence of unusual amino acids (a characteristic feature of lantibiotics). There are two variants of *Lactococcus* produced Nisin (A & Z), which differ by single amino acid substitution at the 27th position. There is no known effect on the antimicrobial activity of Nisin, but it imparts higher solubility and diffusion characteristics to nisin Z when compared with Nisin A.

Production of Nisin

Nisin production is a very tedious process and is affected by various cultural factors such as pH, temperature, and other factors like enzymatic degradation and inhibition. Nisin can be produced using various methods and media such as



fermentation of milk skimmed and milk whey by *L. lactis*, by using culture media like Man Rogosa and Sharpe medium (MRS), Complete Medium (CM). However, all these methods are expensive and cannot be utilized for commercial production. Industrially barley extract, corn soluble and sago starch have been used to reduce production costs. The production of nisin and lactic acid simultaneously using corn stover by simultaneous saccharification and fermentation with *L. lactis* subspecies *lactis* ATCC 11454 is considered as beneficial in industries.

Extraction and Purification of Nisin

Three different isolation methods have been described, i.e.,

- a) Precipitation by ammonium sulphate
- b) Chloroform extraction
- c) Cation exchange chromatography

Out of these three methods, ammonium sulphate precipitations yield the highest amount of the product (experimentally proved).

Mechanism of Antimicrobial Action

The significant steps involved in killing the susceptible cells are

- a) Passage through the cell wall
- b) Interaction with lipid II

A significant advantage of using Nisin is that it does not require any membrane receptor on the target cells.

Antiviral properties of Nisin

- a) Effect on Bovine Viral Diarrhoea Virus - The studies clarified that there was no significant influence on direct contact of virus with Nisin, but it reduced the viral yield at the adsorption stage when Nisin was present in the culture medium throughout the entire course of infection.
- b) According to studies, Lactococcus derived Nisin A and nisin Z effectively interacts with the human endothelial cell surface receptors hACE2 to which SARS-CoV-2 binds to begin the infection. Nisin binds with a higher affinity and could prevent the entry of the virus in the host cell.



Antibacterial Properties of Nisin

Nisin is effective against various bacteria and is used in pharmaceutical industries for different purposes. It is an effective bactericide against Gram-positive bacteria but is ineffective against Gram-negative bacteria due to outer membrane barriers. Nevertheless, if Nisin is combined with substances (cider or cinnamon) or physical treatments, it can quickly destabilize Gram-negative bacterial membranes.

Antifungal Properties of Nisin

It has been suggested that Nisin Z (concentration – 500 µg/ml) leads to ultrastructural disturbances of *Candida albicans*, thus affecting the growth and transition from blastospore to hyphal forms. Researchers have proposed that Nisin can exhibit antifungal activities similar to against Gram negative bacteria if used in combinations. Still, there is no experimental proof for the same yet.

Nisin in Cancer Treatment

Experimentally, it has been explained that the cell viability of SW1088 human cell lines was down-regulated by Nisin, and the level downregulation was dependent on dosage.

Applications and Advantages

- Nisin naturally preserves food, cheese, butter & it can either be used alone or in combinations.
- Medical Applications include effective behaviour in Atopic dermatitis.
- Nisin is widely used in nanotechnology also as a food bio preservative.

Conclusion

Antimicrobial peptides like Nisin have always been an exciting point for researchers as an alternative for antibiotics. Many bacteriocins produced by bacteria are toxic to the cells of mammals, but in Lactic acid bacteria, the bacteriocins produced have negligible or no side effects at all. Nisin is widely used in industries as a food preservative. It holds many chemical and physical properties. The presence of unusual amino acids in the structure possibly imparts antifungal, antiviral, and antibacterial activities. Only Nisin has received GRAS (generally regarded as safe) status by FDA and employed in various sectors. Further research can bring both benefits and flaws in nisin utilization.



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ANTIBIOTIC RESISTANCE IN GRAM POSITIVE BACTERIA

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The accidental discovery of antibiotics was considered as a milestone in the research field. It has established a new era in science. But along with the boon of antibiotics, emergence of resistance against the antibiotic, followed the discovery like a curse. A rise in Multi-Drug Resistant (MDR) bacteria has created difficulty in treating the infections with the currently available antibiotics. Various gram-positive bacteria like Vancomycin resistant *Enterococcus faecium*, Methicillin resistant *Staphylococcus aureus* and drug resistant *Streptococcus pneumoniae* are the major villains in nosocomial infections. The resistance against the antibiotics usually arises due to the genetic changes. Antibiotic resistance has increased the mortality and morbidity rate.

The antibiotic resistance in gram-positive bacteria is usually observed occurring by two major strategies

- a) Enzymatic degradation of antibiotics
- b) Decreasing the affinity and susceptibility of their target site.

Certain mechanisms of antibiotic resistance are explained below and depicted in Figure – 1.



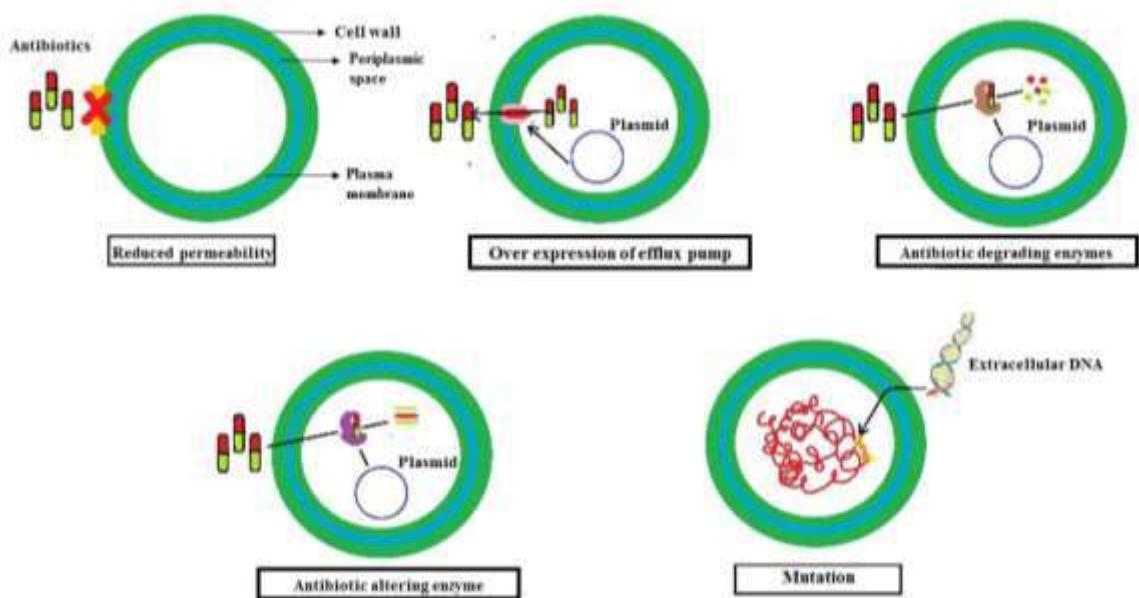


Fig. 1. Mechanism of resistance in Gram Positive Bacteria

- a) β -lactams attack Penicillin Binding Proteins (PBPs) which ultimately lead to cell death. The resistance is acquired by the degradation of antibiotics by β -lactamases.
 - ✓ Penicillin resistance can be plasmid based, bacteriophage based and horizontal gene transfer based.
 - ✓ Methicillin resistance can be acquired from foreign DNA element uptake.
- b) Vancomycin and Teicoplanin inhibit the last stage of cell wall synthesis. Resistance to such glycopeptides is by acquiring related degradative gene cluster.
- c) Mutation of the genes for DNA gyrase and Topoisomerase imparts resistance against Quinolones.
- d) Efflux mechanisms help in providing resistance against Aminoglycosides.

Effect of Biofilms on Antibiotics

The surface associated bacteria along with the surrounding autogenic extracellular matrix that form complex communities are known as biofilms. Biofilm forming bacterial communities can grow adhered almost to every surface and are cause of many bacterial infections. In health-care systems *Staphylococcus* genus is the major reason of biofilm associated infections. Out of which *S. epidermidis* is the most common nosocomial pathogen isolated from medical surfaces and *S. aureus* being associated with serious infections to host cells like osteomyelitis. The Real-time cell



analysis (RTCA) assays that were previously used for analysis of eukaryotic cells were recently used for studying biofilms and effect of antibiotic treatments on their growth. The real-time dose response studies conducted by various researchers on *Staphylococcus* isolated from various clinical lesions elucidated on the inability of a few antibiotics to kill or even inhibit the biofilms providing resistance to the bacteria against that antibiotic. But certain antibiotics like RNA polymerase inhibitors and DNA gyrase inhibitors are found to be more effective. But many of these antibiotics including the ones mentioned above lack the ability to destroy or inhibit already formed biofilms. Though effective on planktonic bacteria, in the case of already formed biofilms, the antibiotics are only able to liberate the bacteria from the biofilm to an extent but are ineffective in inhibiting these biofilms.

Overcoming Resistance in Gram positive bacteria

Given below are a few approaches against MDR Gram-positive bacteria.

- a) ***Novel Antibiotics*** - Teixobactin (from *Eleftheria terra*), Malacidins (from soil microbiome), Antimicrobial peptides, Cannabinoids, Odilorhabdins, etc. are novel compounds highly effective against Gram positive bacteria.
- b) ***Bacteriophage Therapy*** - The idea of bacteriophage therapy subsided the surge of antibiotic discovery during the golden age. Bacteriophages can also be used for disrupting biofilms. Bacteriophage-antibiotics combined therapy has been suggested as it is highly effective against Gram positive bacteria.
- c) ***Probiotic Approach*** - Live microbes (usually from the genera *Lactobacillus* and *Bifidobacterium*) when administered in adequate quantities impart health benefits to the host. They can reduce the spread or evolution of antibiotic resistance.
- d) ***Educational Approach*** - The improper usage or overuse of antibiotics is the major cause of emergence of antibiotic resistance. Educating the prescribers and the general public about the proper usage of antibiotics and antibiotic resistance may help in decreasing the rise of antibiotic resistance.
- e) ***Maintaining Sanitation of Medical Devices*** - Proper cleaning and maintenance of sterility of medical devices can reduce the incidence of biofilm formation by nosocomial pathogens, thereby reducing the overuse of antibiotics in the case of infections.



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ENDOPHYTIC FUNGI AND THEIR SIGNIFICANCE

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Fungi are important group of eukaryotic microorganisms. They are known for their heterotrophic nutrition and survive as saprophytes or symbionts or pathogens. Plants do carry fungi inside their tissues referred as endophytic fungi. The major criterion considered for endophytic fungi is their inability to cause disease in plants. These endophytic fungi are exceptionally significant in determining plant health. In various ways these fungi help the plant to withstand adverse abiotic and biotic factors. They establish themselves in association with plants by colonizing either inter- or intra-cellular regions of the plant tissues. Generally, presence of these fungi never causes any ill-effect on the host plant. Instead, such association proved beneficial in various ways. These fungi are phylogenetically diverse groups of organisms. They establish remarkably stable, long-term relationships with plant hosts. Plant microbiome, genetics of host plant species, cultivation methods and other environmental factors influence the successful establishment of these fungi. Most endophytic fungi are capable of infecting several host plants. Some are host-specific and few are organ-specific. Chemical communication between endophytic fungi and plants is particularly vital in determining successful mutualism. The interaction is highly complex owing to many factors associated.



Mutualistic interactions between endophytic fungi and their host plants have beneficial impact on both. The fungi acquire food and shelter whereas series of useful effect have been assigned to plants. The endophytic fungi trigger good growth in the plants due to production of growth hormones like gibberellins, indole acetic acid and cytokinins. The additional amounts of such growth hormones induce positive response in host plants to survive under adverse conditions.

Presence of endophytic fungi in plant tissue induces general defense mechanisms of plants against many pathogens. Such response can make the plant resistant to infection or show reduced disease symptoms when infected by pathogens. A series of antimicrobial compounds produced by endophytic fungi can inhibit the plant pathogenic fungi and bacteria. Hence, such fungi are also gaining importance in agriculture.

Some of the well-known endophytic fungi are *Phomopsis* sp. from *Tectona* sp., *Cladosporium* from wheat, *Colletotrichum* from Citrus plants, *Phyllosticta* from *Centella* sp., *Penicillium* from tomato, *Taxomyces* and *Acremonium* from *Taxus* sp. Many hydrolytic enzymes like proteases, cellulases, amylases, and pectinases are being produced by these fungi. Mycodiesel is also obtained from endophytic *Gliocladium*. Interestingly; endophytic fungi have been known to produce several types of therapeutic compounds used in human medicine. Arrays of secondary metabolites with various biological activities have been found in such fungi. The biological activities of metabolites include anti-microbial, anti-oxidant, anti-diabetic, anti-pyretic, anti-cancerous, insecticidal, immunomodulatory etc. Enzyme inhibitors are also found in endophytic fungi. Most important biological active metabolites produced by endophytic fungi are Taxol, podophyllotoxin, deoxypodophyllotoxin, camptothecin, hypericin, emdoin, azadirachtin, subglutinols etc.

Medicinal plants are gaining importance due to several phytochemicals associated providing health benefits. Such phytochemicals with therapeutic properties are also produced by endophytic fungi. Hence, isolation and characterization of biological active compounds from endophytic fungi have expanded significance. Different compounds produced by endophytic fungi within the plants may get altered due to complex conditions in the tissues. Endophytic fungi have potential to produce the compounds by themselves or capable of converting precursors from plants. Based on such information protocols have been developed to produce biological active compounds at industrial level. Fungal fermentation technology is already available for such purposes.



Enormous competence of endophytic fungi has been realized. Biotechnological advantages of this elite group of fungi will provide more beneficial compounds. Medicinal properties of endophytic fungi have greater prospective for commercial production. Biosynthetic potential of endophytic fungi needs to be studied in order to make successful production *in vitro*. The molecules produced by endophytic fungi may act as lead molecules in the synthesis of novel biologically active compounds.



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MYCOTOXINS ASSOCIATED PROBLEMS IN SORGHUM

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Sorghum (*Sorghum bicolor*) is the third major staple food crop in India and fifth major cereal in the world. Some of the fungal pathogens and storage fungi associated with seed borne disease of sorghum are capable of producing the secondary metabolites-mycotoxins which are harmful to human and animal health. Hence the study of mycotoxins effects associated with seed borne diseases of sorghum is important in maintaining the grain quality and grain nutrition for the purpose of consumption.

Mycotoxicosis

The diseased or unhealthy condition caused by the consumption of such mold contaminated sorghum grains/products in humans and contaminated feed in animals lead to mycotoxicosis. The seed borne fungi responsible for mycotoxin production in sorghum are listed in the following Table - 1 and the general structures of mycotoxins are represented in Figure - 1.



Table – 1: Mycotoxin producing Seedborne Fungi

Fungi Detected	Mycotoxins produced
<i>Aspergillus</i> spp.	Aflatoxins
<i>Fusarium</i> spp.	Fumonisins
<i>Penicillium</i> spp.	Ochratoxins and Citrinins

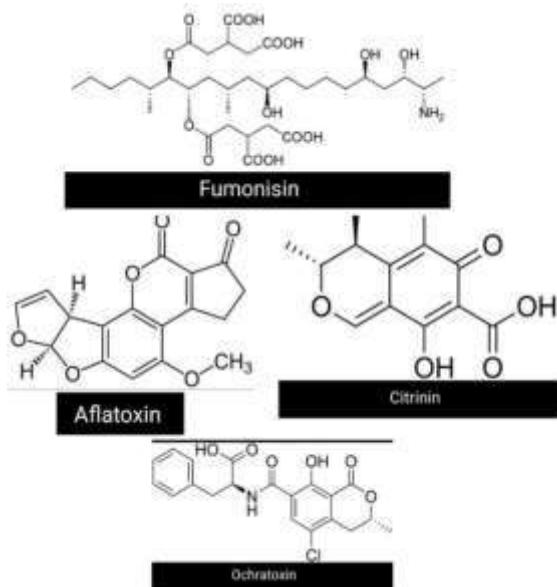


Figure – 1: Structures of Mycotoxins associated with Sorghum

Effects of Mycotoxins

- Aflatoxin:** It causes DNA mutations, post-translation peptide chain modification, protein and nucleic acid methylation, formation of free radicals and is carcinogenic in nature.
- Fumonisin:** It causes cancer and birth defects, induces cell proliferation in liver and kidney leading to cancer, hepatocellular cancer, esophageal cancer etc.
- Citrinin:** It causes nephrotoxicity, hepatotoxicity, embryotoxicity, cytotoxicity, immunotoxicity and carcinogenicity. It also induces reactive oxygen species.
- Ochratoxin:** It is embryotoxic and immunotoxic in nature. Also causes nephrotoxicity and carcinogenicity in human and animals. Also induces renal tumors and chromosomal aberrations in kidneys.





Conclusion

Mycotoxin residues may contaminate the ready-to-eat foods posing health hazards. Mycotoxins may affect infants if such grains are used in the infant food preparation. It is essential to ascertain mold association in sorghum to prevent possible Mycotoxicoses.

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FUNGAL PIGMENTS AND THEIR APPLICATIONS

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Colour of nature is exemplary. Biological pigments provide an array of colours to most organisms. A pigment is a substance emitting the colour by absorbing particular wavelength of light. Most of the pigments play an essential role in the life of organisms. The plants appear green because of chlorophyll pigment which is used for trap solar energy in generation of food during photosynthesis. Haemoglobin protein offers red colour to the blood in most animals and helps in carrying the oxygen through the circulation.

The pigments are produced by plants, animals and microorganisms. In spite of their biological significance pigments are also used by humans for different purposes. Earlier, the plant pigments were considered useful in imparting colour to food. People started using colours in food, cloth, painting and drug. The colour to the range of products enhanced aesthetic beauty and provided additional biological activities in some cases. Ever increasing demand for the pigments made chemists to synthesize various chemical pigments. Of late, it is proved that the synthetic pigments have many adverse effects such as immunosuppressive and carcinogenic in nature. Such chemical pigments act as pollutants in the environment. In view of this, some of the synthetic pigments are now banned. These lead scientists to look for alternate sources of pigments mainly of biological origin. The nature and quantity of plant based pigments may get affected by seasonal variations and place of cultivation. The pigments from microbial sources are gaining importance because



they can be produced easily using simple affordable raw materials and technology. Additionally, microbial pigment production has advantage over plant pigments in terms of isolation and purification protocols.

The microbial pigments are obtained from algae, bacteria and fungi. Among these, the fungal pigments are of great significance in terms of variety and biological activities. The ascomycetes and basidiomycetes fungi are known to produce unique range of secondary metabolites including pigments. The fungi belonging to the genus *Aspergillus*, *Fusarium*, *Monascus*, *Penicillium*, *Paecilomyces* and *Trichoderma* were majorly used for the production of pigments. The pigments produced by fungi belong to aromatic polyketide groups such as azophilones, flavonoids, quinones and melanins.

The fungal pigments are widely used in different industries such as textile, food, leather, cosmetics and pharmaceutical industries. Ideal pigment producing fungus should have characteristics for industrial applications. The pigment should be non-toxic to animals and humans. At the same time the fungus should use simple available raw materials and amenable for large scale cultivation.

In the recent days, many food grade pigments obtained by fungal fermentation process are already available in the market. Among them, ankaflavin and canthaxanthin by *Monascus* sp., Natural RedTM from *Penicillium oxalicum*, riboflavin from *Ashbya gossypii*, lycopene and β-carotene from the *Blakeslea trispora* are greatly useful with commercial value.

Fungal pigments have gained importance in pharmaceutical industry as anti-microbial, anti-oxidants, anti-cancerous, and cytotoxic agents. It has been demonstrated that the pigments produced by the *Aspergillus*, *Fusarium*, *Monascus*, *Penicillium*, *Talaromyces* and *Trichoderma* species possess antimicrobial activity against pathogenic bacteria and fungi. The pigments such as carotenoids, naphthoquinones and violacein are known as potential antioxidants. Pigments from *Chaetomium* sp., *Fusarium* sp., *Penicillium* sp. *Sanghuangporus baumii*, *Stemphylium lycopersici* and *Thermomyces* sp., are known to be potential antioxidant activity to be exploited in healthcare industry. Cytotoxic activity of the pigment produced by *Chaetomium* spp., *F. oxysporum* and *T. verruculosus* provide prospect in pharmaceutical industry. Anti-tumor activity of fungal pigments provided way for drugs to be developed. The pigments such as ankaflavin, monascin, monaphilone A–B, monaphilone A–B, monapilol A–D, and monapurone A–C from *Monascus* species confirmed the anti-cancerous activity against various types of cancers.



The textile industry highly depends on the synthetic pigment for dyeing the fabrics. Recently, the natural pigments from fungi have proven to be good alternative to dangerous synthetic pigments. Fungal pigments are eco-friendly, biodegradable, and non-toxic, provide intensive colour range and show high staining capability. The pigments produced by different fungi such as *Acrostalagmus*, *Alternaria*, *Aspergillus*, *Bisporomyces*, *Chlorociboria*, *Cordyceps*, *Cunninghamella*, *Curvularia*, *Fusarium*, *Monascus*, *Penicillium*, *Phymatotrichum*, *Scytalidium*, *Talaromyces*, *Trichoderma* and *Thermomyces* are being used in textile industry for dyeing fabric such as wool, cotton yarn, silk, polyester and nylon. The red pigment from *Scytalidium cuboideum*, yellow pigment produced by *S. ganodermophthorum* and green pigment from *Clorociboria aeruginosa* have been useful for dyeing different fabrics. The fungal pigments showed no adverse effects on fabric and found to be non-toxic to human skin.

Even in cosmetic industry natural products have to be replaced the synthetic pigments. The fungal pigments such as carotenoids, melanin, lycopene etc., have been found valuable for the anti-aging facials, cosmetics, face creams, sunscreens, sun lotions, sun blocks, etc. Inspiringly, the pigments from fungi like *Monascus* spp., *Penicillium* and other fungi have already entered into the market for their use in cosmetics like lipsticks, skin conditioning and skin care products.

Indeed, fungal pigments as colouring agents in industries have opened up an eco-friendly, sustainable and non-toxic compounds of biological origin. Sooner they should replace the toxic synthetic pigments in order to provide safe colourants. Biotechnological applications of fungal pigments ensure the quality and quantity of them in food and pharmaceutical industries.



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OSMOPHILES: SUGAR LOVERS

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Microorganisms are able to grow over a comprehensive range of solute concentrations. Individually few species are able to grow at high osmotic pressure, characteristic of environments having either supersaturated salts or sugar concentrations. The osmotic concentration of the environment affects the water that is obtainable to a microorganism. The greater the solute concentration of the environment, the less readily obtainable the water. Some prokaryotes can maintain the availability of water in environments with high solute concentration by increasing the solute concentration within the cell.

When water activity of the surrounding environment of cell becomes low enough then it causes osmotic stress/pressure, at that time cell cannot take up water and becomes dormant. All microorganisms have not ability to grow in presence of high osmotic pressure, microorganisms which have ability to tolerate such environment are called as Osmophiles. Osmophilic microorganisms are able to grow at low water activity values, e.g. 0.85 or less. Microorganisms, that are able to grow in this type of environment it's called Osmophiles. Osmophiles are the microorganisms that able live environments with high osmotic pressure. They are loving environments with low water activity like high sugar concentrations. The terms "xerotolerant" and "osmotolerant" have been used in place of "osmophilic" because these organisms do not have an absolute requirement for reduced aw or high osmotic pressure; rather, they only tolerate drier environments better than other microorganisms. However, many microorganisms are not able to grow because these high sugar concentrations serve as a growth-limiting. Nevertheless, osmophiles



principally yeast and some bacteria can able to guard their cells against high osmotic pressure generated by these high solute concentrations.

Natural habitat of Osmophiles is high sugar containing environment. Decaying fruit and cane, floral nectar, honey, dates, sugar beet juice and aphid exudates are examples of natural high-sugar environments. Natural habitats of Osmophiles, high in sugars are mainly limited to dried fruit, floral nectar, honey, sugarcane, dates, and associated soils. Osmophiles are also present in chocolates, sugar confectionery, liquid sugars, sugar syrups, jellies, creams, hard candy, soft candy, caramel, toffee, marzipan, nougats. Honey is recommended to be a reservoir for bacteria with osmophilic nature. A common intrinsic parameter associated with high sugar products is their low water activity, which is known to inhibit the growth of most spoilage bacteria. The water activity range for high sugar products is between 0.20 and 0.80. To consumers, the term sugar is usually associated with sucrose, which is widely distributed in nature and used extensively in products throughout the food industry. However, dextrose, lactose, and fructose are also used. More recently, polyols like sorbitol, mannitol, xylitol, and maltitol have become replacements for sugar in sugar-free confectionery and chocolate products and many other food products. More seriously, Osmophiles can cause spoilage in sugar, fruit juice, concentrated fruit juice, liquid sugars, honey, chocolates, dairy dessert etc. Moreover, they synthesize Osmoprotectants including sugars, alcohol, amino acids, betaines, ectoine, and polyols as an adaptation to these environments. Normally, osmoprotectants compatible solutes, which are either neutral or zwitterionic. They behave as osmolytes, which maintain fluid balance and cell volume. Stable high-sugar environments are uncommon. Commercial food production generates high-sugar streams like sugar thick juice and maple syrup. Osmotolerant and osmophilic bacteria and yeast are known from high-sugar environments. These are environments that can have very high osmotic pressure, with sugar contents as high as 70-80%, thereby creating low water activities.

A lot of researchers are looking forward to express the osmophiles because of their economic importance in industry. Osmophiles have many applications. Osmophiles have probiotic properties with antimicrobial activities. Osmophiles are also able to produce exopolysaccharides for example levan. Osmophiles are also widely used for the production of alcohol.



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GUT FEELING: HOW OUR SECOND BRAIN INFLUENCES OUR MOOD AND BEHAVIOR? A NEUROSCIENTIFIC INSIGHT

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Gut feeling or intuition is a complex phenomenon to study and even trickier to define. A sixth sense which unexpectedly pops into our awareness which we can't describe but that appears reliable and gives confidence that we blindly incline toward that decision making state. This gut feeling likely comes from a mixture of non-conscious sensory perceptions and past experiences. The brain is the site of intelligence, which interprets our sense, initiates the body movement, and controls our behavior. The gut is our Gastro-Intestinal (GI) tract which constitutes several organs that start from the mouth to the anus including our digestive system. It is driven by the supremely powerful ecosystem called Gut Microbiota (GM) which influences our brain as well the metabolic processes. This plays a key role in normal enteric physiological processes, including digestion, motility, epithelial homeostasis, production of vitamins, etc. Possibly, the gut microbiota may affect the immune and endocrine systems, and the dysbiosis of the gut microbiota causes several issues including allergies, functional bowel disorders, asthma, obesity, diabetes, etc. The gut is a highly innervated organ with its neural system known as the Enteric Nervous System (ENS), having an intricate network of over 100 million neurons embedded in the gut wall which communicates with the central nervous system via vagus nerves, which connect parts of the gut to the brain and sends signals in both directions.



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There are various mechanisms which have been found to be connected with the brain, which comprises of production of various neurochemicals as well as neurotransmitters or the variation of host neurotransmitter catabolism, innervations via the vagus nerve, or activation of the Hypothalamic Pituitary Adrenal (HPA) axis. Gut bacteria play a key role in stress-induced increase in inflammation. Stress is a factor preventing the signals sent through the vagus nerve and can cause gastrointestinal glitches. The GM produces neuroactive compounds such as catecholamine, histamine and which can stimulate host neurophysiology either directly through interaction with receptors within the GI tract or indirectly through absorption/passive diffusion through the gut wall and into the portal circulation. Certain gut bacteria can produce a wide variety of physiologically active chemicals such as neuromodulators and neurotransmitters, which our brain uses to control basic physiological processes as well as learning, memory, and mood-like mental processes.

Gut bacteria supply nearly 95% of the body's required serotonin, which can affect both mood and GI activity. They also produce and respond at the same time to other neurochemicals such as Gamma Amino Butyric Acid (GABA), Norepinephrine, Dopamine, Acetylcholine, and Melatonin. These allow the brain to tune its actions to the response it receives from the gut ecosystem. Dopamine is one of the chief neurotransmitters that exhibits a reward-driven behavioral pattern. This is a predecessor for other catecholamines, such as norepinephrine and epinephrine. Norepinephrine acts for arousal and signal detection as well as for reasoning and attentiveness. It is also responsible for happiness and maintains the body clock. Serotonin involves gastrointestinal secretion, respiration, vasoconstriction, behavior, and neurological function. Though serotonin is generally used all over the body, 90 – 95 % of serotonin exists in the gastrointestinal tract only. Our GM also produces a lot of Short-chain fatty acid (SCFA) by digesting fiber we intake, which further affects the brain functionality concerning the offering from the high energy foods with controlling our appetite. GABA is one of the major inhibitory neurotransmitters of the Central Nervous System (CNS). There are substantial numbers of literatures available that depicts the link between GABA and CNS and also shows the consequences including CNS disorders.

Our gut-brain axis is also connected through the immune system. If our immune system keeps turned on for long, then it can cause inflammation which may lead to depression and Alzheimer like diseases. There are some inflammatory toxins made by certain bacteria which are barricaded by GM from the gut to the blood. It has been demonstrated that cerebral metabolites are regulated by normal GM



through the microbiota-gut-brain axis and signified that the GM is strongly related to brain health and illness, development, behavior, memory, and learning. Therefore, the understanding of these peptides and receptors is important for treating pathological diseases caused by stress, including obesity and metabolic syndromes.

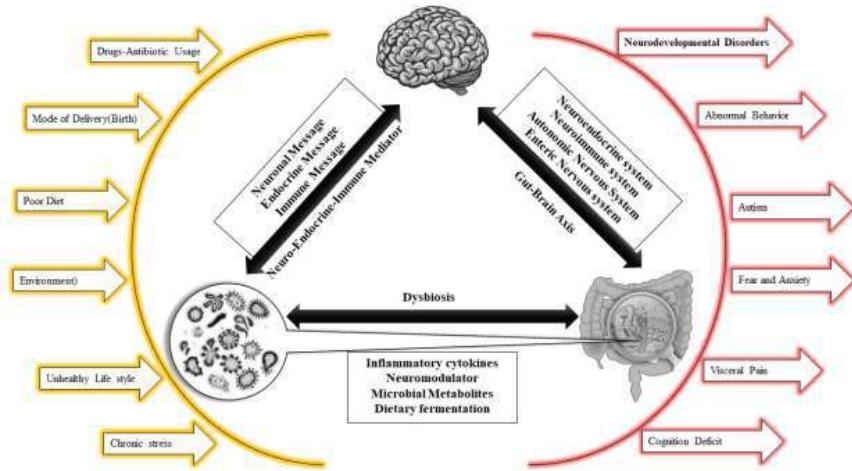


Figure – 1: Human microbiota - gut-brain axis

Modulation of this axis is growing day by day as this is a suitable target for the novel treatments for a wide variety of neurodevelopmental disorders as both mental illnesses and neurological diseases stay associated with abnormality. A Chinese Study showcased how alteration of gut-microbiota affects psychology. Patients with depression had microbial alterations in species from three bacterial phyla (Firmicutes, Actinobacteria, and Bacteroidetes). Further faecal samples from five patients having depression transplanted into healthy mice caused gloomy behavioural patterns which proved the character of GM. Various dietary factors have been showing anxiety and depression-like behaviours. Several human studies revealed how consumption of fermented milk product with probiotics affect the activity of brain sections which control central processing of emotion and sensation using functional brain imaging. Numerous animal studies also confirmed their influence of probiotics on behaviour. Additional evidence advises that probiotics may prevent the development of changes in brain activity in response to chronic stress. A study based on psychology tells how GM alters our mood and emotion, how personality and character are close. There are also evidences showing how stress management is impacted by the gut microbiota which is a part of the stress response system. Psychological stresses neither only trigger the neuroendocrine, immune, and nervous systems, but also influence our mood and behaviour.



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MULITPOTENTIAL BIOPIGMENT SOURCED FROM A BACTERIUM

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A novel psychrophilic bacterial strain, an extremophile that is capable of growth and reproduction in low temperatures ranging from -20 °C to +10 °C called *Rhodonellum psychrophilum* GL8 was discovered at the high altitude Pangong Tso Lake in the Himalayas. The bacterium is gram negative, aerobic, non-motile and rod shaped. The optimum pH required for its growth is 5 - 9. The bacterium was identified based on 16S rDNA sequencing amplicon of 1370bp. Chromatographic and spectrometric analysis of pigment showed that it contains 2-methyl-3-butyl-prodigine, prodigiosin, 2-methyl-3-hexyl-prodigine, 3,4-didehydrorhodopsin, anhydrorhodovibrin, alloxanthin and tetradecanoyl-hexadecanoyl compounds. The pigment possesses antimicrobial, antifungal and skin cell growth stimulating properties. It shows antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Saccharomyces cerevisiae* with Minimum inhibitory concentrations (MIC) from 31.25 µg/ml to 500 µg/ml. The red coloured pigment also shows synergistic antifungal activity when combined with fluconazole and amphotericin B against *Candida albicans* and *Saccharomyces cerevisiae*; vancomycin and erythromycin against *Escherichia coli* and *Staphylococcus aureus*.

The pigment shows growth stimulating properties of murine skin cells, but no toxicity to human cells which opens opportunities for its applications in wound healing. It also shows higher radical scavenging activity. It is useful for the development of antimicrobial smart fabrics, medicated bandages, natural food colouring agents and preservatives.



The toxic chemicals released from artificial products like synthetic dyes have resulted in soil and water contamination. The side effects of the deliberate application of synthetic dyes have led to a 10 – 15 % increase in the demand for naturally occurring non-toxic dyes. Microbes as potential sources of pigments promise to be eco-friendly and economical, besides being non-toxic, inexpensive and readily available. Their mass production is feasible given that production is independent of seasonal fluctuations and the microbes provided with suitable growing conditions.



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ORGANOPHOSPHOROUS PESTICIDES

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Pesticides, the substances which can be used for destroying pests, proved to be both useful and harmful to the environment and mankind. There are many different types of pesticides such as Herbicides (weeds), Insecticides (insects), Fungicides (fungi), Nematicides (nematodes), Rodenticides (rat poisons). The synthetic pesticide's first use was made in the year 1940, which by 2001 reached 2.26 million tons indicating their increase in consumption. Herbicide was the most used pesticide, followed by insecticide, fungicide, and others. China was the major contributing country in the same, followed by USA and Argentina. Though only 25 % of world production of pesticides was used in developing countries, 99 % of the human deaths occurred there itself; the reason being intense, hence, unsafe use of pesticides and weaker health, education, and regulatory systems in developing countries. However, an increase of up to 3.5 million tons was observed w. r. t. usage of pesticides by the year 2020. Mostly, insecticides are used in many developing countries and fungicides or herbicides in developed countries. Out of the many pesticides used, one is Organophosphorous pesticides (an insecticide).

Organophosphorous compounds are organic compounds that mainly contain phosphorous and are produced by the esterification process between alcohol and phosphoric acid. Their capability of killing pests by destroying an enzyme in them called Acetylcholinesterase (AChE) which is a carboxyl ester hydrolase, is inactivated by phosphorylation of the serine hydroxyl group located at its active site. It is mainly found in RBCs, CNS, PNS, and neuromuscular junctions and can degrade neurotransmitter acetylcholine into choline and acetic acid. Some of the



examples of Organo-phosphorous pesticides (OPs) are malathion, parathion, ethion, dimethoate, trichlorfon, carbofuran, diazinon, fenthion, dichlorvos, chlorpyrifos, and fenitrothion, etc.

OPs are mostly used in commercial agriculture, in many different vegetables and fruit crops like corns, soybeans, fruit and nut trees, Brussels trees, cranberries, cauliflower as well as other row crops for controlling pests. The overuse of OPs leads to their accumulation in crops which later runoff along with rainy water or some other medium below the ground, hence causing contamination of soil, turf, water table, sources of drinking water, and other vegetation. All of these factors can affect humans in unfortunate ways. It also affects the environment by persisting there with high resistance, influencing the different populations of microorganisms as well as macroorganisms.

Human exposure to pesticides can be through oral route, inhalation, by reacting with the tyrosine residue present in the keratin of skin epithelium and may give rise to various problems associated with the nervous system such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, issues related to the reproductive system such as menstrual cycle disturbances, miscarriages, stillbirths, developmental defects, etc. The Organo-phosphorous pesticide that is majorly responsible for harmful effects on humans, especially in association with reproduction, survival, and fetal development in both vertebrates and invertebrates is chlorpyrifos.

The use of pesticides, which includes OPs too, started with the need of protecting plants and crops from pests, protection from it have become need now by considering its toxicity. The protection from them achieved is by their microbial degradation. Bacteria *Stenotrophomonas acidaminiphila* (strain G1) is of immense importance in organophosphorus pesticides pollution remediation. It contains inside the cell an enzyme methyl parathion hydrolase which can degrade OPs like methyl parathion, utilizing O, O- dialkyl phosphorothionate & O, O- dialkyl phosphate as substrates. For the degradation of methyl parathion, optimum conditions were also found out by certain experiments and it was concluded that the incubation temperature should be 40 degrees Celsius, substrate concentration should be 50 mg per litre and inoculum volume should be 20% v/v. The initial 50 mg per litre concentration of strain G1 degrades 100% methyl paraoxon, methyl parathion diazinon and phoxim, 95% of parathion, 63% of chlorpyrifos, 38% of profenofos and 34% of triazophos in 24 hrs. All the above compounds are organophosphate insecticides.



There are other strains of *Stenotrophomonas* sp. such as *Stenotrophomonas* sp. PF32, *Stenotrophomonas* sp. SMSP-1 and other microbes like *Serratia* sp. SPL-2, *Pseudomonas aeruginosa* Is-6, etc. that can degrade various pesticides, too. Some factors affect degradation by bacteria of Organo-phosphates including water holding capacity of soil, pH, Temperature and ageing of pesticides in contaminated soil before inoculation. Despite their toxic nature, OPs are still in use for their applications of being used as pesticides because of which crop, food and material preservation by control of weeds, insects, infestations and various diseases is possible.



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GLIMPSES OF CORONAVIRUS

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The Corona virus or SARS-CoV-2 which attacked humans on late 2019 was named by WHO as COVID-19 in which 'CO' stands for Corona, 'VI' stands for virus and 'D' stands for disease. Which caused a great loss to mankind and it proved to everyone that money is not important than one's health. The COVID-19 impacted environment two different ways, one is that the nature and our animals in the environment were free from unwanted anthropogenic activities and pollution. Secondly, the humans were affected physically, economically and personally. Many people went into depression think about their life and future of their family and some people cannot come out of their depression and chose to end their life by committing suicide. To the contrary, many people used this pandemic as an opportunity to grow started their own business for their and their family's future and succeeded. There are many other strains of corona viruses which attacked humans and animals but they had lesser impact than COVID-19.

The most common ancestor of the Corona virus is estimated to be existed before 8000 B.C.E and the alpha corona viruses have evolved in 2400 B.C.E, the beta corona viruses in 3300 B.C.E, gamma corona viruses evolved in 2800 B.C.E and delta corona viruses existed in 3000 B.C.E and the human corona viruses shared a common ancestor with the bat corona virus. There are many types of viruses in this family and has a long history years back in the world. The first known corona virus infection was found and occurred in North America in late 1920s where an acute respiratory infection occurred in chickens and the illness was characterized by gasping and listlessness in young chicks and there were high mortality rates with 40



% to 90 %. These viruses were first isolated by David Bushnell and Alfred Brandly in 1933 and the virus were first named as infectious bronchitis virus or IBV. In the late 1940s other two classes of viruses causing animal infections like murine encephalitis and mouse hepatitis virus which causes hepatitis in mice.

Corona viruses are the class of viruses belonging to the family Coronaviridae and the viruses are classified into four genera namely Alpha Coronavirus, Beta Coronavirus, Gamma Coronaviruses and Delta Coronaviruses. The Alpha Corona viruses has some of the species like Alpha Coronaviruses 1, Human Coronavirus 229E, Human corona virus NL63, Miniopterus bat corona virus HKU8, Porcine epidemic diarrhoea virus, Miniopterus bat corona virus 1, Rhinolophus bat corona virus HKU2, Scotophilus bat corona virus 512. Some of the species present in beta corona viruses are Betacorona viruses 1, Hedgehog coronavirus 1, Human coronavirus HKU1, Middle East Respiratory Syndrome-related corona virus, Murine coronavirus, Pipistrellus bat coronavirus HKU5, Rousettus bat coronavirus HKU9, SARS CoV SARS-CoV-2, Tylonicteris bat Coronavirus HKU4. Some of the species present in gamma coronavirus are Avian corona viruses, Beluga coronavirus SW1 and some of the species present in delta coronavirus genera are Bulbul coronavirus HKU11, Porcine coronavirus HKU15, etc.

Some of the corona viruses are identified in animals like Canine corona virus belonging to alpha corona viruses in dogs, Feline enteric corona virus belonging to alpha corona viruses in cats, Equine corona virus belonging to beta corona viruses in horses, Bovine corona viruses belonging to beta corona viruses, Porcine epidemic diarrhoea virus, Transmissible gastroenteritis virus, Porcine respiratory corona virus these three viruses belong to alpha corona viruses, Porcine corona virus belongs to beta corona viruses and Porcine hemagglutinating encephalomyelitis virus belongs to delta corona viruses in swine, Avian infectious bronchitis virus in chickens and Turkey corona virus in turkeys.

Canine corona viruses are also called as CCoV and these viruses are RNA viruses which belong to the class of alpha corona viruses. There are two types of canine corona viruses one type is the one which is highly contagious and attacks the intestine of dogs and spread worldwide. There is a vaccine available and is given to puppies which are more prone to viral infection and during serious cases of infection intravenous fluids are given for dehydration and commonly the virus is destroyed by common disinfectants. There is second type of canine respiratory corona virus which causes respiratory illness in dogs.



Feline enteric corona viruses are positive stranded RNA viruses belonging to the class alpha corona viruses which affect the cats across the world. There are two species of corona viruses which affect the cats called as canine corona virus (CCoV) and porcine transmissible gastroenteritis (TGEV). The infection spreads from the faeces of the infected cats through orally or through fomites like grooming, litter boxes, housing, etc. When the infection occurs there is a rapid spread in the infection in the oropharyngeal tissues with salivary shedding and also the infection spreads to the epithelial cells of the villi in the intestine which leads to the shortening and destruction of the cells.

Equine corona virus also called as equine enteric corona viruses denoted as ECoV is a RNA viruses belonging to betacorona viruses and it commonly affects the horses of above 2 years of age. The disease is transmitted to the healthy ones by the virus infected faecal matter such that the infection is of oral-faecal transmitted disease. The horses which affected by this disease have symptoms like laziness, anorexia, fever, softness of faeces, colic, etc, death is rare. Treatments include fluid therapy like anti-inflammatory and anti-steroidal fluids and the disease can be prevented by using disinfectants like sodium hypochlorite and the infected animals should be isolated till the disease is cured.

Bovine corona viruses are a positive single stranded RNA virus which belong to class of betacoronaviruses and affects the calves of cattle of age 1 day to 3 weeks old. The bovine virus infects the calves and the infection occurs by oral and respiratory routes. The oral route infection may spread all over the small and large intestine and the villi in the small intestine is infected and destroyed leads to immature villi formation, fusion of villi and in large intestine it may lead to atopy of colonic ridges. Some of the symptoms include dull, anorexia, dehydration, yellow diarrhoea starts after 48 hours of infection and may last to 3 - 6 days and the faeces are contaminated with viral infection. Treatments include giving fluids to treat dehydration, giving kaolin mixtures may reduce the severity of diarrhoea and affected animals should me isolated from healthy ones.

Porcine corona viruses have five strains of viruses belonging alpha, beta and delta corona viruses and the strains are Transmissible gastroenteritis virus (TGEV), Porcine respiratory corona virus (PRCV), Porcine epidemic diarrhoea virus (PEDV), Porcine haemagglutinating encephalomyelitis virus (PHEV) and Porcine deltacorona viruses (PDCoV). These viruses are single stranded positive sense RNA virus with a capsid and pleomorphic which affects the pigs. The symptoms may be varied for various strains like indigestion, respiratory tract infections and some cases may lead



to death. The treatments include giving fluids to treat diarrhoea and dehydration, isolating the affected animals and other simple treatment methods.

Avian corona viruses or infectious bronchitis virus (IBV) are a group of viruses belonging to the class of gamma coronaviruses with a single stranded RNA coronaviruses. They affect mainly chickens and other birds like turkey, duck and goose are also affected. They cause infection mainly in the respiratory tract and may sometimes found in epithelial cells in kidney, ileum, bursa of fabricius and cloaca. Control measures include vaccination with live IB virus, attenuated virus vaccine, identification and isolation of affected chickens and keeping the surrounding clean using disinfectants.

Human coronaviruses have evolved from ages belonging to class alpha and beta coronaviruses. Some of the human coronaviruses include Human Coronavirus OC43, Human Coronavirus HKU1, Human Coronavirus 229E, Human Coronavirus NL63, Middle East respiratory - related syndrome, Severe Acute Respiratory Syndrome (SARS CoV) and Severe Acute Respiratory Syndrome - 2 (SARS CoV-2)

Human Coronavirus OC43 and Human Coronavirus 229E are the responsible for common cold across the world and may also cause severe infections in the lower respiratory tract, common cold, pneumonia and such complications. These viruses are single stranded RNA viruses belonging to class alpha coronaviruses, the OC43 virus enters into cell by binding with N-acetyl-9-O-acetylneuraminic acid receptor and the 229E virus enters the host through binding with APN receptor. Human Coronavirus HKU1 is a group of virus belongs to beta coronaviruses and has a single stranded RNA virus which binds to the host by N-acetyl-9-O-acetylneuraminic acid which cause cold and severe cases may lead to pneumonia, bronchitis and other upper respiratory tract infections. Human Coronavirus NL63 is a virus which belongs to alpha coronavirus which binds to the host by ACE2. The symptoms include fever, cough, sneeze, rhinitis, bronchitis, sore throat, hoarseness, pneumonia and croup which mainly spread through direct person to person transmission. The common preventive measures for all these viruses are washing hands with soap and water and isolating the sick individuals.



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CATACLYSMIC MEET OF HIV-1 AND MOLECULAR MOTOR PROTEINS

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Molecular motor proteins aid in transporting cellular cargo within the cell, along the microtubules. Microtubules are part of the cell cytoskeleton, which function as dynamic scaffolds providing structural support to the cell. Microtubules confer polarity, playing an important role in growth of the polymer and ability to participate in directed mechanical activities. The microtubules network is a dynamic and an intricate arrangement of microtubules inside a cell. It directs the movement of materials and organelles within cells.

Two types of motor complexes, dyneins and kinesins, hydrolyse ATP to convert chemical energy stored in ATP into mechanical energy to generate force for moving unidirectionally along microtubules in a stepwise manner and to transport cellular cargo. Kinesins (except for Kinesin-14) are plus end directed i.e., they exhibit anterograde movement whereas, dyneins move progressively along a MT toward minus end of the polymer i.e., they exhibit retrograde movement. There are 15 different kinesin families, labelled from kinesin 1 to kinesin 14B, based on phylogenetic evaluations. Kinesin-1 is conventional kinesin and is member of a superfamily of related proteins, called Kinesin Related Proteins (KRPs). There are two subsets of dyneins: cytoplasmic and axonemal. To accomplish specificity, dyneins and kinesins interact with various cargo-specific adaptor proteins, which link



the molecular motors to the numerous cargoes. Thus, regulation of transport seems to depend on regulation of motor activity by adaptors. Cytoplasmic dyneins interact with an intervening multi-subunit adaptor, dynactin which links dynein to the membrane bound cargo, regulates dynein activity and increases dynein's processivity while walking along MTs. Endosomes, lysosomes, ER derived vesicles heading towards the Golgi complex, etc. are dynein-driven cargoes. One of the dynein-driven cargo is the Human Immunodeficiency Virus (HIV) which is transported to the nucleus of an infected cell. The mechanisms by which kinesin-1 and dynein activities are coordinated to transport cargo are an ongoing area of research.

The human immunodeficiency virus belongs to the genus Lentivirus of the family Retroviridae, and subfamily Orthoretrovirinae. HIV is classified into the types 1 and 2 (HIV-1, HIV-2) based on genetic characteristics and variations in the viral antigens. HIV-1 infection seems to involve bidirectional transport. For an incoming HIV-1 particle to employ the MT network to reach the nucleus, the motor proteins dynein and kinesin are vital. HIV-1 are proficient at usurping host's MTs and motor proteins for their intracellular transport. The MT network and its associated proteins are involved in several of the early steps which are crucial for HIV-1 to efficiently infect cells:

- **Entry:** Entry of the viral particle is aided by the binding of surface glycoprotein gp120 of the HIV-1 particle to the CD4 receptor on the host cell.
- **Reverse Transcription:** It is the process by which retroviruses such as HIV convert their RNA genome into a double-strand DNA provirus.
- **Uncoating:** It is the disassembly of the HIV-1 capsid which occurs during the post-entry phase of infection.
- **Transport to the Nucleus:** HIV-1 particle must traffic to the nuclear envelope, where the viral genome is translocated into the nucleus for subsequent integration into the host cell chromosome.
- Microtubule network is also utilized by HIV-1 particles for releasing and efficiently assembling particles for budding and propagating.

Studies concerning motor proteins, adaptor proteins and HIV-1 demonstrate that:

- HIV-1 utilizes a dynein-dynactin-Bicaudal D2 (BICD2) complex for infection implying that the depletion of components of the dynein-dynactin-BICD2 complex results in impaired HIV-1 nuclear import. BICD2 functions as a capsid-specific dynein adaptor protein and promotes HIV-1 retrograde trafficking to the nucleus.



- Interference of HIV-1 coating as a result of disruption of the dynein complex. This indicates that dynein motor complexes probably are involved in both, HIV-1 trafficking and in its uncoating. This supports the idea that the two processes may be related.
- The microtubule-associated proteins Dia1 and Dia2 were found to promote uncoating.
- Depletion of siRNA of the dynein heavy chain or inhibition of dynein by ciliobrevin D treatment, demonstrated to reduce viral DNA accumulation in the nucleus and inhibiting HIV-1 infection.
- Microtubule affinity-regulating kinase 2 (MARK2) activates fasciculation and elongation protein zeta 1 (FEZ1) which binds to Kinesin-1 and HIV-1 capsid for transport.
- Overexpression or depletion of FEZ1 in cells, reduce HIV-1 infection indicating that the interactions of FEZ1 with kinesin must be balanced properly.
- Stable subset of microtubules is important for reverse transcription. This suggests potential role for active transport along these tracks for components necessary for reverse transcription.

Capturing sufficient events at the moment of virion transfer to draw persuasive conclusions might be challenging as majority of HIV-1 particles are non-motile and trafficking events occur swiftly. This limitation can be overcome by advances in imaging techniques such as bioluminescence imaging and tyramide signal amplification. Development in imaging technologies significantly enhances the understanding of cellular and molecular interactions in humans and their model organisms of infectious diseases. Taking benefit of the latest innovations in imaging technologies may help resolve critical questions in the HIV field. Fostering multidisciplinary alliances in HIV research is vital. The important roles that dynein, kinesins and the microtubule network play in cell division and homeostasis suggest these specific components will be challenging targets for antiviral therapy. If we target the capsid of HIV-1 to which both the adaptors, one of Kinesin-1 and the other of dynein bind, we can reduce the ill effects which would occur in case the motor proteins/adaptors are altered. The potential for therapeutic targeting can be substantially enhanced with greater knowledge about the mechanisms of the viral transport including initiation to its travel via the microtubule network to the nucleus.



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THE ANTIBIOTIC ERA

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The term antibiotics literally means “against life”; in this case, against microbes. There are many types of antibiotics - antibacterial, antivirals, antifungals, and antiparasitic. The most commonly used antibiotics are antibacterials. Before antibiotics, 90 % of children with bacterial meningitis were died. Strep throat was at times a fatal disease, and ear infections sometimes spread from the ear to the brain, causing severe problems. Other serious infections, from Tuberculosis to Pneumonia to whooping cough, were caused by aggressive bacteria that reproduced with extraordinary speed and led to serious illness and sometimes death.

With the discovery of Penicillin and the dawning of the Antibiotic era, the body's own defenses gained a powerful ally. In the 1920s, British Scientist, Alexander Fleming was working in his laboratory at St. Mary's Hospital in London when almost by accident, he discovered a naturally growing substance that could attack certain bacteria. In one of his experiments in 1928, Fleming observed colonies of the common *Staphylococcus aureus* bacteria that had been worn down or killed by mold growing on the same plate or petri dish. He determined that the mold made a substance that could dissolve the bacteria. He called this substance Penicillin, named after the *Penicillium* mold that made it. Fleming and others conducted a series of experiments over the next 2 decades using penicillin removed from mold cultures that showed its ability to destroy infectious bacteria.



With the success of Penicillin, the race to produce other antibiotics began. Today, Pediatricians and other doctors can choose from dozens of antibiotics now on the market, and they're being prescribed in very high numbers. At least 150 million antibiotic prescriptions are written each year, many of them for children.

Problems with Antibiotics

The success of antibiotics has been impressive. At the same time, however, excitement about them has been tempered by a phenomenon called Antibiotic resistance. This is a problem that surfaced not long after the introduction of Penicillin and now threatens the usefulness of these important medicines. Almost from the beginning, doctors noted that in some cases, penicillin was not useful against certain strains of *Staphylococcus aureus* (bacteria that causes skin infections). Since then, this problem of resistance has grown worse, involving other bacteria and antibiotics. This is a public health concern. Increasingly, some serious infections have become more difficult to treat, forcing doctors to prescribe a second or even third antibiotic when the first treatment does not work.

In light of this growing Antibiotic resistance, many doctors have become much more careful in the way they prescribe these medicines. They see the importance of giving antibiotics only when they're absolutely necessary. In fact, one recent survey of office-based physicians, published in JAMA: The Journal of the American Medical Association in 2002, showed that doctors lowered the number of antibiotic prescriptions they prescribed for children with common respiratory infections by about 40 % during the 1990s.

Antibiotics are also given to animals, particularly those being raised for slaughter on industrial farms. Farms are estimated to account for roughly 70 percent of total antibiotic sales in the United States; the proportion is even higher in some other countries. Experts urge veterinarians to reserve antibiotics for sick animals, but breeders commonly use the drugs to prevent disease and boost animal growth, increasing their profits. This overuse contributes to the spread of resistant bacteria, which can be transmitted from animals to humans through the food chain and in more indirect ways. There are several steps that need to be taken to stop the accelerated increase in antibiotic resistance and to enable the efficacy of antibiotics in the future. Some of them are

- ***Judicious antibiotic use*** – This can be defined as using the right antibiotic agent, at the right time, with the right dose and for the right duration.²² Reaching this goal is obviously not simple. A multidisciplinary approach is needed to improve the quality of antibiotic prescribing.



- **Surveillance and Infection control** - Preventing the spread of resistant bacteria, especially in health-care institutions, is a key element in controlling antibiotic resistance. Surveillance is needed mainly for patients transferred from locations with high resistance rates and for multi-drug resistant bacteria, with strict adherence to infection control guidelines.
- **New vaccines** – As learned from past experience, preventing infectious diseases by vaccines is the preferred way to control infections – an ounce of prevention is worth a pound of cure. Efficacious vaccines against infectious diseases can significantly reduce the prevalence of illnesses caused by a given pathogen, acting somewhat as ‘anti-resistance vaccines’.
- **New antibiotics** – Because of the complex and diverse mechanisms of antibiotic resistance, and because the traditional bacterial targets for the action of antibiotics have already been exploited, very few new antibiotics are currently in the pipeline. Creative approaches are needed to discover new classes of antibiotics, with novel targets and mechanisms.
- **Non-antibiotic Antimicrobial therapy** – Considerable effort has been invested in developing additional antimicrobial products that are safe and effective, such as bacteriophages, agents that will inhibit toxin production by bacteria, biofilm formation, bacterial adherence to mucosal surfaces, translation interference, monoclonal antibodies and novel immunomodulators. An example of unconventional antimicrobial strategy uses synthetic biology, which consists of genetically engineered modifications of biologic systems to perform novel functions that do not exist in nature.

Experts have been warning for years that we're approaching a post-antibiotic era -a time when our antibiotics are pretty much useless and drug-resistant superbugs can all too easily decimate our health. Yet we continue to dole out too many antibiotics, driving the resistance. So, it is very important that we take the required efforts in research related to antibiotics and give our contribution to the antibiotic era.



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CYANOBACTERIA: SCULPTORS AND SAVIOUR OF THE EARTH

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Cyanobacteria, the primary and first oxygen - evolving group of photosynthetic Gram negative prokaryotes, are unique among microbial world and grow in diverse habitats. Around 3 billion years ago the Earth's atmosphere had very little or no oxygen and was mainly composed of Nitrogen and Carbon dioxide. The emergence of Cyanobacteria corresponds to the 'Great Oxidation Event', during which our planet's atmosphere changed to about 20 % oxygen gas. The correlation of Oxygenation and proliferation of cyanobacteria seen in fossilized stromatolites suggests that these little green microbes sculptured the atmosphere and world due to which living organisms can survive.

As this minute organism shaped the world and made life possible they might even be a potential solution to the grand global challenges of the 21st century (energy, climate, and food security). What's so great about this minute creature? It grows 10 times faster than plants, and less than a tenth of the land is required to get the equivalent amount of biomass. Because it doesn't require fresh water, it can be often fertilized more efficiently than land crops, and thus we can avoid the intensive water usage, wasteful fertilizer runoff, and downstream eutrophication related with modern agriculture. These efficient features have collectively offered these bio-agents as the precious bio resource for sustainable development.



As humans have already increased the concentration of CO₂ within the atmosphere by 50 percent, which can further increase in the near future due to industrialization, fuel usage and greenhouse gases. Many climate scientists have invoked the concept of negative emissions—sucking carbon dioxide back out of the atmosphere. This might be done by enhancing natural processes. It's in this context that cyanobacteria have some distinct advantages. Cyanobacteria can use land that's non-productive for agriculture and may even be harvested from the oceans. Algae that are efficient at capturing carbon dioxide have already been used at plants like Sweden's Algoland project to neutralize the CO₂ produced as a byproduct of cement production.

In 2010, two American engineers realized they might use algae biomass to form plastics. They formed an organization called Algix and set up a factory in Mississippi. Algix turns green algal scum into a plastic called ethylene-vinyl acetate or EVA. Shoot air into molten EVA and we get EVA foam that can be used to prepare the soles of the sports shoes. The first shoe company to use Algix's product is the UK Company Vivobarefoot, which makes a shoe entirely out of algae. This lead to an excellent innovation that deals with the matter of synthetic plastic usage and decomposition.

Cyanobacteria might be the solution to all of those challenges providing a valuable source of Carbon-neutral fuels, aqua feeds for the agriculture and aquaculture industries, and high-protein food products for direct human consumption. Many high-value, niche-market products are already being produced because algae possess unusual nutritional, anticarcinogenic, and anti-inflammatory properties. People have been selling these kinds of special algal products for a while, but these markets are relatively small.

Currently, companies are producing an enormous range of products from Algae – antioxidants, protein, flavors, colorants, etc. We can now find Algae-based products within the most exclusive restaurants around the world as part of their menus, in cosmetic creams, and in many other products as well. Within the subsequent three to five years we are going to find algae in many of our everyday products, turning algae into a multi-billion dollar industry.

We have about 10-15 years left to deal with our global climate, energy, and food security problems because it will take two to three decades to implement them at the required scale to unravel these global challenges, then its game over.





But it's tantalizing to think that the descendants of the Cyanobacteria that rescued our planet from the doldrums and paved the way for intelligent life could also provide the tools that can be used to solve the major global challenges and would make sure that life has a future.

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COMMON DISEASES OF GOLDFISH IN INDIAN AQUARIUM CONDITIONS

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Ornamental fish keeping is the second-largest liked hobby after photography. It was first done by Thung dynasty in China. The first time goldfish keeping started in 18th century. In 21st the, ornamental fish keeping got recognition as the industry. It is one of the fastest-growing businesses worldwide, but one thing that prevents this industry from furnishing is a disease, and Goldfish are the most susceptible species for disease. Several conditions of economic importance are recorded, and the common diseases, symptoms, and methods of inspection together with treatment protocols are summarized in this article.

Causative factors for Diseases in Goldfish

The causative factors of fish diseases can be grouped under six broad categories

- Internal to the individual - genetic diseases
- Environment associated factors
- Physical injuries (ex: handling/transporting)
- Nutritional diseases (ex: deficiency syndromes)
- Co-existing organisms (biological entities)



Common diseases and Management in Goldfish

i) Viral diseases

Only one disease which is common in Goldfish is Lymphocystis. The causative agent of Lymphocystis is Iridovirus

Symptoms: Nodular white swellings (cauliflower) on fins or body. It also shows the tumor-like structure and lumps in the body surface

Treatment: no treatment is possible. The best way to control this disease is by removing the fish

ii) Bacterial diseases

a) Fin and Tail Rot

The causative agent of fin and tail rot is *Aeromonas* sp and *pseudomonas* sp.

Symptoms: Disintegrating fins, blood on edges of fins, reddened areas at the base of fins, skin ulcers with grey or red margins, cloudy eyes.

Treatment: Fishes may treat with Antibiotics such as Oxytetracycline at the rate of 20 - 30 mg in water, also mixing it with feed at 1 % in the meal.

b) Scale Protrusion

The causative agent is *Aeromonas* sp. and *Pseudomonas* sp.

Symptoms: Scale erosion and protruded scales are seen in the body of fish

Treatment: Add an antibiotic to the food. With flake food, use about 1 % of an antibiotic such as chloramphenicol or tetracycline. In the water, add about 10 mg per litre of the required antibiotic.

c) Liquid disease

The causative agent is *Aeromonas hydrophilia*

Symptoms: Bloating of the body, protruding scales, and accumulation of liquid in the body cavity

Treatment: Add an antibiotic to the food. With flake food, use about 1 % of an antibiotic such as chloramphenicol or tetracycline. In the water, add about 10 mg per liter of the required antibiotic.



d) Ulcer disease

The causative agent is *Aeromonas*, *Pseudomonas*, *Vibrio* and *Edwardsiella tarda*

Symptoms: Ulcers are present all over the body

Treatment: Disinfect the water with suitable antiseptics such as acriflavine or monacolin (monoamino acridine) with 0.2 % solution @ 1 ml per litre followed by antibiotic treatment.

iii) Fungal Diseases

The fungal disease seen in Goldfish is Saprolegniosis.

Symptoms: Tufts of white cotton-like growth on the skin or fins.

Treatment: In addition, 10 ml of 1.0 % Phenoxethol per litre of aquarium water can be added. It is advisable to repeat for a few days as per the requirement.

4) Parasite disease**a) Ich Disease or Ichthyophthiris Disease**

The organism causing the disease: The ciliate. *Ichthyophthirus multifiliis*.

Symptoms: White glistening spots or Salt-like specks on the body/fins. Excessive slime on the body, difficulty in breathing, clamped fins, and loss of appetite are other symptoms.

Treatment: Quinine hydrochloride or Quinine sulfate at 30 mg per liter (1 in 30,000) can be used. Others such as acridine orange, acriflavine, mild formalin solution, benzalkonium chloride, malachite green or malachite green with copper are effective.

b) Costia

The organism causing the disease: *Costia* sp.

Symptoms: Milky cloudiness on skin.

Treatment: Copper at 0.2 mg per litre (0.2 ppm) to be repeated once in a few days if necessary. Acriflavine may be used at 0.2 % solution (1 ml per litre).



c) *Chilodonella*

The organism causing the disease: *Chilodonella* sp.

Symptoms: Dulling of the colours due to excessive slime, fraying of the fins, weakness and gill damage.

d) *Argulus*

The organism causing the disease: *Argulus* sp.

Symptoms: The fish scrapes itself against objects, clamped fins, visible parasites about 1/4 inch in diameter are visible on the body of the fish.

Treatment: With larger fish and light infestation, the lice can be removed with forceps. Weak formaldehyde is also helpful to remove the parasites.

General methods to detect the Health of Fish in Field conditions

Visual examination is one of the quickest and least costly and requires a well-trained eye. But it need not be highly reliable. Some of the short indicators are given below:

a) External

- Reflexes in healthy fishes, reflexes will be quick: Escape reflex, Eye reflex, and Tail reflex.
- The other types of Symptoms may be: Sluggish behaviour Twirling, Spiral or erratic movements, Faded or darkened pigmentation, Exophthalmus or 'pop eye' condition.
- Hemorrhages Erosion of jaw or mouth Gill parasites, gill erosions, white nodules Tailor fin rot Distended abdomen (Dropsy) Protruded anus (vent) Blood oozing Ulcers/boils (furuncles) External parasites Cotton wool-like growth

b) Internal

- Gas filled hollows Ascitic fluid in the abdominal cavity
- Hemorrhages in the muscle wall/air bladder/ internal organs
- The liquid in the air bladder White nodules in internal organs Swelling organs (Kidneys, liver, etc.)



Diagnosis

Diagnosis of the disease is very easy in aquarium fishes. When any abnormal behaviour is seen in the aquarium, fishes are separated for diagnosis. Some of the diagnosis methods are given below

a) Microscopic and Histologic examination

This quick diagnostic tool is used for the detection of bacterial and parasite pathogens in the aquarium condition. More specific information can be obtained from the histopathological method, in which the tissue sections can be stained and observed. These sections give very specific information about the pathogen and damage caused by them

b) Bacterial isolation

This method is very specific to the bacteria. In this method, bacteria can be isolated with the help of liquid media or solid agar media. These media are may or may not be specified in nature which may facilitated the growth of sapcific bacteria and make them identifiable. These bacteria later used for the identifection of treatment and pathogenicity tests.

c) Tissue culture

This method is used for the detection of the virus. Because they need a living cell for their propagation, the virus is very specific to the cell lines and can be detected in the specific cell line. A sample is homogenized and added to the cells in the tissue culture flask. If the virus is present in the sample, it causes the Cytopathic Effect (CPE).

Rapid Diagnostic Tests for detecting Fish pathogens

This method is very useful for rapid detection and treatment, early detection means early treatment and less loss some of the techniques are given below

- a) Immuno-diagnostic assays such as Monoclonal & Polyclonal antibody assays.
- b) Direct fluorescent antibody test (d-fat)
- c) Enzyme Immunoassays (EIAs) or ELISA
- d) Dot immunobinding assay
- e) Western Blotting technique
- f) The Latex agglutination assay
- g) DNA - based diagnostic tests
- h) Polymerase Chain Reaction (PCR) tests.



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MICROBE FROM COW STOMACH CAN UNDERGO PLASTIC DEGRADATION

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The most useful domestic animal that benefits the human life in many ways as dairy products and as fuel from cow dung and as so on all over the country is cow. To the extreme recently, researchers at the university of natural resources and life science in Vienna hypothesized that one of the four compartments of cow stomach can contain enzyme that can digest certain types of man-made polyester plastics, including poly (ethylene terephthalate, or PET, since cow already have natural polyester cutin as its diet which can be found in peels of tomatoes and apples, said by Dr. Doris Ribitsch and his team. More than 8 tonnes of plastics have been produced-which equals to 1 billion elephants from which only 21 % are tackled, whereas, other rest polluting the land. This is due to practice of usage of single-use plastics where people unwittingly consume and breathe micro plastic particles that causes life threat to all living beings. There are many ways used to reduces the threat like chemical degradation, production of bioplastics, usage of enzymes, bacteria and fungi which all isn't sufficient. Hence, the researchers examined the effect of the liquid from the rumen by collecting the sample form the slaughterhouse, and analyzed its DNA and identified that 98 % of the microbes involved in the degradation process with bacteria. Then they incubated each plastics for three days. It was tested on three plastics such as PET, PBAT, PEF in both film and powder form of plastics. Out of three PEF broken down the best. And importantly breaking down of plastic is quicker in powder form than the film. The rumen fluid may be cheaper but the process that has to made in laboratory is costly. As an emergence





Genetic engineering of those bacteria to assist in the recycling process would be the next step.

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USES OF MICROBES AS FERTILIZER IN AGRICULTURE

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Biofertilizers are basically microbial preparations or microbial products, mainly used as growth enhancer. Basically, microbes like bacteria, fungi and blue green algae, which have manorial value, are used as biofertilizer in agriculture. They are mainly applied to seed, soil and seedlings, plant surface for the enhancement of plant growth by several mechanisms such as increasing necessary nutrients, increasing root biomass, nutrient uptake capacity etc.

Based on beneficial activity and the microbial agent used in their production of biofertilizers, it is divided into several categories. Some of them are discussed here.

Nitrogen fixing biofertilizers mainly convert atmospheric gaseous N₂ Ammonia (NH₃), which plants can easily use. Nitrogen fixing organisms like species of *Rhizobium*, *Azotobacter*, *Azospirillum*, *Acetobacter*, *Azolla* and blue green algae are commercially used biofertilizers. These microorganisms mainly fixed N₂ through symbiotic, non-symbiotic and associative relationship. *Azolla* is inoculated in rice paddy fields, when the fields are flooded in the spring. They quickly multiply to cover the water and suppressed the growth of weeds. According to a given data the rotting plant material releases nitrogen to the rice plants, providing up to nine tones of protein per hectare per year. Blue Green Algae also play a great role in N₂ fixation by Heterocyst formation.



Phosphate is another major nutrient in Crop productivity. Generally, *Bacillus polymyxa*, *Achromobacter* sp., *Arthrobacter* sp., *Aspergillus awamori*, *Penicillium digitatum*, *Streptomyces* sp. etc are used as Phosphate solubilizing microbes. They solubilize fixed phosphorus in soil and make it available to the plants. Vesicular Arbuscular Mycorrhizae (VAM) enhance essential micronutrients uptake of crop plants and help in better utilization of applied Phosphatic fertilizers, particularly in the soils subjected to rapid fixation of Phosphate.

Potassium (K) is another essential macronutrient plays an important role in the growth and development of plants. It also helps in improvement crop quality. Microorganisms play a key role in natural K cycle. Few species of rhizobacteria are capable of mobilizing potassium in accessible form in soils. Some other biofertilizers are sulphur oxidizing biofertilizers, silicate solubilizing biofertilizers, decomposing cultures etc. Besides some limitations like short shelf life (only about 6 months) for transport, store and distribution, low level of acceptance, no large scale extension, biofertilizers are advantageous for us. Some advantages of biofertilizers are

- It increases soil fertility; improve physical properties, soil health.
- These can be alternative of chemical fertilizers as it contributes for plant nutrients through biological nitrogen fixation and solubilization of fixed phosphate, potassium and sulphur.
- These are cost-effective and pollution free fertilizers, highly demandable at present situation.
- It (*Azotobacter* sp.) helps to increase drought tolerance in plants and seed dormancy.
- Help in weed suppression (*Azolla* sp.).
- Some inoculants produce antibiotics and antifungal compounds (*Azotobacter* sp.) which reduces soil borne diseases.

Hence, we should increase the uses of biofertilizers and reduce the use of chemical fertilizers. Providing proper knowledge to farmers and training to workers is important. We should pay more attention on the regular supply and quality assurance.



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MECHANISMS OF IRON OXIDE NANOPARTICLES INTERACTION WITH BACTERIA

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Iron oxide nanoparticles (IONPs) have been of great interest, not only for fundamental properties caused by their multivalent oxidation states but also for their superparamagnetic, high force, low Curie temperature, high magnetic susceptibility, etc. IONPs have attracted much attention in the biomedical field. These nanoparticles have many different 'mechanisms of action' that converge to irreversible lethal effects. Therefore, it is crucial to know IONPs mechanisms of interaction with bacteria. Identification of morphological features IONPs and bacteria are important for understanding the interaction of IONPs with bacteria. Gram-positive and Gram-negative are the two main types of bacteria divided based on the cell wall structure, which is used for the evaluation of antibiotics. The cell wall size of Gram-positive bacteria is 20-80 nm which contains a thick layer of peptidoglycan and cell membrane. Gram-negative bacterial cell wall which is of 10-30 nm contains thin peptidoglycan with the plasma membrane and outer membrane. Both structurally and chemically, Gram-positive cell wall is more complex as compared to Gram-negative cell wall. The outer membrane LPS increases the net negative charge of the cell membrane present in Gram-negative bacteria, makes the cell wall impermeable to lipophilic solutes and this will also limit the penetration of negatively charged free radicals. Thus, IONPs sensitivity is less against Gram-negative bacteria. As compared to Gram-negative bacteria, due to the less



permeability barrier Gram-positive bacteria have a peptidoglycan layer and are much more sensitive to lower concentrations of IONPs. Due to the properties of the cell wall and cell membrane polarity Gram-positive bacteria become sensitive and therefore, Gram-positive bacteria are more sensitive to IONPs than Gram-negative bacteria.

The negative charge of *S. aureus* cell membrane is less than that of *E. coli* which causes more penetration of the negatively charged IONPs. Hence, the effectiveness of Fe_2O_3 nanoparticles is higher against Gram-positive bacteria. The interactions of a bacterial surface with IONPs and the attachment to various substrates is mediated by bacterial cell has adhesion factors. The bacterial response to antibacterial agents such as IONPs can be determined by the properties of the charge. Due to the anionic properties of teichoic acids and LPS in their cell surface, both Gram-positive and Gram-negative bacterial cell wall surface is negatively charged. The adhesion of IONPs to bacterial cells is governed by the surface charge of the bacterial cell wall. IONP only acts when it comes in contact with the cell wall of the bacteria. Vander Waals forces, electrostatic attraction, hydrophobic interactions and receptor-ligand are various interactions that promotes IONPs-bacterial contact. After coming in contact with the bacteria, IONPs can cross microbial cell membranes, interfere in metabolic pathways and changes in the membrane shape and function may induce. The inhibition of enzymes, deactivation of proteins, induction of oxidative stress and electrolyte imbalance is due to the interaction of IONPs with the microbial cellular machinery and when these nanoparticles are inside the microbial cell, they modify gene expression levels.

The release of positive ions such as Fe^{2+} , Fe^{3+} can adsorb on the bacterial surface of the cell. This happens when oxidation of IONPs takes place. In most Gram-negative bacteria, these ions damage the cell wall by forming holes. The electrostatic interaction between the negative charge of cell envelopes and the positive charge of iron oxide metal ions changes the efflux and influx of biomaterials from bacteria. Other interactions of IONPs with bacteria are bacterial cytosol leakage, blockage of electron transport chain, nucleic acid and protein damage, blistering or blebs and clumping of membranes. In the inner surface of bacteria, 10 to 12 nm-sized IONPs bind to sulfur-containing amino acids specifically to cysteine, homocysteine and methionine. For attachment of the metal ions, high affinity is seen in cysteine with the thiol functional group. Inside the bacteria, there will be the change in enzyme function such as NADH dehydrogenases, because of the attachment of cysteine.



Another antibacterial aspect of IONPs is Reactive Oxygen Species (ROS) formation, this is due to the higher activity of IONPs. Examples of ROS include superoxide radical (O_2^-), singlet oxygen (O_2), hydroxyl radical ($\cdot OH$), and hydrogen peroxide (H_2O_2). They are produced by the active Fe ions such as Fe^{2+} , Fe^{3+} by reacting with H_2O_2 . In the case of IONPs, the photocatalytic property effect was observed on antibacterial activities. The photocatalysis of IONPs is caused by electron-hole formation by electron excitation from the valence to conduction band under light or UV radiation. The electron holes react with H_2O molecules for ROS production. The bacterial membrane permeability is altered by oxidative stress and damages the cell. The effective factors in the rising of antibacterial activity are surface properties of IONPs which involve a high surface to volume ratio and low degree of crystallinity.

Future progress in genomics, metabolomics, and proteomics will help in the clear understanding of biochemical networks involved in cellular responses to oxidative stress. Improved understanding of these mechanisms of interaction will be helpful for clinicians regarding the usage of IONPs against certain bacteria in different situations.



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CYBORG BACTERIA - AN EMERGING NATURAL SOLAR CELL

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With the increase in industrialization, people started exploiting the natural sources to fulfill their energy requirements that is by machines powered by fossil fuels – coal, petrol, natural gas. But burning of these fossil fuels leads to release of the harmful carbon dioxide and other greenhouse gases that are largely responsible for atmospheric pollution and ozone layer depletion. This results in excessive global warming. These harmful effects of global warming can be reduced by the application of environment friendly sources of energy- creation of artificial photosynthetic system to generate renewable source of energy and simple organic and inorganic substances using sunlight.

Genetically engineered Cyborg bacteria can be used to solve this problem more efficiently. The natural carbon dioxide harvesting molecules in plants, that is Chlorophyll according to scientists, the process of photosynthesis is relatively inefficient.

The new approach seeks to improve the efficiency by equipping bacteria with some chemical compounds that act like a solar panel. Another added advantage of using these bacteria is that these Cyborg bacteria have a natural defense mechanism against heavy metals like cadmium, mercury or lead to protect themselves in adverse situations. These metals are arrested in the form of their sulphides and thereby



preventing the spread of these metals in their bodies. These sulphides in turn behave like small crystallized nano particles acting like semiconductors on the surface of the bacteria supercharging them. These cyborg bacteria have as a part of their respiratory process to convert water and carbon dioxide in the presence of sunlight to acetic acid which can be utilized for industrial purposes – manufacturing food, pharmaceuticals, fuels, plastics etc.

Dr. Kelsey Sakimoto first developed these cyborg bacteria that could act as living solar cells- this process is economically favorable as it does not require any expensive electrodes like other biological processes (requires only vats of culture broth). He worked with non-photosynthetic bacterium - *Moorella thermoacetica*.

In culture of broth of the bacteria Cadmium and sulfur containing amino acid cysteine was added and the suspension was kept out under sunlight and the bacteria naturally produced a compound cadmium sulphide which is an efficient light absorber which acted as semiconductor devices and increased the efficiency of the bacteria to produce acetic acid. On top of that the process was self-replicating and regenerating-potentially a low waste technology and unlike most of the other biological systems it is stable and works for an extended period of time.

The efficiency of these hybrid bacteria *Moorella thermoacetica* - Cds is around 90 % which is approximately four times of commercially available solar panels and six times more than artificial photosynthesis. The acetic acid is produced is given to some modified strains of *Escherichia coli* that use acetic acid as their food source. These *Escherichia coli* strains can now produce butanol and Polyhydroxy butyrate (PHB) a polymer used to produce biodegradable plastics, surgical implants for tablet packaging etc. If this prototype can be implemented on a large commercial scale the excess rate of carbon dioxide in the atmosphere, which causes global warming can be reduced, excessive toxic heavy metals harmful for the environment could be trapped for producing essentially important acetic acid and then the process is economic as well.



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THE BIOACTIVE COMPOUND OF ENDOPHYTIC ACTINOMYCETES

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Bacon and white proposed endophytes are the Microbes that colonize living, internal tissues of plants without carrying any immediate over negative effects. Endophytes could be better protected from biotic and abiotic stresses like Bacteria, Fungi, and Actinomycetes etc. Actinomycetes were also known as actinobacteria, derived from the Greek word were refers to lightning. Actinomycetes are considered as a unique group of microorganisms which placed between the true bacteria and true fungi. Actinomycetes are Gram positive, filamentous, spore forming, bacteria that have high G-C content in their DNA and cell wall containing L-L diaminopimelic acid. Endophytic actinomycetes produce different structural types such as catecholates, hydroxamates, and citrate based polycarboxylates. Moreover, all plant species serves as a source for endophytes and accepted as a source of novel bioactive compounds. Many studies reported that Endophytic actinomycetes are cable of producing a wide range of pharmaceutically relevant bioactive compounds such as antimicrobial, antitumor, anti-inflammatory, antiviral agents, etc., 24,000 active secondary metabolites was produced from microorganisms, In that 10,000 were isolated from actinomycetes, of these *Streptomyces* species alone contributed 7,600 bioactive compounds. Endophytic actinomycetes, especially those belonging to the genus *Streptomyces* have become an important microbial source for application in various crops such as *Lycopersicon esculentum*, *Triticum aestivum*, *Oryza sativa*, *Medicago sativa*, *Citrus reticulata*, *Brassica repa*, *Zea mays*. Various studies indicate that endophytic



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actinomycetes contribute of agriculture crops by employing production of plant hormone, Enzyme (chitinase, amylase, cellulase,) ammonia, antibacteria, solubulation of phosphate, nitrogen fixation, Biofertilizer and Biocontrol properties. Further studies are needed to evaluate the functions of these Endophytic actinomycetes enhancing host plant growth, that inducing systemic resistance and antipathogenic activity in field's traits. Some of the examples are tabulated below.

Table – 1: The Application of Endophytic actinomycetes and their Bioactive Compounds.

S.No	Endophytic Actinobacteria	Isolated plants	Application	Effect
1	<i>Microbispora</i> sp., <i>Streptomyces</i> sp., <i>Micromonospora</i> sp.	<i>Brassica rapa</i>	Biological control	Suppressed the occurrence of a post-inoculated strain of <i>Plasmoidiophora brassicae</i>
2	<i>Streptomyces</i> spp.	<i>Medicago sativa</i>	Growth promotion and N2-fixation	Improved shoot weight and the number of nodules
3	<i>Actinoplanes campanulatus</i> ,	<i>Zea mays</i>	Production of IAA	suppressed pathogenic activity
4	<i>Streptomyces</i> sp. and Non- <i>Streptomyces</i>	Spontaneous plants of Algerian Sahara	Production of IAA	Promoted seed germination and root elongation
5	<i>Streptomyces</i> spp.	<i>Triticum aestivum</i>	Solubilization of phosphate, production of phytase, chitinase, IAA, siderophore and malate	Improved plant growth, biomass and mineral
6	<i>Streptomyces</i> sp. MBR-52	<i>Rhododendron ferrugineum</i>	Production of Rooting promoting plant hormones	Accelerated emergence and elongation of plant adventitious roots



7	<i>Streptomyces</i> sp. and <i>Leifsonia xyli</i>	Medicinal plants	Solubilization of phosphate, production of siderophores, HCN, ammonia, chitinase, IAA, antifungal activities	Improved a range of growth parameters in <i>Capsicum annuum</i> L.
8	<i>Streptomyces</i> sp., <i>Nocardia</i> sp., <i>Nocardiopsis</i> sp. and <i>Spirillospora</i> sp.	<i>Citrus reticulata</i>	Production of IAA	Promoted shoot height, fresh shoot weight and fresh root weight of seedlings
9	<i>Streptomyces</i> sp. GMKU 3100	<i>Oryza sativa</i> L. cv. KDM105	Production of Siderophores	Increased root and shoot biomass and lengths of rice and mungbean plants
10	<i>Streptomyces diastaticus</i> , <i>Streptomyces fradiae</i> , <i>Streptomyces olivochromogenes</i> , <i>Streptomyces collinus</i> ,	Medicinal plants	Production of chitinase; Plant growth promoting abilities and antagonistic potential	Protect chickpea against <i>Sclerotium rolfsii</i> infestation; increased the biomass and reduced plant mortality of chickpea



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DROSOPHILA OF THE PLANT KINGDOM -

Neurospora

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Neurospora is commonly called as red or pink bread mould, some species of it are also known as Orange bread mould of the Phylum from 'Ascomycota'. The genus name "*Neurospora*", meaning 'nerve spore' in Greek, reference to the characteristic bands on the spores that look like axons. The first study of sexual reproduction was done by B. O. Dodge. Late on it was used by George Wells Beadle & Edward Laurie Tatum in X ray mutation experiments according to discover mutants that would serve as a source nutritional requirements. They have observed failures in metabolic pathway, caused by flaws in specific genes. This led them to propose the "One gene, one enzyme". The science what Beadle and Tatum have worked on is called "Biochemical Genetics".

There are several reasons that why the genus *Neurospora* are the choice for genetic research. *Neurospora crassa* is the best-known species in this genus, which is commonly used as a model organism in Biology. Most of the genetic studies for plant are done on *Neurospora* because it is easy to grow, easy to culture and it can also survive on Minimal media (Inorganic salts, Glucose, Water and Biotin etc. in agar) and has haploid life cycle that makes genetic analysis simple since recessive traits will show up in progeny. Analysis of genetic recombination is facilitated by the ordered arrangement of the products of meiosis in ascospores of *Neurospora*. The genome is about 43 mega base long with about 10,000 genes. It is having 7 chromosomes, which has been sequenced.

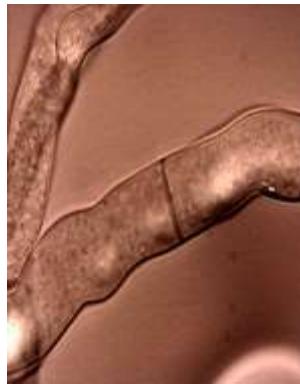




The filamentous structure of
Neurospora crassa



Orange Bread Mould –
Neurospora crassa



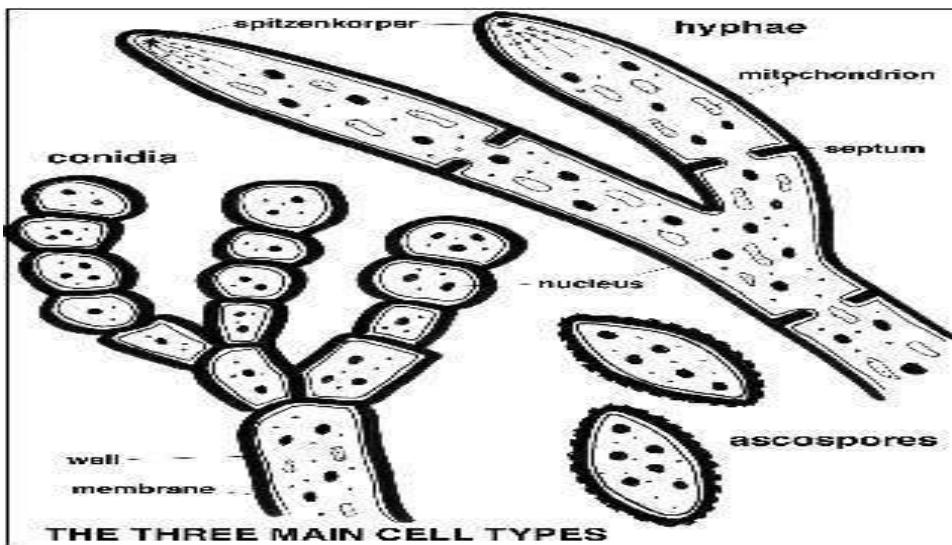
Neurospora crassa
hyphae (axon)

Since both have almost the same function, it's not wrong to say, the *Neurospora* as a Drosophila of plant kingdom. It is also important as it can be used to study the mechanism of molecular events like circadian rhythms, epigenetics, gene silencing, cell polarity, cell fusion, development as well as many phases of Biology and Biochemistry.

Neurospora crassa has a long history as an outstanding model for biochemical, genetic and cellular research. Although this fungus is known as a Saprotophrophs. Generally, their natural habitats are tropical and subtropical regions but they were also found in temperate climate. *Neurospora crassa* shows the site of colonization that can be easily seen on burning vegetations or trees after forest fires. *Neurospora* grows at an extraordinary rate the mycelium advances at 4 mm/hr in reasonably warm (32°C) environment if given some nutritional cultures of neurospora are easily identifiable by their orange aerial vegetative spores (conidia). Conidium-aseexual spores produced on a Conidiophore of certain fungi.



Morphology

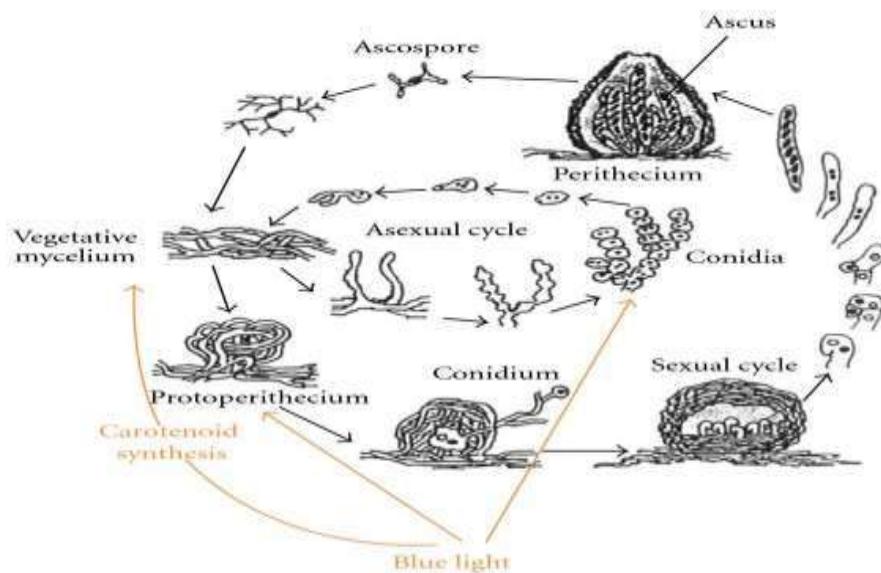


Basic Cell Structure - Hyphae, Conidia and Ascospores

Neurospora crassa is a heterothallic filamentous fungus and the name came from the nerve like bands found on its sexual ascospores. The haploid vegetative filaments, which is known as hyphae also look slightly like axons. It is morphologically complex multicellular organism with many more cell types than the unicellular yeast *Saccharomyces*. Most of workers are familiar with mycelia, macroconidia, perithecia and ascospores but the variation of cell type's produced by *Neurospora* may not be fully appreciated. All cells contain the basic components of a eukaryotic cell like nuclei, mitochondria, endoplasmic reticulum, golgi body, vacuoles, various types of vesicles, cytoskeleton, ribosomes and others. The cytoplasm is closed all round by a cytomembrane and outside this is a stiff cell wall largely composed of the polysaccharide chitin. At least a dozen specialized cell types can be special at various stages of the life cycles, but three cells types are the most famous at different phases of the life cycles, but 3 cell types are the major: (i) The hyphae "cell"; (ii) The conidium and (iii) The ascospores. Hyphae are tubes with walls that are stiff without at tips, which is where growth occurs. The conidia are mostly dealt with macroconidia. It also acts as agnatic fertilizing elements (spermatia) in Heterothallic species. And these football (rugby ball) - shaped cells with obese black walls developed from the products of meiosis. Each has numberless similar haploid nuclei.



Life Cycle



Life cycle of *Neurospora crassa* depending on Environmental conditions

Neurospora complete their life cycle in nearly 10 to 15 days. They are haploids, spending most of their life cycles in the haploid stage. The haploid mycelium reproduces asexually through 2 processes: (i) Simple proliferation of current mycelium, and (ii) Formation of conidia (macro & micro) which can be depressed and then germinate to produce new mycelium. This type of life cycle completely depends on environmental conditions; the vegetative mycelium can go through the asexual sporulation processes (Macroconidiation & Macroconidiation) which enters in sexual cycle by forming protoperithecia. Upon fertilization, initiate development leading to the formation of meiotically originated ascospores. The Blue lights show the input arrows.

Neurospora crassa, recessive mutations affecting the haploid phase of the life cycle are quite often in natural population. These mutations, when homozygous in the diploid phase, often cause spores to have maturation defects or to produce desolate fruiting bodies with few ascospores (sexual spores). This Genera is parasitize higher plants include *Ophiostoma* (the cause of Dutch elm disease), *Gnomonia* (leaf spots), *Diapoetha* (stem and leaf blights), *Cryphonectria* (chestnut blight), *Claviceps* (ergot of rye) and the powdery mildew fungi, fungal genetics have been widely studied in *Neurospora* & *Glomerella*.



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***Anoxybacillus* SPECIES FROM INDIAN HOT SPRINGS**

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Hot springs water has more than ambient atmospheric temperature and is important from social, cultural, ecological and biological point of view. These are the places where warm or hot groundwater comes out from the Earth and some of them can be found near volcanic region. Since ancient times, it is believed that these hot springs have medical importance mainly due to presence of sulfate like chemicals and are thought to cure many skin ailments, cardiovascular and other type of diseases. They are phenomena of worldwide occurrences and are present near religious places like temples. Many tourists visit them for spa and bathing owing to religious and medicinal value. From scientific angle they are important ecologically and microbiologically since they harbor many different and novel microbes due to unique ecosystem. There is famous Yellowstone national park in USA and first microbial inhabitant studies of the hot springs by culture-dependent method started from here with the isolation of thermophilic bacteria by Marsh and Larsen. Many hot springs has been reported to be niche for Thermophilic i.e., microbes capable of withstanding high temperature ($\geq 45^{\circ}\text{C}$) bacteria, fungi and archaea. The search for thermophiles started with isolation of *Thermus aquaticus* by Brock T. from Yellowstone national park, Taq polymerase enzyme isolated from it revolutionized the concept of polymerase chain reaction. In India GSI [geological survey of India] has identified seven geothermal provinces containing nearly 400 thermal springs, which are distributed in six geothermal provinces following in-depth geophysical



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explorations of hot springs based on the tectonic elements and geothermal gradients across India. Among those, ≥ 28 hot springs have been explored microbiologically, the microbes isolated from these have special qualities and have thermostable bioactive molecules like enzymes, antimicrobials, pigments, etc. The culture-dependent studies showed that Indian hot springs are diverse with respect to their content and microbial diversity. Thermophilic bacteria include mainly gram-positive *Bacilli* and Actinomycetes as well gram-negative *Pseudomonas* kind of species. The Bacillaceae family members are a good source of bacteria for bioprocessing and biotransformation involving whole cells or enzymes.

In contrast to *Bacillus* and *Geobacillus*, *Anoxybacillus* is a relatively new genus that was proposed in the year 2000. *Anoxybacillus flavothermus* (note: some publications use *A. flavithermus*) was formerly known as *Bacillus flavothermus*. The strain was discovered in a New Zealand hot spring and was characterized as a yellow-pigmented facultative anaerobe that grew in the range of 30–70 °C. Later, several strains with close sequence identity with *B. flavothermus* strains were found in Yellowstone National Park (Octopus Spring) and the Australian Great Artesian. In the year 2000, Pikuta and co-workers isolated the new anaerobic strain K1T from animal manure. Based on the 16S rRNA, DNA–DNA hybridization analyses and the phenotypic features of strain K1T, *Anoxybacillus* was proposed as a new genus of the Bacillaceae. The name *Anoxybacillus* was proposed based on its anaerobic property. Anoxic or anoxia refers to a condition wherein the dissolved oxygen is depleted; hence *Anoxybacillus* refers to a small, rod-shaped *bacilli* microorganism that can survive without oxygen. Because these bacteria are alkali-tolerant thermophiles, they are suitable for many industrial applications. The similarity of the 16S rRNA sequences throughout the genera is generally higher than 80 %. The *Anoxybacillus* species clustered together (92 – 99 % similarity) and their nearest neighbors are *Geobacillus*, *Saccharococcus*, and *Aeribacillus* in the range of 90 – 95 %. Strain K1T was thus named *Anoxybacillus pushchinensis*. As *A. pushchinensis* K1T and *B. flavothermus* are phylogenetically clustered together and are distinct from *Bacillus*, *B. flavothermus* was reclassified as *Anoxybacillus flavothermus*. Additional aerobic *Anoxybacillus* species were identified. The genus name remained *Anoxybacillus* despite the fact that the members are not strict anaerobes; they can be aerobes, facultative anaerobes, or facultative aerobes, temperature range of 50 – 65 °C, *Anoxybacillus* genome is small, it may serve as a thermostable expression host for certain thermozyme i.e. enzymes withstanding high temperature. The majority of *Anoxybacillus* species are moderate thermophiles (grow in the temperature range 30 – 75 °C, with the optimum at 50 – 62 °C).



Scientific reports of *Anoxybacillus* species from hot springs are few till date and in India they are even less. From Surajkund hot spring, a place in Jharkhand state there is report of *Anoxybacillus* species, out of which one was found to be novel and was named as *Anoxybacillus suryakundensis* JS1T. *Anoxybacillus gonensis* was reported in the same study and these species were found to be positive for enzyme amylase and cellulase. Due to thermophilic nature of these species enzyme produced would be thermally stable and can find application in industrial sector. *Anoxybacillus gonensis*, *Anoxybacillus kamchatkensis* were isolated from Sangameshwar-Tural Hot spring and in future they can be found in other hot spring location as well. *Anoxybacillus* cells are alkaliphilic or alkali tolerant, and most of the species are able to grow at a neutral pH. Vegetative cells are rod-shaped or straight or slightly curved, sometimes with angular division and Y-shaped cells, often in pairs or short chains, with rounded ends. The cells are motile or nonmotile. Endospores are round, oval, or cylindrical and have a terminal location. Colony morphology and size are variable. Most of the species produce cellular carotenoid like pigments, which yields yellow colonies. They are catalase variable. Many members of the genus are alkaliphilic, but most of the species can grow at neutral pH. Only *A. amylolyticus* grows optimally at slightly acidic conditions (pH 5.6). *Anoxybacillus* species are chemoorganotrophic, with a fermentative or aerobic respiration metabolism. They can use oxygen or nitrate as electron acceptors. Exploration of *Anoxybacillus* species is in the beginning phase and future research can bring many more novel and important species in focus. In India very few reports are there about *Anoxybacillus* species from hot spring location and can be a subject of intense exploration bringing new understanding and applications in the field.



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AMAZING MICROBIAL COMMUNITIES AS POTENT POLLUTION CONTROL AGENTS

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Carbon monoxide is one of the pollutants released by industries and domestic sources. It has negative impacts on the environment as well as human health. Thus, the emission of this toxic pollutant gas needs to be checked and controlled effectively when the planet is already struggling with global warming and poor atmosphere quality which also affects public health.

The use of microbes in bioremediation is not an alien concept today. Various microorganisms depending on the needs are appointed for the task. The problem associated with carbon monoxide is that it is generally unavailable/non-usable or toxic to the majority of organisms. Thus, many of them can 'act upon' this or 'utilize' it. But certain amazing microbes have the ability to 'act upon' or 'utilize' carbon monoxide. These are called 'Carboxydrotrophs'. Carboxydrotrophs comprise some bacteria, eubacteria and archaea. These microbes can convert the otherwise non-usable carbon monoxide. This conversion is facilitated by an enzyme named CODH (Carbon Monoxide Dehydrogenase). This enzyme can convert carbon monoxide into carbon dioxide. A substrate can be used to be reduced i.e. accept the electrons while on the other hand carbon monoxide is oxidized to give carbon dioxide. Water can also serve as the substrate to be reduced. This carbon dioxide can then enter various pathways and be utilized resulting in different products. Some



microbes can utilize this carbon monoxide as a carbon source. While some microbes can generate energy or reducing equivalents by this and require a carbon source other than carbon monoxide. Some researchers state that the possible reason behind this ability is that these microbes can be evolved long back when the earth's atmosphere was anaerobic and rich in carbon monoxide.

Carboxydrophic microbes can be found in vivid habitats such as hot water springs, oceanic thermal vents, free-living in soil, some in symbiotic association with plants, marshy areas, volcanic springs, volcanic soils. Some are thermophiles, some halophiles, some aerobic while some can be anaerobic too. If classified based on the important product or metabolite of interest produced, Carboxydrophic comprise organisms from various communities such as methanogens (methane producers), hydrogenogens (hydrogen gas producers), acetogens (acetate producers). Some are metals or sulphate reducers. Not only these, but some can also produce malate, butanol and ethanol. Different organisms can have different tolerance levels of this carbon monoxide.

Few examples of Carboxydrophic are *Carboxydothermus hydrogenoformans* (thermophilic eubacteria in volcanic hot spring), *Rhodospirillum rubrum* (phototrophic), *Rhodococcus gelatinosus* (anaerobic and grows in dark), *Rugeria pomeroyi* DSS-3 (marine bacterioplankton), *Archeoglobus fulgidus* VC-16 (hyperthermophilic sulphate-reducing archaeon), *Methanothermobacter marburgensis* (methanogen), some members of *Oscillospira*, *Pleomorphomonas*, *Acetobacterium*. *Acetobacterium woodii* is also a Carboxydrophic of growing interest.

Recent studies are also conducted with the microbes enriched from wastewater treatment plants. They were supplied with different concentrations of carbon monoxide. The organisms were able to consume the off-gases containing carbon monoxide and yielding the products such as ethanol and acetate. Thus, it is really amazing that these Carboxydrophic coming from the above-mentioned groups can give industrially important or comparatively less hazardous products by utilizing this pollutant carbon monoxide. When observed, it is clear. For example, hydrogen gas, ethanol, butanol can be used as fuels. Acetyl CoA is an important metabolite that can enter pathways leading to other important products, malate, fumarate, butyrate, acetate also such important metabolites, reduction of metals or sulphur can also be obtained. Consortia or mixed fermentation can be done with suitably selected organisms too.



The phenomenon itself is fascinating that such products can be obtained using this waste or pollutant gas. This usually non-usable gas is fixed and enters the carbon cycle. Thus, this strategy can also be used for the treatment of syngas which is difficult to degrade. It is a double benefit that the carbon monoxide is not only degraded but also such important products are obtained. Thus, the growing field of green energy, green fuels are the area where these organisms can be appointed. A large area for further research is now open in this field of using Carboxydotrophs. Ever advances in technologies such as in R-DNA technology is a booster as further enhancement and application of this characteristic can be done. This quality of organisms can be utilized as a potent cleansing action. Bioremediation can be taken to a step ahead and coupled with industries to treat the effluents or off-gases which again, in turn, give products.



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INDUSTRIAL APPLICATION OF ALKALINE PROTEASES

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Enzymes are the cornerstones of metabolism and constitute the fundamental basis for existence of life. However, recently enzymes are being implicated in diverse industrial processes because of their specific and fast action for efficient bioconversion of substrate to product, and their capability to save raw materials, energy and chemicals for various manufacturing processes. Enzymes are considered as environment-friendly (green) chemicals that may potentially help replacing completely or reducing the usage of hazardous chemicals for industrial processes, thus promising sustainable production and manufacturing. Among various industrial enzymes microbial proteases dominate the world enzyme market due to their multifaceted application potential in varied bioindustries like food, pharmaceutical, textile, photographic, leather and detergent. Promising applications of proteases in agricultural sector for instance may include biocontrol of pests, degumming of silk, selective delignification of hemp and wool processing. However, for successful industrial applications the proteases must be robust enough to suit the process conditions which are generally hostile. Proteases intended for industrial applications must have activity and stability over wide range of temperature and pH extremes for prolonged time periods and even in the presence of various potential enzyme inhibitors. Of various microbial proteases those from *Bacillus* spp. have got special significance because the latter are known for their ability to produce sturdy enzymes



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that might have suitability for industrial process conditions. The current article presents

Cleansing efficacy, especially for rigid biomaterials, but also allows the washing to be accomplished at relatively low temperatures, thus saving energy and making the process economic. Supplementation of enzymes in detergents helps in reducing quantities of other hazardous chemical-based detergent components like soaps, oxidizing agents, chelating agents and surfactants, thus making detergents more eco-friendly. But there may be some limitations of enzymes usage in detergents, i.e. enzymes may be expensive to produce, may exhibit some allergic reactions, may get denatured at elevated temperatures and pH extremes and may digest some fabrics (wool). However, these limitations can be readily overcome by selecting the most apt detergent-compatible enzymes from the vast diversity of microbial Protease. Fibrin is an insoluble protein derived from its soluble precursor, fibrinogen, which is involved in blood clotting. Fibrin plays a vital role in health and healing; however, formation of inappropriate clot especially under certain pathophysiological conditions in the body is a major risk factor for heart disorders.

Homoeostasis of formation and dissolution of fibrin maintain appropriate viscosity in the vascular system. A shift in balance towards fibrin overproduction leads to unwanted clotting resulting in cardiac complications like acute myocardial infarction, ischaemic heart diseases, valvular heart diseases, peripheral vascular diseases, arrhythmias, high blood pressure and stroke. Enzyme-based method may be developed that may not only recover silver efficiently but might have minimal impact on the environment (Nakiboglu et al., 2013). Proteases have been reported to possess excellent gelatinolytic activity for successful recovery of silver from X-ray film. *Bacillus* proteases viz., *Bacillus licheniformis* (*B. licheniformis*, *B. subtilis* and *B. licheniformis*), have been demonstrated to possess good gelatinolytic activity for efficient silver recovery from X-ray films. Proteases from other sources like *Aspergillus versicolor* and *Purpureocillium lilacinum* have also shown good gelatin hydrolyzing ability. High temperature and slightly alkaline conditions favour stripping off of the gelatin layer. Thus, thermostable alkaline proteases from *Bacillus* spp. are well suited for the process. Nutritionally enriched medium was used for the production of proteolytic enzyme that was capable of efficiently hydrolysing X-ray-bound gelatin (Kumaran et al., 2013).

Considering the eco-unfriendly nature of chemical based methods of silver extraction from X-ray films, a greener approach that is based on application of enzymes is gaining attention. The enzyme-based silver extraction from X-ray films relies more on renewable energy resources than on fossil fuel and, thus, might offer



an overall eco-safe Proteases are used for a wide range of food processing applications, e.g. in dairy, bakery, fish and seafood processing, animal protein processing, meat tenderization, plant protein processing and generation of bioactive peptides. Major aim for application of enzymes in food processing is to enhance the nutritional and functional properties of foods such as improved digestibility, modifications of sensory quality, improvement of antioxidant capability and reduction of allergenic compounds. However, the choice of enzyme and the desired degree of hydrolysis must be realized by taking into account the taste, solubility and specific application properties of the hydrolysate product.



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MICROORGANISMS LED REDUCTION OF GREEN HOUSE GASES

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Emission of greenhouse gases e.g., carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (NO_2) increase global temperature thereby leading to global warming. These gases emanate from the use of fossil fuel, deforestation, faulty disposals of industrial waste in in-land water bodies, decay of plant matters and biomass burning amongst others etc. Microbes have been proved to play a dual role of producer as well as consumer of greenhouse gases in line with their functional nature. While imbalance in microbial function leads to release of gases in atmosphere, microbes take part in biogeochemical cycle and natural nutrient recycling process facilitating growth of mineral nutrient in the soil.

Carbon dioxide is produced by increase in microbial activity through soil respiration. This process can potentially double with 5 to 10° C increase in temperature. On other hand, research has discovered that two Cyanobacteria i.e., *Prochlorococcus* sp. and *Synechococcus* sp. are capable of remove 10 billion tons of carbon from air and fix 2/3rd of total carbon concern in atmosphere. In earth's biogeochemical cycle, microorganism is involved in transformation of decay organic matter into harmless products. Best example of process is photosynthesis by cyanobacteria (blue green algae) and degradation of hydrogen sulphide by green and purple sulphur bacteria.



Methane is released by daily activity of combustion, landfills, waste deposition, livestock and farming amongst others. Bacteria release and use methane as an energy source for metabolism. As an example, Methanogenic bacteria include both *Methanosarcina barkeri* which convert carbon dioxide into methane and methanotrophs like *Methylocella silvestris*, *Methylocapsa aurea* that convert this back into carbon dioxide. Another example of methanogen bacteria present in Rumenant animal is *Butyrivibrio fibrisolvens* which release methane gas because bacteria acted on their decomposed food. As per research report, 20 % of global methane is produced from farm animals. In practice, vaccines are being given to ruminant animals to remove their methanogen bacteria.

Nitrogen constitutes 78 % of earth's atmosphere available in compound like, ammonia, nitrous oxide and dinitrous oxide. *Rhizobium* sp., fixes nitrogen either in symbiotic or Asymbiotic condition as well as transform fixed ammonium ion to nitrogen oxides and amino acids into proteins and alkaloids molecule. Apart from nitrogen fixation; nitrification by *Nitrosomonas* sp., converts ammonia into nitrites. Denitrification by *Nitrobacter* sp. oxidise nitrites into nitrates. Ammonification is release of ammonium from nitrogen after decomposition by fungi and bacteria, which will again re-enter normal Nitrogen cycle.

Halocarbon gases, ozone, water vapour and aerosols are among the greenhouse gases released in lesser quantity. Future research is based upon detailed meta-analysis of microbial action against them. Efforts will also be made to explore options of modification in these micro-organisms through genetic engineering to boost their efficiency against above mentioned harmful green-house gases.



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MICROBIOLOGY OF CORONAVIRUS

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Coronaviruses is the group of viruses causing respiratory infections. COVID-19 (Coronavirus disease) caused by severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2).

Virology of Coronavirus

Realm	-	Ribovira
Order	-	Nidovirales (Viruses replicate using set of mRNAs)
Sub-order	-	Cornidoviridaceae
Family	-	Coronaviridae
Sub-family	-	Orthocoronaviridae
Genera	-	On the basis of genus evolving the Coronavirus becoming variants such as Alpha, Beta, Gamma and Delta.

The diameter of the positive stranded RNA Coronavirus ranges from 20 nm to 500 nm. It has a characteristic crown like appearance. It is largest known viral RNA genome with a length of 27 to 32 kb. Its membrane studded with Glycoprotein spikes and surrounds genome roughly spherical in shape. Virus genome encodes 4 or 5 Structural proteins S, M, N, HE & E. The Spike (S) protein projects through the viral envelope and forms the characteristic spikes in coronavirus "Crown". It is heavily glycosylated, probably forms a homotrimer and mediates receptor binding and fusion with the host cell membrane. The major antigens that stimulate neutralizing antibody as well as important targets of cytotoxic lymphocytes are on S protein.



Coronaviruses is widespread among Birds and mammals. Bats being host to the largest genotypes. Seven coronavirus serotypes associated with disease are HCoV-229E, HCoV-NL63, HCoV-HRO1, SARS-CoV, and MERS-CoV.

- **Alpha Coronavirus Genus:** There are 2 human coronavirus HCoV-229E and HCoV-NL63. Transmissible gastroenteritis virus of pigs and feline infections peritonitis. There are also several related bat Coronaviruses among the Alpha Coronaviruses.
- **Beta Coronaviruses Genus:** HCoV-OC43 and HCoV-HKU1 contains several bat viruses. Important animal beta coronavirus is mouse Hepatitis virus.
- **Gamma Coronavirus:** Most prominent in birds. Avian Infectious Bronchitis Virus (AIBV) an important veterinary pathogen causing respiratory and reproductive tract disease in chickens.
- **Delta Coronavirus Genus:** It contains recently discovery avian coronavirus found in several species of songbirds.

None of common cold human coronaviruses HCoV-OC43, HCoV-NE63, HCoV-HKU and HCoV-229E replicate easily in tissue culture, and until recently, this impeded progress in their study. Both HCoV-229E and HCoV-OC43 were discovered in 1960 and were shown in volunteer experiments to produce common colds in adults studies in the 1970 and 1980 linked them to as much as one third of upper respiratory tract infectious during winter outbreaks 5 – 10 % of lower respiratory illness in children.

Vaccines

At the end of 2020, 40 vaccines for human trials and 150 vaccines ere for preclinical trials. Three distinct development process for vaccine production

- **Phase - I:** It is designed to test vaccine safety although immunogenicity is also measured: dose ranging studies are also after included.
- **Phase - II:** These expand the safety profile and immune response assessment in larger number of participants.
- **Phase - III:** These are designed to determine efficacy in preventing a predefined endpoint, usually laboratory conditioned disease in glance among those who received vaccine versus those who received the control product and is calculated with the following formula.

In COVID-19 vaccine initiative Phase - I, II and III studies have frequently been combined with a seamless transition from one phase into the next.



Antigenic target

Major ionogenic target for both SARS-CoV-I and MERS vaccines large surface spike protein.

Vaccine - enhanced disease

Vaccinated animals developed non-neutralizing antibody and Th2 cell responses that were associated with eosinophilic lung inflammation. It shows no enhanced disease in humans. Include criterial for neutralizing antibody and Th1 Polarized Cellular immune responses. Vaccine delivered intramuscular or intradermally Live attenuated COVID-19 vaccines /administrated to the respiratory tract are undergoing clinical research. Vaccine developed using platforms are:

- ***Traditional approach:*** (a) Inactivated virus and (b) Live attenuated virus
- ***Newer Platform:*** Recombinant proteins (Papillornavirus) and Vectors (Ebola).

The vaccine developed in India and currently used are Covaxin developed by Bharat Biotech and Covishield by Serum institute, India.

Treatment

Remdesivir is an antiviral medicine used in treatment. Hydro chloroquine drug was also initially used for treatment.



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ALGAL BLOOM: HARMFUL EFFECTS AND CONTROL

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Algal bloom is defined as an enormous growth of cyanobacteria in entire range of water, from salt to freshwater. They have been observed as foam, mats, scum or clumps. These microscopic algae are known as marine or water blooms (phytoplankton). Excess of nutrients like phosphorous or nitrogen, high temperature, sunlight, hydrology and change in climate condition are believed to be the reasons behind growth of these phytoplankton. Eutrophication is a consequence of an enrichment of nutrient or dead organic matter inside water leading to death of fishes because of depletion of oxygen consumed by bacteria present in these aquatic systems. Different algae types have been reported based on colours produced by their pigments such as blue-green, red or yellowish brown.

Algal bloom has serious impact on environment, aquatic ecosystem and economies. They produce neurotoxin which has a severe impact on wildlife consuming them. Fishery are strictly banned in these areas because neurotoxins are exposed to fishes making them unedible for human and animals. *Alexandrium*, (red algae) released toxins that mixed with seafood results in paralysis. Another red algae, *Pseudo-nitzschia* secrete domoic acid as toxins which can cause vomiting, diarrhoea, seizures, altered gene expression, impaired cell function, damage brain function and short term memory loss if consumed at a high level. *Microcystis* (cyanobacteria) produced toxin impacts liver leading to symptoms of gastrointestinal illness.



Excessive nutrients from fertilizers or sewage water by runoff end up bringing even more warmer water to water bodies in the summer season.

As a control measure, steps have been taken to either proactively detect or stop algal growth. Phosphorous free fertilizers and detergents are used to limit nutrient rich runoff. Researcher of NIEHS have used remote sensing technology for prediction of algal bloom. They have monitored water quality by detecting algae chlorophyll through satellite. Currently, ultrasound radiation has been identified as most impactful method of controlling algae producing algal bloom. These radiations cause structural and functional destruction of algal cell by preventing them from absorbing sunlight at the water surface, thereby preventing algae from entering the blooming stage. To efficiently clean the water environment, the frequencies of these ultrasonic radiations have to be specifically aligned to the types of algae.

Going forward, studies in this area needs to focus on standardizing method of controlling an Algae in line with climate conditions. Water ecosystem needs to be conserved by proactively estimating the pollution challenge which can be identified through the foul smell emanating from the overgrowth of new algal species. The procedure will require higher quantum of field trials by employing biocontrol agents to scavenge biotic growth of bad algae.



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ENDOPHYTES: A PROMISING SOURCE OF BIOACTIVE COMPOUNDS

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Introduction

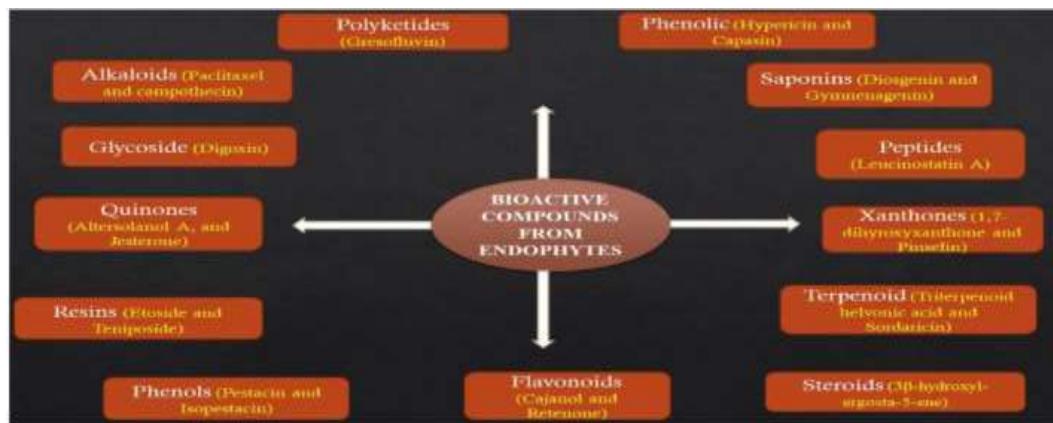
Endophytes are an endosymbiotic group of microorganisms that colonize in plants and microbes that can be readily isolated from any microbial or plant growth medium. They act as reservoirs of novel bioactive secondary metabolites, such as alkaloids, phenolic acids, quinones, steroids, saponins, tannins, and terpenoids that serve as a potential candidate for antimicrobial, anti-insect, anticancer and many more properties. While plant sources are being extensively explored for new chemical entities for therapeutic purposes, endophytic microbes also constitute an important source for drug discovery.

Plants have served as a source of medicinal bioactive compounds against numerous forms of ailments for centuries. Ironically, in recent years, microorganisms associated with plants rather than plants themselves have proved to offer material and products with high therapeutic potential. Endophytes are an endosymbiotic group of microorganisms often bacteria or fungi that colonize the inter- and/or intracellular locations of plants. For these organisms, all or part of their life cycle occurs within their hosts, without causing any apparent symptoms of disease. They are ubiquitous in nature and exhibit complex interactions with their hosts, which



involve mutualism, antagonism and rarely parasitism. Endophytes are known to enhance host growth and nutrient gain. They may improve the plant's ability to tolerate various types of abiotic and biotic stresses, and enhance the resistance of plants to insects and pests. They produce phytohormones and other bioactive compounds of biotechnological interest (enzymes and pharmaceutical drugs).

Researchers have indicated the presence of one or more types of endophytes in every single plant studied to date. Endophytes can colonize in the stem, roots, petioles, leaf segments, inflorescences of weeds, fruit, buds, seeds and also dead and hollow hyaline cells of plants. The population of endophytes in a plant species is highly variable and depends on various components, such as host species, host developmental stage, inoculum density and environmental condition. However, very few studies have exploited these symbiotic groups of organisms and their bioactive metabolites. For the past few decades, it has become evident that the discovery rate of active novel chemical entities is declining. While plant sources are being extensively explored for the discovery of new chemical entities for various therapeutic purposes, endophytic microorganisms play an important role in this search for natural bioactive compounds, with potential use in the health sector and in drug discovery. This review highlights the various sources of endophytes, their secondary metabolites and role as source of drugs. Such studies may improve the understanding of endophytes and address the need for new and useful compounds necessary to combat various pathogens associated with human health and other possible medicinal uses.



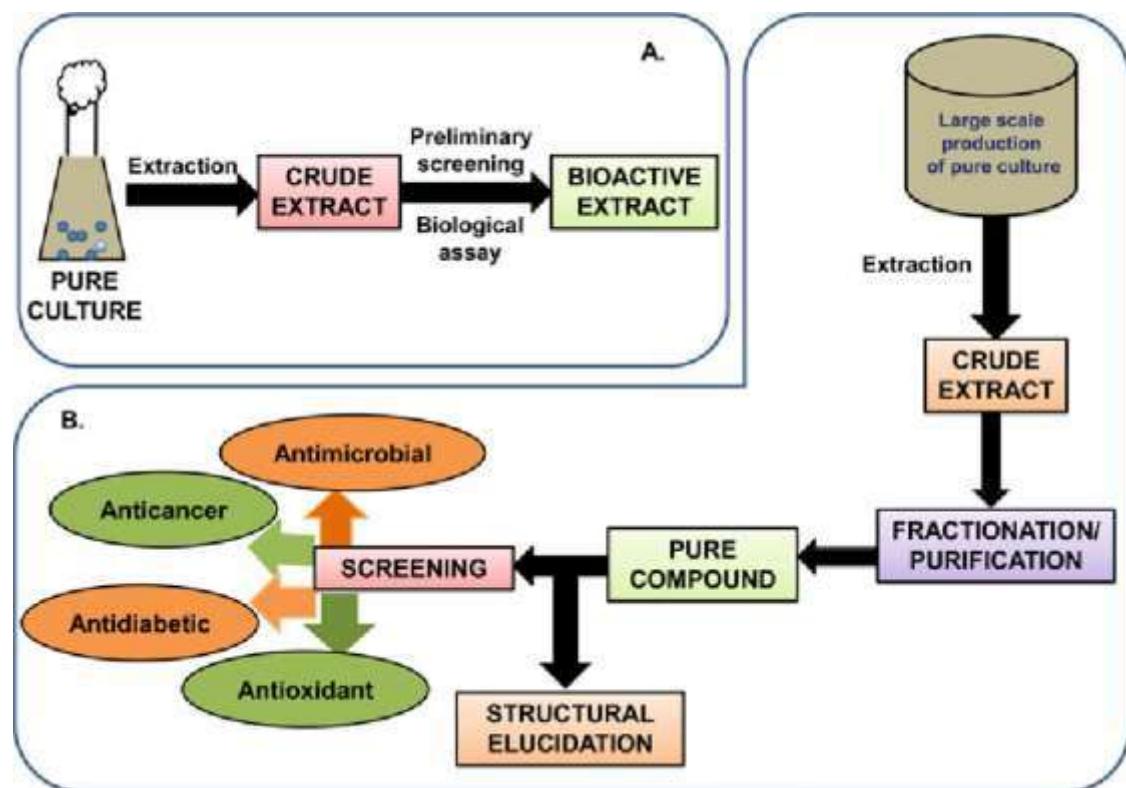
Bioactive Compounds from Endophytes

Endophytes are reported to produce a number of bioactive metabolites in a single plant or microbe which served as an excellent source of drugs for treatment against various diseases and with potential applications in agriculture, medicine,



food and cosmetics industries. These secondary metabolites were categorized into various functional groups, alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, saponins, tannins, terpenoids, tetralones and xanthones.

Extraction of metabolites from endophytes is affected by various factors, such as the season of sample collection, climatic condition and geographical location. However, with a revolutionary synthetic process that has been developed during the past few years, extraction from plants and other natural sources has now become more feasible, efficient and convenient. The production of bioactive substances by endophytes, has been directly associated with the evolution of the host microorganisms, which may have incorporated genetic information from higher plants, allowing them to better adapt to the host plant and perform some functions, such as protection from various types of pathogens, insects, and grazing animals. Some of the commonly found secondary bioactive compounds from endophytes are described below.



Taxol (paclitaxol), a complex diterpene alkaloid produced by the endophyte *Metarrhizium anisopliae* found in the bark of *Taxus* tree, is one of the most promising anticancer agents developed or synthesized to date. Camptothecin, from *Nothapodytes foetida* is known to have cytotoxic and antifungal properties.



Huperzine A (HupA), from *Huperzia serrata*, can act as a cholinesterase inhibitor. Lignans, such as cathartics, emetics and cholagogue, isolated from endophytic *Podophyllum hexandrum*, are reported to act as anticancer agents. Resins, such as etoposide and teniposide extracted from *P. emodi*, possess strong anticancer activity. Compounds such as oxacillin, ampicillin, catechin, gallic acid, and cefalexin are known to possess bactericidal activities. Terpenoids possess antineoplastic, antibacterial, and antiviral effects as well as gastrointestinal stimulation. The endophytic fungus, *Cytonaema* sp., produces triterpenoid helvolic acid with strong antibacterial activity.

Microbial Endophytes as Source of Drug Against Various Diseases

Infectious and parasitic diseases account for approximately half of the deaths worldwide. Although it is the generation of nano to pico drugs, natural sources have been proven as the best source for drug discovery. Medicinal plants and their endophytes are an important source of precious bioactive compounds and secondary metabolites that contribute to more than 80% of the natural drugs available in the market. Endophytic microorganisms are the storehouse of novel secondary metabolites that can serve as an excellent source of drugs for antiarthritic, antimicrobial, anticancer, antidiabetic, anti-insect, and immunosuppressant activities. To date, only a few plants have been investigated for their endophytic diversity and potential to produce bioactive secondary metabolites. The discovery of novel antimicrobial secondary metabolites and bioactive compounds from different types of endophytic microorganisms is an important alternative to overcome the increasing levels of drugs resistance to various pathogenic microorganisms. There are a number of bioactive compounds, such as camptothecin, diosgenin, hypericin, paclitaxel, podophyllotoxin, and vinblastine, which have been commercially produced by different endophytic fungi present in respective plants and they are of both agricultural as well pharmaceutical importance. These compounds are analogs of various types of phytohormones, essential oils etc. isolated from various endophytes.



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CARCINOGENIC IN COSMETICS

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Cancer is an abnormal and uncontrolled growth of cell in the body and it's termed as malignancy. Worldwide, cancer is one of the leading causes of mortality, morbidity, and decreased quality of life. A carcinogen is an agent which has the capacity to cause the cancer in human beings. It may be a substance in the environmental exposure, a product we use, or a chemical in foods and drinks. Just because we had contact with a carcinogen doesn't mean that we will get cancer. Our chance of getting sick depends on many things such as immune system status, genetic factors and age. The main mechanism of carcinogens is the activation of proto-oncogenes (unmutated cell) into oncogene (mutated). Approximately, 50 different types of proto-oncogenes that can be activated into oncogenes. Proto-oncogenes are not abnormal genes, but a normal part of every cell makeup, and are important in early embryonic development. Oncogenes lead to cancer development only if they are activated after development is complete. As a result of exposure to carcinogenic agents. Both internal and external conditions that are able to activate oncogenes and causes cancer. Internal factors are unique to the individual influence a person's chance of developing cancer. External factors are such as physical carcinogens, chemical carcinogens and viral carcinogens.

In our day-to-day life cosmetics became a part of it. Some of these can cause health problems in some people, such as skin or eye irritation or allergic reactions. There have been numerous controversies surrounding cosmetics and their chemical constituents with carcinogenesis. In our day-to-day life, unknowingly we use many products which contains carcinogens, it includes shampoo, conditioner, soap, face



wash, hair dye, hair straightening products, body lotion, toothpaste, eye shadow, hand wash, talcum powder and make-up products.

In recent years, a random test conducted on two batches of famous private company baby shampoo samples in Rajasthan has found trace of the chemical formaldehyde, a known carcinogen. Most of the fears around the chemical arise from the fact that the International Agency for Research on Cancer (IARC) classifies formaldehyde as a carcinogen. Formaldehyde is everywhere around us, including in the food we eat such as fruit & fish and household products like furniture polish. Not just shampoo, but several personal care products have chemicals that release formaldehyde such as quarternium-15 & methenamine. These are added because formaldehyde kills bacteria & fungi, which are also a risk to cosmetic users.

Parabens is another group of chemicals widely used as artificial preservatives in cosmetics and body care products. These chemicals are added to prevent and reduce the growth of harmful bacteria and mold, increasingly the shelf life of the product. It causes the endocrine disruption, reproductive harm and breast cancer in women. Parabens can act like the hormone (estrogen) in the body and disrupt the normal function of hormone system and affects the reproductive system functions. There were several types of parabens are used in cosmetics they are, propyl paraben, methyl paraben, butyl paraben, ethyl paraben, isobutyl paraben and isopropyl paraben.

Talcum powder is a popular and effective ingredient in keeping the skin dry and protected. Talc is a naturally occurring mineral that contains a combination of magnesium, silicon, oxygen and hydrogen. When talc is mined and milled, it becomes talcum powder, which is commonly used in cosmetics. Usually talc is not a carcinogenic, but in its natural form, some talc contains asbestos (a substance known to cause cancer). Talcum powder is linked to cancer, it is important to distinguish between talc that contains asbestos and talc that is asbestos free. Talc that has asbestos is generally accepted as being able to cause cancer if it is inhaled. In some studies, talc miners and millers have suggested an increased risk of lung cancer and other respiratory diseases. Women who apply talcum powder regularly in the genital area have an increased risk of ovarian cancer.

Triclosan and triclocarban are another commonly used antimicrobial agents found in many soaps and detergents. Triclosan was initially developed as a surgical for medical professionals, but in recent years it has been added to a host of consumer products. In 2005 the FDA found triclosan and triclocarban was unnecessary for plain soaps. Triclosan in toothpaste, soap and cosmetics may accumulate in the



body, which could have an adverse effect on thyroid hormones and genitalia, and risk of developing breast cancer.

In our regular life-style chemicals plays a vital role, because every single product in the market contains at least one chemical. We must aware of the chemicals in the product which we use, because some of the chemicals are carcinogens, we should not to take risk on life. When our world grows faster the risk of life also increased. There are several other carcinogens also used in our cosmetics, but without our knowledge we bring our life under the risk. The concentration of carcinogen used in products may less but when we frequently use it there is an increased chance getting sick.



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NATURAL THERAPEUTICS AGAINST COVID-19

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Nowadays global world has been facing the pandemic issue COVID-19, a disease induced by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2). In recent days extensive research is going on COVID19 for development of therapeutics, but there is still no approved therapy against SARS-CoV-2. The utility nature of plant derived components may provide novel therapeutics to treat the symptoms of this disease. Enormous number of natural therapeutics are suggested for the treatment of various viral pathogens that stimulated analysis of natural metabolites against SARS-CoV-2. Moreover, interferon therapies, monoclonal antibodies, oligonucleotide-based therapies, peptides, small-molecule drugs, and vaccines, are regarded as some strategic approaches for controlling or preventing COVID-19. Existing drugs like favipiravir, remdesivir, lopinavir, ritonavir, nebulized α -interferon, chloroquine, hydroxychloroquine, ribavirin, and interferon (IFN), can be used as the first-line treatment for coronavirus outbreaks, but there is no ultimate solution to eradicate the disease. *In silico* analysis showed that different plant derived component are the most valuable drug targets against SARS CoV-2. Plant-based products affect several key events in the pathogenic process. This study is mainly focusing on some of the plants that have antiviral activity.



Curcuma longa

It is effective for its antineoplastic, anti-proliferative, anti-aging, anti-inflammatory, anti-angiogenic, antiviral and anti-oxidant effects, and can regulate redox status, protein kinases, transcription factors, adhesion molecules, and cytokines in the human body.

Nigella sativa

Nigella sativa seed oil presented both antiviral and immunomodulatory effects against cytomegalovirus, reducing viral loads to an undetectable level. It can also enhance the immune response by increasing CD3 and CD4 counts, as well as up-regulating Interferon-gamma (IFN- γ) release from Natural Killer T-cells and Macrophages.

Vernonia amygdalina

Aqueous extracts of *Vernonia amygdalina* showed positive effects in enhancing immune response by increasing the levels of White blood cells and CD4 $^{+}$.

Azadirachta indica

A popular Indian plant, is traditionally boiled and consumed for treatment of fever with reported anti-inflammatory effects and have direct antiviral effects against various viruses including dengue.

Allium sativum

Pre-treatment with aqueous garlic extract showed notable antiviral effects mainly by reduction in infectivity by reduction in serum TNF- α , ICAM-1 and immunoglobulin (G and M) levels confirming the enhancement in immune system activity.

Cinnamomum verum

It's essential oil and powder exhibits anti-oxidant, immunostimulant, and antiviral activity. Its bark extract exhibits immunomodulatory activity and significantly increased serum immunoglobulins, phagocytic index, neutrophil adhesion, and antibody titer.

Nigella sativa

Nigella sativa bioactive compounds have been observed as potential inhibitors of COVID-19 in molecular docking studies. Nigellidine gave energy complex at active site with energy scores closest to chloroquine and better than



hydroxychloroquine and favipiravir whereas α -hederin gave energy complex at the active site with energy scores better than chloroquine, hydroxychloroquine, and favipiravir.

Ocimum sanctu

Hydro-alcoholic extract of *Ocimum sanctum* inhibits intracellular multiplication of virus.

Phyllanthus emblica

Amla has been reported to significantly relieve chromium-induced immunosuppressive effect on lymphocyte proliferation and led to restoration in production of IL-2 and INF γ .

Withania somnifera

Multiple studies have proved that Ashwagandha has antiviral and immunomodulatory potential. Very recently, an *in silico* study concluded that Withaferin-A exhibits antiviral potential against SARS-CoV-2 through inhibiting RNA polymerase with higher binding energy than hydroxychloroquine and other drugs used against SARS-CoV-2.

Zingiber officinale

Fresh ginger aqueous extract showed antiviral activity against Human Respiratory Syncytial Virus in human Respiratory tract Cell lines (HEp-2 and A549) and decreased the plaque counts in a dose-dependent manner. It also stimulated the secretion of IFN- β that contributes to counteracting against viral infection.



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HUMAN PAPILLOMAVIRUS (HPV) AND CERVICAL CANCER

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Cervical cancer is a type of cancer that occurs in the cells of the cervix—the lower part of the uterus that connects to the vagina. Cervical cancer is the fourth most common cancer among women globally with an estimated 570,000 new cases in 2018. The main types of cervical cancer are:

- **Squamous Cell Carcinoma:** This type of cervical cancer begins in the thin, flat cells (squamous cells) lining the outer part of the cervix, which projects into the vagina. Most cervical cancers are squamous cell carcinomas.
- **Adenocarcinoma:** This type of cervical cancer begins in the column-shaped glandular cells that line the cervical canal.

Sometimes, both types of cells are involved in cervical cancer. Very rarely, cancer occurs in other cells in the cervix. It is seen that various strain of the Human papillomavirus (HPV), play a role in causing most cervical cancer. Human papillomavirus is a group of viruses that are extremely common worldwide. Human papillomavirus is mainly transmitted through sexual contact and most people are infected with Human papillomavirus shortly after the onset of sexual activity. Papillomaviruses are members of the Papovaviridae family. Human papillomavirus is a relatively small, non-enveloped virus, 55nm in diameter. Human papillomavirus has its own number or type. There are more than 100 kinds and some are riskier than



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other. About 60 of 100 Human papillomavirus types cause warts on areas like hand or feet. The other 40 or so enter the body during sexual contact. They are drawn to the body's mucous membranes, such as the moist layers around the anus and genitals. Not all of the 40 sexually transmitted Human papillomavirus cause serious health problems. High risk Human papillomavirus strains include Human papillomavirus 16 and 18, which cause about 70 % of cervical cancers. Other high risk Human papillomavirus include 31, 33, 45, 52, 58 and a few others. Low risk Human papillomavirus strains, such as Human papillomavirus 6 and 11 cause about 90 % of genital warts, which rarely developed into cancer. These growths can look like bumps. Sometimes, they are shaped like cauliflower.

The Human papillomavirus genome consists of a single molecule of double-stranded, circular DNA containing approximately 7,900 bp associated with histones. Human papillomavirus has an icosohedral capsid composed of 72 capsomers, which contain at least two capsid proteins, L1 and L2. The E6 and E7 viral protein of Human papillomavirus is mainly responsible in causing cervical cancer. The E6 and E7 genes in the viral genome codes for the viral protein. The genome contains genes for capsid protein L1 and L2 as well as genes that involved in replication of the virus such as E2,E6,E7. The E6 protein blocks apoptosis, as it is a protective adaptation of the virus, normally the host cell would undergo apoptotic death in response to viral infection. On the other hand the E7 protein activates genes that initiate the cell cycle, allowing the infected cells that proliferates well and has lost the ability to die by apoptosis, in other words it becomes a potential cancer cell. When Human papillomavirus infects a cell it shades its capsid and the circular genome becomes linear and the L1 gene is deleted.

Before an Human papillomavirus infection can cause Cervical cancer, Human papillomavirus DNA is integrated with the DNA of the infected cell and the E2 gene is suppressed or deleted which deregulates the E6 and E7 oncogenes. Many viral genes are deleted following integration but E6 and E7 must be consistently expressed in order for the infection to progress to cancer. The viral DNA is transcribed to produce Messenger RNA (mRNA) which is then translated to produce viral protein. The E6 and E7 mRNA creates proteins that interferes with genes responsible for normal cell reproduction because the E6 and E7 gene plays an important role in dysregulation of cell growth. They are important to identify Human papillomavirus infection.



Role of E6 and E7 Protein in Oncogenesis

Both E6 and E7 contribute to achieve uncontrolled proliferation through deregulation of growth suppressors. E6 targets an important growth suppressor, p53, while pRb is one of the major targets of E7 among few others. It has been diagrammatically illustrated in Figure – 1.

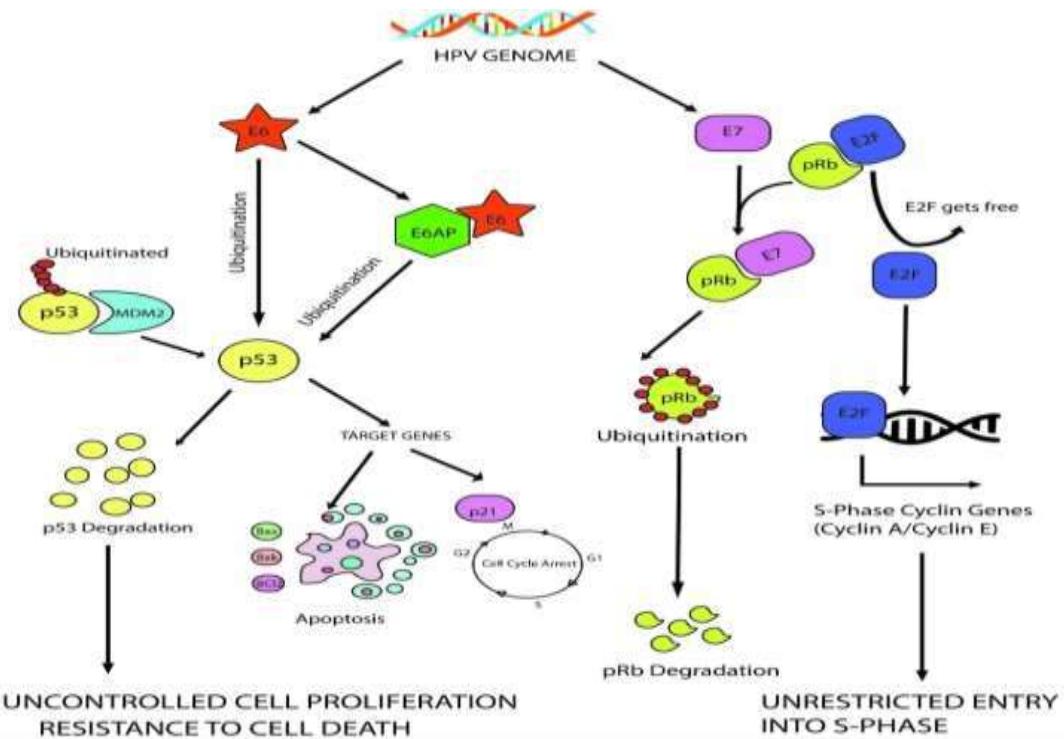


Figure – 1: E6 mediated manipulation and E7 mediated inhibition of pRb protein leading to sustained cell proliferation and resistance to apoptotic barrier

The E6 has been found to degrade p53 through ubiquitination with the help of E6AP (E6-associated protein also known as UBE3A). Human papillomavirus E6 can bind to the LxxLL consensus sequence in the conserved domain of E6AP to form a heteromeric complex of E6/E6AP/p53, ultimately leading to the degradation of p53. This forces the cells through uncontrolled cellular division, evading the preventive checkpoints. Similarly, E7-mediated inhibition of retinoblastoma protein (pRb) is also a significant step toward achieving unrestricted cell proliferation. pRb-E2F interaction is a mandatory checkpoint for the cells to travel through G1-S phase transition. When the cells are not prepared to enter the S-phase, pRb protein remains bound to the E2F family of transcription factors to prevent them from transcribing the genes required in S-phase. In Human papillomavirus infected cells, E7 targets pRb for ubiquitination, leading to the release of E2F transcription factors, which



transcribe cyclin E, cyclin A and p16^{INK4A}, an inhibitor of CDK4/6, forcing the cells through premature S-phase entry. CDK inhibitor p16^{INK4A} (Tumour suppressor protein) is an important target of Human papillomavirus E7 to regulate the cell cycle. Human papillomavirus E7 triggers the expression of p16^{INK4A} not only through pRb disintegration but also by epigenetic derepression through KDM6B (H3K27-specific demethylase 6B).

Comprehensive cervical cancer control includes Primary prevention (Vaccination against Human papillomavirus), Secondary prevention (Screening and treatment of precancerous lesions), Tertiary prevention (diagnosis and treatment of invasive cervical cancer) and palliative care. Vaccines that protect against Human papillomavirus 16 and 18 are recommended by WHO and have been approved for use in many countries. Screening and treatment of pre-cancer lesions in women is a cost-effective way to prevent cervical cancer. Cervical cancer can be cured if diagnosed at early stage and treated promptly.



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METHODS INVOLVED IN DETECTING ANTIMICROBIAL RESISTANCE

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During these pandemic situation multidrug resistant bacteria is threatening to serious problem worldwide. Importantly, secondary bacterial infections are a common result of a weakened immune system following a viral illness. Therefore, therapeutic success is becoming difficulty in treating infection by antibiotic therapy. Many methods are available for detection of antimicrobial resistance.

Phenotypic Methods for Detecting Antimicrobial Resistance

In vitro procedures like disk-diffusion method, dilution method (broth and agar dilution method), E-test and automated methods are mainly available to detect phenotypic antimicrobial resistance in individual bacterial isolates.

i) Disk-diffusion method

Disk diffusion is also known as Kirby-Bauer antibiotic testing. The application of commercially available drug-impregnated filter paper disks to the surface of an agar plate that has been inoculated to confluence with the organism of interest is called disk diffusion.



ii) Dilution Method

The minimal concentration of antimicrobial agents that kill (bactericidal activity, MBC) or inhibit the growth (bacteriostatic activity, MIC) of microorganisms can be determined by quantitative methods like agar dilution and broth dilution in which the bacterial isolates are subjected to a series of concentrations of antimicrobial agents in a broth environment

iii) Epsilometer Test (E-Test)

Epsilometer test (E- test) is an ‘exponential gradient’ method provides a direct quantification of antimicrobial susceptibility of microorganisms by applying both the dilution of antibiotic and diffusion of antibiotic into the medium.

iv) Automated Reading Method

The results of disk sensitivity tests and breakpoint sensitivity tests can be read using a camera interfaced to a computer system. through measurement of Turbidity (Nephelometry) or the production of CO₂.

Genotypic Methods for Detection of Antimicrobial Resistance

Molecular methods such as PCR, hybridization techniques, whole genome sequencing and Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDITOF MS) are used for molecular characterization of the gene.

i) Polymerase Chain Reaction (PCR)

PCR method is mainly use to detect genes present in bacteria for detecting antimicrobial resistance when DNA sequence is available for the whole or partial gene which can be used to design the PCR primers. The PCR-amplified PCR gene products can be visualized by running agarose gels and staining DNA with ethidium bromide or other fluorescent DNA chelating dyes.

ii) Hybridization Technique

FISH (Fluorescence *in situ* Hybridization) is a highly specific method to visualize the presence of the target organism in a quantitative manner. The PNA-FISH technology applies peptide nucleic acid probes which allow more rapid and specific binding than DNA or RNA probes which performs by targeting 16S rRNA sequence.



iii) Whole Genome Sequencing

Whole genome sequencing method provides a feasible system for identification of pathogen and for detection of antimicrobial resistance. Third generation sequencing systems such as Illumina miniseq, PacBio sequel system, Oxford Nanopore seq, MinION nanopore sequencer and PromethION can provide fairly long reads at high speed.

iv) DNA Microarray Technology

The main principle involved in DNA microarray technology is the comparison of presence or absence of genes to a reference strain or genes for which whole genome sequence was available. The DNA microarray technology was initially based on glass slides that were spotted with thousands of specific DNA probes based on genes present in one reference strain for which a whole-genome sequence was available.

MALDI-TOF MS (Matrix Assisted Laser Desorption and Ionization Time of Flight Mass Spectroscopy)

MALDI-TOF MS is a technique used to analyze biomolecules such as DNA, carbohydrates, proteins, and peptides by their ability to become ionized and enter gas phase and then measuring their time of flight. The principle used in this is the mass/charge (m/z) ratio of the resulting molecular fragments is analyzed to produce a molecular signature. Databases containing species specific information is needed for analysis of biological samples.

Electro Chemical Detection of Antimicrobial Resistance

The principle of this method is mainly based on the electrochemical measurement that occurs during the molecular bonding on the surface. Resistance determination can be made as a result of changing the surface chemistry with molecules such as oligonucleotides, proteins etc. belonging specifically to a particular bacterium.

Current Technologies in Detection of Antimicrobial Resistance

i) Machine Learning Methods

Supervised machine learning machines are mainly used for the prediction of bacterial resistome mainly based on WGS data. This is mainly based on machine learning algorithms which are trained on known data and they can predict unknown data by using training models. Data used for ML training is mainly based on AI application and includes information about infectious diseases. Commonly used ML



models for AMR are Naive Bayes (NB), Decision Trees (DT), Random Forests (RF), Support Vector Machines (SVM), and Artificial Neural Networks (ANN).

ii) AI based Mobile Application for Antimicrobial Detection

Antibiogram is one of the image processing techniques can operates on a desktop computer onto which images need to be previously transferred. Smartphone based app combines machine learning algorithms and image processing with a rule based expert system for automatic reading of plates for the measurement of inhibition zones. This application captures images with phone's camera, and the user is guided throughout the analysis on the same device by a user friendly graphical interface. It mainly involves the steps of preparation of antibiogram, data acquisition, image processing and categorization of bacterial isolates into antibacterial resistant and antibacterial sensitive.



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FOOD TOXICOLOGY

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Food is perhaps the most vital resource for the survival of living organisms after oxygen and water. But, not all of the available foods are safe for consumption and hence, they must be tested for safety. The study of the adverse effects of chemicals on living organisms is defined as “toxicology”. Paracelsus (1493-1541) is accredited with the basic concept of toxicology, which is stated as: “All substances are poisons; there is none that is not a poison. The right dose differentiates the poison from remedy”. Toxicology is the science of poisons, toxicants, or toxins. A poison, toxicant, or toxin is a substance capable of causing harm such as seriously injuring or ultimately, causing the death of an organism when they are administered to an organism. There are generally five types of toxic entities: chemical, biological, physical, radiation and behavioral toxicity.

Branches of Toxicology

- a) Clinical toxicology
- b) Forensic toxicology
- c) Occupational toxicology
- d) Environmental toxicology
- e) Regulatory toxicology
- f) Food Toxicology

Food toxicology is defined as the study of nature, properties, effects and detection of toxic substances in food and their disease manifestation in humans. The term “toxin” refers to a poison derived from a protein or conjugated proteins which



are produced by some higher plant, animal, or pathogenic bacteria that is highly poisonous for other living organisms, for e.g., botulinum toxins, aflatoxins, ergot etc. Many times common foods are contaminated with unacceptably high levels of toxicants which includes substances that are inherent toxicants, naturally found in foods, or contaminants. The chemical agents can be man-made (e.g., pesticide residues, food additives, contaminants originating with processing machinery, or packaging materials) or of natural origin (e.g., microbial, animal or plant toxins). They can, also be generated in the course of preparing, processing, and preserving foods (e.g., mutagens and carcinogens).

Determination of Toxicants in Food

a) Qualitative and Quantitative analysis of toxicants:

- ✓ Gas chromatography (GC)
- ✓ High performance liquid chromatography (HPLC)
- ✓ Column and Thin layer chromatography (CC, TLC)
- ✓ Distillation
- ✓ Extraction

b) Biological determination of toxicants:

- ✓ Acute Toxicity
- ✓ Genetic Toxicity
- ✓ Bioassay
- ✓ Metabolism
- ✓ Sub-chronic Toxicity
- ✓ Teratogenesis
- ✓ Chronic Toxicity

Foods are susceptible to toxic contamination from microbial, environmental, and natural toxins so most toxicologists deal with exogenous compounds, or those compounds that are not part of the normal metabolism of organisms, i.e., xenobiotic or foreign compounds. Food toxicologists deal with toxicants in food, the health effects of high nutrient intakes, and the interactions between toxicants and nutrients.



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ROLE OF MICROBES IN FORENSIC INVESTIGATION

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Microbial forensics has been defined as “a scientific discipline dedicated to analyzing evidence from a bioterrorism act, biocrime, or inadvertent microorganism/toxin release for attribution purposes”. This emerging discipline is still in the early stages of development and faces substantial scientific challenges to provide a robust suite of technologies for identifying the source of a biological threat agent and attributing a biothreat act to a particular person or group. The unlawful use of biological agents poses substantial dangers to individuals, public health, the environment, the economies of nations, and global peace. It also is likely that scientific, political, and media-based controversy will surround any investigation of the alleged use of a biological agent, and can be expected to affect significantly the role that scientific information or evidence can play. Microbial forensics combines the practices of epidemiology with the characterization of microbial and microbial-related evidence to assist in determining the specific source of the sample, as individualizing as possible, and the methods, means, processes and locations involved to determine the identity of the perpetrator(s) of an attack.

Microbial forensics is an interdisciplinary field that involves scientists, public health, law enforcement, the intelligence community, and policy and decision makers. Together they provide the interconnected system that helps protect us from



naturally occurring disease outbreaks and acts of biological terrorism and biocrime. New advancements in molecular techniques, especially sequencing technologies, provide tools for the microbial forensic scientist to extract more information at dramatically reduced costs and faster turnaround times than previously possible. High quality and confidence of results are essential since microbial forensic interpretations can have a large impact on society, regarding safety, political policy, and economics. Challenges will continue to exist in microbial forensics, however, implementation of new technology and continued communication across the scientific, public health, law enforcement, intelligence and policy communities will contribute towards the advancement of the microbial forensics field.

Geolocation

In the past few years, intensive work has been carried out to characterize environmental microbiome, particularly in urban environments and transit systems. These studies have demonstrated that unique community profiles may exist in certain areas of a city as well as “molecular echoes” of environmental events, and even a forensic capacity for geospatial microbiomic data.

Substrate Analysis

The potential of analyzing microbial profiles from the soil is increasingly being recognized in forensic microbiological research. Both the rhizosphere and bulk soil microbiomes exhibit a high level of heterogeneity between different sites. As such, with methodological refinement, soil microbiome samples could provide valuable biogeographic data to localize the origin of the soil sample. Another potential application is the acquisition of information to help determine the provenance of an item(s) associated with a crime.

Personal Identification

A growing body of evidence suggests that human individuals may be uniquely identified based on stable autochthonous (i.e., native to a given environment) microbial profiles. This could have a substantial impact on forensic science—for example, in situations where the investigator cannot retrieve sufficient amounts of human DNA (i.e., from human somatic and germ cells). Yet it is unknown whether the variation in microbial communities between people is sufficient to identify individuals within large populations uniquely or stable enough to place them over time. To answer some of these questions tested different body site-specific microbial profiles and attempted to match them with 25–105 microbiome profiles during the person’s first and second visits to the sampling site.



Biological Sex Determination

Recent evidence supports another contribution of microbiomics toward personal identification – the discrimination of biological sex, which could be useful where sufficient quantities of human DNA are unable to be retrieved. For example, airborne bacteria communities have previously been characterized in indoor environments.

Trace Evidence

There is an increasing interest in studying forensically relevant microbial profiles left behind on objects and surfaces. For instance, several studies showed that there is often a high level of bacterial presence on personal objects such as mobile phones. Furthermore, human-associated items such as shoes and mobile phones have been shown to support distinct microbiomes.

Manner and Cause of Death

The ‘manner of death’ is a determination made by an expert following an investigation (e.g., a coroner, the police, or a medical examiner). Five manners of death are generally considered: natural, accidental, suicide, homicide, and undetermined.

Postmortem Interval

Determining the PMI (the time elapsed since a person has died) is often an essential part of a criminal investigation. To improve PMI prediction accuracy, researchers have begun examining the thanatotrichome. Postmortem, these communities overwhelm the immune system allowing for subsequent colonization. Preliminary studies suggest that these microbial communities may undergo important successional changes in organs that could aid in determining the PMI.

Conclusion

Over the last decade, advances in genomic sequencing and bioinformatics have given rise to microbiomics, which fructified in a growing compendium of tools seeking to explore the panoply of microorganisms present in our bodies and environment. The evidence examined in this chapter indicates that microbiomics could be a forensically relevant and promising discipline with a multitude of applications—from determining substrate provenance and acquiring trace evidence to identifying individuals and estimating PMI.



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SARS-COV-2 JABS: A BOON TO COVID-19 OUTBREAK

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The early vaccination trials of Edward Jenner and Louis Pasteur laid the groundwork for immunology. They both played key roles, and as a result of their groundbreaking work, vaccinations for many diseases that were formerly major ailments of mankind have been developed. Vaccination is undoubtedly a budget-friendly way of preventing disease. The creation of successful new vaccines is a time-consuming, complex, and expensive procedure that seldom reaches the point of years-long clinical studies. There is a long history of pandemics in the world, originating with Plague (430 – 26 B.C), Spanish flu from 1918-1920, and MERS. Currently, the world is encountering SARS-CoV 2, also known as COVID-19, 2019 novel corona virus, or 2019-nCoV, because it was found in 2019. Many vaccines for the COVID-19 virus have been developed in many countries all over the world. This review focuses on several SARS-CoV-2 Jabs and their mechanisms, which are incredibly important during this pandemic. Vaccines is an antigenic preparation or biological preparation of microorganisms such as bacteria, viruses or their products administrated for prevention or treatment of infectious diseases and also improves immunity to a particular disease. COVID-19 was found in Wuhan, China on December 31, 2019. It belongs to the member of SARS and of the MERS families. COVID-19 was identified as epidemic and as a global public health emergency by the World Health Organization (WHO). Owing to fast transmission, COVID-19 was declared by the WHO as a global pandemic on 11 March 2020. WHO often studies whether mutations in SARS-CoV-2 induce changes in transmissibility, clinical



presentation and lethality, or whether they affect counter measures such as diagnosis, therapy and immunization. The COVID-19 jabs provide disease protection by eliciting an immune response to the SARS-CoV-2 virus. Several COVID-19 vaccines have shown effectiveness as high as 95% in preventing symptomatic COVID-19 infections in Phase III studies. As of April 2021, at least one national regulatory authority has approved 16 vaccines for public use:

- a) Two RNA vaccines (Pfizer–BioNTech and Moderna)
- b) Seven conventional style inactivated vaccines (BBIBP-CorV, CoronaVac, Covaxin, WIBP-CorV, CoviVac, Minhai-Kangtai, and QazVac)
- c) Five viral vector vaccines (Sputnik Light, Sputnik V, Oxford–AstraZeneca, Convidecia and Johnson and Johnson)
- d) Two protein subunit vaccines (EpiVacCorona and RBD-Dimer)

Covaxin (BBV152) is India's first native, whole-virion, inactivated vaccine established in conjunction with the Indian Medical Research Council (ICMR) and the National Institute of Virology (NIV) for the treatment of Covid-19, a highly contagious illness. COVAXIN has contributed to acceptable safety findings as well as improved humoral and cell-mediated immune responses. The phase three research for Covid-19, which began in mid-November 2020, is India's first and largest third phase efficacy project. According to preliminary data from its phase 3 investigation, the vaccine has an 81 percent efficacy rate.

The CoronaVac, an inactivated vaccine, is manufactured by Sinovac, a Beijing-based biopharmaceutical company. It works by exposing the human immune system to the virus through dead viral fragments without causing substantial sickness. According to preliminary findings from late-stage studies in Turkey and Indonesia, the immunization was successful in 91.25 percent and 65.3 percent of instances, respectively. The University of Oxford worked with the British-Swedish pharmaceutical company AstraZeneca to develop and evaluate ChAdOx1 nCoV-19, also known as AZD1222. Large clinical research indicated that the immunization gave significant protection, with an overall efficacy of 76 %. The coronavirus spike protein gene was inserted by the scientists into another virus known as an adenovirus. ChAdOx1, a modified form of a chimp adenovirus, was used by the Oxford-AstraZeneca group. It can enter cells but not multiply inside them. The Oxford-AstraZeneca vaccine for Covid-19 is more lasting than the mRNA vaccines developed by Pfizer and Moderna.



Russia's Sputnik V has been deemed safe, and it works in a manner similar to the Oxford-AstraZeneca Covishield jab, which is made in India. According to findings from a late-stage study published in The Lancet, Sputnik V gives around 92 percent protection against Covid-19. For either of the two doses in a vaccination regimen, Sputnik deploys a new vector. The Russian Direct Investment Fund claims that this provides longer-lasting protection than immunizations that employ the same delivery mechanism for both shots (RDIF). According to the researchers, introducing a new adenovirus vector may help produce a stronger immune response, when compared to using the same vector twice. Since it lowers the likelihood of the immune system developing tolerance to the original vector.

EpiVacCorona, a COVID-19 preventative vaccine, was discovered by Russia's Vektor State Research Center of Virology and Biotechnology. The vaccine is made up of SARS-CoV-2 protein peptide antigens that have been chemically synthesized, coupled to a carrier protein, and attached to an aluminum-containing adjuvant (aluminum hydroxide). The EpiVacCorona vaccine, administered in two intramuscular injections spaced 21-28 days apart, results in the formation of protective immunity against the SARS-CoV-2 coronavirus. Nonetheless, for the time being, the only way to protect ourselves and others from the deadly virus is to get vaccinated. While immunizations may or may not be effective against the variants, they have unquestionably lowered the severity and fatality rate associated with illness. So, let us all get COVID-19 shots and work together to eradicate COVID-19 from the globe!



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MICROBES - BOON OR BANE

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Microorganisms or microbes are the moment living ones in the universe which are imperceptible to the bare natural eye, and are just noticeable with the assistance of a compound magnifying lens. Microorganism is an expansive term comprising of bacteria, microscopic fungi or molds, protists and even viruses. Virus are significantly more modest than bacteria and are viewed as non-living creatures, still their illness causative activity remembers them for the compass of microorganisms. Research in most recent couple of many years has demonstrated that these microorganisms are really useful in keeping up with the biological equilibrium in the climate by their significant job in separating the dead natural make a difference to their fundamental constituents, subsequently returning it to earth's outside for conceivable reusing. Microorganisms are fundamentally of two sorts: beneficial microorganisms and harmful microorganisms. Microorganisms are extensively grouped by their tendency and activity. Numerous microorganisms are critical to the climate because of their useful capacities without which some other life cannot endure. A few microorganisms are destructive to other life because of them being causative specialists for different illnesses that influence the two people and creatures.



Microbes are bane and boon because some microbes like bacteria, viruses etc. are bad for our body and we become sick. A microorganism, or microbe, is a microscopic organism, which may exist in its single-celled form or a colony of cells. Microbes are important in human culture and health in many ways, serving to ferment foods and treat sewage, and to produce fuel, enzymes, and other bioactive compounds. Microorganisms are fundamental tools in science as model organic entities and have been put to use in natural fighting and bioterrorism. Organisms are an essential part of fruitful soil. In the human body, microorganisms make up the human microbiota, including the fundamental gut greenery. The microorganisms liable for some, irresistible illnesses are organisms and, all things considered, are the objective of cleanliness measures. The human microbiome is the total of all microbiota that dwell on or inside human tissues and bio liquids alongside the relating anatomical destinations in which they live, including the skin, mammary organs, fundamental liquid, uterus, ovarian follicles, lung, spit, oral mucosa, conjunctiva, biliary parcel, and gastrointestinal plot. Kinds of human microbiota incorporate bacteria, archaea, fungi, protists and viruses. Microorganisms are little living things that are seen surrounding us and are as too little to even consider being seen by the unaided eye. They live in water, soil, and noticeable all around. The human body is home to a large number of these organisms as well, additionally called microorganisms. A few organisms make us debilitated; others are significant for our wellbeing. The most widely recognized sorts are bacteria, viruses and fungi. There are likewise microorganisms called protozoa. These are minuscule living things that are answerable for illnesses like toxoplasmosis and intestinal sickness. Present day globalization has heightened human-centric hotspots for hefty metal tainting in different normal natural surroundings. Malicious nature of substantial metals represents a significant danger to all life structures in the climate. Weighty metals are not degradable like the natural toxins but rather could be changed to be steady in a less poisonous structure. Organisms are the modest device as they advance quickly to battle weighty metal pressure by creating different endurance methodologies, for example sequestration or dynamic transportation of metal. Their short generation time, large surface area and ease of genetic manipulation makes them ideal candidates to use for the bioremediation process. Many heavy metal resistant microbes such as species of *Bacillus*, *Pseudomonas*, *Acidothiobacillus*, *Saccharomyces*, *Geobacter* and *Rhizopus* have been used for remediation of heavy metal contaminated sites.

The effectiveness of bioremediation procedure relies on different variables, including biotic just as abiotic, which basically decide bio – accessibility of metal for remediation. The distinctive metal microorganism cooperation's like sorption,



amassing, mineralization, change and solubilization are active for tightening weighty metal fixation at different loci or destinations. The audit stresses on the various connections of the organisms with substantial metals, their endurance systems and the uses of the safe strains in remediation. We can't actually say Whether organisms are help or plague to humanity. On the helpful exercises of microorganisms', our everyday lives priceless assume a significant part in the environment and their capacity make a difference to an inorganic structure is important to keep up with life, on earth. Without the microbial activity, the carcasses. Microorganisms are engaged with the breakdown of dead creatures and supplements will be delivered in the climate for reusing. They might be engaged with such countless sicknesses and contaminations, and afterward as long we can't envision a daily existence on earth without them. In this way, some aspects it may be a boon or in some aspects it's a curse. But we need them.



Figure – 1: Mass Multiplication of Various Beneficial Microbes (*Azospirillum*, *Phosphobacteria*, *Pseudomonas*, *Trichoderma*, *Pacilimycis*, *AMF*, *Bacillus spp.*, etc.)

As result, the overall idea that any microorganisms are destructive to human existence which was generally gone on through ages has been refuted absolutely by various scientists since most recent couple of many years. Destructive microorganisms should be checked by embracing distinctive preventive and chemotherapeutic measures; and yet the better capability of microorganisms can be effortlessly tapped to further develop the way of life of individuals specifically and the general ecosystems overall.



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ANTIMICROBIAL RESISTANCE

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Antimicrobial Resistance (AMR) is a global health and development threat. It requires urgent multi-sectoral action in order to achieve the Sustainable Development Goals. Antimicrobial resistance occurs when microbes evolve mechanisms that protect them from the effects of antimicrobials. The term antibiotic resistance is a subset of AMR, as it applies to bacteria that become resistant to antibiotics. Without effective antimicrobials, the success of modern medicine in treating infections, including during major surgery and cancer chemotherapy, would be at increased risk.

Antimicrobials and Antimicrobial Resistance

Antimicrobials including antibiotics, antivirals, antifungals and antiparasitics used to prevent and treat infections in humans, animals and plants. Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to medicines making infections harder to treat and increasing the risk of disease spread, severe illness and death. As a result of drug resistance, antibiotics and other antimicrobial medicines become ineffective and infections become increasingly difficult or impossible to treat. Examples of antimicrobials are

- Penicillin
- Valacyclovir
- Flucanozole
- Praziquantel



A microbe has five goals once it enters your body:

- To reach the target site (your lungs, for instance).
- To attach to the target site.
- To multiply.
- To take nutrients from you, the host.
- To avoid and/or survive any attacks by your immune system.

Role in Increasing the Rate of Antimicrobial Resistance

- a) ***Health care providers*** - Healthcare providers sometimes prescribe antimicrobials that are not needed, at the wrong dose or for an inappropriate length of time. Some healthcare providers give in to pressure from patients to “try something” even when the exact cause of symptoms is unknown. For example, viral infections such as the common cold should not be treated with antibiotics, because antibiotics kill only bacteria.
- b) ***Broad-spectrum medications*** - Sometimes a healthcare provider may treat an infection with a broad-spectrum antimicrobial that works against a variety of microbes instead of one specific germ. This can increase the risk of antimicrobial resistance.
- c) ***Close contact at hospitals*** - The close contact among hospital workers and sick patients creates a situation that makes it easy for microbes to spread.
- d) ***Using Antibiotics in Agriculture*** - The use of antibiotics in agriculture to promote growth in food and animals is viewed by some scientists as a major problem. Meat-producing animals given antibiotics can develop resistant bacteria. These resistant bacteria may contaminate meat or other food products from the animals. The resistant bacteria are then passed along to people who eat these foods.

Prevention of Antimicrobial Resistance

- It's not possible to eliminate antimicrobial resistance, as microbes will always be able to modify themselves and adapt to their environment.
- Work closely with a healthcare provider to discuss symptoms and decide on the correct medicine to treat any illness.
- Follow the directions exactly for any prescription medication.
- Never take another person's prescription medication or share yours with them.
- Never save old prescription drugs for use at a later time.
- Get vaccinations as recommended.



- Follow good general health practices such as a proper diet, exercise, getting enough sleep and good hygiene (especially frequent hand-washing) to prevent illness and the need for antimicrobial drugs.

Antimicrobial Resistance as a Global Concern

- The emergence and spread of drug-resistant pathogens that have acquired new resistance mechanisms, leading to antimicrobial resistance, continues to threaten our ability to treat common infections.
- The clinical pipeline of new antimicrobials is dry. In 2019 WHO identified 32 antibiotics in clinical development that address the WHO list of priority pathogens, of which only six were classified as innovative. Furthermore, a lack of access to quality antimicrobials remains a major issue.
- Antibiotic shortages are affecting countries of all levels of development and especially in health-care systems. The cost of AMR to the economy is significant. In addition to death and disability, prolonged illness results in longer hospital stays, the need for more expensive medicines and financial challenges for those impacted.
- Without effective antimicrobials, the success of modern medicine in treating infections, including during major surgery and cancer chemotherapy, would be at increased risk.



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ANTIFUNGAL TRETMENT OF MUCORMYCOSIS ASSODIATED WITH COVID-19

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Mucormycosis is a rare but serious angioinvasive infection caused by a group of fungi called Mucormycetes. Recent reclassification has abolished the class zygomycetes and placed the order mucorales in the sub-phylum Mucormycotina. Spores of these ubiquitous fungi can be inhaled and then infect the lungs, sinuses and extend into the brain and eyes. Mucormycosis mainly affects people who are immunocompromised or patients already infected with other diseases. High risk groups include people with other diabetes (especially diabetic ketoacidosis), solid organ transplantation, neutropenia (low neutrophils, a type of white blood cells), long-term systemic corticosteroid use and iron overload (hemochromatosis). The risk high for people living with HIV and those using immunomodulating drugs.

Fungal infections, including Mucormycosis, aspergillosis and invasive candidiasis, have been reported in patients with severe COVID-19 or those recovering from the disease and have been associated with severe illness and death. India has reported a recent surge in Mucormycosis cases. The increased incidence with a fairly severe course of Mucormycosis was reported in patients with a history of COVID-19 and received systemic corticosteroid therapy. Prevention of COVID-19 associated Mucormycosis needs to focus on aiming for better glycaemic control in COVID-19 patients and monitoring the use of systemic corticosteroids in treating severe cases. The symptoms of Mucormycosis depend on where in the body the



fungus is growing. The most common presentation is a sinus infection (sinusitis) that is accompanied by nasal congestion, nasal discharge and sinus pain. A fever and headache may also occur. The management of patients with Mucormycosis, with a considered rate before the COVID-19 pandemic, had not been optimal as described in a case report. Optimal antifungal therapy has become an important on considering the rate of increased Mucormycosis patients.

Primary antifungal therapy for Mucormycosis mainly based on polyene. Even though amphotericin B deoxycholate (AmB) is the predominant therapy for decades, lipid formulation of AmB is significantly less nephrotoxic and administration of High dose for long period is safer than AmB. Moreover, the survival rate of patients treated with liposomal amphotericin B (LAmB) is higher than patients treated with AmB. Amphotericin B deoxycholate (AmB) is replaced by lipid formulations of AmB that include liposomal AmB (L-AmB), AmB lipid complex (ABLC), and AmB Colloidal Dispersion (ABCD) because of its significant toxicity. Based on the survival rate, either LAmB or ABLC is used as the first line treatment for Mucormycosis, taking into account the approach to Mucormycosis should always be multi-modal.

Posaconazole exhibits significant activity against Mucormycosis agents. Compared to itraconazole and isavuconazole, posaconazole enhances high minimum inhibitory concentration. Comparatively higher MIC values were found for *Mucor circinelloides*. Fungicidal activity of posaconazole has been demonstrated against *Rhizopus* and *Mucor* spp., But fungicidal activity of amphotericin (95 % killing noted at as early as 6 hrs) is higher than posaconazole (70 % killing at 6 hrs). Animal studies were conducted to explore the in vivo efficiency of posaconazole in diabetic ketoacidotic and neutropenic mice with disseminated Mucormycosis. But posaconazole monotherapy did not improve survival or reduce fungal burden as compared to L-AmB. Although posaconazole monotherapy cannot be used as primary antifungal therapy, it used as an option for patients with Mucormycosis who are refractory to or intolerant of AmB or who in need of prolonged continuation or maintenance therapy. Fluconazole and voriconazole have no effective activity against agents of Mucormycosis. Based on the studies no other azoles are effective than posaconazole. Echinocandins such as Caspofungin, anidulafungin and micafungin have no meaningful effect against Mucormycosis agents as single agents. *Rhizopus oryzae* expresses the target enzyme of echinocandins, 1,3-D-glucan synthase, and caspofungin has shown some effect in an animal model of infection but with an unexplained inverse-dose response relationship. Mortality rate is higher when lower dose is administered than the higher dose. This inverse dose relationship may be



similar to the paradoxical effect with caspofungin against *Candida albicans*. Flucytosine and terbinafine lacks activity against agents of Mucormycosis.

Combination antifungal therapy mainly involved in AmB formulation and either posaconazole or eshinocandin. Combination of AmB lipid complex and caspofungin improved survival of diabetic ketoacidotic mice infected with *Rhizopus oryzae*. L-AmB combined to anidulafungin or micafungin improved survival in mice infected intravenously with *Rhizopus oryzae* compared to monotherapy. The combination therapy did not show effective result than LAmB alone or posaconazole alone. In the treatment of murine Mucormycosis, triple combination therapy (L-AmB, micafungin and deferasirox) shows effective result than all other therapy. However, combination therapy cannot be used as first line treatment for Mucormycosis, it can be used as an option in salvage therapy after failure of appropriate first-line therapy. There are many unresolved issues as epidemiology, diagnosis and treatment of Mucormycosis. Mortality will be reduced when patients with Mucormycosis diagnosed and treated earlier.



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ANTIBACTERIAL ACTIVITY OF METAL OXIDES NANOPARTICLES

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Bacteria are generally defined by the plasma membrane, cell wall, and cytoplasm. The cell wall lies outside the cell membrane and consists primarily of a homogenous peptidoglycan layer (which consists of amino acids and sugars). Both the osmotic pressures of the cytoplasm and the unique cell shape remain on the cell wall. The gram-positive bacteria are equipped with one cytoplasmic membrane and thicker cell wall (20 – 80 nm) with a peptidoglycan multilayer. The Gram Negative Bacteria wall is comprised of two cells' external membrane, an external membrane and a plasma membrane that has a 7 – 8 nm thin peptidoglycan covering. The NPs [silver (Ag), cuprous oxide (Cu_2O), cupric oxide (CuO), zinc oxide (ZnO), titanium dioxide (TiO_2), tungsten oxide (WO_3), Ag + CuO composite and Ag + ZnO composite etc.] in these ranges may simply pass-through peptidoglycan and are hence extremely harmful. All but the biological elements of the nucleus are in the jelly-like fluid cytoplasm which fills a cell. The tasks of this organelle include growth, metabolism and reproduction. This includes proteins, carbs, nucleics (*80 %), salts, ions and water. The cytoplasm consisted of proteins, ions. It adds to the electrical conductivity of the cellular structure. Bacterial walls have overall negative charge. The Figure - 1 shows the common bacterial cell structures. According to the American Heritage Medical Dictionary 2007, anti-bacterial activity is defined as the action to kill or inhibit bacterial development. A function is also described as the surface area in contact with the microorganisms. While antibacterial drugs in certain



concentration medicines may harm or obstruct bacterial growth, they are not detrimental to the host of bacterial infection (Saunders Comprehensive Veterinary Dictionary 2007). An antibacterial agent is considered to be killing bacterium or preventing its development. Various methods have been used for measuring and researching in vitro antibacterial activity. These procedures include the diffusion of discs, the dilution of broths, the agar dilution method and the microtiter plates. The parameters investigated depend on additional approaches. For example, Gunalan *et al.* found metal oxides are more effective when created from green synthetics than suspending them from other produced materials. when made from a green synthesis technique. The oxygen generated on the surface of metal oxides, which causes harm to micro-organisms can be explained. The response is hydrogen ions to the creation of H₂O₂ molecules. The production of H₂O₂ can penetrate and kill cell membrane microorganisms. The H₂O₂ generation depends substantially on the surface area of the Metal Oxides, which results in enhanced surface oxygen and antibacterial activity of nanoparticles.

Mechanism of Antibacterial Activity of Metal Oxides - NPs

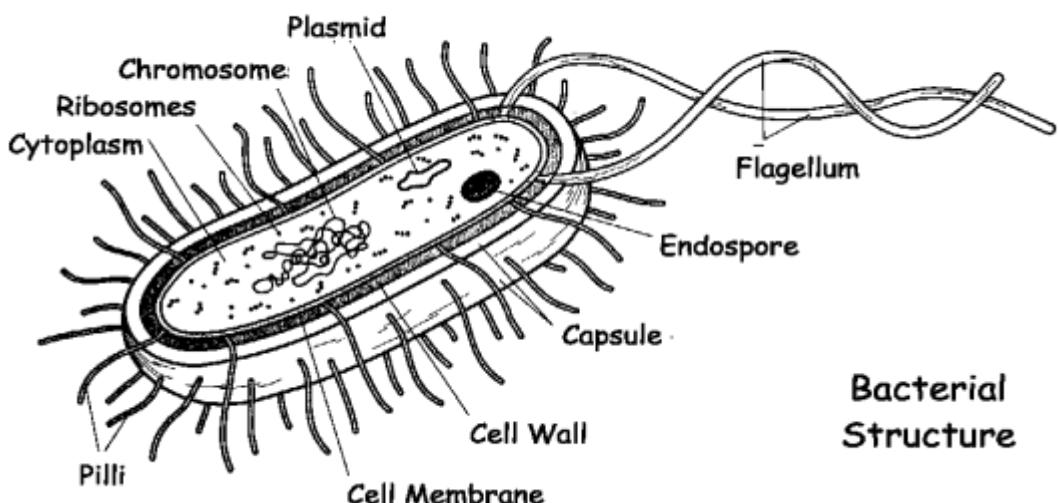


Figure – 1: Bacterial Cell Structures

In order to quantify different processes, Researchers studying Metal oxides induced morphological bacterial alterations with SEM or FESEM. However, the antibacterial activities of Metal Oxides - NPs have been raised with certain concerns, although the specific mechanism of toxicity is not fully illuminated and is still contentious, given that there are certain questions within the range of antibacterial activity requiring profound explanations. The following are defined mechanisms that



were presented in the literature: Direct interaction with the cell walls of Metal Oxides -NPs, which leads to bacterial integrity destruction. Antimicrobial ion releases primarily from metals, However, the toxicity mechanism varies in various media as the species of dissolved Metal oxides may change according to the medium components besides the physiochemical properties of Metal Oxides - NPs.



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THE ROLE OF ANTIVIRAL FUNCTIONAL FOODS IN THE MANAGEMENT OF COVID-19

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The novel Coronavirus Disease 2019 (COVID-19) is the major public health burden in the world. The morbidity and mortality of the global community due to this disease is dramatically increasing from time to time. Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome (SARS-CoV-2). The first known case was identified in Wuhan, China in December 2019.

Functional foods naturally possess active ingredients or “nutraceuticals” that are associated with disease preventative health benefits are now widely accepted for the prevention and management of major non communicable diseases, especially those characterized by inflammatory and oxidative stress disorders such as diabetes and cardiovascular disease. However, less is known about the role of functional foods in communicable diseases, especially on the immune system defense against viral infections such as COVID-19.

The antiviral functional foods include fermented products, probiotics which enhance gut bacteria and gut-lung axis-related respiratory fitness. Herbs & roots which prevent viral replication, enhance anti-influenza virus IgG and IgA antibodies production and T cell function. Dairy products which lower viral replication, reduce



infection rate & lung pneumonia. Fish Chicken and Meat used in immune defense; peptides enhance Monocytes and Macrophages functions and prevent infected lung injury. Fruit and vegetables contain vitamins and minerals which provides antioxidant and immune protection of the respiratory system. Plant cyclotides prevent T-cell malfunction. Coffee which decreases progeny virus yield, Neutrophil and Monocyte chemotaxis lipopolysaccharide and prevents mucosal response to influenza pathogens. Nuts and Seeds having immunoprotective phenolic compounds for high-risk groups. Olive oil that prevents Respiratory Syncytial Virus and Influenza A & B and Parainfluenza 1, 2 & 3 viruses.

A variety of fruits, vegetables, oily fish, olive oil, nuts, legumes are all considered functional foods based on their natural contents of nutraceuticals, including polyphenols, terpenoids, flavonoids, alkaloids, sterols, pigments, and unsaturated fatty acids. Polyphenol-rich herbs, especially coffee, differently fermented teas (green, black) and yerba maté, have also shown to have various effects on metabolic and microvascular activities, cholesterol and fasting glucose lowering, anti-inflammation and anti-oxidation in high-risk populations. Bioactive peptides, naturally present in food proteins or formulated as nutraceuticals based on their molecular weight, amino acid chain length, or peptide composition, have also been postulated to elicit versatile physiological responses associated with immunological, antimicrobial, cardiovascular, gastrointestinal, neurological, and other hormonal activities of the human system. Such functional food benefits can be translated to protect against viral infections and COVID-19. Viral infections are characterized by a compromised immune function and deficient micronutrient stores, particularly vitamins, including vitamins A, B6, B12, C, D, E, and folate, and trace elements, including zinc, iron, selenium, magnesium, and copper. Evidence already supports an efficient function of the immune system through consuming those various nutraceuticals within a variety of functional foods including essential fatty acids, linoleic acids, essential amino acids, and the aforementioned vitamins and minerals, especially where forms of immunity may be affected by deficiencies in one or more of these nutraceuticals. Adequate dietary intake, and supplementation of such functional foods, contribute to maintaining optimal levels in the human body, which enhances several aspects of the immune system, and provides an important antiviral prevention of COVID-19. Conversely, less robust immune responses have been shown to be the primary risk factor for COVID-19, which makes it timely to describe the protective role of functional food component benefits in the context of preventing COVID-19 and seasonal infections. In terms of jointly addressing non communicable diseases and communicable diseases prevention within high-risk populations, investigating the functional foods effects on communicable diseases



including COVID-19 is particularly important. Higher infection and mortality rates related to COVID-19 have been documented among older adults and patients with obesity, cardiac diseases, hypertension, or diabetes.

Various dietary patterns contain functional food components that have been promoted in the past for NCD prevention, especially the vegetarian diet, the Nordic diet, or the Mediterranean diet (MD), and its combination with other lifestyle approaches. Common functional foods within those diets include plant-based fruit and vegetables such as olive oil and tree nuts, seeds, fish, dairy products, and herbs, teas, and fermented products, which contain key nutraceuticals with disease protective anti-inflammatory and anti-oxidation properties. Established health protective functional components include Monounsaturated Fatty Acids (MUFA) such as oleic acid in olive oil, omega-3 polyunsaturated fatty acids (e.g., alpha-linolenic acid) found in tree nuts such as walnuts, Eicosapentaenoic acid (EPA), and Docosahexaenoic acid (DHA) found in oily fish, high amounts of polyphenol flavonoids and antioxidants found in fruit and vegetables, and high amounts of fiber found mainly in cereal and whole-grain foods. Consuming those functional foods, and their components vary across geographical global regions, but what is agreed on is their cardiometabolic protective benefits of reducing major NCDs and mortality risks. The challenge is to translate such functional effects towards enhancing and protecting the immune system and its antiviral defense response into the prevention of emerging CDs such as COVID-19.

Micronutrients such as Vitamin A (orange, flesh fruits, milk, green leafy vegetables, pumpkins and egg yolk). Helping a body's natural difference against illness and infection (The immune system) works properly. Keeping skin and the lining of some parts of the body, such as the nose, healthy. The Vitamin B6 (Chicken, Liver, Fish, Potatoes, Maize and Cereals). Vitamin B6 is used for preventing and treating low levels of pyridoxine and the anemia that may result. It is also used for heart disease Pre-menstrual syndrome, depression and many other conditions. Vitamin C (oranges, gooseberries, tomatoes, lemon and amla). An antioxidant powerhouse, effective in strengthening and boosting the immune system Vitamin D (oily fish, red meat, egg yolks and liver). It is involved in calcium absorption, immune function and protecting bone, muscles and heart health. Vitamin E (sweet potato, avocado, spinach, nuts, mango and kiwi). It is another potent antioxidant that does a great job in fighting off infection. It stimulates the production of natural killer T-cells that destroy germs and cancer cells. Elderberry is full of nutrients including minerals like phosphorus, potassium, iron, copper and vitamins such as vitamin A, B and C, proteins and dietary fiber. Flax seed, walnuts,



soybean oil, chia seeds, and Omega-3 fatty acids are thought to help reduce the risk of heart disease. They have been used along with diet and exercise to help lower levels of a certain blood fat (triglyceride) and to raise levels of "good" cholesterol (HDL). Zinc Meat, nuts, whole grains, chickpeas, lentils, dark chocolate, It is an essential mineral that a body does not make on its own. It helps the immune system fight off invading bacteria and viruses.

Conclusion

Functional foods may provide a further effective diverse antiviral approach and could have a joint prevention of both non communicable diseases and communicable diseases among diverse populations. Dietary intake of foods rich in vitamins and minerals can be increased to provide an immune boost, especially in individuals with deficiency in these micronutrients. Increased intake of probiotics, omega-3 from fish, protein peptides from chicken and fish, and olive-based products are also recommended. There is no specific model to follow to enhance the immune system against COVID-19. However, the more varied the dietary sources, the better the protection is against all viral infections. Enhanced dietary intake of functional compounds may contribute as a preventative medicine against emerging viral infections.



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IMMUNE BOOSTING INDIAN HERBS AND SPICES AS POTENTIAL ANTIVIRAL PREVENTIVES IN COVID-19

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The COVID-19 pandemic has now risen to a global health crisis across the globe. This novel virus outbreak has challenged India's economic, medical and public health infrastructure. Health care professionals and researchers around the world are looking for an effective treatment regime for COVID-19. The number of people infected by COVID-19 in India crossed 9.74 million; nearly eleven - months after the country reported its first case. The Ministry of Health and Family Welfare of India (MOHFW) has taken numerous measures to raise awareness on COVID-19 and the necessary actions to control the spread of the virus. The central and state governments are formulating several wartime protocols to achieve this goal. The MOHFW has implemented the new clinical management protocol to treat COVID-19. Besides, the Ministry of AYUSH has also provided guidelines to use conventional preventive and treatment strategies to enhance immunity. The national recovery rate has increased to 94.66 % and the reported fatality rate is down to 1.45, due to "test, track and treat". MOHFW and Ministry of AYUSH are the two pillars of health care to prevent and manage the current pandemic outbreak in India. Since, there is no specific drug or vaccine effective against COVID-19 infection, exploring every possible option for prevention and treatment is of great importance.



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Ayurveda, Chinese traditional medicine and naturopathy promote use of many herbs in treatment of COVID-19. A notable use of spices and herbs in very early times were used in medicine in the making of holy oils and ointments. Traditionally India was known as the legendary land of spice and compounds derived from nature are a worthy therapeutic alternative for several infectious diseases as they are innately better tolerated in the human body.

Spices and herbs have high antioxidant and antimicrobial activities and therefore are extensively studied worldwide. Spices contain bioactive compounds such as tannins, alkaloids ,phenolic diterpenes and so on. Some of the promising immunity-boosting herbs are garlic, black cumin, ginger, and liquorice. Fumigation of homes, by Ayurvedic herbs such as garlic (*Allium sativum*) peel, turmeric (*Curcuma longa*) powder, Carom (*Trachyspermum ammi*) seeds is used for disinfection. Tulsi has anti-inflammatory effects, increases the anti-oxidant activity and protects the cells and its membrane from being damaged by the toxic substances. Mushroom have been found to have multiple powerful antiviral actions against several viruses that cause influenza and other infections. The herbs showing encouraging results include Harsingar (*Nyctanthes arbor-tristis*), giloy (*Tinospora cordifolia*), neem (*Azadirachta indica*), aloe vera (*Aloe barbadensis miller*), cannabis (*Cannabis sativa*), turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), ashwagandha (*Withania somnifera*), red onion (*Allium cepa*), tulsi (*Ocimum sanctum*), and black pepper (*Piper nigrum*). Experimental scientific evidences has shown that traditional herbal remedies tested with respiratory viruses can bring about the following mechanisms in respect to the immune system. Reduction in viral replication, enhancement of specific anti-influenza Ig A and Ig G antibody production, stimulation of interferon gamma production by T cells, neuraminidase inhibiting properties, virus budding prevention, inhibition of viral RNA and viral protein synthesis, inhibition of viral binding and penetration into host cells. The antiviral immunological defense capacity could be enhanced using functional foods, nutraceuticals, and physical activity behaviors, whether such behaviors are alone or combined. Functional foods and nutraceuticals can be safe and cost-effective strategies to enhance the immune system and provide protection from pathogenic viral infections.

The Indian herbs and spices are Tulsi, Salt, Rose water, Liquorice, Oregano, Thyme dry leaves, Betel leaves, Dry ginger, Flax seed, Basil seed, Basil leaves, Nigella seed, Parsley, Fennel seed, Caraway/Kala jeera/Shahi jeera, Vinegar, Fenugreek, White sesame seeds, Black sesame seeds, Poppy seed, Pomegranate seed, Star Anise, Nutmeg, Mace, Saffron, Cinnamon, Cardamom Green, Clove, Bay leaf,



Big Brown Cardamom, Pepper, Asafoetida, Cumin, Turmeric, Chilli, Coriander leaves, Mint leaves, Curry leaves, Black salt, Garlic Chukku, Thippili, kirambu, Akkirakaram, karpooravalli, Koshtam, Seendhil, Siruthekku, Vatta Thiruppi ver, Koraikizhangu, Nilavembu, Aadathodai, Kadukkai, Neermulli ver, Sirukanchori ver.

Conclusion

In the current pandemic scenario, precautions and boosting immunity are one of the best choices to get away from COVID-19 infection. We conclude that the uses of spices and herbs may play a significant role against viral infections. In India, people have been using spices as well as herbs since ancient times due to their taste, antiviral, antimicrobial, antioxidant, and immunity-boosting properties. Since ages, Indians have a habit of taking these natural products that have conferred immunity in the Indian population, which probably is the major cause for low mortality in India.



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ADVANCES IN THE SCREENING OF BACTERIAL CELLULOSE PRODUCERS

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Cellulose is the most abundant biopolymer that is naturally found on earth and is mainly harvested from higher plants such as wood and cotton. Plant-based cellulose is a renewable resource that is widely used in multifarious applications such as paper, textiles and construction materials. However, it consists of hemicellulose, lignin, waxy aromatic substances and other impurities that require additional energy- or chemical-intensive purification processes in order to obtain pure cellulose. In contrast, bacterial cellulose while identical to plant cellulose in chemical structure is produced without contaminant molecules and morphologically differs from plant cellulose. Bacterial cellulose, also known as microbial cellulose, is a promising natural polymer synthesized by certain bacteria. Bacterial cellulose due to its supramolecular structure and high purity, cellulose content demonstrates unique properties. Microbial cellulose is entirely free of "contaminants" such as lignin, hemicelluloses and pectin typical components in plant cellulose of wood. Thus, bacterial cellulose is a very useful biomaterial, which could be used in many different industrial processes.

Bacterial cellulose is produced by species of various genera of bacteria including *Acetobacter* (renamed *Gluconacetobacter*), *Agrobacterium*, *Achromobacter*, *Aereobacter*, *Acanthamoeba*, *Achromobacter*, *Sarcina*, *Rhizobium*, *Alcaligenes*, *Zoogloea* and others. The most widely studied bacteria is strains of *Acetobacter xylinum* or nowadays *Glucoanacetobacter xylinus* due to its high efficient productivity of bacterial cellulose.



Earlier the Bacterial cellulose producers were identified based on their typical milky white swollen colonies when grown on Buffered Schramm and Hestrin's medium. Bacterial cellulose forms as a white leathery pellicle at the air-liquid interface. This characteristic of the Bacterial cellulose has been used for screening and detection of cellulose producers from different samples. In most of the methods, potential biocellulose producer organisms are selected by growing into standard HS medium for 2 weeks at 30 °C under static conditions. Only the bacterial isolate that shows the pellicle formation in the culture medium are used for further production of Bacterial cellulose.

Screening media incorporated with fluorescent brightener dyes which binds to cellulose have been used for detection of bacterial cellulose producers. Since the fluorescent dye binds to the cellulose content in the organism, Cellulose producing bacterial colonies fluoresces when observed under UV light. The same screening media have been used to isolate and screen mutants of *Gluconacetobacter xylinus* NCIM 2526 for higher cellulose productivity.

A Modified, Efficient and Sensitive pH Indicator Dye Method have been for the Screening of Acid-Producing *Acetobacter* Strains Having Potential Application in Bio-Cellulose Production. In order to screen cellulose-producing *Acetobacter*, the isolated cultures from vinegar/rotten fruits were inoculated in Hestrin-Schramm (HS) medium containing ethanol and CaCO₃. After the desired incubation, the positive cultures form a zone, which is observed around the bacterial growth, resulted from the solubilization of CaCO₃ by acetic acid produced from the oxidation of ethanol during fermentation. However, in this method, the clarity of the solubilized zone is not very sharp and distinct. Therefore, an improved method for screening, of the microorganisms producing acetic acid has been developed. In this method, Methyl red (MR) is incorporated as a pH indicator in Hestrin-Schramm medium containing ethanol and CaCO₃. Plates containing Methyl Red at alkaline pH are yellow and turn dark red at acidic pH. Thus, a distinctive, clear zone is formed around bacterial colonies producing acetic acid and is easy to differentiate between acid producers and non-producers. The present method is more rapid, accurate, and sensitive and can be successfully be used for the detection of acetic acid-producing bacteria particularly for the screening of potent cellulose producer *Acetobacter* sp.





Future Perspective

There are different fluorescents dyes are available like calcofluor white M2R, fluorescein isothiocyanate and rhodamine B which has ability to bacterial cellulose. Such dyes can be incorporated in Hestrin–Schramm (HS) medium to develop a medium for effective screening of biocellulose producers.

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DIETARY MICRONUTRIENTS AS IMMUNOMODULATORY TOOLS FOR THE PREVENTION OF COVID-19

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Coronavirus disease -19 is a highly contagious disease caused by a single-stranded RNA virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Coronavirus disease-2019 (COVID- 19) may cause severe illness in 20% of patients. This may be in part due to an uncontrolled immune-response to SARS-CoV-2 infection triggering a systemic hyperinflammatory response, the so-called “cytokine storm”. Host immunological response against SARS-CoV-2 can affect the disease outcome. Higher levels of interleukin-6, interleukin-10 and tumour necrosis factor- α are associated with poor outcome.

Micronutrients are essential elements which are required in varying quantities to bring about tissue maintenance and repair. They play a central role in metabolism and improving the overall health and immune status of an individual. Micronutrients include vitamins and minerals, which are vital to healthy development, disease prevention, and wellbeing. With the exception of vitamin D, micronutrients are not produced in the body and must be obtained from the diet. Micronutrients are antioxidants which enhance natural killer cells and lymphocytic activity and increase interleukin-2 production. Adequate dietary intake and supplementation with



functional foods contribute to maintaining optimum levels of immunity in addition to providing an antiviral-like state helpful in the prevention of COVID-19.

Vitamins

Vitamin A is often mentioned as the "anti-inflammation vitamin" as a result of its significant role in improving immune capacity. It has a focal role in the improvement of the immune system and assumes administrative roles in cell immune reactions and humoral immune processes. β-carotene is a powerful antioxidant that can reduce inflammation and boost immune function by increasing disease-fighting cells in the body. Vitamin B6's role in immunity examines that Vitamin B6 inadequacy impact both humoral and cell mediated immune reactions and in this way disables immune reaction. Vitamin D cooperates with Folate and Vitamin B6, to help support blood homo-cysteine levels.

Research shows vitamin D supplementation may reduce the hazard for viral infections, including respiratory tract diseases, by diminishing creation of pro-inflammatory compounds in the body. Vitamin D is vital for the protection of the respiratory tract through its role in preserving tight junctions, killing enveloped viruses and inducing cathelicidin and defensins. It also reduces the risk of cytokine storm by decreasing the production of pro-inflammatory cytokines. Vitamin B12 has an important role as an immune modulator for cellular immunity. Vitamin C is a powerful antioxidant and secures against harm induced by oxidative pressure. For serious infections, including sepsis and Acute Respiratory Distress Syndrome (ARDS), high portion intravenous vitamin C treatment has appeared to essentially improve indications in patients. Vitamin E goes about as an amazing cancer prevention agent preventing cells against oxidative harm from free radicals. Vitamin E has been shown to have a significant role in regulating immune capacities. Indeed, even a single vitamin E inadequacy has appeared to weaken the immune reaction and its role in advancing an immune system especially obvious in aged people.

Minerals

Zinc is a trace mineral that has been hypothesized to inhibit viral replication and attachment to the nasopharyngeal mucous, and through this mechanism, has been implicated in the management of the common cold. In vitro studies suggest that zinc modifies effects of several respiratory pathogens, including rhinovirus, respiratory syncytial virus, and SARS-CoV-2.



Omega-3 fatty acids can influence antigen presenting cell, T-cell and B-cell function, although their effects on these cell types in humans is not consistently reported. They bring about treg cells formation, B cell activation and upregulate CCR5 expression, which regulates trafficking and effector functions of memory/effector T-lymphocytes, macrophages, and immature dendritic cells. However, they are clearly demonstrated to be anti- inflammatory and to be converted to specialised pro-resolving mediators such as resolvins, protectins and maresins.

Conclusion

Nutrients and minerals play an imperative role in the control and prevention of this newly emerged viral infections. The patients' nutritional status with COVID-19 must be analyzed before administering any treatment, and nutritional supplements should be given to the affected individuals along with routine treatment. We suggest a potential interventional role of nutrients to strengthen the immune system against the emerging infection caused by COVID-19. Supplementation with vitamins, omega 3 fatty acids and zinc appears to be a safe and low-cost way to support optimal function of the immune system, with the potential to reduce the risk and consequences of infection, including viral respiratory infections. Supplementation should be in addition to a healthy diet and fall within recommended upper safety limits set by scientific expert bodies. Therefore, implementing an optimal nutrition, with micronutrients and omega-3 fatty acids supplementation, might be a cost-effective, underestimated strategy to help reduce the burden of infectious diseases worldwide, including coronavirus disease 2019.



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INCREASED FUNGAL INFECTION IN POST-COVID DIABETIC PATIENTS

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The COVID-19 pandemic has struck the world so hard that a complete recovery from this disease is still beyond our imaginations. While this disease is reaping hundreds of lives throughout the world, another complication has emerged which makes the current situation more depressing. Now, diabetes patients are in the spotlight. After recovering from the SARS-CoV-19 virus infection, a sudden peak of fungal infection was observed in diabetes patients. The fungal infection not only targets the diabetes patients but also affects those who are recovering from COVID-19 and are immunocompromised, even those who take in steroids in large sums. Everyone inhales a numerous fungal spore in their day-to-day life. But our body does not provide the perfect condition for them to grow and flourish within our body. The immune system in our body will fight and destroy these spores and contain their infection. It's not the case when it comes to diabetes patients who has been infected by the novel corona virus. Their body will be weak and their immune system will be incompetent. Fungus needs a moist damp surface enriched with glucose for their survival and growth. All these criteria for the fungal growth are provided by a diabetes patient. The uncontrolled level of blood sugar is utilized by the fungus to multiply and proliferate. The weakened immune system which is a result of the Covid infection fails to attack and destroy these spores, creating the situation more complicated.



Not every diabetes patient will experience this. The fungal spores are entered into the body if the people are treated in poor hygienic conditions. Use of unsterilized oxygen masks, oxygen cylinders and over exposure to soil are all reasons for the infection. One such example is the black fungus disease caused by Mucormycosis. These fungal moulds grow in damp and wet areas such as damp walls, soils, decayed matter, etc. Diabetes patients who are severely affected by COVID-19 are treated using drugs like corticosteroid and tocilizumab. These reduces their immunity and makes them prone to Mucormycosis infection. The increased level of blood sugar provides an acidic media which is favourable for the growth of black fungus. The combination of COVID-19 infection, corticosteroid treatment and diabetes make a perfect combination for the fungus to proliferate. Rhino-oculo cerebral mucormycosis (ROCM) is the most common fungal infection found in post-covid patients. This is a rare, rapidly growing fungal infection which mainly affects the nose, paranasal sinuses and often extending to orbit brain and palate. The COVID-19 infection damages the respiratory tract and the eye which thus increases its susceptibility to fungal infection. They might even block the blood vessels and cause ischaemia, tissue infarction and necrosis. Symptoms involves unilateral swelling of the face, blackish discharge from the nose, blurred or doubled vision, teeth loss and so on. Serious symptoms include vomiting blood, decreased vision, blindness, altered sensorium and seizures. The treatment for such fungal infection will be the consumption of antifungal medicines such as amphotericin B, Posaconazole or isavuconazole. Removal of the infected region from the body through surgery can also prevent further complications related to these fungal infections.



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TRADITIONAL INDIAN HERBAL MEDICINE IN THE PREVENTION, TREATMENT AND MANAGEMENT OF COVID-19

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Coronavirus disease 2019 (COVID-19) is a highly contagious infection caused by Severe Acute Respiratory Syndrome Coronavirus – 2 (SARS-CoV-2). The first known case was identified in Wuhan, China in December 2019. The disease has since spread worldwide, causing large scale damage due to different waves of infection and leading to an ongoing pandemic. Severe Acute Respiratory Syndrome related Coronavirus (Sars-cov-2) has become a Pandemic Hazard to Global Public Health Worldwide.

The current strategy for treatment of COVID- 19 includes supportive care, supplemented with a combination of broad spectrum antibiotics, antivirals and corticosteroids. Taking into consideration the current situations, various treatment approaches have been sought out including traditional medicine, which have been already used to combat epidemic outbreaks such as SARS and H1N1 influenza. Traditional Indian medical systems (Ayurveda, Siddha, Unani, and homeopathy) are a vast repertoire of knowledge about medicinal plants. The promising phytochemical and pharmacological characteristics of these plants have paved the way for developing therapy against novel Coronavirus (CoV) infection.



The Indian traditional system of medicine is one among the oldest systems of medical practice in the world similar to Chinese traditional medicine. The current use of Alternative medicines, which was already on the rise worldwide, has soared even higher during the pandemic. India, actually has an entire ministry devoted to promoting use of these traditional remedies that have been used for centuries: the Indian ministry for Ayurveda, Yoga, Unani, Siddha and Homeopathy (AYUSH). These systems of medicine rely on medical philosophies and represent a holistic and healthy way of achieving good lifestyle and prevention of diseases. Traditional formulations of AYUSH are well recognized immune modulators that have been used for many centuries to cure respiratory ailments and disorders. The AYUSH ministry (Government of India) has recommended their use for prophylaxis in containment zones and to the general public.

The ayurvedic approaches include Ayush Kwath, Samshamani Vati, Ayush-64, Agastaya Hareetaki and Anuthail. The Unani approaches include Triyaq-e-araba, Roghan-e-baboona, Arq-e-ajeeb, Khamira-e-banafsha laooq-e-sapistan, Sharbat-e-sadar, Khameera Marwareed, Asgandh Safoof, Habb-e-bukhar, Sharbat-e-toot Siyah and Laooq-e-katan. The Siddha Approaches include Nilavembu Kudineer, Ahatodai Manapagu, Kabasuara Kudineer. The following preparations are commonly used for the management of COVID-19.

Kabasura Kudineer

It is Siddha medicine and used in the reduction of SARS-CoV2 and in COVID-19 when compared to Vitamin C and zinc. It has various immunomodulatory effects. It is used twice or thrice daily according to the severity of fever and other respiratory symptoms. It is a potent preparation with anti-viral and Immuno-modulatory properties. All the ingredients have been proved to possess anti-inflammatory activities.

Nilavembu Kudineer

Nilavembu kudineer is widely used in siddha medicine to combat, prevent and manage all types of viral infections and fevers. Nilavembu kudineer is made using various herbs like Nilavembu, vetiver, vilamichai ver, korai kizhangu, black peppercorn, ginger and sandal powder. The herbal decoction has various medicinal properties, which include antioxidant, antiviral, antibacterial, anti-inflammatory, antipyretic, analgesic, etc. According to Agathiyar gunavagadam, Nilavembu kudineer is a more potent therapeutic drug in treating diseases like vathasuram, suranoigal (Febrile illness), neercovai (Sinusitis) and mayakam (Syncpe, or shock).



AYUSH-64

AYUSH-64 is an Ayurvedic formulation which was developed by the Central Council for Research in Ayurvedic Sciences. (CCRAS), the apex body for research in Ayurveda under the Ministry of AYUSH, for management of Malaria. This drug was repurposed as the ingredients showed notably antiviral, immune modulator and Antipyretic properties. The *in silico* study done on AYUSH-64 showed that about 35/36 of its Phyto-constituents have high binding affinity against COVID-19 virus. The formulation has also shown very promising results in Influenza like illness. With the scientific evidence generated from different clinical studies across India, AYUSH-64 has been identified as a potential adjunct to standard care in the management of Asymptomatic, mild and moderate COVID-19 to improve the clinical recovery and quality of life.

Conclusion

Sars-cov-2 has become a Threat to Human Population due to non-availability of Approved drugs for its treatment. The potential of AYUSH Medicines and Medicinal Plants of India, The Herbal Drug, Manufacturers, and The National and Global Research Organizations should develop necessary strategies for furtherance of Preclinical and Clinical Research with these Promising Therapeutic Leads.



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THE POWER OF HUMUS

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The humus is uniform dark, spongy, jelly like appearance, and is amorphous. "humus" sometime also is used to describe mature or natural compost extract from woodland or other spontaneous sources for use as a soil conditioner. Humus serves as a source of nitrogen, phosphorus, and sulfur for higher plants. The humus play of major role in soil fertility a true architect to soil construction, it stabilize its texture, it promotes air and water circulation, it acts on it water holding capacity. This function of humus improves the structure of the soil and in conjugation with humus capacity to retain important nutrients, humus rich soil helps plants grow more easily. These are easily available in our environment and used as a nutrient for crop. Humus is hence considered the backbone of crop production as it has a major role in their growth.

The microflora of a soil is an intimate part of soil organic matter. In fact, much of the colloidal portion of humus consists of living and dead microbial cells or their disintegrating residues. The decomposition process of organic matter has a direct impact on the oxidation process of complex organic compounds such as the lignin-like humus. These compounds are broken down into simple sugars, amino sugars, aliphatic, and a type of acid referred to as phenolic.

Humus supports the growth of an all-important organism called mycorrhizal fungi. This fungi forms a symbiotic relationship with many plants and is an important factor in the soil food web. This fungi help bind the soil particles to form good soil structure. adding humus in the soil will increase soil fertility and enhance



the growth of seedlings and other vegetation while also reduce the need for watering to a minimum and help make plants more resilient to disease.

- Humus is one of nature's best compost. It can be used as Biofertilizer in Agriculture. It increase soil quality and do not produce any harmful effect.
- It is also one of the best waste management options.
- It minimizes the environmental pollution and increase crop yield and gives good result.
- Composting of organic wastes is an environmentally sound means of diverting organic waste from landfills and producing valuable soil amendments.
- The production of humus from garden waste produces humus from this waste.

Humus allows soil organisms to feed and reproduce, and is often described as the "life-force" of the soil. The process that converts soil organic matter into humus feeds the population of microorganisms and other creatures in the soil, and thus maintains high and healthy levels of soil life.



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SOIL HEALTH USING COMPOST

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"Composting is the need of future".

Composting is a natural decomposition process stemming from microbial succession, which results in the degradation and stabilization of organic matter. Composting is a biochemical process that involves a large variety of mesophilic and thermophilic aerobic microorganisms, including *Bacteria*, *Actinomycetes*, *Yeast*s and *Fungi*, in transforming low-value undecomposed materials into a high-value humified products. composting is an important approach to process biodegradable wastes by transforming potential organic waste fractions into a useful product such as compost.

Gardeners and farmers add compost to soil to improve its physical properties. They may even use compost instead of soil to grow plants. Mature compost is a stable material with a content called humus that is dark brown or black and has a soil-like, earthy smell. Organic waste in landfills generates, methane, a potent greenhouse gas. By composting wasted food and other organics, methane emissions are significantly reduced. Compost improves downstream water quality by retaining pollutants such as heavy metals, nitrogen, phosphorus, oil and grease, fuels, herbicides, and pesticides

Composting process of farm waste material and its proper use in crop production minimizes, the chances of pollution hazard and sustain the environmental stability. The micro-organisms are mainly responsible for further breaking down part of the humus into carbon dioxide, water and nutrients for the



plants. This process is called mineralization: nutrients are released and can be taken up directly by plant roots. Composting reduces the amount of waste to be transported and disposed of thus also reducing negative effect to the environment converting organic waste into compost eliminates pollution of the air water and soil. Composting can offer an endless possibility in organic, farm, and kitchen waste management. Only composting is the way to achieve a sustainable organic waste management. Composts is an effective way to increase healthy plant production, help to reduce the use of chemical fertilizers, and conserve natural resources. Compost provides a stable organic matter that improves the physical, chemical, and biological properties of soils, thereby enhancing soil quality and crop production.

Composting is the transformation of best from the waste. Converting waste into compost eliminates pollution of the air, water, and soil. Home composting is the most environmentally-friendly way of dealing with kitchen and garden waste, plus it produces compost that can be used as an excellent soil improver. All the human and animal manure which the world wastes, if returned to the land, instead of being thrown into the sea, would suffice to nourish the world. Composting is a practical and environmentally friendly way of caring for your vegetable garden, flower beds, and lawn



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BIOGAS: AN ENDLESS SOURCE OF BIOFERTILISER, ELECTRICITY AND FUEL

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Biogas is an environmentally-friendly, renewable energy source. It's produced when organic matter, such as food or animal waste, is broken down by microorganisms in the absence of oxygen, in a process called anaerobic digestion. Jan Baptita Van Helmont first determined in 17th century that flammable gases could evolve from decaying organic matter. Biogas has gained popularity in recent years as a "greener" fuel.

Biogas, also known as renewable natural gas, is "renewable" in the sense that humans and animals will keep producing waste – but we don't want to encourage generating more waste for the sole purpose of creating more biogas. After all, though capturing and using methane is better than allowing it to escape to the atmosphere, burning the gas still has a climate impact. We could further reduce this impact by capturing and storing the CO₂ from biogas combustion.

Biogas is a gas created through the biological introduction to organic matter even without the oxygen. Organic waste such as dead plant and animal material, animal feces, and kitchen waste can become a gaseous fuel known as biogas. Biogas arises from biogenic material and is a kind of biofuel.



Kitchen waste is very useful to our day life in different conditions. It can be used for biogas, an organic acids, biofertilizers, and biomass production as humus which contain biological activity and associated with many major roles for fixation of environmental parts. Due to its degradation is becoming a great challenge because kitchen waste made up of biological polymer substances which provide nutritional substances for growing pathogenic microorganisms. The cheapest, eco-friendly and acceptable method is aerobic degradation by aerobic microbes. The anaerobic microbes release the extracellular enzymes such as hydrolytic enzymes involve in degradation of kitchen waste in anaerobic conditions and produces methane gas act as a global warming factor.

A complex with consortium of microorganisms participates in the hydrolysis and fermentation of organic material most of the bacteria are strict anaerobes such as *Bacteriocides*, *Clostridia*, *Enterobacteriaceae* and *Bifidobacteria*. The higher volatile fatty acids are converted into acetate and hydrogen by obligate hydrogen producing *Acetogenic bacteria*.

The gases methane, hydrogen, and Carbon monoxide (CO) can be combusted or oxidized with oxygen. This energy release allows biogas to be used as a fuel; it can be used for any heating purpose, such as cooking. It can also be used in a gas engine to convert the energy in the gas into electricity and heat. Biogas comprises mainly methane (CH_4) and carbon-di-oxide (CO_2) and could have small quantities of hydrogen sulphide (H_2S) and moisture. Biogas is really a renewable fuel, therefore it qualifies for alternative energy subsidies in certain parts around the globe.

Biogas is a renewable, as well as a clean, source of energy. Gas generated through biodigestion is non-polluting. It actually reduces Greenhouse emissions (i.e. reduces the greenhouse effect). No combustion takes place in the process, meaning there is zero emission of greenhouse gasses into the atmosphere. Therefore, using gas from waste as a form of energy is a great way to combat global warming.

The gas is useful as a fuel substitute for firewood, dung, agricultural residues, petrol, diesel, and electricity, depending on the nature of the task, and local supply conditions and constraint, thus supplying energy for cooking and lighting. Biogas from wastes, residues, and energy sources, which can be used for replacement of Fossil fuels in power and heat production, and it can be used also as gaseous vehicle fuel. The by-product of the biogas generation process is enriched organic digestate, which is a perfect supplement to, or substitute for, chemical fertilizers. The fertilizer discharge from the digester can accelerate plant growth and resilience to diseases,



whereas commercial fertilizers contain chemicals that have toxic effects and can cause food poisoning, among other things.

Biogas technology with the concept of zero waste is expected to reduce the rate of global warming and eco-friendly renewable energy. In addition to being an alternative energy, biogas can also reduce environmental problems, such as air pollution, soil pollution, and global warming, and its easy operation and a wide selection of organic wastes feedstock, with its dual benefits as a waste management tool and simultaneous energy production. Biogas technology is the biochemical conversion technology of bio-energy conversion where decomposition or degradation of organic matter occurred in the absence of oxygen by microorganisms. In the end, while biogas today is a more sustainable solution than traditional natural gas, we should consider it as an important transition fuel on the road to completely decarbonizing our energy supply.



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MICROORGANISMS IN GANGES PURIFICATION

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The Ganga is a major river in the Indian subcontinent, It begins its journey from the Gangotri Glacier of the central Himalayas and drain into the Bay of Bengal through its vast delta in the Sunder bans. It is a sacred river in Hinduism and worshipped as the goddess Ganga. There is a strong belief that this sacred river has extraordinary powers like self-cleaning and healing properties. In the ancient times the Ganga water was also referred to as 'The water of immortality' and 'Mother Ganga'. These unique properties of Ganga water is due to the presence of significantly higher properties of organisms with antimicrobial properties. Other Indian rivers also contain these organisms but the Ganga particularly in its upper Himalayan stretches has more of them.

Today the river is among the world's most polluted (filled with untreated sewage, industrial wastes, pesticides and also polluted due to various spiritual activities performed). In spite of these, the Ganga river supports a surprising amount of biodiversity which is essential for its self-purifying and regeneration properties.

Various pathogenic species of bacteria are isolated from the Ganga water, they include Escherichia, Enterobacter, Salmonella, Shigella, Vibrio e. t. c. Along with bacteria, huge populations of bacteriophages are also present in the river water. Bacteriophages are a kind of virus that kills bacteria and these phage's are harmless to humans as they are highly strain specific.



In 1896, British physician E Hanbury H akin observed that cholera microbes died within three hours in its waters, but thrived in distilled water. The bacteria that cause cholera, the phage that will kill this life- threatening bacteria will be specific to it and won't be effective towards any other cells. Studies by the Institute of Microbial Technology – IMTech (one of the laboratories of the C SIR) revealed that more than 20 types of bacteriophages are found in water of Ganga river which often target bacteria that are responsible for causing deadly diseases (like tuberculosis, pneumonia, cholera and urinary tract infections).

In the river Ganga the bacteriophages were detected to be approximately 3 times more in proportion than bacterial isolates. The samples drawn from the Ganga contained almost 1,100 kinds of bacteriophages, and proportionally there were less than 200 species detected in the samples obtained from other rivers (Yamuna and N armada). Hence, it was suggested that Ganga may contain unique microbial life, which helps in self- purification of the river. The super phage isolated from Ganga and decoded for its Lysine gene and cloned to produce lysine protein at IIT Roorkee, holds great potential as an antibacterial pharmaceutical.

Excessive oxygen content in water is one of the reasons for self- purifying property of river Ganga. Due to high level of oxygen in the waters of Ganga gives it the unique ability to remain fresh over a prolonged period of time. Despite of pollution, the water of the Ganga still possess 'Medicinal qualities'. Some microbiologists working on to develop new 'anti- microbial compounds' with the help of Ganga water. There by the sacred river Ganga is known for its characteristic water quality in terms of Physic o- Chemical property and Microbial diversity which play a major role in the self- purification process.



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MICROORGANISMS IN SPACE

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Staying clean in a world full of germs is very important. As we know microorganisms are omnipresent in nature. They are found in soil, water, air, and even in extreme conditions such as hot springs, deep seas, polar regions (Extremophiles) etc. Then why not in space? Microbes generally thrive to live, die and multiply in any kind of environment. The microbial population found on the International Space Station are no different. On Investigation, it was revealed that the bacteria actually originate on Earth which are similar to those found in gyms, hospitals, offices etc. on Earth. These could have been carried into space either on the equipment brought along or from the astronauts themselves. Bacteria were some of the first organism investigated in 1960. A Russian satellite carried *Escherichia coli*, *Staphylococcus* and *Enterobacter aerogenes*. Conditions in space can shift rapidly from one extreme to another. For example, one side of the Space Station facing the sun reaches 250 degrees F, while the side facing away from the Sun is -250 degrees F. NASA conducted studies on the bacteria to find out how they can survive in the harsh conditions of space. The answer was the anatomy of certain bacteria whose unique characteristics make it possible for them to stay in vacuum without getting destroyed. These Extremophiles are required for studying the physiological requirement of survival in space. They can also be used as the model organism to understand the fate of biological systems in the environment. The study of these microbes is important, because astronauts during space flight have altered immunity and do not have access to the sophisticated medical intervention available on earth .we usually think its toilets that are the grossest, even the space stations are super



gross. Even after a shower there are many billions of bacteria and fungi present on your skin, so when you're trapped in a space station above the earth they may exist there too. Because of their ubiquity and resistance to spacecraft decontamination, bacterial spores are considered as the contaminants in the space. In 1998 US astronauts opened a rarely accessed panel and found large free floating mass of water without gravity, moisture had began to coalesce forming brownish or cloudy white colored spheres. When the samples taken from the sphere are examined they had found amoebas, protozoa and dust mites.

Space is a crazy place and various studies prove that bacteria love space, they're even better at forming bio-films, higher density populations. And on the other hand they are even more capable of causing diseases. *Escherichia coli* and *Staphylococcus* are found in micro-gravity which are usually found on human palms and cause some nasty illnesses. Another study in microbiome found Actinobacteria is important for soil systems on earth which causes skin irritations and inflammations. *Salmonella* is a type of enterobacteria and in tests done with mice, salmonella flown into space killed the mice 2 days earlier than the regular salmonella. Hence, studying microbes in space could be very helpful for us in the future, its also to search for the microbial species we've never seen before, in the hope that they may have useful characteristic. This is a vital research if human ever hope to travel to various distant planets safely. Therefore, the development of space microbiology in the future would help in future space exploration and which could be interesting.



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ANTIMICROBIAL RESISTANCE IN *Stenotrophomonas maltophilia*

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Stenotrophomonas maltophilia is a motile, aerobic, glucose non-fermenting, Gram negative bacteria that belongs to the Xanthomonadaceae family. It is found on wet surfaces and habitats in aquatic environments, soil, plants, foods, and on the surface of human skin. After the 1970s it has been more frequently reported as a nosocomial pathogen in immunocompromised patients having previous exposure to broad-spectrum antibiotics (especially with carbapenems), prolonged hospitalization (especially in ICU), prolonged use of mechanical ventilation and intravascular devices, respiratory tracts infections (cystic fibrosis), bloodstream infections, and the patients suffering from cancer and HIV. Infections caused by *Stenotrophomonas maltophilia* include septicemia, endocarditis, conjunctivitis, skin and soft tissue infections, bone and joint infections, gastrointestinal infections, meningitis, postoperative wounds, abscesses, urinary tract infections, and pneumonia which is associated with a high mortality rate with uncontrolled clinical trials. Over time large surveillance studies such as SENTRY studies during 1997~2003, the CANWARD surveillance study during 2007 – 2012, and the SENTRY antimicrobial surveillance program reported the high frequency for occurrence of *Stenotrophomonas maltophilia* infections ranged from 0.8 % - 1.68 % that indicated the increasing trend in infections in the general population. The World Health Organization recently classified *Stenotrophomonas maltophilia* as one of the leading Multidrug-resistant organisms (MDROs) in hospital settings.



Stenotrophomonas maltophilia is a low-grade pathogen having extracellular and cell associated virulence factor. The cell associated virulence factors included lipopolysaccharides, flagella, fimbriae, adhesins and a diffusible signal molecule which contributed to their adherence to biotic and abiotic surfaces, colonization, biofilm formation, and cellular invasion into eukaryotic cells. The extracellular virulence factors included the production of extracellular enzymes (Proteases, Lipases, Esterases, Deoxyribonuclease (DNase), Ribonuclease (RNase), Hyaluronidase, Phospholipases, and Heparinase), haemolysins, cytotoxins and siderophores. Extracellular virulence factors enable the *Stenotrophomonas maltophilia* to invade the host cell by degrading the host tissue and their survival inside the host by providing essentials.

They intrinsically encode the production of β -lactamases, several aminoglycoside-modifying enzymes, quinolone target protection proteins, and multidrug efflux transporters which enables them resistant to a variety of antimicrobial agents, including β -lactams (even carbapenems), aminoglycosides, quinolones, macrolides, and tetracyclines regardless of its clinical and/or environmental sources. Trimethoprim/sulfamethoxazole (TMP-SMX) also known as co-trimoxazole remains the most effective antimicrobial agent against *Stenotrophomonas maltophilia*, with an overall susceptibility rate higher than 90 % and have been approved by FDA for the prevention and treatment of chronic bronchitis, Traveler's diarrhea, Urinary tract infections, Shigellosis, Toxoplasmosis, *carinii* pneumonia and *Pneumocystis jirovecii* pneumonia/ *carinii* pneumonia. In 1974, TMP-SMX professionals began prescribing as medication by healthcare, and now this drug took place in the list of the World Health Organization's (WHO) essential medicines. But in the last few years, maltophilia having resistance to Trimethoprim/sulfamethoxazole have been reported all over the world such as *Stenotrophomonas maltophilia* isolated from clinical samples collected from different geographical sites all over the world shows TMP-SMX resistance ranged 2 % - 100 %. TMP-SMX resistant maltophilia has been also identified from environmental samples. The presence of TMP-SMX resistance in environmental maltophilia isolates possess a great public health threat to hinder therapeutic options for community-acquired infections.

Ceftazidime, Chloramphenicol, Levofloxacin, minocycline is also considered the drug of choice to treat maltophilia in patients having side effects of TMP-SMX. Different strains of maltophilia having resistance for these antibiotics have been also isolated from both clinical and environmental samples from different geographical locations all over the world.



The treatment of *Stenotrophomonas maltophilia* is challenging due to its intrinsic resistance pattern to multiple antibiotics including carbapenems as well as its ability to form a biofilm that protects it from natural immune defenses or the action of antimicrobial compounds. trimethoprim-sulfamethoxazole fluoroquinolones, levofloxacin, minocycline and tigecycline are generally the drug of choice to treat *Stenotrophomonas maltophilia* infection but the global emergence of acquired resistance to these antibiotics has been reported. However now a day the clinical infections caused by *Stenotrophomonas maltophilia* have become a global problem associated with both hospital-associated and community-acquired infections.



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HEALTH BENEFITS OF FERMENTED RICE

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Fermented foods share an integral part of age-old wisdom from ancient Indian civilization. They play imperative roles in providing the health promoting macronutrients, micronutrients, phytochemicals. One among them is "FERMENTED RICE". Fermented rice, also known as Tapai, is a fermented food made from inoculating a carbohydrate like rice with plain yoghurt, with a starter culture. It may include acidic fermentation, alcoholic fermentation or both. *Pediococcus*, *Leuconostoc*, *Bacillus*, *Gluconobacter* and *Lactobacillus* were the dominant bacteria whereas *Rhizopus*, *Saccharomyces* and *Saccharomycopsis* were the predominant fungi detected during fermentation of rice.

Fermented rice is rich in probiotic bacteria and enzymes, increasing the health of gut microbiome and digestive system and enhancing the immune system. The fermented rice restores healthy intestinal flora and can prevent gastrointestinal ailments like duodenal ulcers, infectious ulcerative colitis, Crohn's disease, irritable bowel syndrome, celiac disease, candida infection etc. The process of fermentation increases the availability of several nutrients such as calcium, magnesium, iron and potassium - for example, Iron in 100 gm of fermented rice will be increased from 3.14 mg to 73.91 mg on overnight fermentation, which is a best treatment to treat anemia naturally. Eating fermented rice reduces fatigue due to the high availability of vitamin B12. It aids in speed recovery due to high availability of nutrients. It maintains pH balance in the stomach.



Certain researchers claim fermented rice produces collagen, which is necessary to maintain the elasticity of the skin. It is also said that fermented rice water can act as a natural hair conditioner and since it is rich in vitamin E and ferulic acid, it acts as an important antioxidant. Lactating mothers facing the problem of low milk secretion, fermented rice helps secret more milk and is a good source of lactic acid.

Malnutrition and poor health are the major burdens in the developing countries like India. The fermented rice-based food formulation may be the gift, as this improves the overall nutritive capacity and has an added advantage of physiological functions due to the bioavailability of essential amino acids, minerals, phytic acid and other nutritional factors. Further, the wet processing of rice with cultures like LAB improves fortification, energy density etc. The Standardization of process parameters and the adaption of newer technologies for fermentation of rice by mixing with other cereals, considering acceptability by the end users, are also essential to improve individual nutritional status as well as community health.

“HEALTH IS WEALTH”



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CORDYCEPS MILITARIS MUSHROOM

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What is Cordyceps militaris Mushroom?

Cordyceps militaries is species of fungus in the family Cordycipitaceae, and the type species of the genus *Cordyceps*. It is potential harbour of biometabolites for herbal drugs. *Cordyceps militaries* is an important medicinal mushroom useful for extraction of several bio metabolites for natural drugs to revitalize the various physiological system of the body from ancient era. The *Cordyceps militaries* mushroom has been used for long time in eastern Asia as a nourishing food and in traditional Chinese medicine as treatment for cancer patients. Among all the species *Cordyceps militaries* is considered as the oldest source of some useful chemical constituents. Different cordyceps species, most notably *Ophiocordyceps sinensis*, have a long history of use and have been found growing only from head of a subterranean caterpillar above 3000 m altitude on the Qinghai Tibetan plateau. *Cordyceps militaries* are also good act as anti-inflammatory, anti-oxidant, anti-leukemic, anti-proliferative, anti-bacterial, anti-fungal, anti-metastatic, anti-HIV, anti-malarial, anti-fatigue, anti-diabetic, anti-fibrotic, steroidogenic, hypoglycaemic, hypolipidemic, liver protective, reno protective, as well as pneumonia protective. It is 1 lakh to 4 lakh per 1 kilogram of *Cordyceps militaries* mushroom.



How to Production of *Cordyceps militaris* Mushroom?

- The *Cordyceps* mycelium will grow in dark, they are sensitive to lights, they grow between 55-75 Fahrenheit. Recants day to grow cordyceps militaris mushroom can be hard process.
- There are four steps to produce cordyceps militaris mushroom
- Make potato starch
 - ✓ Requirements
 - 250 gm potato
 - 1 litter RO water or bottled water
 - Sterile knife
 - Slicer
- First of take 250 grams of potato and wash it properly then peel of outer side of potato and slice in small pieces. Piece of slice should be 1 cm. Take one litter water, add 200 gm potatoes pieces and boil it. Make sure potatoes is not cook well but it should be semi cook. Then take that boil potato water it is our potato starch.
- Make potato dextrose for liquid tissue culture (spawn)
 - ✓ Requirements
 - 500 potato starch
 - 2.5 teaspoon glucose
 - $\frac{1}{4}$ teaspoon peptone
 - $\frac{1}{4}$ teaspoon yeast extract
 - 0.150 gm magnesium sulphate
- First of take 500 ml potato starch, add 2.5 tea spoon glucose, add $\frac{1}{4}$ teaspoon peptone, add $\frac{1}{4}$ teaspoon yeast extract, add 0.150 gm magnesium sulphate and mix well. Then add this mixture in conical flask and closed cotton plug. Autoclave this mixture in 15 psi for 40 min.
- After autoclave mixture colour was yellow-orange. then cooldown in AC room for two hours. Our potato dextrose are ready. Then cut one cm piece from tissue culture by sterile knife, take this piece by sterile forceps and add this culture in potato dextrose. All this work must be doing in LAF (Laminar air flow). Place the conical flask in a rotary shaker for four days. After four days our spawn is ready
- Substrate
 - ✓ Requirements
 - 500 gm brown rise
 - One letter potato starch
 - 4 teaspoon glucose
 - 2 teaspoon peptone
 - 1.5 teaspoon yeast extract
 - 0.600 gm magnesium sulphate
 - 2 pcs vitamin B1
 - 2 pcs egg brown or normal



- Take 500 gm brown rice add some water as per brown rice and soaking for 30 minutes. after 30 min remove water, spread brown rice on cotton cloth for drying.
- Take 1 letter potato starch add one by one 4 teaspoon glucose, 2 teaspoon peptone, 0.600 gm magnesium sulphate, 1.5 teaspoon yeast extract. Take 2 pcs vitamin B1 tablet add substrate let melt it for 5 to 10 minutes. Then add the vitamin mixture in substrate. Take electric mixer jar, add 2 pcs egg and substrate mixing on high speed for 40 second.

Bottle Filling and Packing

- Take bottle, add 20 gm brown rice at the bottom, fill up by 40 gm substrate and close by bottle cap. On cap should must be 5 to 6 mm hole and the hole fill up by cotton plug. Then autoclave the bottle for 40 minutes at 15 psi (121 C). after autoclave cool down the bottle at room temperature for 2 hours. Take sterile syringe fill up by spawn. Add 10 ml spawn on substrate in bottle. This work must do in LAF.

Storing

To put the bottle in dark room for 9 days. In dark room temperature must be 20 C to 22 C and humidity must be 65% to 70%. After 9 days we see mycelium on substrate and the colour was white. After that to put in the room light for 7 days. The result was mycelium colour change white to orange. The next step was to put in the bottle in direct light after 90 days cordyceps militaris mushroom are ready for harvesting.

Benefits of Cordyceps militaris Mushroom

- Immune system booster: it contains compound like cordycepins and adenosine which fight bacteria and help in immune system.
- Increase stamina, energy and immunity.
- Control cholesterol and glucose level; cordycepins have a liquid lowering effects.
- Improve lung health; it helps to hold oxygen capacity by 40%.
- Improve brain function; cordyceps militaris improve learning function of brain.
- Give relief from asthma and chronic bronchitis; it is attributed to presence of bioactive compound like cordycepins and adenosine, which play vital role in immune system of human body.
- Anti-aging; cordyceps militaris is having very rich antioxidants.
- Improve heart health; it prevents cholesterol accumulation in arteries and also help to manage blood pressure.
- May help in type 2 diabetes; it keeps blood sugar in control by mimicking action of insulin.
- Improve liver problem; its help to prevent hepatic cell degeneration.



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IMPACT OF COLD PLASMA TREATMENT ON QUALITY OF MILK AND MILK BASED FOOD PRODUCT

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Dairy products which are commonly known as milk based products are the food products produced from milk. Milk is a natural, nutrient rich product and an easy supplement for human dietary requirements. The dairy industries processes raw milk into various milk based products which are rich source of energy such important milk based products are ghee, butter, cream, yogurt, cheese, paneer etc. But Infectious diseases caused by the ingestion of pathogenic bacteria in contaminated milk are still a major health risk of consumers. Recently, consumers prefer minimally processed, preservative free, healthy and safe food products for consumption. Generally dairy industrialists used the conventional thermal processing like pasteurization and sterilization for of raw milk due to their effectiveness in improving the product safety and shelf life. But these conventional methods of heat treatment can be caused for undesirable protein denaturation, non-enzymatic browning, loss of vitamins and flavor changes, freezing point depression etc. which may results the depletion of sensory quality as well as physicochemical properties of final product. For this reason non-thermal processing may important for decontamination of milk. In this recent era, application of cold plasma treatment as a non-thermal processing technology gaining interest day by day.



Plasma is the fourth state of matter which is defined as a partial ionized gas, composed by positively and negatively charged ions, electrons and neutral atoms, molecules and radicals. It is generated naturally or artificially and can exist in various forms. In nature, it is found as the aurora borealis or might appear for a short while during lightnings or thunders and artificially plasmas are generated by supplying energy to a neutral gas causing the formation of charge carriers. Plasma is classified into two groups one is thermal plasma where the electrons and ions have a high level of energy to influence plasma behavior and another is non-thermal plasma or cold plasma referred as a partially ionized gas which is comprised of several excited atomic, molecular, ionic and radical species, co-existing with numerous reactive species including electrons, positive and negative ions, free radicals, gas atoms, molecules in the ground or excited state. Generally cold plasma was generated by three different methods which are Dielectric Barrier Discharge (DBD) method where self-sustainable electric discharges were generated in between two electrode configurations separated by an insulating material, Radio frequency discharge (RF) method where cold plasma is generated at high frequency and high voltage using a generator that provides the energy to ionize the gas and microwave discharge method.

Currently, Cold plasma treatment has gained attention to decontaminate the milk. Plasma treatment can reduce more than 50 % population of most common fecal bacteria *Escherichia coli* in raw milk. However cold plasma treatment did not affect the lipid composition of milk and not significantly increased the aldehyde content of milk based product. Dielectric Barrier Discharge Cold Plasma (DBD-CP) treatment can effective for sterilization and the most important physicochemical values including color, viscosity, pH, and texture of milk treated by DBD-CP showed acceptable changes. Not only milk, cold plasma treatment has been also used to decontaminate the milk products. High voltage cold plasma treatment can inactivate and reduced *Listeria innocua* bacteria from cheese. The basic mechanisms that have been attributed to the inactivation of microorganisms by plasma are the destruction of DNA, volatilization of compounds, and etching of the cell surface by adsorption of reactive species formed during plasma generation. Milk is one of the fourteen major allergenic foods identified in Annexure II of Regulation (EU) No 1169/2011.

Milk allergy occurs through the ingestion, inhalation and skin contact makes it an important consumers or public health issue. The major milk allergens are Casein, β -lactoglobulin and α -lactalbumin. Cold plasma treatment can also be reduced the allergenicity of milk. Day by day this novel technology is gaining interest





and acceptance among dairy processing sectors for surface sterilization, decontamination of milk and improving the sensory and nutritional quality of the milk based product.

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CHROMATOPHORES - THE SHINING CELLS

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A wide range of animals, including amphibians, fish, reptiles, crustaceans, and cephalopods, have Chromatophores, pigment-containing cells or groups of cells. Mammals and birds, in contrast, have a class of cells called melanocytes for colouration. Chromatophores are produced in the neural crest during embryonic development and are largely responsible for ectothermic animals' skin and eye colour. Mature chromatophores are grouped into subclasses based on their colour; Xanthophores (Yellow), Erythrophores (Red), Iridophores (Reflective / Iridescent), Leucophores (White), Melanophores (Black/Brown), and Ctenophores (Blue). Colour-bearing cells derived from the neural crest of cold-blooded vertebrates and cephalopods were given the name chromatophore. The word comes from the Greek words chroma, which means "colour," and photos, which means "bearing". The term chromatocyte, on the other hand, was coined for the colour-producing cells found in birds and mammals.



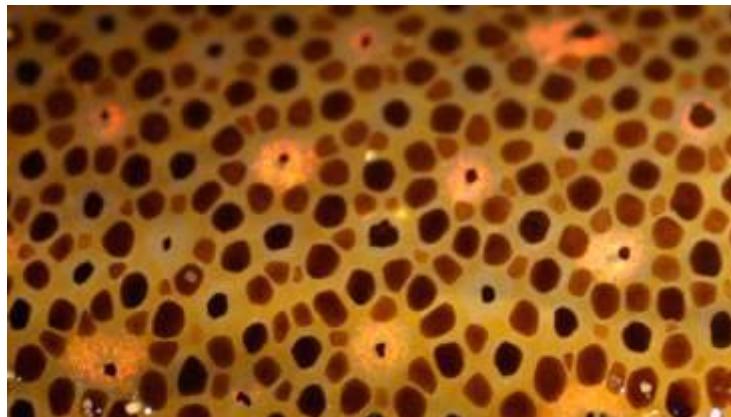


Figure – 1: Chromatophores on the skin of a squid

Some of the main purposes of such colour changes are camouflage, signalling, and temperature regulation. Only one such cell type, the melanocyte, has been identified in these animals. Biochromes and structural colours, or "schemochromes," are two types of colour-producing molecules. True pigments, such as carotenoids and pteridines, are among the biochromes. These pigments selectively absorb portions of the visible light spectrum that make up white light, allowing other wavelengths to reach the observer's eye. Different combinations of diffraction, reflection, and scattering of light from structures with a scale around a quarter of the wavelength of light produce structural colours. Many such structures interfere with some wavelengths of light and transmit others, simply because of their scale, so they often produce iridescence by creating different colours when seen from different directions. Whereas all chromatophores contain pigments or reflecting structures (except when there has been a mutation, as in albinism), not all pigment-containing cells are chromatophores.

Xanthophores and Erythrophores

Chromatophores that contain large amounts of yellow pteridine pigments are named xanthophores and those with mainly red/orange carotenoids are termed erythrophores. However, vesicles containing pteridine and carotenoids are sometimes found in the same cell, in which case the overall colour depends on the ratio of red and yellow pigments. Therefore, the distinction between these chromatophore types is not always clear. Most chromatophores can generate pteridines from guanosine triphosphate, but xanthophores appear to have supplemental biochemical pathways enabling them to accumulate yellow pigment. In contrast, carotenoids are metabolised and transported to erythrophores. This was first demonstrated by rearing normally green frogs on a diet of carotene-restricted crickets. The absence of carotene in the frogs' diet meant that the red/orange



carotenoid colour 'filter' was not present in their erythrophores. This made the frogs appear blue instead of green.

Iridophores and Leucophores

Iridophores, sometimes also called guanophores, are chromatophores that reflect light using plates of crystalline chemochromes made from guanine. When illuminated they generate iridescent colours because of the constructive interference of light. Fish iridophores are typically stacked guanine plates separated by layers of cytoplasm to form microscopic, one-dimensional, Bragg mirrors. Both the orientation and the optical thickness of the chemochrome determines the nature of the colour observed. By using biochromes as coloured filters, iridophores create an optical effect known as Tyndall or Rayleigh scattering, producing bright-blue or -green colours. A related type of chromatophore, the leucophore, is found in some fish, in particular in the tapetum lucidum. Like iridophores, they utilize crystalline purines (often guanine) to reflect light. Unlike iridophores, however, leucophores have more organized crystals that reduce diffraction. Given a source of white light, they produce a white shine. As with xanthophores and erythrophores, in fish the distinction between iridophores and leucophores is not always obvious, but, in general, iridophores are considered to generate iridescent or metallic colours, whereas leucophores produce reflective white hues.

Melanophores

Melanophores contain eumelanin, a type of melanin, that appears black or dark-brown because of its light-absorbing qualities. It is packaged in vesicles called melanosomes and distributed throughout the cell. Eumelanin is generated from tyrosine in a series of catalysed chemical reactions. It is a complex chemical containing units of dihydroxyindole and dihydroxyindole-2-carboxylic acid with some pyrrole rings. The key enzyme in melanin synthesis is tyrosinase. When this protein is defective, no melanin can be generated resulting in certain types of albinism. In some amphibian species, there are other pigments packaged alongside eumelanin. For example, a novel deep (wine) red-colour pigment was identified in the melanophores of phylomedusine frogs. This was subsequently identified as pterorhodin, a pteridine dimer that accumulates around the eumelanin core, and it is also present in a variety of tree frog species from Australia and Papua New Guinea. While it is likely that other lesser-studied species have complex melanophore pigments, it is nevertheless true that the majority of melanophores studied to date do contain eumelanin exclusively. Humans have only one class of pigment cell, the mammalian equivalent of melanophores, to generate skin, hair, and eye colour. For this reason, and because the large number and contrasting colour of the cells usually



make them very easy to visualise, melanophores are by far the most widely studied chromatophore. However, there are differences between the biology of melanophores and that of melanocytes. In addition to eumelanin, melanocytes can generate a yellow/red pigment called pheomelanin.

Cyanophores

Nearly all the vibrant blues in animals and plants are created by structural colouration rather than by pigments. However, some types of *Synchiropus splendidus* do possess vesicles of a cyan biochrome of unknown chemical structure in cells named cyanophores. Although they appear unusual in their limited taxonomic range, there may be cyanophores in other fish and amphibians. For example, brightly coloured chromatophores with undefined pigments are found in both poison dart frogs and glass frogs, and atypical dichromatic chromatophores, named erythroridophores have been described in *Pseudochromis diadema*.

Practical Applications

- Chromatophores are sometimes used in applied research, zebrafish larvae are used to study how chromatophores organize and communicate to accurately generate the regular horizontal striped pattern as seen in adult fish. This is seen as a useful model system for understanding patterning in the evolutionary developmental biology field. Chromatophore biology has also been used to model human conditions or diseases, including melanoma and albinism. Recently, the gene responsible for the melanophore-specific *golden* zebrafish strain, *Slc24a5*, was shown to have a human equivalent that strongly correlates with skin colour.
- Chromatophores are also used as a biomarker of blindness in cold-blooded species, as animals with certain visual defects fail to background adapt to light environments. Human homologues of receptors that mediate pigment translocation in melanophores are thought to be involved in processes such as appetite suppression and tanning, making them attractive targets for drugs.



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THE OFFBEAT MICROBE: *Caulobacter crescentus*

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Caulobacter crescentus is a unique gram-negative freshwater microbe which is being extensively studied and applied for understanding of many biological processes. The organism is straight or curved rod with a stalk-like extension at one end. The cell remains attached to rocks, plants or other cells by means of an adhesive holdfast at the tip of the stalk.



The stalk is very thin and is an extension of the cell body. It comprises of a narrow cytoplasmic core. It is also surrounded by Gram-negative cell envelope. There are two mechanisms which control the stalk synthesis which are totally independent of each other. The stalk is initiated during the swarmer to-stalked cell transition and then continues to elongate slowly with each subsequent round of division by the stalked cell. Apart from this developmental control, stalk is also found to elongate during nutrient limiting conditions like when cells that are starved



for phosphate. Keeping this view, it is also being suggested in scientific community that *Caulobacter* stalk might specialize in nutrient uptake.

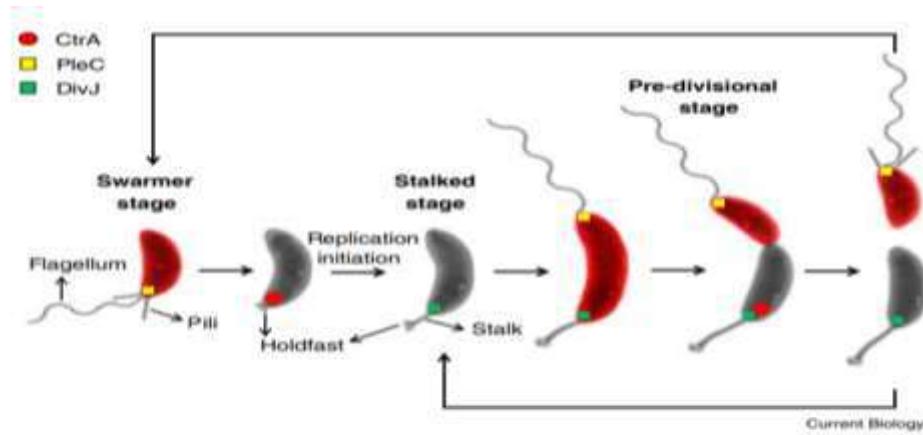


Figure 1. The dimorphic cell cycle and asymmetric division of *Caulobacter crescentus*.
Precise spatiotemporal patterning of key regulatory proteins coordinates development and cell polarity during cell cycle progression. Cells are false colored red to indicate the presence of dispersed, active CtrA.

The asymmetric cell division process makes it stand out from other bacteria. It results in two non-identical daughter cells, one of which shows presence of flagellum while other shows presence of stalk called holdfast. The daughter cell with flagellum is called as Swarmer cell while that with holdfast is called Stalk cell. The hold fast helps the cell to attach firmly to the surfaces even in aquatic environments. The swarmer cell however remains motile for 35 to 40 minutes then settles on a new nutrient rich substrate. As the time passes, the flagellar pole of the swarmer cell undergoes differentiation. It secretes a polysaccharide adhesin known as the holdfast, which mediates permanent surface attachment of the cell. It thus sheds the flagellum and gets differentiated into stalk cell. The stalked cell is known to be reproductively mature and it gives off daughter swarmer cells, marking the completion of the dimorphic life cycle.

Swarmer and stalk cells show totally different regulation of gene expression. Replication of genetic material is turned on immediately in stalk cells but is kept repressed in swarmer cells. This makes the transition of swarmer cells to stalk cells important for division. This transition is controlled by around 500 genes. The protein CtrA plays a crucial role in not only the prevention of any extragenous initiation of DNA replication but also in undergoing timed degradation during transition. Shortly after DNA replication initiation in the stalked cell, CtrA is synthesized and activated. Two important regulatory proteins, the histidine kinases (DivJ at the stalked end) and (PleC at the flagellum end), localize to opposite poles of the cell as the stalked cell progresses towards division.



The process of chromosome duplication being expensive, occurs only once per cell cycle in *Caulobacter* which is thought to be playing important role for them to thrive in nutrient-starved environments. Stalked cells work for maximizing reproductive yield through dedicated use of resources for daughter cell production while swarmer daughters colonize new grounds having ample amount of nutrients. *Caulobacter* can form biofilms. Permanent attachment of *Caulobacter* cells within these communities occurs using their holdfast polysaccharide. It is found that this adhesive is the strongest of all known biological adhesives till date.

Caulobacter thus is that off-beat microorganism which needs to be explored in detail. Scientists from all over the world are doing extensive research using this organism as model system to understand key points of developmental biology. The adhesive produced by stalk is finding its applications in underwater works. All the microbiologists need to put more attention to such unique organisms existing in nature.



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QUORUM SENSING IN MICROORGANISMS

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Communication or the fundamental act of communicating and exchanging information, is a sign of life and one of the distinguishing characteristics of living species. Bacterial communication does not take the form of physical contact; instead, it is accomplished by the production of tiny extracellular biochemical molecules that aid in identification and subsequent reaction. This type of communication occurs in bacterial colonies to react and reflect on a defined environment with a specific threshold of signalling molecules and the 'quorate' population of the bacterial population conducting activities in a coordinated manner and making decisions in high cell density. A group-based choice and high cellular density dependent communication are terms used to describe such decisions. Auto inducers are extracellular signalling molecules like this (AIs). This quorum sensing has also been discovered as a major component in the creation of pathogenic conditions in a specific host.

Quorum sensing's major action has been observed in the bacterial gene regulatory mechanism via cell density. As a result, three quorum sensing models have been developed: one for Gram negative bacteria, one for Gram positive bacteria, and one for all bacteria. The quorum sensing signalling pathway is only observable in bulk populations, or, to put it another way, quorum sensing is only effective in high cell density. The quorum sensing circuit in bacterial populations has been extensively researched under the following headings: quorum sensing in Gram



positive bacteria, quorum sensing in Gram negative bacteria, and quorum sensing in relation to interkingdom communication.

The quorum sensing method can be broken down into four key steps: i) The bacterial cell produce tiny biochemical signal molecules. ii) The active or passive discharge of signal molecules into the external environment. iii) Once these secreted signalling molecules pass a threshold concentration, that is, in bulk cellular density, they are recognised by particular receptors on cell membrane receptors. iv) This is a crucial phase because once these AIs enter the cell; they alter gene expression and express specific cell behaviour. Having said that, studying quorum sensing and its evolutionary aspects aids us in gaining a better awareness of the world around us and, as a result, a better understanding of ourselves.



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SULPHATE RESPIRATORS

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Sulphate respirators are unique in their characteristics to respire sulphate instead of the oxygen. The sulphate reducing bacteria have the ability to carry out the sulphate respiration. They are small, curved, obligate Gram-negative anaerobes. This microorganism use sulphate as a terminal electron acceptor and produce hydrogen sulphide. The production of the hydrogen sulphide recognized as a biological sulphate reduction. Sulfide appears sole final product in the culture of sulphate reducing bacteria. Step of sulphate reduction to sulfide occur in the cytoplasm, sulphate has to cross the cytoplasmic membrane. The first demonstration of the microbial sulphide production resulted in the isolation of first sulphate reducing bacteria.

Two most important pathway involve in the sulphate reduction are:

- a) Assimilatory sulphate reduction
- b) Dissimilatory sulphate reduction.

Assimilatory sulphate reduction pathway catalyze reduction of sulphate to sulphide for synthesis protein and amino acid. Plant, fungi and bacteria reduce inorganic sulphate to sulphide to fulfill their need for amino acid and proteins. In the Assimilation pathway, the formation of APS (adenosine 5'- phosphosulphate) to form Phosphoadenosine phosphosulphate (PAPS). PAPS are then reduced to sulfite by PAPS reductase. Then the reaction is catalyzed by the enzyme sulfite reductase and the reaction ends with production of sulfide. Dissimilatory sulphate reduction is also known as respiratory sulphate reduction. It includes the pathway of sulphate



reduction which proceed with energy production. In the dissimilatory sulphate reduction, APS (adenosine 5'-phosphosulphate) reduces to sulfite (SO_3^{2-}), reaction catalyzed by APS reductase enzyme. The sulfite reductase is then carried out further steps in the process result in the formation of sulphide from the sulphite. In the dissimilatory reduction, the sulfide is excreted, while in the assimilatory reduction, the sulphide is incorporated into organic sulfur compounds.

Sulphate reducing bacteria can also use Lactate, acetate, pyruvate, ethanol and amino acids as electron donor for their growth. They can oxidize organic compounds to carbon dioxide completely or incompletely with the formation of acetate. They can use ammonium salt as a nitrogen source for their growth. They have capability of developing patchy biofilms and also have an affinity for adhering to available surfaces. Recently sulphate reducing bacteria have been used to remove heavy metal such as (Cr, Zn, As) from waste water by the formation of metal sulphides. The ability of the sulphate reducing bacteria to generate large quantity of H_2S make them important in industrial and economic application. Sulphate reducing bacteria have ability to create extremely reducing condition which can reduce wide variety of metal by this ability. In addition, this ability of sulphate reduction eliminates acidity from the system as well as increasing the sulfide precipitation. Sulphate reducing bacteria are applied to treat specific type of industrial waste water. Application of sulphate reducing bacteria in the waste water treatment is great interest now days, because of it improve the removal of heavy metal, pathogen removal, also serve as a pre-treatment before digestion that result in the high production of biogas. The aim is not only to remove heavy metal from waste water, but also to recover metals to reduce depletion. Heavy metal precipitation with sulphide, which product from Sulphate reducers activity, is reported as an efficient method to remove heavy metals. The SANI process is a novel method for sulphate containing waste water treatment, in this process 3 major benefits of sulphate reducing bacteria for waste water are combined: a) Pathogen removal; b) Metal removal and c) Decreased sludge production.

The produced sulphide is not only beneficial for pathogen removal and metal recovery but is also used for autotrophic denitrification, result in nitrogen removal and sulphide oxidation. Sulphate reducing bacteria such as *Desulfovibrio*, *Desulfomicrobium*, *Desulfovibrio*, *Desulfobacter*, *Desulfomonas* and *Desulfotomaculum* make an important component of human intestine. An increased number of these organism found in people with periodontitis, inflammatory bowel disease, ulcerative colitis and many other diseases.



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HYBRIDOMA TECHNOLOGY

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Introduction

Hybridoma Technology is a method for producing a large number of identical antibodies, also called Monoclonal antibodies. The hybrid cell has the capacity of antibody production derived from B cells, At the same time it can divide continuously by the quality derived from Myeloma cells. By combining the desired qualities of both cells, the technology ensures large scale Antibody production of single specificity called Monoclonal antibodies.

Methodology

There are six steps involved in Hybridoma technology.

- a) Immunization of specific animals.
- b) Isolation of myeloma cells.
- c) Fusion between spleen cell & myeloma cell.
- d) Selection of HAT medium.
- e) Isolation of Hybridoma cells.
- f) Screening of Hybridoma cells.

a) Immunization of Specific animals

- An antigen immunized to an animal (mice) *via* intravenously by injection.
- Where in the spleen it activates B cell, which produces plasma cells.
- Plasma cell to produce monoclonal antibodies.
- Isolation of plasma cells.



b) Isolation of Myeloma cells

- Myeloma cells are cancerous cells which are isolated from bone marrow.
- Myeloma cells are generally immortal in nature (which never dies) and have multiplication properties.

c) Fusion between Spleen cell and Myeloma cell

- It requires a PEG (poly ethylene glycol) medium for fusion.
- It can also be done by electro fusion.
- Fusion between spleen cell & myeloma cell produced 5 different types of cells.
- Fused plasma, Fused myeloma, Hybridoma, Unfused plasma and Unfused myeloma.

d) Selection of HAT medium

- Before multiplication of antibodies, it has to synthesize a new copy of DNA and for that it requires synthesis of nucleotides.
- For synthesis of nucleotides mainly two pathways are there i) Salvage synthesis and ii) De Novo synthesis.
- In the Salvage synthesis, it requires degraded parts of old nucleotides to produce new nucleotides.
- In De Novo synthesis, it synthesizes completely new nucleotide by small molecules (sugar and amino acid).
- In HAT medium cells are not synthesized by de novo synthesis ,due to presence of aminopterin in HAT medium ,which blocks dihydro folate enzyme ,which is necessary for this synthesis.
- For synthesis in the Salvage pathway, it must require HGPRT (Hypoxanthine genuine phosphoribosyl transferase). Where HGPRT & TK (Thymidine kinase) are used as precursors.

e) Isolation of Hybridoma cells

- Fused myeloma and unfused myeloma didn't have HGPRT enzyme so can't survive in HAT medium.
- Fused plasma and unfused plasma have HGPRT enzymes but don't have long life.
- Hybrid cells have HGPRT enzymes from the spleen cell as well as they have the ability to multiply rapidly and produce. So, isolation of hybrid cells is the only cell which survives in HAT medium.



⑨ Screening of Hybridoma cells

- ELISA screening method which is done by incubating hybridoma culture, in which secondary enzymes get conjugate and form of coloured product shows positive hybridoma.
- Two techniques are used for multiplying the hybridoma cells (i) *In vivo* and (ii) *In vitro*.
- *In vivo* procedure involves introduction of hybridoma cells in to the animal spleen.
- *In vitro* method involves culturing of hybridoma cells in suitable culture media and then antibodies are isolated and purified.
- Once a Hybridoma colony is established, it will continuously grow in culture medium like RPMI-1640 and produce antibodies.
- Storage in liquid nitrogen.

Applications

- Used in Serological, Diagnosis, Immunopurification, Therapy, etc.



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DENIZENS OF MICROPLASTICS

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Plastic particles with a size of <5 mm is called as 'micro plastics' which results in severe marine pollution. Plasticsphere constitutes a colony of complex microorganisms inhabiting the surface of plastics. Microbes accumulate plastics because of its weathering Degradation and Surface embrittlement. With varying geographical areas and plastic substratum, the composition of microbial colony varies. The micro plastics has varying effects on bacterioplankton colony affecting their structure and function differing from those occupying higher food web position.

Inhabitants of Plastics

Biofilm formation in the plastics occurs mainly by surface fouling caused by the discrepancy in the degradation rates of plastics. Thus, floating plastics develop algal mat followed by a colony of invertebrates. Plastics are found more in benthic regions because of its increased surface density by the inhabitants as well as by increased fouling processes. But when the defouling happens by other foragers then the density decreases as a result the plastics return back to the surface.

Main effects of Plastics on Microbes

Plastics increases microbial adhesion and colonization via formation of biofilms. The spreading of bacterial pathogens in the sea happens because plastics serve as potential carriers. '*In situ*' monitoring experiments enable us to know about the taxonomic composition and metabolic profiles of the microflora present in micro/nano plastics. Wherein, the microbes will vary qualitative & quantitative.



Degradation

Each microbe has unique enzyme secretion and different enzymatic action that degrade various types of plastics. The most specific extracellular enzymes are oxygenases, lipases, esterases. Fungus has been found to have higher micro plastic degradation ability. Microbes alter the micro plastic interaction with metals or other organic compounds via differential degradation, which changes pollutant adsorption on plastics which in turn increases their pollutant carrying capacity. Since MP pollution have become Ubiquitous, it become an urgent need to look for various degradation aspects as MP serve as a novel ecological niche for the microbes. However, when pure strains are used to study the degradation mechanisms it provides convenient way to investigate metabolic pathways.

Negative Effect of Plastics

The micro/nano plastics affect the structure as well as metabolic profile of microbes in terms of glycolytic, proteolytic and lipolytic activity. It also has detrimental effects on the major ecosystem driving force namely Biogeochemical cycles.

Action of Microbes

Pure culture of the microbes adheres to the micro plastics and damages it. Where they reduce the weight percentage of micro plastic. They alter the surface properties where it becomes rough and processed. Cracks and erosions seen in plastic surface also indicates that it have underwent degradation. It also causes development of numerous cracks and grooves that affects its stability. Microbes can also alter the functional group of plastics, ex. Disappearance of carbonyl bond in PE by bacillus cereus. Some bacteria are found to live in the gut of wax worms, ticks and insects capable to eating Polyethylene type of plastics. On the other hand, fungus can alter not only morphology but as well as internal factors. An enzyme namely serine hydrolases secreted by fungus plays main role in MP degradation. It forms different bonds with MP and thus at last use MP as a carbon source also. Biofilm mediated degradation of MP includes 4 main steps: Adhesion and change in surface properties, leaching of monomers and additives, enzymatic action and embrittlement. This microbe mediated degradation is affected by environmental factors as well as physical and chemical properties of MP like crystalline nature, surface density and functional groups. The microbial colony and its characterization also influences the degradation at high level. Thus, the microbes provide an insight of knowledge that they can be further used in future to treat MP to reduce its pollution.



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AMYLASES: ENZYME OF CURRENT NEED

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Amylases have received a great deal of attention because of their significance especially in biotechnology. Amylase constitutes a class of industrial enzymes having approximately 25 % of the enzyme market world-wide. Amylases are enzyme which hydrolyze starch molecule to give diverse product including dextrin and progressively smaller polymer composed of glucose units. Alpha amylase acts on starch and breaking them up into sugars (hence the term saccharification). Starch is a carbohydrate source consisting of two molecules amylose and amylopectin. Amylase is formed from chains of glucose linked α 1, 4 and amylopectin is formed from α 1, 4 linked chains of glucose with 1, 6 linked branch points. The amylases are enzymes that work by hydrolyzing the straight chain bonds between the individual glucose molecules that make up the starch chain. A single straight chain starch is called an amylose. A branched starch chain (which can be 10 considered as being built from amylose chains) is called an amylopectin. These enzymes are of great significance in present day biotechnology with applications ranging from food, fermentation, and textile to paper industries, starch syrup industry, distillery, and detergent industry. Amylase was the first enzyme to be discovered and isolated. Although it can be derived from several sources, including plants, animals and microorganism, microbial enzymes generally meet industrial demands.

Many *Bacillus* species and thermostable *Actinomycetes* like *Actinomycetes thermomonospora* and *Actinomycetes thermoactinomyces* are versatile producers of amylase. The genus *Bacillus* produces a large range of extracellular enzymes of which amylases and proteases are of Industrial importance. Currently two types of amylases



glucoamylase and alpha-glucosidase are important for starch hydrolysis. Glucoamylase attacks - 1, 4-bonds, releasing D-glucose molecules This enzyme also attacks -1, 6 bonds at branching points in the amylopectin molecule but much more slowly than -1, 4 linkages. Alpha amylases are produced endogenously in many different organisms e.g., *B. subtilis*, *B. stearothermophilus*, *B. licheniformis*. Besides *Aspergillus niger*, *A. oryzae*. Today many microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry. This is because the amylase effects a rapid reduction on the length of the starch polymer. The resulting fragments are oligosaccharides that are readily soluble in water and are too short to retain significant adhesive capability. Thus, to cope up with the increasing demand, a variety of projects have been focused on increased production, activity, and stability of these enzymes. The major advantage of using microorganisms for the amylase production is economical bulk production capacity and microbes are also easy to manipulate to obtain enzymes of desired characteristics.

The history of Amylases began in 1811 when the first starch degrading enzyme was discovered by Kirchhoff. Ohlsson suggested the classification of starch digestive enzymes in malt as alpha- and beta-amylases according to the anomeric type of sugars produced by the enzyme reaction. These enzymes can be divided basically into four groups: end amylases, exo-amylases, debranching enzymes and transferases. Endo amylases: cleave internal alpha-1, 4 bonds resulting in α-anomeric products, exo-amylases: cleave alpha-1, 4 or alpha -1, 6 bonds of the external glucose residues resulting in alpha or beta anomeric products.

Amylase constitutes a class of industrial enzymes having approximately 25% of the enzyme market world-wide. Many *Bacillus* species and thermostable *Actinomycetes* like *Actinomycetes thermomonospora* and *Actinomycetes thermoactinomyces* are versatile producers of amylase. The genus *Bacillus* produces a large range of extracellular enzymes of which amylases and proteases are of Industrial importance. Currently two types of amylases glucoamylase and alpha-glucosidase are important for starch hydrolysis. Glucoamylase attacks - 1, 4-bonds, releasing D-glucose molecules This enzyme also attacks -1, 6 bonds at branching points in the amylopectin molecule but much more slowly than -1, 4 linkages.

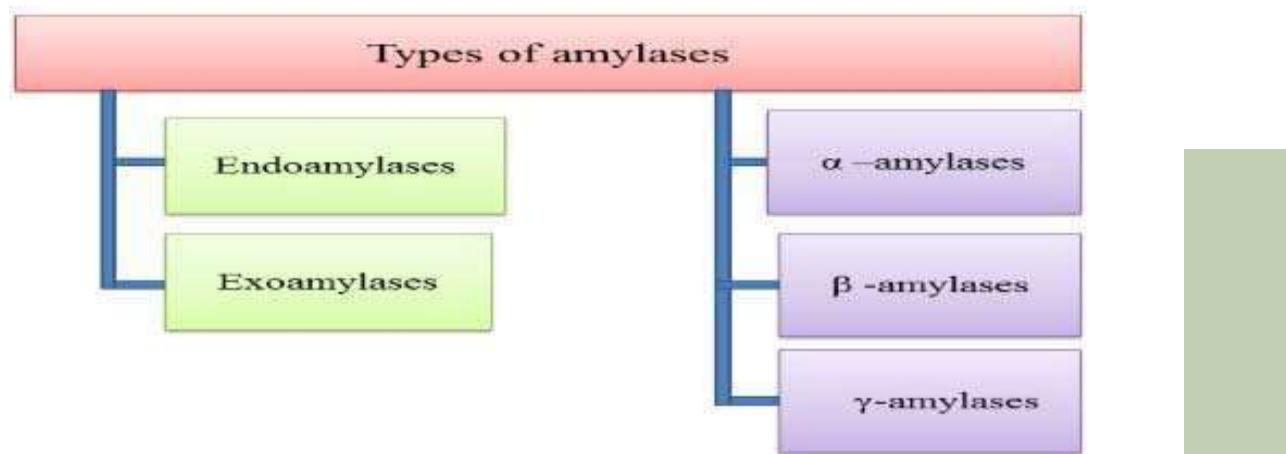
Distribution of Amylase among Microorganisms

Amylases are universally distributed throughout the animal, plant and microbial kingdoms. Over the past few decades, considerable research has been undertaken with the extracellular amylase being produced by a wide variety of



microorganisms. Amylase has been derived from several fungi, yeasts, bacteria and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in industrial sectors.

Types of Amylases



α-Amylases

The α -amylases (EC 3.2.1.1) (CAS 9014- 71-5) (alternative names: 1,4- α -D-glucan glucanohydrolase; glycogenase) are calcium metalloenzymes. By acting at random locations along the starch chain, α -amylase breaks down long-chain saccharides, ultimately yielding either saccharides, ultimately yielding either maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. They belong to glycoside hydrolase family. Because it can act anywhere on the substrate, α -amylase tends to be faster acting than β -amylase. In animals, it is a major digestive enzyme, and its optimum pH is 6.7 – 7.0. In human physiology, both the salivary and pancreatic amylases are α -amylases. The α -amylase form is also found in plants, fungi (ascomycetes and basidiomycetes) and bacteria (*Bacillus*).

β-Amylase

Another form of amylase, β -amylase (EC 3.2.1.2) (alternative names: 1,4- α -D glucan maltohydrolase; glycogenase; saccharogen amylase) is also synthesized by bacteria, fungi, and plants. Working from the non-reducing end, β -amylase catalyzes the hydrolysis of the second α -1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time. During the ripening of fruit, β -amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. They belong to glycoside hydrolase family.



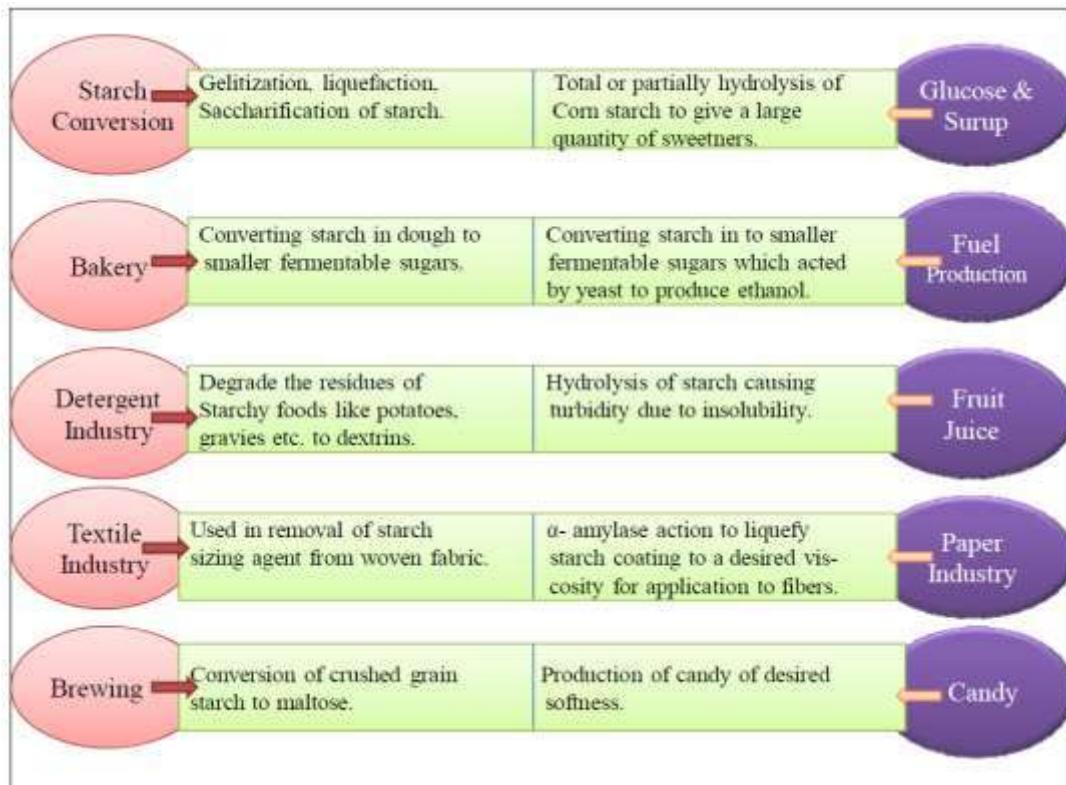
Both α -amylase and β -amylase are present in seeds; β -amylase is present in an inactive form prior to germination, whereas α -amylase and proteases appear once germination has begun. Many microbes also produce amylase to degrade extracellular starches. Animal tissues do not contain β -amylase, although it may be present in microorganisms contained within the digestive tract. The optimum pH for β -amylase is 4.0–5.0.

γ -Amylase

γ -Amylase (EC 3.2.1.3) (alternative names: Glucan 1,4- α -glucosidase; amyloglucosidase; *exo*-1,4- α -glucosidase; glucoamylase; lysosomal α -glucosidase; 1,4- α -D-glucan glucohydrolase) will cleave.

α (1–6) glycosidic linkages, as well as the last α -1,4 glycosidic bond at the nonreducing end of amylose and amylopectin, yielding glucose. The γ -amylase has most acidic optimum pH of all amylases because it is most active around pH 3. They belong to a variety of different GH families, such as glycoside hydrolase family 15 in fungi, glycoside hydrolase family 31 of human MGAM, and glycoside hydrolase family 97 of bacterial forms.

Industrial Applications of Amylase



Amylase, a starch degrading enzyme have gained importance in various industrial process like pharmaceutical, food, brewing, paper, textile, and chemicals. It is extensively used in pharmaceutical industries in digestive tonics, for hydrolysis of starch to produce different sugars like glucose and maltose which have several applications. The most widespread applications of α -amylases are in the starch industry, which are used for starch hydrolysis in the starch liquefaction process that converts starch into fructose and glucose syrups.



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A SHORT INTRODUCTION ON SOLID STATE FERMENTATION

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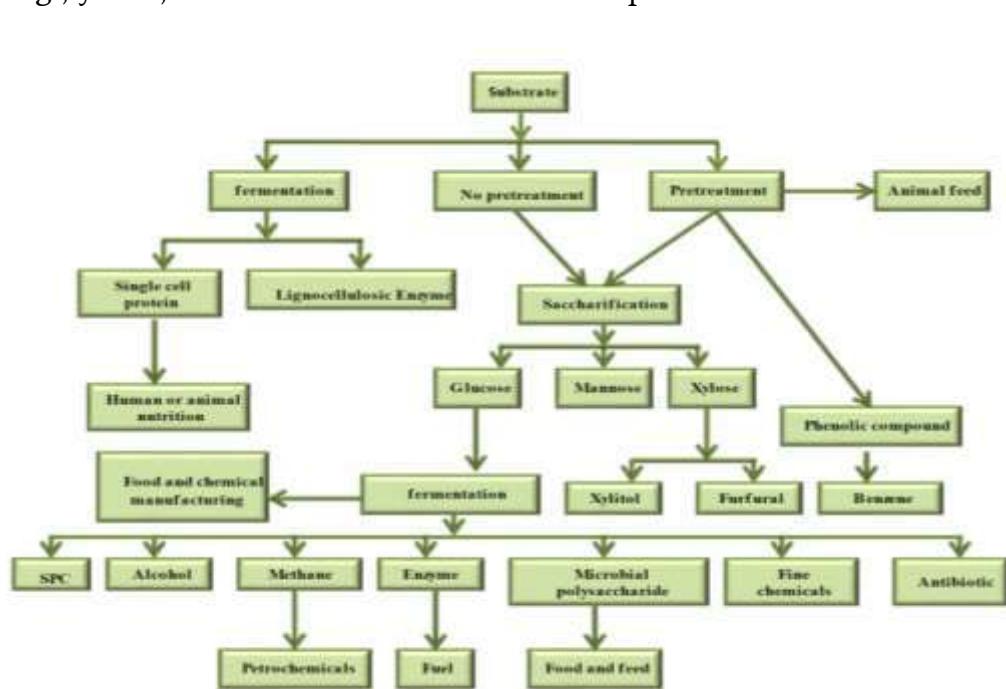
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In this era there is high demand of products like food and chemical from food, agro-industrial residue, other solid wastes by a sustainable technology. Sustainable production is described as production that is efficient and effective in the use of raw materials and energy, generates a minimum of waste or low-value streams, and leads to new and current goods and materials with the needed functionality, safety and integrity. Solid State Fermentation (SSF) and related technologies offer alternate manufacturing paths for such biotechnology-based products. Solid state fermentation has been given attention by industries and research around the world. Solid State Fermentation is basically used for food and feed production but its contribution can be harnessed by its use in production of enzymes, biologically active metabolite and other chemicals like biofuel etc. Any biotechnological processes in which organism grow on non-soluble material or solid substrates in the absence or near absence of free water is recognized as Solid State Fermentation (SSF).

Commonly used in SSF substrates are polymeric and remain insoluble or sparingly soluble in water but most of them have low cost and easily obtainable and represent a concentrated source of nutrients for microbial growth. Substrates in SSF are cereal grains (rice, wheat, barley, and corn), legume seeds, wheat bran, lignocellulose materials such as straws, sawdust or wood shavings, and a wide range of plant and animal materials. SSF offer several advantages such as easy product



recovery, low cost of complete production process, smaller fermenter-size, reduced downstream processing, and also reduction of energy requirements for stirring and sterilization. Process of SSF, different factors like microorganisms, solid support used, water activity, temperature, aeration, and type of fermenter used should be considered before going to start any fermentation process. . Some SSF processes, e.g., tempeh production, require selective growth of microorganisms such as molds that need low moisture levels to carry out fermentation with the help of extracellular enzymes secreted by fermenting microorganisms. Different microorganisms like fungi, yeasts, and bacteria that are used in SSF processes.



Solid State Fermentation (SSF) is a process in which microorganisms grow in an environment without free water, or with very low content of free water. The concept of using solid substrates is probably the oldest method used by man to make microorganisms work for him. There are some processes in which solid-state fermentation cannot be used as in bacterial fermentation. In recent years, SSF has shown much promise in development of several bioprocesses and product. The aim of SSF is to bring cultivated fungi or bacteria in tight contact with the insoluble substrate and to achieve the highest nutrient concentration from the substrate for fermentation. Two types of SSF systems have been distinguished depending on the type of solid phase used. The most used system involves cultivation on a natural material and less frequently on an inert support impregnated with liquid medium.



Solid state fermentation (SSF) is presented as a promising technology for waste valorization through the bioconversion of organic wastes used as either substrate or inert support. SSF shows sustainable characteristics in the bioconversion of solid wastes that have been proved to be able to give high efficiency in terms of product yields and productivities, low energy consumption, and solving disposal problems. SSF is not a new technology in bio processing since it has been mainly applied in the Asian region from ancient time, but recently it is gaining a lot of attention due to the increasing use of different types of organic wastes and the larger production of added-value products. The table below shows various products obtained by using SSF. SSF is a process carried out with microorganisms growing on solid and moist substrates that act as nutrient sources and support the microbial growth in the absence or near absence of water. The table below shows the few products achieved by SSF of substrate mentioned

Enzymes	Microorganism	Substrate
Amylase	<i>Bacillus subtilis</i> D19	Wheat bran, Banana peel, Orange peel, Rice bran and Pine apple peel
Cellulose	<i>U. maydis</i> MB215	α -cellulose
Pectinase	<i>Aspergillus niger</i>	Wheat bran
Lipase	<i>Panicillium simplicissimum</i>	Coconut oil cake
Protease	<i>Bacillus circulans</i>	Vegetable waste
Pigment	<i>Aspergillus carbonarius</i>	Apple, Black carrot, Red beet pulp

Advantage of SSF

- The main advantage of such method is that it produces a minimum amount of waste and liquid effluent thus not very damaging to the environment.
- Less contamination by low water content.
- Oxygen-supply, CO₂-removal, and temperature -control by aeration.
- Process is simple.
- Cost Effective.
- Less Effluent release reduces pollution.
- High products yield.
- Aeration Process is easy.
- Resembles the natural habitat of some fungi and bacteria.
- Easier downstream processing.



Disadvantage of SSF

- Not suitable for bacteria that require high moisture content.
- Controlling and measuring of parameters is difficult than SSF.
- Higher impurity is present.
- Slower microbial growth.

Application of SSF

- Production of enzyme by solid state fermentation- protease, lipase, cellulase, pectinase.
- Production of Organic Acids under solid state Fermentation- citric acid, lactic acid.
- Secondary Metabolites production Under SSF Condition – antibiotics, quinolines, growth factors, terpenoids.
- Production of biocontrol agent under solid state fermentation- e.g., Biocontrol agent –microbial agent, fungal agent.
- Production of Biofuel by Solid state fermentation e.g., Ethanol.
- Its application in bioprocesses such as bioleaching, bio-beneficiation, bioremediation, bio-pulping, etc.
- It is widely applied to producing several enzymes, organic acids, flavoring compounds etc.



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AN INSIGHT ON BIOSURFACTANTS

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Surfactants are amphiphilic compounds, have properties such as surface and interface activity, emulsification, foaming, wetting, and density reduction of hydrophobic compounds. Secondary metabolites produced by Microorganism are called as biosurfactants.

Biosurfactants produced by a variety of microorganisms mainly bacteria, fungi and yeasts are diverse in chemical composition and their nature and the amount depend on the type of microorganism producing a particular biosurfactant. Many microorganisms for industrial utilization for waste products have been isolated from contaminated soils, effluents and waste water sources. Thus, these have an ability to grow on substrates considered potentially noxious for other non-producing microorganisms.

The composition and emulsifying activity of the biosurfactant not only depends on the producer strain but also on the culture conditions, thus, the nature of the carbon source, the nitrogen source as well as the C:N ratio, nutritional limitations, chemical and physical parameters such as temperature, aeration, divalent cations and pH influence not only the amount of biosurfactant produced but also the type of polymer produced

The biosurfactant producing microbes are distributed among a wide variety of genera. The nature of biosurfactant varies from strain to strain; therefore, it is needed and demanded by the industrial R&Ds, to screen the production strain for their biosurfactant potential and its characterization. As among the biological surfactants,



the surfactant: Rhamnolipids have acceptance by the Environmental Protection Agency (EPA) for its use in food industry and pharmaceutical industry. Rhamnolipids have been lucrative surfactant molecule with the view of green technology platform. It is reportedly be a alternative for the industry to implement , as renewable resource-based surfactant and exhibit several promising applications in multiple fields. Thus ,these have an ability to grow on substrates considered potentially noxious for other non producing microorganism.



184

HAY FEVER AND ANTIHISTAMINES, THE OVER THE COUNTER

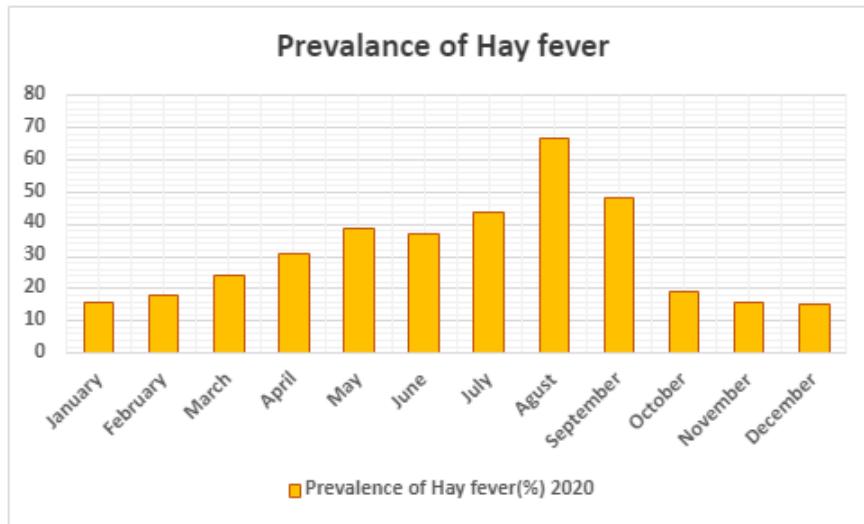
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We are living in the contemporary 21st century where vaccines and medicines for various diseases and medical complications are being developed and produced at a high rate but still some allergies are just inevitable. Allergies can be especially frustrating in this pandemic year, when most people have been limited to the confines of their homes for months on end. One among the exasperating allergies is the Hay fever or Allergic rhinitis, an allergic reaction to tiny particles in the air called allergens which can be pollen, dust mite, animal hair or even a direct air flow. It is an unfavourably susceptible response with upsetting manifestations including irritated, watering eyes, running nose, constant sneeze, and skin rashes which is much similar to a common cold. It is often an inherited trait. So, it's more likely to have it if it runs in the family. Hay fever usually starts during childhood but people show symptoms later on in life. It can be developed too but experts are not sure what triggers it.

The Bar graph depicts the percentage Hay fever prevalence over a period of 12 months in the year 2020. Overall, there is a steady increase of Hay fever sufferers till the month of July and reached the peak in August when the allergy season usually starts. The percentage of allergy prevalence dipped in the following months and remained stable. Research also shows that warmer temperatures caused by climate change are pushing plants to produce more pollen for longer periods of time. This in turn causes these allergies to now kick off 20 days earlier than in 1990 and last for ten more days with 20 percent more pollen.





Being the uninvited guest, it can be seasonal especially in the spring which gives the name Spring hay fever. When symptoms occur all year round, it is known as perennial allergic rhinitis caused by indoor allergens. Identifying the allergen is an important part of managing the Hay fever. In some cases, the cause could be obvious but in others your doctor will need to consider your medical history. Hay fever has no cure so far. However, Antihistamines stand as the best choice to ease the symptoms.

Antihistamines are meds frequently used to give a temporary relief for hypersensitivities like conjunctivitis, hives, allergic rhinitis, and responses to bug nibbles and stings. It blocks the impacts created by histamines in the body. Histamine is normally produced from Mast cells when an allergen which is identified as destructive is encountered by the body/ is inhaled.

Non-sedating antihistamines such as loratadine, fexofenadine, mizolastine, cetirizine, ebastine are the most ideal alternative, as they are less inclined to cause tiredness as opposed to other antihistamines that cause drowsiness. Yet, the types that cause you to feel drowsy might be a better choice if it ceases the symptoms. It's important to note that food and different beverages don't influence most antihistamines. However, they can collaborate with different drugs that you are taking. So, it is better to keep a watch on that. It's important to remember that over-the-counter allergy medications only relieve symptoms; they don't cure the problem, so they may be less effective if your allergies are intensifying. In conclusion, it's necessary to try a few different types to find the one that works for you. As Hay fever is usually not severe or life threatening, drugs can allow patients to lead a normal life.



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WONDERS OF GUT MICROBIOME

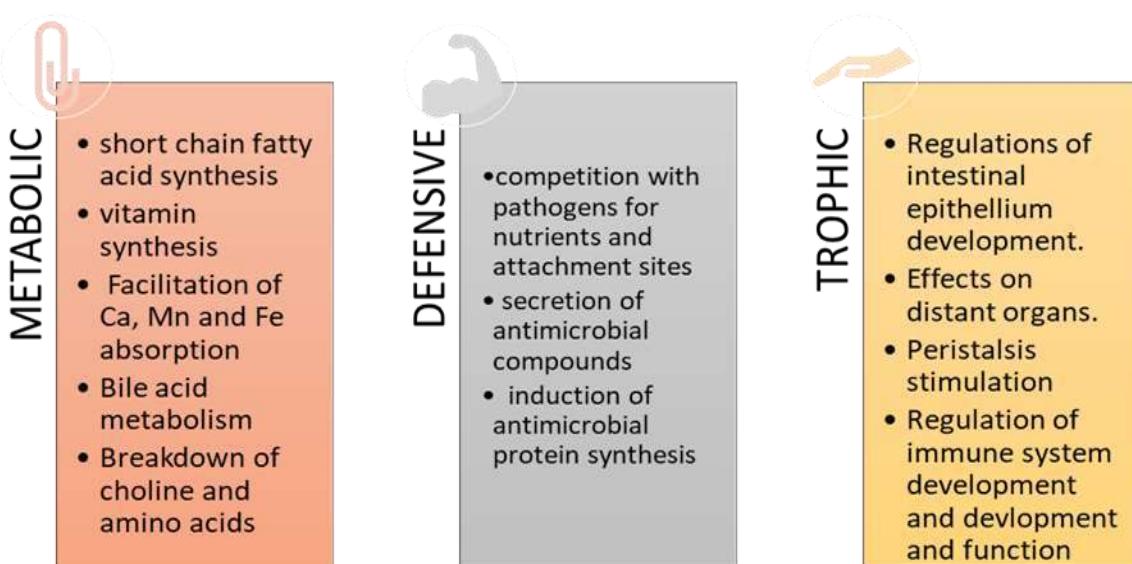
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Gut microbes are microbes which are present in the digestive tract of humans, animals and insects. Our gut is the main location for the human microbiota. It is important because it helps in our digestion, improves our immune system and it also plays a major role in our health too. Our gut is made up of many microbes like bacteria, fungi, viruses, protozoa and archaea. We have to maintain a proper gut health and balance it properly if its not balanced we will have to face many problems since our gut health affects everything, it can damage our skin causing irritation, discomfort in digestion, our weight changes unexpectedly without our knowledge, mood swings etc. The gut microbiome in our body is unique, it occupies our body when we are born and changes throughout our lifetime. Good gut microbes help us absorbing nutrients from the food we intake and it also teaches immune system about harmful microbes, toxins, mutagens and other diseases.

It was found that microbes in the gut can reduce inflammation by producing regulatory T cells and dulling the response of inflammatory T cells, thereby creating a balanced system that can fight off salmonella infection and also protect the intestinal tissues from strong damaging inflammatory immune response. Scientist found out that these gut microbes signal the antigen presenting cells to release an anti-inflammatory molecule cytokine IL- 10.





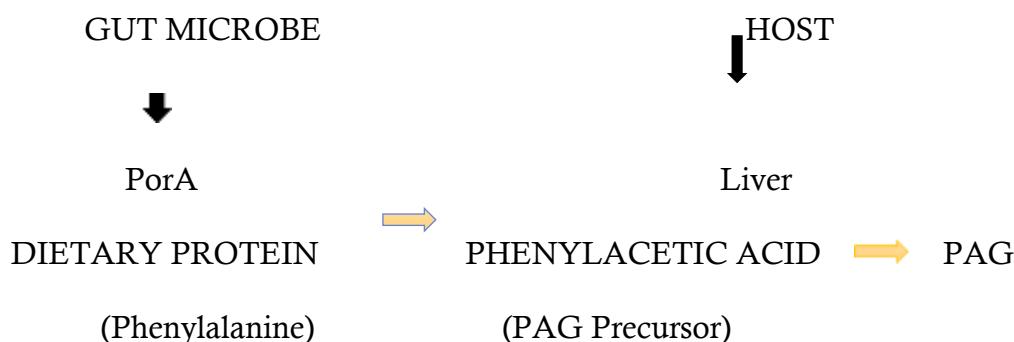
The composition of our gut microbiome is associated with the overall composition of the diet. So, we can modify our gut microbiome by choosing certain food and optimize our health. A person with more *prevotella copri* bacteria in their gut are more likely to have healthy blood sugar levels. We can also see the difference of composition of gut bacteria community in healthy individual and colon cancer patients. The higher levels of *Prevotella* species which is obtained by fiber rich plant-based diet helps to maintain a healthy colon. Research conducted in King's College London with one thousand individuals showed that the human microbiome is closely related to metabolic disease than genetics.

The microbes communicate with brain's central nervous system through immune, nervous and endocrine signaling mechanism. Changes in gut flora composition can lead to increased intestinal permeability which allows neuroactive compounds in blood. Microbiome affects gene expression, changes in it causes depression and change in one's behavior. Enteric nervous system is called the 2nd brain since it has 500 million neurons governing the gastrointestinal tract, it communicates with the central nervous system through the vagus nerve and prevertebral ganglia, this biochemical signaling is called the Gut Brain Axis, missing any microbes causes stress it was proved in 2004 when they experimented this on laboratory germ free and infected mice which is missing gut microbes was seen under stress and it also affected its memory. After this they found that the (BDNF) Brain-Derived Neurotrophic factor was important in developing stress tolerance, improves neuronal development and stress induced damage was lacking in those mice. There are also discussions taking place about the relation between Autism and the gut



microbe, but it is found that 70 % of people who suffer with Autism also suffer from gastrointestinal problems, these problems may be associated with altered gut microbiome. Example of microbe which affects the brain is *Lactobacillus helveticus R0052*. Taking few fermented foods like yogurt etc, can improves the brain and gut connection.

Gut microbe plays a major role in person's overall health especially heart health and may contribute to residual risk. A compound named Phenylacetylglutamine (PAG) is created by gut bacteria digests, dietary protein. It was used as a chemical signature in blood that tracked cardiac risk and heart disease. Researchers identified that microbial PorA gene cluster as a contributor to the precursor of phenylacetylglutamine (PAG) production. Through gene engineering and microbe transplantation, Researchers revealed that microbe PorA gene participates in PAG production in vivo and also contributes to thrombosis potential (like clot generation in heart attack or stroke).



PAG was shown to specifically interact with Adrenergic receptors. Both the genetic and pharmacological studies in animal have proved that PAG induced heightened thrombosis is mediated by Adrenergic receptors. So, individuals with higher levels of PAG are more likely to experience cardiovascular disease, thrombosis, myocardial infarction, stroke and type 2 diabetes. A common drug which blocks the beta-adrenergic receptor which are used by patients with heart disease can reverse the adverse effects of the high PAG level. This could explain why the beta-blockers reduce the risk of heart attack, stroke and death in heart disease patients. So, it shows that gut microbes via the generation of PAG play an important role in heart disease risk. It provides a new potential pathway to identify patients at risk for cardiovascular diseases and new treatment options that target the gut microbiome pathway. So, gut bacteria are found to be directly or indirectly involved with many diseases and the composition of gut bacteria in a person can indicate the risk of disease. To conclude, if gut bacteria undergo some imbalance, several diseases may occur. So, it's more valuable to know about the important impacts of gut





bacteria on human health and use it to prevent and treat many diseases. We can take proper care of our gut by maintaining a proper diet, sleep well, avoid unnecessary medications, avoid stressing yourself, drink plenty of water and also by taking supplements containing proper prebiotics and probiotics.

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THE CONVERSION OF BITTER CACAO BEAN TO DELICIOUS CHOCOLATE

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Everyone's Favourite is Chocolate but what most of us don't really know is that this sweet delight begins as a bitter bean. These beans go through multi-step modifications before becoming the chocolate we know and love. Chocolate comes from the seeds of the cacao or chocolate tree, *Theobroma cacao* is named by the famous botanist Carl Linnaeus. In Greek, *Theobroma* Means 'food of the gods'. Chocolate's origins can be traced back to Mesoamerica since 450 BC. Cacao seeds were considered to be a gift from Quetzalcoatl and the seeds were valued as a sort of payment by the Mexican. *Theobroma cacao* are grown best in tropical regions (65 - 90 °F) and at lower elevations (2,000 ft). Cacao requires deep and well drained soils and grows well in the ph range of (6.5 to 7.0). Cacao is now grown in 50 countries. About 70 percent of the world's cacao beans come from four West African countries: Ivory Coast, Ghana, Nigeria and Cameroon. The Ivory Coast and Ghana are by far the two largest producers of cacao.

The *Theobromo* cocoa tree gives flowers which produces pods pollinated by midges. These large pods contain an average of 30 bitter seeds embedded in a sticky sweet pulp in a covered Box. Each pods are opened as a bean and pulp is stored in a heap. When the pulp becomes a cloudy broth and the cacao seeds start absorbing flavours from the surrounding. Fermentation lasts a total of 7 days and are dried in the sunlight. Initially small amount of microbial growth starts growing.



Fermentation of cocoa beans is a process in which growth of yeasts and bacteria occur in the pulp in this stage, breakdown of sugars and mucilage occurs. It consists of three phases:

Phase	Microbes	Duration
First phase	Anaerobic yeasts	24 - 36 hours
Second phase	Lactic acid Bacteria	48 - 72 hours
Third phase	Acetic Acid Bacteria	76 - 96 hours

The cacao pulp contains maximum of water with 10 – 15 % sugars which are glucose, sucrose and fructose. The higher sugar content in the pulp helps in the growth of yeasts which ferments sugar into ethyl alcohol at the temperature of 25 – 35 °C where the cacao pulp becomes anaerobic with low pH and temperature. Yeasts are also the major contributors to Pectinolysis which allows entering of air in the cacao pulp. Yeast such as *Hanseniaspora opuntiae* because of its low ethanol and heat tolerance is considered as predominant during fermentation process and the yeast *Saccharomyces cerevisiae* when acts up makes the cacao less bitter in taste.

During the fermentation heat reaches 20 – 50 °C on rise in temperature the yeast are killed by the alcohol produced by themselves. As the pulp drains away, more air enters the fermenting cocoa pulp-bean mass, providing ideal conditions for bacteria like *Lactobacillus* and *Streptococcus* to grow. Lactic acid bacteria do not require oxygen to grow; instead, they absorb fructose and convert it to citric acid, at this process the pH rises. Making the environment more favourable for acetic acid bacteria, which converts some of the alcohol that has been formed into vinegar. Acetic acid bacteria, such as *Acetobacter* and *Gluconobacter*, are grown by the oxygen and in lower pH. The beans are dried, and moulds such as the *Geotrichium* starts growing. The Lactic acid is oxidized by *Geotrichium* to acetic acid and succinic acid. The acetic acid kills the bean, causing its cell walls to break down and separated substances to mix. The curing process causes changes within the bean as a result of this process. Enzyme activity, oxidation, and the breakdown of proteins into amino acids are all involved. This stage develops important flavour precursors, which begins to add a unique chocolate taste and aroma.

If the fermentation is carried out longer than 6 days the microorganism may develop on the beans than the pulp. When *Bacillus* and filamentous fungi such as *Aspergillus*, *Penicillium*, and *Mucor* hydrolyze lipids in beans to produce short-chain



fatty acids, off-tastes results. *Pseudomonas*, *Enterobacter*, or *Escherichia* may grow and produce off-tastes and odours as the rise in pH as 7.

Pulp composition	Before Fermentation	After fermentation
pH of pulp	3.7	3.9
Sucrose	12 %	0
Citric acid	24 %	11 %
Ethyl alcohol	0.5 %	0.1 %
Acetic Acid	0.4 %	12 %

The beans are well dried and the first step includes roasting beans at 121°C. Being killed most of the microbes by roasting, some *Bacillus* species may survive the roasting process. This process converts the flavour precursors within the bean into compounds such as aldehydes, esters, lactones, and pyrazine, which give chocolate its aroma and flavour. Next includes few processes such as winnowing removal of shells, nibs grinding, the alkalizing process to modify the flavour and colour of and cocoa powders, liquor pressing and last comes the manufacturing of chocolate.

Researchers discovered that cocoa has health benefits in both animal and human studies. After eating cocoa, people had lower rates of cardiovascular disease, diabetes, and vasodilation, cocoa also has a positive impact on learning and memory. The chocolate contains Theobromine which are stimulant effects on the human brain as caffeine which release ‘feel good’ effects. Excess intake of theobromine may also lead to problems.

Researchers are still studying how many pods to put in the heap, how long to ferment, how to cover the heap, and when to stir the pile. Microbiologists are examining the role of each microbe in fermentation to inoculate with one or more species and also the taste and flavours of chocolates in order to produce consistent and enriched quality flavour of chocolate.



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VACCINE IN RECENT DEVELOPMENT FOR EMERGING DISEASES

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Vaccine plays an important role in resistance and protection from microbial infectious diseases. Though some vaccine can only prevent the spread of infectious and not cure in all but due to recent development vaccine have improved a lot and more virulence to microbial infection. Newly emerging and reemerging infectious diseases have threatened humanity throughout history.

Development of new Vaccine method

- a) ***Development of DNA Vaccine:*** In recent development of vaccine, engineered DNA vaccine are used from infectious organism. By identifying the antigen DNA sequence can be inserted. Once inserted into the host the organism undergo replication and protein of interest is produced and then the host develop immune response against the protein.
- b) ***Development of Therapeutic vaccine:*** It prevents specific infectious diseases by delivering immunogenic antigen from infectious agent resulting immunity against foreign organism.



Improvement in Vaccine by using Microbes

The bacterium *Streptococcus pneumoniae* and Influenza viruses account for considerable morbidity and mortality worldwide. It is shown that it reduces the infection of *S pneumoniae* diseases in infants and young adults.

Outburst of New infectious diseases

The appearance of new infectious diseases has been recognized by the past recent years before the identification of causative agent. Despite development of countermeasures like vaccine, therapeutic and diagnosis. Emerging infectious diseases (EIDs) are threats to human health and global stability. Emerging pandemic diseases now like SARS CoV -2 pandemic.

Vaccine for Emerging infectious diseases

Vaccines are cornerstone of the management of infectious disease outbreaks and are the surest means to defuse pandemic and epidemic risk. The faster a vaccine is deployed, the faster an outbreak can be controlled. Like the outbreak of Covid 19 have led to the development of vaccine using mRNA technology (Pfizer and Moderna). Viral vectors such as Ad5, Ad26 and MVA have been used in HIV as well as in Ebola vaccines. Each vaccine is different.

Vaccine development is a long process and involves large sample sizes for conducting research; it takes years to establish vaccine. Since its associated with high costs and failure the developers take extra precaution and follow strict sequence of steps involving multiple rounds of data analysis. Developing vaccine in outbreak requires new strategy of executing multiple steps before conformation.

Results and Improvement after Vaccine is developed

It's not only challenge to come up with effective vaccine for COVID-19 in short period but most significant problem would be enough doses of the vaccine to be supplied to countries globally. Therefore, along with focusing on vaccine development, we should focus on the spread of virus. Economically weak countries as Africa would face problems in accessing vaccines, as happened with anti-HIV drugs. Therefore, there should be fair distribution of vaccine. Thus, the lessons of COVID-19 pandemic need to be compiled and applied to development of future vaccines against emerging infectious diseases and pandemic pathogens. The future of vaccinology provides tremendous promise for controlling diseases. Vaccines will be delivered orally, by nasal spray. However, despite rapid advance in the development of new vaccines, concerns about vaccine safety and a rise in anti-vaccine thus as advance vaccine technology improve public education is required. Thus, as new



microbial infectious diseases appear the development of vaccine of that certain will emerge faster due to advance technology.

The use of vaccine is most fastest and effective methods to stop the spread of infectious diseases and harmful microbes. The development of vaccine has been advanced as there is identification of new infectious diseases. Though it takes time to get an vaccine approved but it's plays the most important role in boosting our immune response against foreign photogenes. As new infectious disease emerges , new vaccine for that emerging infectious diseases is developed.



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ROLE OF MICROBES IN COFFEE FERMENTATION AND THEIR IMPACT ON COFFEE QUALITY

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Introduction

Coffee is one of the most important and widely used commercial crops in the world. After ripe coffee cherries are harvested, coffee must pass through several steps to become (green) raw coffee beans. There are three different processing methods used to obtain green coffee beans from coffee cherries, namely, the wet, dry, and semidry methods. Microorganisms (yeasts and bacteria) play a major role in the coffee fermentation process by degrading mucilage by producing different enzymes (pectinase), acids, and alcohols. The culture development is crucial and is done by selecting microorganisms that have certain characteristics, such as mucilage degradation ability, tolerance to stress during fermentation, the ability to suppress the growth of pathogenic fungi, and a positive impact on the sensory quality of the coffee.

Coffee Fermentation Process

Fermentation is a chemical process in which complex molecules are broken down into simpler molecules, producing liquid products and gases (volatile compounds). Coffee fruits are processed by one of three methods immediately after harvesting to allow spontaneous or indigenous fermentation to occur. The three



different coffee processing methods are referred to as dry, wet, and semidry. The main purpose of the fermentation process in all methods is to remove the mucilage layer, which is rich in polysaccharides (pectin), and to decrease the water content of the coffee beans. However, if carefully managed, fermentation also has a positive impact on the coffee's quality attributes.

Microbial Enzymes and Starter Cultures for Coffee Fermentation

i) Enzymes produced during Coffee Fermentation

Previous reports have identified over 50 yeasts and bacterial species that are present during coffee fermentation. mention that the microorganisms in coffee fermentation contribute to the production of ethanol and lactic, butyric, acetic, and other higher carboxylic acids during the fermentation of pectinaceous sugars. Coughlan and Mayer report that a variety of extracellular enzymes are generated by cellulolytic *Bacillus* species, which potentially contribute to the breakdown of cellulose and pectin substances that exist in the skin, pulp, and mucilage of coffee cherries.

The three most important enzymes produced by microorganisms for degrading pectin substances during coffee fermentation are pectin lyase, polygalacturonase, and pectin methyl esterase. Pectin lyase catalyses pectin degradation by trans-elimination, releasing unsaturated galacturonic acids. Polygalacturonase is the main enzyme involved in coffee fermentation. It catalyses the hydrolysis of α -1,4 glycosidic bonds into pectic acid (polygalacturonic acid). Pectin methyl esterase is responsible for the de-esterification of the methoxy group of the pectin, forming pectic acid and methanol.

Impact of Fermentation of Coffee on Flavour and Aroma

i) The Positive Impact

Coffee is popular as a non-alcoholic drink because of its pleasant aroma and refreshing flavours. The fermentation process can increase the diversity of coffee aroma and flavour compounds. More than 700 volatile and non-volatile compounds that contribute to coffee flavour have been identified. During coffee fermentation, microorganisms produce diverse metabolites. Microbial activity and the extent of fermentation determine the concentrations of free sugars (e.g., glucose and fructose) and free amino acids that continue to surround the bean and subsequently contribute to the production of Maillard compounds and volatiles during the roasting process. Pederson and Breed summarized early studies on the implication that fermentation improves coffee quality. Wet-processed coffee has superior aroma qualities to dry-



processed coffee because of the aromatic compounds produced during the removal of the mucilage layer in wet processing. The selection of appropriate microorganisms that have a positive impact on coffee flavour and aroma during fermentation is critical, and the fermentation process should be controlled to achieve this positive impact.

ii) The Negative Impact

The major challenge in coffee fermentation, according to several studies, is the difficulty of controlling the process. The fermentation process must be well controlled to ensure the development of beneficial microorganisms that produce a high-quality beverage with a good aroma. When fermentation fails, it results in the development of spoilage microorganisms that adversely affect the coffee's aroma and flavour. Coffee beans resulting from such fermentations are often referred to as "stinkers" Under Fermented coffee beans contain residual mucilage and sugar that prevent drying and create a conducive environment for the development of spoilage bacteria and fungi. Over Fermentation encourages the production of undesirable chemical compounds, notably propionic and butyric acids, which confer off-flavours, such as an onion taste. Species of the *Bacillus* genus, especially *Bacillus megaterium*, may be responsible for the propionic acid found in coffees processed via dry or natural processing. Propionic acid is detected in high concentrations only when the fermentation process proceeds for longer than its optimum duration. Enterobacteriaceae and acetic acid bacteria lead to the production of excessive acetic acid during prolonged fermentation in dry processing state that over fermentation may also produce short-chain fatty acids and their esters, such as 2-methyl butanoic acid ethyl ester, 3-methyl butanoic acid ethyl ester, and cyclohexanoic acid ethyl ester. However, it has a disadvantage that if fermentation proceeds beyond the recommended time, microorganisms can also reduce the quality by creating off-flavours and undesirable characteristics of the diverse microflora found in natural coffee fermentation.



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WHEN YOUR DNA GETS DAMAGED?

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Deoxyribose nucleic acid (DNA) is a molecule composed of two polynucleotide chains that coil around each other to form a double helix that carries genetic instructions for the development growth reproduction and functioning of all organisms and viruses. There are two DNA strands they are known as Polynucleotides as they are composed of simpler monomeric units called nucleotides. And the nucleotides have two components a backbone that is made from sugar. Deoxyribose and phosphate groups, and nitrogenous base, known as cytosine, thymine, adenine and guanine. The genetic code is formed through different arrangements of the bases. A segment of DNA that codes for the cell's synthesis of a specific protein called as a gene. The DNA gets damaged via environmental factors as well environmental agents such as UV light, ionizing radiation and Geno toxic chemicals replication fork can be stalled due to damaged DNA and double stand breaks are also formed by the DNA damage. The DNA in just one of your cells gets damaged tens of thousands of times per day and they multiply that by our body's hundred trillion or so cells, and we got have a quintillion DNA error every day and because DNA provides the blue print for the proteins your cells need to function and the damage causes serious problems such as cancer. The error comes in different forms sometimes, nucleotides, DNA's building blocks, get damaged other times, nucleotides get matched up incorrectly causing mutation and they nicks in one or both strands can interfere with DNA replication and or in even cause sections of DNA to get mixed up fortunately, our cells have ways of fixing most of the problems these problems most of the time, these repair pathways all rely on a specialized enzymes different one respond to different type of



damage. One common error is base mismatches and each nucleotides contains a base, and during the DNA replication the enzyme DNA polymerase is supported to bring in the right partner to pair with every base on each template stand. They seem like Adenine with thymine, and guanine with cytosine but about once every hundred thousand additions it makes mistake, the enzyme catches most of these right away and cuts off a few nucleotides and replaces them with the correct ones and just in case it missed a few a second set of protein comes behind in to check if they find a mismatched and replace it and this is called mismatch repair, together these two systems reduce the number of base mismatch errors to about one in one billion but DNA can get damaged after replication too. Lots of different molecules can cause chemical changes to nucleotides some of these come from the environment exposure, like certain compounds in Tobacco smoke but others are molecules that are found in cells naturally like, Hydrogen Peroxide.

Certain chemical changes are so common that they have specific enzymes assigned to reverse the damage but the cell also has a more general repair pathway, if just one base is damaged it can usually be fixed by a process called base excision repair. One enzyme spins out the damaged base and the other enzyme come in to trim around the site and they replace the nucleotides and the UV light can cause damaged and that is harder to fix, and sometimes, it causes two adjacent nucleotides to stick together distorting the DNA's double helix shape. This damage requires a more complex process called a nucleotide excision repair. In this process a team of protein remove a long strand of 24 or so nucleotides, and replace them with the fresh ones and very high frequency radiation like gamma rays and x-rays causes a different kind of damage they can actually serve one or both strand of the DNA backbone. The double strand breaks the most dangerous, but even can cause cell death. The two most common pathways few repairing double strands breaks are called homologous combination and non-homologous end joining. Homologous recombination uses an undamaged section of similar DNA as a template. Enzyme interlace the damaged and undamaged strands and they get them to exchange sequence of nucleotides and they finally fill in the missing gaps to end up with two complete double stranded segments non-homologous end joining on the other hand, and they dose not rely on a template, instead of cancer. So, if you're looking for a Fountain of youth its already operating in your cells billion and billions of times a day.



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PHAGE THERAPY

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Bacteriophages are considered as a potential alternative to fight pathogenic bacteria during the antibiotic resistance era. With their high specificity, they are being widely used in various applications: medicine, food industry, agriculture, animal farms, biotechnology, and diagnosis.

Bacteriophages (Phages) are bacterial viruses that infect a bacterial cell and reproduce inside it. They differ a lot in their shape and genetic material. They are abundant; there are thousands of millions of bacteriophages around us, although we cannot see them. They live everywhere: in the soil, in the oceans, in our bodies (feces, saliva, sputum, blood, and urine), and also in food. However, we should not worry about bacteriophages as they are harmless to humans.

As bacteria have a rapid defense against antibiotics, they can adapt to such a degree that the antibiotic can no longer harm them but phages operate differently. Each type of phage is species-specific, that it infects one particular species of bacteria. Simply as with bacteria, the number of bacteriophage species is extraordinarily large. Bacteria cannot develop resistance to those bacteriophages.

Phage therapy has several advantages over antibiotics. Though bacteria develop resistance to phages, it is easier to develop a new phage than a new antibiotic. The basic principle of phage therapy is the use of bacteriophage as a possible method of treatment and prevention of bacterial infection. Phage therapy is comparatively safer than antibiotics as there are minimal side effects to the patients.



Bacteria that are infected by phages are incapable of regaining their viability. Phages are versatile agents and thus, can be used in combination with antibiotics and can also be converted into different forms like liquid, creams, or solids. Phages are found throughout nature. This means that it is easy to find new phages when bacteria become resistant to them. This indicates that a phage can be directly used to target a disease caused by bacteria. Phage therapy would be a great option in treating infections caused by antibiotic resistant bacteria as the phages work against both treatable and antibiotic-resistant bacteria without affecting the host body.

Despite all their advantages, there are few drawbacks to being treated with bacteriophages. As phages are highly specific and only infect a few strains of bacteria, they have a narrow host range. As a result, different phages are required for different bacterial infections. As phages are protein-based live biological agents, there is a possibility of the interaction between the phage and the immune system of the patient and result in undesired effects.

Bacteriophage therapy has brought a revolutionary change in medical science and helps a lot in combating against many infectious diseases. Due to bacteriophage discovery human and animal health are considered safer as compared to the past. Although even after a lot of success there is still a gap for all researchers of the world to work on it and sort out the problems for the welfare of mankind.



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DIAGNOSIS AND MANAGEMENT OF INFECTIONS CAUSED BY MULTI - DRUG - RESISTANT *Staphylococcus aureus*

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Introduction

Staphylococcus aureus are considered as the golden cluster seed; they are Gram positive bacterium which is cocci in shape frequently habituating the nose, throat, intestine, vagina, and skin of human body. This spherical bacterium is of prime concern because of its ability to cause wide range of life-threatening infections and their nature to get adapted to varied environmental conditions. These distinct characteristics of *S. aureus* make them difficult to treat the associated infections and they tend to develop resistance to common antimicrobial agents. Multiple antibiotic resistances is of major public health concern in the treatment of *Staphylococcal* infections, specifically Methicillin Resistant *Staphylococcus aureus* (MRSA) infections because of extensive use of antimicrobial agents. Thereby an efficient control of indiscriminate antibiotic use and prevention of the contact transmission of these resistance strains are very important to completely eradicate these potent infectious organisms.

Antimicrobial agents

The Greek words Antimicrobial means anti (against), micro (little) and bios (life) which acts against microbial organism. There are two modes by which



antimicrobial agents work - microbiocidal and microbiostatic. Alexander Fleming was the first to discover *Penicillium rubens*, a naturally powerful antimicrobial fungus, in 1928. The substance extracted from the fungus was used to treat *Staphylococcus* infections. Antimicrobial medications have revolutionized not only the treatment of infectious diseases, but also humanity's fate. Antimicrobial chemotherapy made significant progress, leading to an excessively optimistic belief that infectious diseases would be eradicated in the near future. However, developing and re-emerging infectious diseases have left humans vulnerable to infection in the near future.

Infections with drug-resistant organisms are still a challenging to treat in clinical practice. When an improper antimicrobial agent is used to treat an infection caused by drug-resistant bacteria, the treatment may not be effective. Furthermore, in a setting where multidrug-resistant organisms have become widespread, antibiotic treatment options may be limited. There are few newer antibacterial agents available in the market currently. In light of the growing awareness of medication safety, we are now facing with a scenario where antimicrobial agents have extremely restricted alternatives. Due to the development of resistance by the microbes, some of the antimicrobial agents have lost their activity which has caused many health problems.

Antimicrobial Resistance

Due to the exposure to many antimicrobials, microorganisms develop resistance which is more difficult to treat than non-resistant bacteria. This kind of antibiotic resistance raises medical expenses, prolongs stay in hospitals and increases mortality rates. In recent years, there is lot of research happening on multidrug – resistant microbes. World Health Organization (WHO) has listed the most harmful microbes which are resistant to prevailing treatments as a result of genetic mutations and acquired genomes. eg; Methicillin-resistant *Staphylococcus aureus* and drug-resistant *Streptococcus pneumoniae*.

Antimicrobial Resistance to Gram – Positive Bacterial Infections

Gram positive bacterial infections (GPIs) specifically, Methicillin-Resistant *Staphylococcus aureus* (MRSA) causes various disease like skin and soft tissue infections, surgical and trauma wound infections, urinary tract infections, gastrointestinal tract infections, pneumonia, osteomyelitis, endocarditic meningitis, septicemia, and infections of indwelling medical devices (Catheters).



Mechanisms of *Staphylococcus aureus* resistance

- a) **Beta – Lactamase Enzyme – mediated:** Production of beta lactamase (Penicillinase) which is plasmid coded and transferred by either transduction or conjugation. Beta lactamase inactivates penicillin by splitting the beta lactam ring. This type of resistance also occurs in β - lactamase resistant Penicillin.
- b) **Alterations in Penicillin – binding protein PBP_{2a}:** Methicillin resistance correlates with the presence of the resistance genes *mec A* which codes for unique penicillin binding protein PBP_{2a} (PBP2'). This type of resistance does not occur in β - lactamase resistant Penicillin.

Methicillin Resistance *Staphylococcus aureus* (MRSA)

Initially Methicillin was used as an antibiotic to treat Staphylococcal infections because they are susceptible towards this antibiotic till 1961. Because of indiscriminate usage of methicillin, these strains acquired resistance towards this antibiotic. A set of chromosomal genes controls this condition which are termed chromosomal *mec* genes (SCC *mec*) specifically the *mec A* gene. *Staphylococcus aureus* acquires this gene due to gene transfer mechanism.

Evidences from recent studies have shown that the resistance mechanisms of *Staphylococcus aureus* are very cumbersome, mainly for MRSA, which seems to be resistant to varied groups of antibiotics. Therefore, proper understanding of this multi-drug resistance of MRSA in correct timing and elucidating its mechanism at the molecular level are of great significance for the effective treatment of infections caused by *Staphylococcus aureus*. Analyzing the molecular characteristics of *Staphylococcus aureus* can be of great help and will be a basis for designing effective prevention strategy and control measures against these hospital infections caused by *Staphylococcus aureus* and further monitor the evolution of *Staphylococcus aureus* in nature. These MRSA strains are divided into two classes based on source of transmission.

- a) Hospital – acquired MRSA (HA MRSA)
- b) Community –acquired MRAS (CA MRSA)

Vancomycin

It has long been considered as an efficient drug for the treatment of severe MRSA infection, including both HA-MRSA and CA-MRSA which is responsible for many life-threatening, invasive infections such as pneumonia and sepsis. Vancomycin has also been recognized as the last line of defense against this Gram-



positive *Staphylococcal* infection. Surprisingly these MRSA strains started showing resistance towards vancomycin and became a widespread concern in the field of medicine. Currently, these vancomycin-resistant *Staphylococcus aureus* were classified into three types: Vancomycin-Resistant *Staphylococcus aureus* (VRSA), Vancomycin-Intermediate *Staphylococcus aureus* (VISA) and heterologous vancomycin resistant *Staphylococcus aureus* (hetero-VRSA).

Vancomycin Resistance Staphylococcus aureus (VRSA)

In recent years, these strains started developing resistance to vancomycin which was considered to be an alternative drug for treating *Staphylococcus aureus* infections. These strains have been found to possess both *van A* and *mec A* genes. This has lead to major therapeutic challenges all around the world because they are sensitive only to Linezolid.

Vancomycin Intermediate Resistant Staphylococcus aureus (VISA)

In vitro micro-dilution studies have shown intermediate resistance in some strains of *Staphylococcus aureus* to vancomycin, because of the absence of resistance genes in their cell wall. This has led to clinical failure in patients who had been on long – term vancomycin treatment.

Prospects for the future - New Antimicrobials

Daptomycin a cyclized lipopeptide drug obtained from the fermentation broth of *Streptomyces roseosporus*. Mainly due to their unique mode of action and absence of cross-resistance with other antibiotics, it can be considered as an alternative drug to treat skin soft tissue infections and bloodstream infections caused by MRSA. Many scientific studies revealed that daptomycin has a faster bactericidal effect than vancomycin, linezolid or quinupristin/dalofopine Furthermore, daptomycin also possess the ability to resist most clinical gram-positive bacteria *in vitro*; therefore, daptomycin was universally accepted for the treatment of infections caused by many drug-resistant bacteria, such as Vancomycin-Resistant *enterococci*, MRSA, glycopeptide-sensitive *Staphylococcus aureus*, coagulase-negative *Staphylococci*, and penicillin-resistant *Streptococcus pneumoniae*.

Linezolid

Linezolid is a synthetic, new class of oxazolidinone antimicrobial agents. It is mainly used to treat systemic infections such as sepsis, and pneumonia caused by vancomycin-resistant *Enterobacter faecium*. This unique mechanism of inhibitory action helps them to eradicate cross-resistance between linezolid and other



antibiotics. Moreover, the survival rate and recovery rate of MRSA associated infections with linezolid were significantly higher than that of with vancomycin. Based on clinical trials, the oral and injection dosage forms of linezolid were quite effective in treating MRSA, and infections such as vancomycin-resistant *enterococci*, penicillin-resistant *pneumococcal*, and macrolide-resistant bacteriostatic *streptococci*.

Teicoplanin

Teicoplanin, a glycopeptide is used to treat Gram-positive infections, especially infections caused by *Staphylococcus aureus*. Teicoplanin can be administered intravenously or intramuscularly. It has an advantage of just requiring one dose per day.

Novel Therapeutic Strategies for MRSA Treatment

MRSA are multidrug resistant strains resistant towards β -lactam antibiotics and also to several other antimicrobial agents such as aminoglycosides, quinolones, and macrolides. The mortality rate of systemic infections caused by MRSA is more than 50%, and this become a public health concern in clinical and community and is difficult to treat. In addition, the hospitals act as the prime habitat for MRSA which may lead to severe nosocomial outbreaks. The only way to prevent *S. aureus* infections is to incorporate infection control measures. More efficient diagnostic procedures should be included to the program to screen for the presence of *S. aureus*. Therefore, many new drugs against MRSA and some novel methods have to be explored in this current scenario.

Consideration to be taken

New drugs such as Endopeptidase, Lsostaphin, or Phage lytic enzymes can also be taken into consideration. Quorum Sensing Inhibition, Lectin Inhibition, Iron Chelation, Phage Therapy and Nanoparticles are some of the newer technologies which could be suggested in the upcoming years. With Capsular Polysaccharide Protein Conjugated Vaccine have given good results in clinical studies with hemodialysis patients.

