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1

UNDERSTANDING REAL-TIME REVERSE IMPACT OF OBESITY ON THE INNATE IMMUNE RESISTANCE PATTERN RECOGNITION

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In India, prevalence of obesity and overweight is elevating quicker than the world average. It can be inferred from the prevalence of overweight that increased from 8.5 % to 16.5 % between 2000 and 2018 and the prevalence of obesity that increased from 2.5 % to 5.5 % over the same period. Such increasing rates correlate to diets loaded with saturated fats. Apart from Indian diets being supersized, lack of physical exercise is another factor contributing it. Recently a number of studies have revealed the impact of obesity on the functioning of immune system. it is innate immune arm that gets most influenced. Innate immune system is a versatile system of sensory and response molecules that is initially ready to take a note of invasion by foreign microbes. Binding of microbial conserved motifs termed Microbe associated molecular patterns (MAMP's)for example, LPS, lipoteichoic acid, peptidoglycan, mannose-rich glycans and host cell damage products termed Damage associated molecular patterns (DAMP's) to Pattern recognition receptors (PRR's) for example toll like and NOD like receptors that are present on the main player cells of innate immune system (e.g. Macrophages, Dendritic cells, Neutrophils) triggers the gene activation in them to code inflammatory mediators so as to upregulate the inflammatory process. The main

signaling molecules among them are cytokines that signals and alerts the whole immune system to recruit army of cells and proteins for combating the foreign invader. Scientists from Batson to Tokyo revealed a series of papers which tell the amazing story of how the elegant system of detecting MAMP's and DAMP's by phagocyte cell PRR's gets upset when we 'Supersize'.

Visceral (belly) fat originally regarded simply as an energy reserve, is actually a specified tissue having macrophages, innate lymphoid cells, preadipocytes and other related cells held in a net of matrix proteins extracellularly along with lipid-laden adipocytes (fat cells). Normally adipocytes prepare pro- and anti-inflammatory cytokines to maintain a balance between rival inflammatory functions to keep a homeostasis which is prerequisite for tissue repair and regeneration functions. However, now studies have revealed a concreate correlation between elevating visceral fat (adipose) as seen in obese conditions and enhanced serum level of pro- and anti-inflammatory cytokines (adipokines). This is because visceral adipocytes along with their associated macrophages recognize saturated fatty acids as DAMP's. This disturbs the normal inflammatory process which leads to elevation of one of the pro-inflammatory adipokine, interleukin (IL) 18 and macrophage marker of inflammation, C- reactive protein (CRP) in obese persons. This in turn leads to low grade systemic and chronic inflammation which can pose a threat to cardiovascular system. Understanding the mechanism of how systemic inflammation is triggered and regulated by MAMP's and DAMP's offers an insight of how human diseases and disorders reflect lifestyle choices.

In general, to reverse the effect of visceral fat on innate immune cell pattern recognition, physical exercise and antioxidant rich low-fat diet intake plays a cardinal role as both strategies lead to a leaner mass that is all the way beneficial for sound health.

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2

ANTIFUNGAL ACTIVITY OF NATURAL SUBSTANCE AGAINST NORMAL FLORA OF SKIN AND SCALP

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The term normal microbial flora refers to the population of microorganisms that inhabit the skin and mucous membranes of healthy normal persons. Human microbial flora is sort of complex and consists of several hundred species. The balance of the flora can change as a result of disruption of bacterial community by a mess of internal and external factors leading to opportunistic pathogens that are normally found during a suppressed from multiplying rapidly and causing secondary infection. Due to its constant exposure to and get in touch with the environment, skin is consistently populated with microorganisms that reflect the contacts, inhibits and profession etc of the host.

Normal microbial flora present

Microorganisms associated with the skin are *Staphylococcus aureus*, *Staphylococcus epidermidis*, Diphthroids, *Pseudomonas aeruginosa*, Anaerobes, *Candida*, *Torulopsis* and *Pityrosporum*. Important factor which help in elimination of non-resident microorganisms from the skin include low pH, fatty acids in sebaceous secretions and presence of lysozyme.

The number of superficial skin microorganisms are often diminished by vigorous daily scrubbing with soap containing disinfectants. The nature of the skin microbial flora is additionally depending on one's occupation and environment and degree of clothing used. The pathogenic organisms harboured on the skin can play a crucial role in nosocomial infections.

The composition of the scalp micro flora is assessed quantitatively in normal individuals and in patients with dandruff and seborrheic dermatitis, disorders characterized by increasing scaling. Dandruff is a shedding of dead skin cells from the scalp as skin cells die and normally released after treatment. Dry skin is the commonest explanation for flaking dandruff. It is a common scalp disorder affecting people of any age and gender. According to a study, dandruff has been shown mostly due to three possible factor, 1) Skin oil commonly referred to sebum, 2) Individual susceptibility and allergy sensitivity and 3) Metabolic by products of skin microorganism. Three organisms are constantly found: 1) *Pityrosporum*, 2) Aerobic cocci and 3) *Corynebacterium acnes*. *Pityrosporum* made up 46 % of the total microflora in normal, 74 % in dandruff, and 83 % in seborrheic dermatitis. The cocci are dominantly Baird-parker type and no quantitative or qualitative change occurred in scaling disorders. *Corynebacterium acnes* comprises 26 % of the flora on normal scalp, 6 % in dandruff and only 1 % in seborrheic dermatitis. Another fungus of *Malassezia* species such as *Malassezia globose* and *Malassezia furfur* (*Pityrosporum ovule*) are the cause of dandruff. These fungi normally occur on the skin surface of both healthy people and those with dandruff. During dandruff the level of these fungus increase 1.5 to 2 times.

Activity of Natural substances

Different natural substance like garlic, lemon, turmeric, cloves, neem, onion, cinnamon, aloe Vera etc are used as antifungal agents which are easily available in our home. Garlic is one among the best antifungal agent. Its sulfuric compound stops infection and stop fungi from spreading to other areas of skin. Ajoene is a lively compound found in garlic which plays an excellent role as a topical antifungal agent. Garlic has been shown to inhibit the expansion of fungal disease as equally as the drug Ketoconazole when tested on the fungi *Malassezia furfur*, *Aspergillus* and *Candida* sp. *Aloe vera* leaves is superb for naturally calming irritation and redness cause by fungus. *Aloe vera* has inherent ability to induced toxic effects on mycelial growth and proliferation of fungi. *Aloe vera* compounds including Anthraquinone, Saponin, *Aloe* - emodin have both direct and indirect antimicrobial properties. Citric acid in lemon juice helps in restoring the normal pH balance of the scalp that helps in overgrowth of the dandruff causing yeast on the scalp. So, the home products are very useful and we can easily treat our fungal skin and scalp infection by using these products.

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3

PARTICIPATION OF GUT MICROBIOME IN DIABETES AND OBESITY

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Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder characterised by problems with sugar metabolism. Today, the prevalence of Type 2 diabetes mellitus is increasing at an alarming rate worldwide (with approx. 90 % cases). Also, the majority of T2DM patients are obese. Dysbiosis in the gut microflora is found to be strongly associated with the pathogenesis of obesity and T2DM. The difference in gut microbiome could presumably be related to the distinct lifestyle and dietary habits (high carbohydrate and fat intake, low fiber intake) and uncontrolled antibiotic consumption. In addition to being governed by genetic and environmental factors, T2DM is also influenced by the patient's gut microbiome specifically, their abundance and diversity. The differences in the gut microbiome could be influenced by cultural, environmental, dietary, and socioeconomic factors. Therefore, the pathology of obesity is recognised to be associated with changes in the gut microbiome diversity and composition (i.e., changes in abundance at the level of phyla, genus or species).

More than 1000 species of bacteria belonging to different phyla reside in the human gut, the dominant ones being Firmicutes and Bacteroidetes together with Actinobacteria and Proteobacteria. Most of the gut microorganisms reside in the large intestine, which contains an estimated $10^{11\text{-}12}$ bacterial concentrations per gram of content. These gut microbiota play a number of physiological roles involving digestion, metabolism, extraction of nutrients, synthesis of vitamins, and prevention against colonization by pathogens, and immunomodulation. Components of microbiota are mostly bacteria, with a minority of viruses, fungi, and eukaryotic cells. The disturbance

in relative bacterial abundance and diversity eventually results in metabolic disorders, such as obesity, chronic kidney disease, arteriosclerosis, type 2 diabetes, etc., by hampering the production of carbohydrate metabolism end products.

As fibers cannot be digested by the human digestive fluid, they are fermented by the gut microbiota, thereby generating short-chain fatty acids (SCFAs) as metabolites. SCFAs employ systemic anti-inflammatory effects by producing immunoglobulin A and immunosuppressive cytokines. More early-life use of antibiotics and a decrease in fiber intake results in dysbiosis, which is implicated in the increased incidences observed in inflammatory diseases, including diabetes. SCFAs promote secretion of glucagon-like peptide-1, an important incretin hormone, which is made by enteroendocrine L cells. Glucagon-like peptide-1 obstructs secretion of glucagon, hampers gluconeogenesis in the liver, improves insulin sensitivity and augments central satiety, thereafter resulting in bodyweight loss. These numerous interactions amongst gut microbiota-derived metabolites, gut microbiota-host immune system is communicated through various signalling pathways. These biological signals impacts the body system-wide, and directly influence organs as metabolic reactions are controlled by microbes producing choline, phenols, bile acids, and SCFAs by both the gut microbiome and host genome, which are vital to health.

Moreover, gut microbial communities (*Clostridium hystoliticum*, *Eubacterium rectale*, and *Clostridium coccoides*) can affect neurotransmitters involved in gut–brain signalling pathways, and thus, regulate food intake and body weight. These neurotransmitters directly control the intestinal transit time, serotonin levels, physical activity, and intestinal permeability. The lack of diversity in the gut microbiome is implicated in an underdeveloped immune system, resulting in the host being susceptible to a range of opportunistic diseases. Experimentally, It is observed that germ-free mice (mice raised in the absence of any microorganisms) are leaner compared with mice that harboured obesity causing microbiota since birth. Thus, the ability of bacteria to modulate host signalling pathways could influence the host energy balance and host metabolism. Here, below given are the common predominant bacteria in the human gut (Table - 1). During birth and till 1 year of age the gut is predominated mostly by Actinobacteria and Proteobacteria which then changes more towards Firmicutes and Bacteroidetes later in life.

Table - 1: Bacteria predominant in human microbiome

Firmicutes (60 – 80 %)
Ruminiococcus
Clostridium
Lactobacillus
Bacteroidetes (20 - 30%)
Bacteroides
Prevotella
Xylanibacter
Actinobacteria (<10 %)
Bifidobacterium
Proteobacteria (<1 %)
Escherichia
Enterobacteriaceae

Additionally, the gut microbiome might be a new biomarker for type 2 diabetes prediction, as gut metagenome-based computational models could predict the type 2 diabetes-associated phenotype in glucose-intolerant patients. As diabetes is multifactorial and can progress to other related metabolic diseases, such as cardiovascular problems, it is of utmost importance that the delicate interrelationships between gut microbiota and host metabolism are well understood in order to suggest appropriate lifestyle and nutritional interventions by engineering an optimal gut environment towards the prevention and maintenance for remission of diabetes. Currently, use of pro and prebiotics and other new techniques such as gut microbiota transplant, dietary changes or even antibiotic therapy, has been postulated to be useful tools to modulate the development of obesity and insulin resistance.

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4

METAGENOMICS: AN APPLICATION BASED PERSPECTIVE

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Metagenomics is the study of microbes in their natural living environment, which encompasses complex microbial communities in which they usually exist. The study scrutinizes the genomic configuration of an entire organism, including each of the microbes that exist within it. It is a significant concept for the microbes and the host to be thought of as codependent and observed as a community, rather than considered to be distinct entities.

The arena of metagenomics is relatively new because microbes have traditionally been studied in a laboratory-based setting, rather than within the host as a combined entity. Therefore, the current knowledge of microbes in their natural habitat is scarce. Metagenomics aims to make progressions in environmental and clinical microbiology, despite significant barriers such as difficulty to make a culture and the genomic diversity of microbes. It is hoped that increased understanding of the nature of microbes in the environment could have a significant impact on other sciences and research areas, such as medicine, biology, biotechnology and ecology.

The term metagenomics, the genomic analysis of a population of microorganisms, was coined by Handelsman with a notion to analyze a collection of similar but not identical items, as in the statistical concept of meta-analysis. The idea that the whole environmental microbiome can be explored and analyzed together has revolutionized our understanding of the ecology around us. It has opened new horizons in the development of biotechnology based on the exploitation of uncultivated microbial

species. The vast majority of microorganisms being unculturable, metagenomics has resulted in discoveries that remained hidden from the traditional culturing techniques. Though a multifaceted approach, the crux of applied metagenomics is to express recovered genes in a cultivable heterologous host. A booming area of biotechnology is the industrial use of microorganisms to produce antibiotics, enzymes, and other bioactive compounds. The demand for the commercial production of enzymes that are used in large-scale industrial processes is growing rapidly. The industrial applications of metagenomics include identification of novel biocatalysts, discovery of new antibiotics, personalized medicine, and bioremediation. In addition, biosurfactant producing bacteria have been successfully used for the bioremediation of industrial, agricultural, and domestic wastes, resulting in a reduction of the environmental pollution. A wealth of information has been uncovered by metagenomics, such as microbial diversity, vast swathes of uncharacterized metabolism, and increased complexity of biogeochemical pathways and it promises to provide new enzymes and molecules with diverse applications. In fact, the crystal structure of metagenomics derived RNase H1 has also been determined which indicated structure-based mutational shift at the active site of the motif. López-López also discovered metagenomic extremophilic esterases having a different active site compared to the esterases produced by culturable microorganisms. Schallmey and coworkers also harvested novel metagenomics Polyhydroxy Alkanoate (PHA) synthase encoding genes. The DNA sequence analysis of the studied clones indicates that the complementing genes are homologous but substantially different from the known Polyhydroxy Alkanoate synthase - encoding genes. Such genes are of great potential for the industrial production of bioplastics. Additionally, a metagenomic library constructed from crude oil-contaminated soil encoding Polyhydroxy Alkanoate synthase showed 76 % identity with the synthase of *Alcaligenes* sp. Moreover, revealing the importance of metagenomics and unculturable microorganisms, microbiome project, such as the Human Microbiome Project and Gut Microbiome Projects, has been initiated. The ultimate goal of the project is to understand the changes in the human microbiome which are associated with human health or disease.

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5

BACTERIAL FERTILIZER

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Microbial fertilizers are attractive replacement for chemical fertilizer. Chemical fertilizers are polluting environment thereby they cause harmful health issues. Microbial fertilizer is the best alternative source for chemical fertilizers. Microbial fertilizers are naturally active products they have inoculants like bacteria and algae. Some bacteria are commonly used as biofertilizer such as *Anabaena*, *Azotobacter*, *Rhizobium*, *Azospirilium brasilense* and *Pseudomonas* sp. Bacteria helps plants to get some nutrients from soil. Bacteria can form a symbiosis with a plant's roots. Basically, they can fix nitrogen, it is important for the plant metabolisms.

Some direct and indirect mechanism by which bacteria promote plant growth. They are

- Phyto hormones production such as Indole acetic acid
- Siderophores production
- Fixation of nitrogen and
- Solubilization of phosphate

Most bacteria support plant roots to grow. They can have either deleterious or beneficial impact on plant growth and health. Some bacteria are naturally occurring in soils or they supplied as biofertilizers. They increase nutrients bioavailability and improve soil structure. Bacterial inoculants and organic amendments could be considered as a potential option to incorporate in crop Integrated nutrient management strategy of degraded soils.

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6

IMPORTANCE OF MICROBIOLOGY IN EVERYDAY LIFE

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Microorganisms and their activities are vitally important to virtually all processes on Earth. Micro-organisms matter because they affect every aspect of our lives – they are in us, on us and around us. Microbiology is used in our daily life and plays an important role in it. Some of the main features of the microbiology which are used in our daily life are discussed here. Microbiology applied in everyday life; in food production, biodegradation, commercial-product production, biotechnology and genetic engineering. There are various dishes in which microorganisms are needed. For example, for the making of curd and cheese, microorganisms are needed. A bacterium called *Lactobacillus* converts the lactose sugar present in milk to lactic which results in the conversion of milk into curd. Moreover, yeast can be used to make bread, bacteria is important during the process of making yoghurt. Also, vitamin K is only synthesized by the microorganism in the human body. Apart from this, bacteria are used to synthesize the commercial valuable products like hydroxybutyric acid which is used in the manufacture of disposable diapers and plastics. Also in ethanol which is a biofuel. They also synthesize amino acids which are very common dietary supplements.

Thus, the science of microbiology aims to gain and expand our fundamental understanding of microorganisms by studying their morphology, metabolism, physiology, reproduction and genetics. This is all how microbiology plays a significant role in the different sectors. In the next couple of years, we will see various other uses of the microbiology which will be very beneficial for us in all aspects.

During this Pandemic situation, the value of Microbiology and every Microbiologist's has increased

While the epidemiologists are examining the numbers and modelling the spread of disease, the laboratory-based microbiologists are paying attention to the diagnostic tests being used to identify people testing positive for Coronavirus.

Microbiologist 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.

Now the world is in the middle of a pandemic and responsibility of microbiologists becomes even more important. They play an instrumental role in setting up validated test systems and developing whole new testing algorithms for COVID -19 in their respective laboratories. This is not an easy task as it needs to be done quickly at the same time in an efficient manner.

Managing the supply chain of kits, reagents and consumable is also an uphill daily battle as there is increased demand all across the country. Many of them are facing supply shortages. The capacity building with limited resources has been a big challenge for most of them.

Our microbiologists are gathering information that can be used to inform trials assessing disease transmission and potential new treatments. This includes providing guidance on which specimens should be collected and the best diagnostic tests to use.

Microbiologists are essential in helping us to treat diseases. Many work as biomedical scientists in hospitals and laboratories: testing samples of body tissue, blood and fluids to diagnose infections, monitor treatments or track disease outbreaks. Some microbiologists work as clinical scientists in hospitals, universities and medical school laboratories where they carry out research and give scientific advice to medical staff. Other microbiologists work on disease-causing microbes, such as flu or tuberculosis, and the information they find is used to develop vaccines and improve current treatments.

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7

MIRACLE BENEFITS OF DATES SEED

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The date palm is from family of Arecaceae which have seeds with one institution (monocots). Date seed do not have a smell or odorless and has a slightly bitter taste bland. In general, it has a light and dark brown. Today, a various study regarding date seed have been published in order to determine the functional properties of date seed is used for food and non-food items such as thermal properties, treatment and diet, the composition of macro and micronutrients, the composition of phenolic acids, as an ingredient of bread, and protein solubility. Date seed are highly recommended for use in foods and dietary supplements. Because, it is a very good source of dietary fiber. Total mineral content found in single seed is comparable to the mineral content of barley. The minerals contained in date seed include sodium, potassium, calcium, iron, copper, magnesium, manganese, zinc, phosphorus, lead, cadmium. This suggests that a single seed is a good source of minerals, and also can be used to replace barley in food products. The amount of dietary fiber found in date seed about 58 % of which 53 % soluble dietary fiber as hemicellulose, cellulose and lignin. In comparison, the higher dietary fiber was detected in other studies conducted in three different varieties of date seed, the number of fibers between 65 to 69 percentage. This shows the high content of lignin and resistant starch.

On the other hand, protein is also found in date seed with a sizeable amount. Albumin, globulin, prolamin and glutelin are soluble protein which found in seed of the current date, the number of 5 - 6 percentage of the total protein level. Total phenolic content which is found in date seed is 48.64 mg/100 g.

Date pits are a major waste product of the date industry that could offer potentially valuable material for the production of useful food ingredients. This waste product of date processing industries could be regarded as an excellent source of food ingredients with interesting technological functionality that could also be used in food as an important source of dietary fiber without any negative impact on sensory quality of end-products if the pits are properly milled. The polysaccharide content is often expressed in terms of the fiber content, and is divided into crude fiber, Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF).

Resistant starch is used in food products due to its physicochemical properties including swelling, increased viscosity, gel formation, and water-binding capacity. Due to the resistance to digestive enzymes, resistant starch can be used as a dietary fiber. There is also an evidence that resistant starch may have prebiotic activity due to the resistance to digestion and subsequent fermentation, resulting in an increase in bowel health. It stimulates the growth of probiotic bacteria and improves culture viability.

Date seeds/kernels are currently used in the feeding of animals such as cattle, sheep, and camel, and in the poultry and fish industries as well. Due to the presence of a large quantity of total dietary fibre they are considered to have potential health benefits for human as prebiotics. Hence, further investigations should be carried out to identify active biological molecules in date seeds and their potential application in encouraging the growth of probiotics and other health benefits.

The amazing Health Benefit of Date seed powder

Useful in treating blood sugar problems

Date seeds are very useful for diabetics and control blood sugar. This incredible powder has Insulin production properties. If a person has diabetes and high blood sugar, it's best to try a date kernel powder for a week.

Prevent DNA damage

Date seeds are rich in antioxidants that cause prevents damage to the body's DNA structure. further, date seeds are effective in boosting the immune system and nerves because they have numerous minerals. The nutritional value of dates and their kernels has made this fruit a valuable food source.

Prevent Kidney stones and Liver damage

Dates seeds help treatment for kidney stones and bladder diseases. Date kernels contain several medicinal compounds, one of which acts like corticosteroids and is used

to treat kidney and bladder disorders, inflammation, and infectious diseases. The palm kernel has a crushing property and helps to break it down more easily by breaking and crushing the stones. Date seed full of Proanthocyanidins which helps to keep safe liver and kidney from any damage.

Antioxidants

Despite the small size of seeds but Date seeds are good sources of antioxidants. Research shows that the antioxidant of date seed protein hydrolysates can use as a potential functional food ingredient for health improvement. we can consider Iranian date seeds as a good source of natural antioxidants for medicinal and business uses.

Date seeds fiber

The date seed, which makes up 10 -15 % of the date's weight, contains an average of 1.73 % dietary fiber. We can say date kernels are a rich source of dietary fiber. This remarkable fiber's source After processing dates for date syrup, date paste, date sauce, or seedless dates, don't go back to the food cycle, and usually, factories use seeds as waste for livestock feed. Therefore, date seed powder can treat gastrointestinal disorders, especially chronic diarrhea, and act as a powerful disinfectant and kill bacteria that enter the digestive system.

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8

STATISTICAL EXPERIMENTAL DESIGN FOR DECOLORIZATION OF REACTIVE RED 120 BY *Trametes versicolor (Linnaeus et Fries)* Pilat 1086 FRI 165 (1973)

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Objectives

Synthetic dyes are extensively used in different industries. Dyes have adverse impacts such as visual effects, chemical oxygen demand, toxicity, mutagenicity and carcinogenicity characteristics. The conventional treatment of dark coloured textile wastewater using chemical coagulation generates large amount of sludge which requires further treatment and disposal. White rot fungi due to extracellular enzyme system are capable to degrade dyes and various xenobiotics.

Method

The present study was to check the effectiveness of Statistical experimental design for decolorization of Reactive red 120 by *Trametes versicolor* Pilat 1086 Fri 165 (1973). Response Surface Methodology (RSM) was used to study the effect of independent variables. RSM involving a Central Composite Design (CCD) was applied to evaluate the interactive effects of 3 significant factors namely lactose, ferrous sulphate and percent inoculum in different ranges.

Results

The results demonstrated the effectiveness of the statistical experimental design and the ability of *Trametes versicolor* for maximum dye decolorisation (>60 %) at the optimum conditions of the significant factors.

Conclusion

In the proposed model azo dyes are degraded without the direct cleavage of the azo bond through a highly non-specific free radical mechanism forming phenolic type compounds, thereby avoiding the formation of toxic aromatic amines, which might be useful to control environmental pollution.

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9

BIOFUEL FROM ALGAE: AN ALTERNATIVE FUEL

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Algae are organisms that grow in aquatic environments and use light and carbon dioxide (CO_2) to create biomass. There are two classifications of algae: macroalgae and microalgae. Macroalgae, which are measured in inches, are the large, multi-cellular algae often seen growing in ponds. These larger algae can grow in a variety of ways. The largest multicellular algae are called seaweed; an example is the giant kelp plant, which can be more than 100 feet long. Microalgae, on the other hand, are measured in micrometers and are tiny, unicellular algae that normally grow in suspension within a body of water. Microalgae have long been recognized as potentially good sources for biofuel production because of their relatively high oil content and rapid biomass production. Microalgae grow very quickly compared to terrestrial crops; the practice of algal mass culture can be performed on non-arable lands using non-potable saline water and waste water. Thus, use of microalgae as an alternative Biodiesel - biofuel feedstock is gaining increasing interest from researchers, entrepreneurs, and the general public.

The requirement of energy for the mankind is increasing day by day. The major source of energy is based on fossil fuels only. Thus, the scarcity of fossil fuels, rising price of petroleum-based fuels, energy protection, and increased global warming resulted in focusing on renewable energy sources such as solar, wind, hydro, tidal, and biomass worldwide.

Normally algae have 20 % – 80 % oil contents that could be converted into different types of fuels such as Kerosene oil and Biodiesel. The diesel production from algae is economical and easy. Different species such as *Tribonema*, *Ulothrix* and *Euglena* have good potential for biodiesel production.

The production of biofuel is a complex process. The process consists of following stages: a) Stage 1 - Microalgae cultivation, b) Stage 2 - Harvesting, drying & cell disruption (cells separation from the growth medium), c) Stage 3 - Lipid extraction for biodiesel production through transesterification and d) Stage 4 - Starch hydrolysis, fermentation and distillation for bioethanol production. However, these processes are complex, technologically challenges and economically expensive. A significant challenge lies ahead for devising a viable biofuel production process.

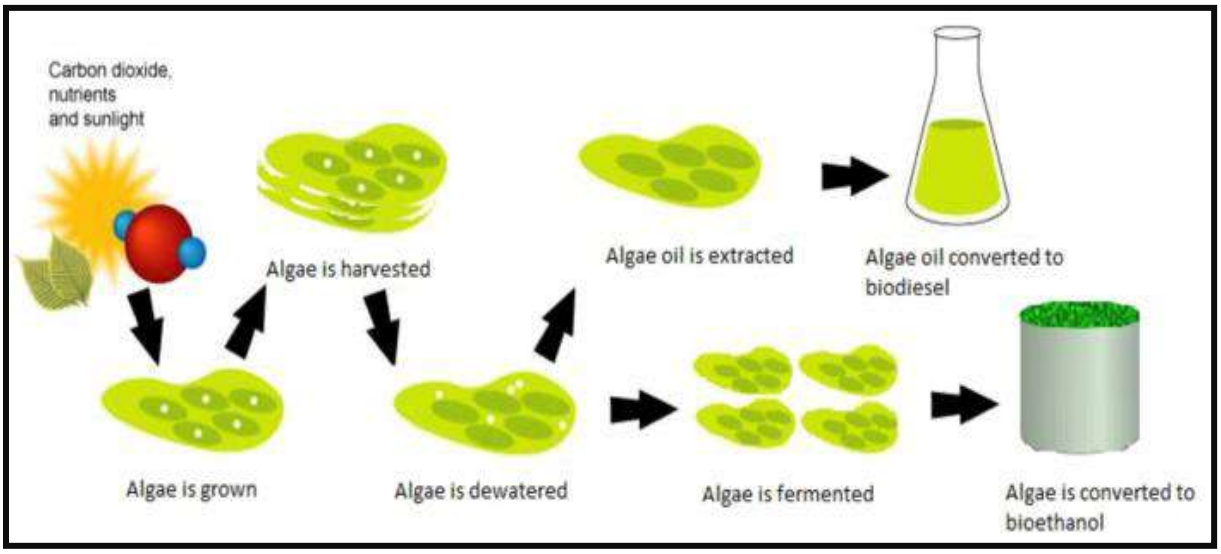

Figure - 1: Process of Biofuel production

There are several advantages of algal biomass for biofuels production: (a) ability to grow throughout the year, therefore, algal oil productivity is higher in comparison to the conventional oil seed crops; (b) higher tolerance to high carbon dioxide content; (c) the consumption rate of water is very less in algae cultivation; (d) no requirement of herbicides or pesticides in algal cultivation; (e) the growth potential of algal species is very high in comparison to others; (f) different sources of wastewater containing nutrients like nitrogen and phosphorus can be utilized for algal cultivation apart from providing any additional nutrient; and (g) the ability to grow under harsh conditions like saline, brackish water, coastal seawater, which does not affect any conventional agriculture. However, there are several disadvantages of algal biomass as feedstock such as the higher cultivation cost as compared to conventional crops. Similarly, harvesting of

algae require high energy input, which is approximately about 20 – 30 % of the total cost of production. Several techniques such as centrifugation, flocculation, floatation, sedimentation, and filtration are usually used for harvesting and concentrating the algal biomass.

Microalgae have immense potentials for biofuels production. However, these potentials largely depend on utilisation of technology, input feedstock (CO_2, wastewater, saltwater, natural light), barren lands and marine environment. Based on energy content, available technology, land, it is hard to overemphasize that biofuels are a realistic short-term, but definitely not a long-term and large scale solution to energy needs and environmental challenges. Microalgae can be temporary sources of energy, and with the appropriate growth protocols they may address some of the concerns raised by the use of first and second generation biofuels.

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10

BIOWEAPONS: AN EMERGING THREAT

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Biological warfare (BW) also known as Germ warfare is the use of biological toxins or infectious agents such as bacteria, viruses, and fungi with the intent to kill or incapacitate humans, animals or plants as an act of war (often termed "bio- weapons", "biological threat agents", or "bio-agents") are living organisms or replicating entities (viruses, which are not universally considered "alive") that reproduce or replicate within their host victims. Entomological (insect) warfare is also considered a type of bioweapon. This type of warfare is distinct from nuclear warfare and chemical warfare. Several species of bacteria and viruses and some bacterial toxins are perceived to be of potential use by bioterrorist groups. The agents believed to pose the greatest threat to humans are *Bacillus anthracis* (Anthrax), *Yersinia pestis* (plague), *Francisella tularensis* (Tularemia), *Variola major* (Smallpox), Hemorrhagic fever viruses and *Clostridium botulinum* toxin (botulism). Many of these agents have been released intentionally. Other bacteria and viruses have also been the subject of research as agents of biological warfare. In addition to microbes that target humans, agents that kill animals have been studied for use in crippling a region's food supply or economy.

Infectious disease agents have been harnessed for centuries as weapons to neutralize armies, decrease the size of enemy populations, and create a state of terror that destabilizes societies. Such s research continued in many "civilized" nations until very recently, and clandestine work may be ongoing despite being banned by international treaty. Even though many known stocks of bioweapons have "disappeared," government groups around the world continue to prepare for attacks that hopefully will never occur.

The use of biological agent is not a new concept and history is replete with examples of such use. Attempts to use biological weapons date back to antiquity. Scythian archers infected their arrows by dipping them in decomposing bodies or in blood mixed with manure as far back as 400 BC. Persian, Greek and Roman literature from 300 BC quote examples of the use of animal cadavers to contaminate wells and other sources of water. In 18th century AD, British forces distributed small pox infected blankets to native Americans to create transmission of disease. During First World War, Germans developed anthrax, glanders, cholera and a wheat fungus for use as s.4 Likewise, during Second World War, Japanese operated a secret biological warfare research and carried out human experiments with plague, anthrax, syphilis on Chinese prisoners.4 In 1940s and 50s, United States and Britain continued research on various offensive s like anthrax and botulinum toxin and also continued to the 60s. In 1970s, USSR and allies were suspected of having used yellow rain (trichothecene mycotoxins) during campaigns in Cambodia and Afghanistan, which caused alimentary toxic aleukia (ATA) in civilians. In 1979, 66 people were killed due to accidental release of anthrax from a weapons facility in Sverdlovsk, USSR.6 Since the 1980s, terrorist organizations have become users of biological agents. The most frequent bioterrorism episodes have involved contamination of food and water. In September, 1984, international contamination of restaurant salad bars in Oregon by followers of Bhagwan Rajneesh infected 751 persons with Salmonella typhimurium. Recently, in a short span of time, i.e. from Sept. to Nov. 2001, 23 cases of bio-terrorism occurred in US which mostly involved, postal workers, where letters contaminated with anthrax were handled or opened. The recent case seems to be spread of corona virus across the world.

There are three broad categories which specify the term bioterrorism. The categories are based on the threat ranging from highest to moderate and eventually to gradual (A, B, and C). Category A consists of those disease-causing agents which can be easily released and can spread from one person to another. Example of Category A is *Bacillus anthracis* and *Yersinia pestis.* Category B includes those agents that are moderately easily transmitted and do not cause deaths, but some people are affected. Example of Category B is *Salmonella* and virus such as alphaviruses. Category C includes those pathogens that are being genetically engineered to cause effect in future and they have a potential to result in high death too. For example, *Naegleria* and Nipah viruses.

Biological warfare agents are unconventional weapons and can be delivered by unconventional means. The most effective method is aerosol sprays because of their particle size (1-5μm) due to which they are most efficiently delivered to their target (air sacs of lung). Other modes of delivery are food and water contamination, conventional explosive munitions and by covert injections.

These Biological warfare agents mainly enter through respiratory tract (following inhalation of aerosolized Biological warfare agents). Others routes are exposed mucosal surfaces, (nose/mouth/eyes), GIT (through contaminated food and water), intact skin (barrier against most Biological warfare agents except mycotoxin) and injection (traumatic wounds).

Currently no reliable detection system exists for Biological warfare agents.

Successful management of exposure to Biological warfare agent relies on early recognition. Medical units should rely on information not only from detectors and intelligence sources, but also from casualties themselves. This applies particularly to Biological weapons/agents since at present there are no rapid methods of identification or detection.

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IMPORTANCE OF *Azotobacter* spp.

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Azotobacter spp. are Gram negative, free–living, aerobic soil dwelling, oval or spherical bacteria that form thick–walled cysts (means of asexual reproduction under favorable condition). There are around six species in the genus *Azotobacter* some of which are motile by means of peritrichous flagella, others are not. They are typically polymorphic and their size ranges from 2 – 10 μm long and 1 – 2 μm wide. The *Azotobacter* genus was discovered in 1901 by Dutch Microbiologist and Botanist Beijerinck (founder of Environmental Microbiology). *Azotobacter chroococcum* is the first aerobic free-living nitrogen fixer. Lipman (1903) described *Azotobacter vinelandii*, and a year later *Azotobacter beijerinckii*, which he named in honor of Beijerinck. Thompson and Skerman1981 described *Azotobacter armeniacus*. Page and Shivprasad (1991) reported a microaerophilic and air-tolerant type *Azotobacter* saline stress which was dependent on sodium ions. A phylogenetic study revealed that *A. vinelandii* belongs to the same as the bacterium *Pseudomonas aeruginosa*.

Azotobacter species have a full range of enzymes needed to perform the nitrogen fixation ferredoxin, hydrogenase, and an important enzyme nitrogenase. *Azotobacter* improves seed germination and has beneficiary response on Crop Growth Rate. It helps to increase nutrient availability and to restore soil fertility for better crop response. It is an important component of integrated nutrient management system due to its significant role in soil sustainability. These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. This cell protein is then mineralized in soil after the death of *Azotobacter* cells thereby contributing towards the nitrogen availability of the crop plants. *Azotobacter* also synthesizes some biologically active substances, including some phytohormones such as auxins, thereby stimulating plant growth. They also

facilitate the mobility of heavy metals in the soil, thus enhancing bioremediation of soil from heavy metals, such as cadmium, mercury and lead.

Azotobacter genus are reported to exploit a broad range of organic substrates like mannitol, various organic acids, benzoic acid, phenolic compounds of soil, etc. as a source of carbon and energy and form several biologically active compounds that instigate the proliferation of rhizospheric microorganisms. The capability of *Azotobacter* sp. to use aromatic compounds has been known for several years. It is able to degrade the derivatives of aromatic compounds like benzoate, p-hydroxy benzoate, protocatechuic acid, etc. *Azotobacter* spp. are characterized by Nitrogen fixation, Siderophore production, IAA and exopolysaccharide production that improve the plant health and Indole-3-acetic acid and exopolysaccharides production. In addition to its beneficial impact on plant growth promotion, *Azotobacter* is also known to be associated with the suppression of pathogenic diseases of plants. Several examples are present in the literature advocating the importance of disease suppression by different species of *Azotobacter.* The *Azotobacter* provided good protection to the plants by aggressively colonizing the roots of wheat crops. These may include the production of siderophores, antimicrobial substances, toxins and also the growth hormones like Auxins, Gibberellins and Cytokinins. Several strains of *Azotobacter* are capable of producing amino acids when grown in culture media amended with different carbon and nitrogen sources. Substance like amino acid produced by these rhizobacteria are involved in many processes that explain plant grown promotion. Biochemical analysis of chlorophyll, nitrogen, phosphorous, potassium and protein content were higher in *Azotobacter* inoculated plants as compared to non–inoculated control plants.

Azotobacter is one of the best options to be used as biofertilizer for eco-friendly and sustainable crop production. Understanding and manipulating all these beneficial properties of *Azotobacter* may prove to be a key interest for the future endeavors of crop improvement.

"*Azotobacter* is great source of nitrogen for plant growth and use of biofertilizers"

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PULLING MILLIONS OF YEAR-OLD LIVING MICROBES FROM BENEATH THE SEA

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The microbial life is very persistent, and often finds a way to survive. Microbes are known to live in very hot or toxic environments, but can they live where there's little food to eat? To find out, South Pacific Gyre, a site of intersecting ocean currents east of Australia that is considered the deadest part of the world's oceans, almost completely lacking the nutrients needed for survival was selected for research. The cores of clay and other sediments from 5700 m below sea level were extracted. The samples did indeed contain some oxygen, a sign that there was very little organic material for bacteria to eat.

The extracted small clay samples from the centers of the drilled cores and simple compounds like acetate and ammonium, that contained heavier forms or isotopes of nitrogen and carbon that could be detected in living microbes were added in glass vials. There are at least 100,000 cells/cm³ of seafloor mud. But in these samples, there were no more than 1000 bacteria in the same amount of sediment. So, the specialized techniques such as using chemical tracers to detect whether any contaminating seawater got into the samples and developing a way to analyze very small amounts of cells and isotopes were used. The added nutrients woke up a variety of oxygen-using bacteria. In samples from the 101 million year-old layer, the microbes increased by four orders of magnitude to more than 1 million cells/cm³ after 65 days.

Genetic analysis of the microbes revealed they belonged to more than 8 known bacterial groups, many of which are commonly found elsewhere in saltwater where they play important roles in breaking down organic matter. Also bacteria in oxygenated sediments under the sea floor were found. Research says that, most of the species do not form spores the inactive phase which some bacteria form in unfavorable conditions. It could be that bacteria have been dividing very slowly all this time, which would make those species distant descendants of ancestors millions of years-old or may be there's another unrecognized source of energy perhaps radioactivity down there that allows slow division by the bacteria, which likely got trapped in these sediments as they were buried by other settling sediments.

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FROM MICROORGANISMS TO MICROBIOMES: A HISTORICAL OVERVIEW

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Historically, the field of microbiome research has emerged from environmental microbiome research (Microbial Ecology) and provides an interdisciplinary platform for many fields, e.g., Agriculture, Food science, Biotechnology, Bioeconomy, Mathematics (Informatics, Statics and Modeling), Plant Pathology and especially human medicine. The new field has already delivered novel and important concepts for describing host-microbial interactions such as the Holobiont theory or meta-organism concept. Further, principles of co-evaluation, co-selection and stress response within, Microbiomes go far beyond the traditional scope of these concepts. Significantly, there has been a fundamental paradigm shift in our understanding of microorganisms and it is now accepted that all eukaryotes are meta-organisms and must be considered together with their microbiota as an inseparable functional unit. This Concept also considers the fact that pathogens represent only a tiny fraction of microorganisms; diversity loss can result in a so-called "Dysbiosis" that describes the altered composition of microbes, which has a cascading impact on the immune system& offers an advantage for emergence & outbreak of pathogens. Taking major historical developments into account it is important to understand how microbiome research has manifested itself as core discipline in modern life. The field of microbiome research originated in Microbiology and started back in the 17th century. Research progress has often been driven by the development of new techniques and equipment. Interestingly many technological inventions have boosted microbiological research in such a manner and caused paradigm shifts in our understanding of health and disease. Since infectious diseases

have affected human populations throughout most of history, Medical Microbiology was the earliest focus of research and public interest.

Current discussions on the microbiome might make you think that concepts of microbiome are a relatively recent discovery however, despite the new predominantly medical attention, the concept actually has its root in the early days of microbial ecology. A popular assumption, is that Nobel Laureate and Microbiologist, Joshua Lederberg, first coined the term "Microbiome" in 2001. This has led to debates over when and how the term "Microbiome" is appropriate. One argument is that because the word microbiome is derived from the 'OMICS' family of terminology, it should be used to describe the collective genomes of microbial species while the collection of organisms themselves should be termed "Microbiota". While this argument sounds reasonable, the word microbiome, isn't actually a derivative of the 'omics' naming scheme. One can find older uses, for e.g, in 1988, Whipps and colleagues used the term microbiome to describe the collection of microbes & their activities within a given environment. They state: "A convenient ecological framework in which to examine biocontrol systems in that of the microbiome. This may be defined as a characteristic microbial community occupying a reasonably well-defined habitat which has distinct physio-chemical properties. This term thus not only refers to the microorganisms involved but also encompasses their theatre of activity".

Indeed, "Microbiome" is a portmanteau of microbe and biome, describing the microbial ecosystem, inhabitants and all, and not just genomes. While the term is relatively new to our Science vocabulary, the underlying concept and importance of microbiome work back to the very beginning of Microbial Ecology and to Sergei Winogradsky in the 1800's. Winogradsky argued for the need to study microbes in their natural contexts. Thus, founding Microbial Ecology as an avenue of research, which studies microbes as they interact with each other and within the context of their environment whether it be aquatic, terrestrial, atmospheric, a living host, or a mixture of any of these. The infamous Winogradsky column, a culturing device that permits researchers to study microbes as they interact with each other in a natural setting, was developed from this understanding. While one may find Winogradsky Columns decorating the offices of Microbial Ecologists due to their fascinating color changes and the historical significance to the field, these devices are still used to isolate environmental microbes with desirable traits and to teach students about microbial communities while investigating the impact of human activities on different ecosystems. The study of microbiomes also utilizes this fundamental ecological concept to the point that a great number of prior microbial population and microbe-environment studies could be rebranded as microbiome work. It's important to know the true history of microbiome

research, because it leads researchers to the host of Statistical techniques and experimental methods that have been developed over the years to specifically address microbiome-related questions. As Winogradsky accurately points out, a microbe in continuous culture behaves differently from its natural habitat. In 1949, he stated "Condition of pure culture in an artificial environment is never comparable to that in a natural environment. One cannot change the notion that a microbe cultivated sheltered from any living competitors and luxuriously fed becomes a hot-house culture, and is induced to become in a short period of time a new race that could not be identified with its prototype without special study" (Winogradsky, 1949). One of the most exciting outcomes of microbiome research is the opportunity to strengthen ties between the distant poles of medical and environmental/ecological microbiology, so that both tools and collaborations can be strengthened and lead to better understanding for all.

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ISOLATION, SCREENING OF ACTINOMYCETAL METABOLITES AND ITS APPLICATION

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The *Actinomycetes* are a various group of Gram positive, branching, filamentous, obligatory aerobic and relatively slow growing bacteria. They can be terrestrial or aquatic, they are having great economic importance to humans. The *Actinomyces* are much smaller and its properties lies between bacteria and fungus. Some soil *Actinobacteria* (such as *Frankia*) live symbiotically with plants roots for fixing of nitrogen. Beyond the great interest in *Actinobacteria* for their soil role its universally habitat in soil and mostly abundant in fresh water. *Actinobacteria* is one of the dominant bacterial phyla and contains one of largest bacterial genera, Streptomyces and other *Actinobacteria* are major contributors to biological buffering of soil. In soil they help to decompose the organic matter of dead organism, which is useful for the plants. Most of *Actinobacterial* species have medical and economical significance while many cause diseases in human.

I carried out the isolation of *Actinomycetes* species by the Selective media (Starch Casein Agar) method. Then for separating its metabolites I was performed the broth method. The metabolites are small organic molecules produced by organism in which some are for growth and reproduction and some are for other purpose, in which the secondary metabolites are important for defence against various other microorganism. The Actinomycetal metabolites are source of many of antibiotics (*Streptomyces*)

chemotherapeutic drugs, immune suppressant and other medicine. Thus separated metabolites of *Actinomycetes* can be used against various pathogenic microorganism carried out an experiment to check its antagonistic property against pathogenic microbes such as *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*, the results were extremely grateful showing wide zone of inhibition thus it's a great medical application.

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THE ROLE OF RELATIVE HUMIDITY IN BACTERIAL GROWTH

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In simple word humidity is amount of water vapor present in the air and that we all know. But it is important for us to know that humidity plays an important role not only on bacterial growth but also for the whole microbial growth. Though, it affects the growth of bacteria by different ways. Like all the organism bacteria too need a certain amount of water for growth. In case of bacterial growth relative humidity is important which is same as humidity but the one major difference is Relative humidity depends on temperature. High RH influence the water content and the water content promote microbial growth. When food stored in high relative humidity spores of different bacteria germinate easily. Different groups of bacterial species bear different mechanism for handling the changes in relative humidity. It is a fact that relative humidity is a measure of water activity of the gas phase.

Requirement of water by the microorganisms is expressed as water activity (a_w). a_w and relative humidity are interrelated. As water activity was reduced below an optimum level, there was an increase in the lag phase, a decrease in growth rate and a decrease in the amount of cell substance synthesized. When water activities are lower than the minimum for growth, cells remain dormant. Suppose a food has low water activity and that food is somehow placed under high relative humidity atmosphere then it is definite that the osmotic pressure channels the gaseous water to the food. And the next thing that will going to happen is germination of the dormant spores which are already present in the food. When the bacterial spores and bacteria itself gets their

desired water content then they start for germination and growing respectively. When water activity outside the cell becomes low enough, it causes osmotic stress: the cell cannot take up water and becomes dormant. Once the bacterial cells get the required water in a high Relative Humidity atmosphere, they start to grow and they usually produce water as an end product of respiration. Thus they increase aw of their own environment.

Water Activity

Water activity has effective ranges where it may be compared to pure water as essentially a control. The water activity (a_w) of pure H_2O is set at 1.0 as a standard (100 % water). The a_w of most fresh vegetables is 0.99 or greater. The a_w of human blood is 0.99; seawater = 0.98; maple syrup = 0.90; Great Salt Lake = 0.75. Water activities in agricultural soils range between 0.9 and 1.0. Microorganisms will thrive over a range of aw from 1.0 to 0.7. The proliferative activity of microorganisms is halted at 0.50.

Water activity (a_w) may be defined as:

$$a_w = \frac{p}{p^0}$$

Where p is the vapor pressure of water in the substance, and p^0 is the vapor pressure of pure water at the same temperature.

The relative humidity of the manufacturing environment is important as products are affected by the amount of water in the atmosphere making it critical that water and relative humidity are monitored. Cytoplasm is an aqueous solution for this it has a lower water activity than pure water. So the micro-organisms in a place of High Relative Humidity (pure water which is in aqueous form) experience net-flow of water molecules into the cytoplasm from the outside. To save itself from excessive water gaining and bursting, bacteria has rigid cell wall that can withstand the osmotic pressure of cytoplasm which may be as high as 30 atm (approximately 3 MPa) in a gram-positive bacterium or as little as 5 atm in gram-negative bacterium. At an a_w of 0.6 or below, macromolecules present in bacterial cell can't function properly in such situation growth of bacteria ceased. Even if the active growth is ceased bacteria still can survive.

The relative humidity of air in equilibrium with a sample is called the Equilibrium Relative Humidity (ERH).

$$ERH = a_w \times 100$$

At a 0.6 a_w corresponding to a water potential of -68MPa, the cytoplasm of bacteria would certainly need to contain a very high concentrations of an appropriate compatible solute (such as amino acids and amino acid derivatives). The relationship between water activity and chemical reactions (Maillard browning, lipid oxidation) impacts the longevity of dormant stage and also to the complex behavior with maxima and minima. Requirement for gaseous water by different types of bacteria to reproduce and grow is very different.

At lower Relative Humidity, Microorganisms on surfaces experiences desiccation, which inhibits their growth and reduces their metabolic activity. This effect is similar to how bacteria may behave when present in a low moisture or low water activity food, though the chemicals in foods may make those interactions more complex. Microorganisms are unable to grow without the presence of water regardless of other environmental conditions, and a higher relative humidity has been shown to better support the growth and survivability of microorganisms in closed environments. The effects of temperature and relative humidity appear to play important roles in understanding how bacteria survive on surfaces.

The spores of different bacteria can survive 0 % relative humidity. Several studies confirmed that in the bacterial growth Relative Humidity plays an important role along with other major factors (mostly Temperature).

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PRODUCTION OF α-AMYLASE BY DIFFERENT KIND OF ORGANISMS

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Amylase is an enzyme that breaks down starch into sugar. Starch, the primary storage polysaccharide, is an important constituent of the human diet and, for this purpose, it is enzymatically processed into a variety of different products. It is degraded by amylolytic enzymes. Although amylases originate from different sources (animals, plants and microorganisms), microbial amylases generally meet industrial demands best, due to their short growth period and productivity. Its extensive application in food, starch liquefaction, saccharification, detergent, brewing, paper, textile and distilling industries, has led to a greater stress for the increase in the indigenous production of α-amylase. With the advent of new frontiers in biotechnology, the spectrum of amylase application has expanded into many other fields, such as clinical, medical and analytical chemistry. Many of the enzymes used in the industries are extracellular derived from microorganisms. Among various extracellular enzymes, α-amylase ranks first in terms of commercial exploitation. Bacteria and fungi secrete amylases to the outside of the cells to carry out extra cellular digestion. When they have broken down the soluble starch, the soluble end products such as (glucose or maltose) are absorbed into their cells. Demand for microbial amylases has increased, due to their specificity of reaction, mild conditions required for the reaction, and less energy consumption than the conventional chemical methods. The industrially important *Bacillus* strains, which are extensively used to produce alpha amylase, using different are *B. amyloliquefaciens*, *B. licheniformis*, *B. stearothermophilus*, *B. subtilis*, *B. megaterium* and *B. circulans*.

Previously many works have been carried out for the production of α-amylase media compositions with varying substrates using the *Bacillus* sp. The important substrate used was starch with varying media compositions. The other substrates included banana peel wheat bran and rice flake manufacturing wastes. *Bacillus licheniformis* is a Gram positive endospore forming organism that can be isolated from soils and plant material all over the world. This organism is used extensively for large-scale industrial production of exoenzymes as it can secrete large quantities of proteins of up to 20 - 25 g/L. The use of the submerged culture is advantageous because of the ease of sterilization and its process control.

Material and Method

Microorganisms and Maintenance of Culture

Bacillus licheniformis strain MTCC 2617 and MTCC 2618 were procured from Microbial Type Culture Collection (MTCC) Chandigarh and grown on Nutrient agar slants at 37 ºC and sub-cultured every month.

Inoculum and Production Media

The inoculum was prepared by the addition of sterile distilled water in to the freshly grown agar slants, from this 0.5 ml of suspension was inoculated in to 100 ml of sterilized fermentation medium and incubated in a shaking incubator at 100 rpm at 37 ºC. The composition of media was (g/L) Bacteriological peptone – 6 g, $MgSO_4.7H_2O$-0.5g, KCl - 0.5 g, Substrate – 1 g. For the production of the enzyme, broths were prepared using four different substrates Starch, Rice, Wheat and Ragi powder.

The activity of the enzyme produced in the media was checked using enzymatic assay method at regular time intervals of 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs respectively, to find the time period and the medium with the substrate that showed the highest enzyme production.

Extraction of the α-Amylase Enzyme

After incubation, the culture broth was centrifuged at 5000 rpm for 20 minutes at 4 ºC. The supernatant was collected and the amylase activity was estimated. The supernatant was further purified to obtain the crude enzyme. The crude enzyme was further purified by ammonium sulfate precipitation, dialysis and ion- exchange chromatography to obtain pure enzyme.

Results and Discussion

Starch Hydrolysis Test

The strains showed formation of a halo zone, which indicated the degradation of the starch from the starch agar by the enzyme α-amylase produced by *Bacillus licheniformis*.

Enzyme Production

In submerged fermentation, the production of the α-amylase by the *B. licheniformis* MTCC strain 2617, using four different substrates as carbon source, reached maximum on the third day (Starch 2.59 IU/ml/minutes, Rice powder 2.4 IU/ml/minutes, Wheat powder 2.23 IU/ml/minutes, Ragi powder 0.89 IU/ml/minutes) and the production declined by reaching the minimum on the fifth day (Starch 1.92 IU/ml/minutes, Rice powder 1.75 IU/ml/minutes, Wheat powder 1.13 IU/ml/minutes, Ragi powder 0.73 IU/ml/minutes). Of the four substrates used, starch gave the highest production of the enzyme on all the days. Similarly by submerged fermentation the production of α-amylase by the *B. licheniformis* MTCC strain 2618, using four different substrates as carbon source, reached maximum on the 3rd day (Starch 3.64 IU/ml/minutes, Rice powder 2.93 IU/ml/minutes, Wheat powder 2.67 IU/ml/minutes, Ragi powder 2.36 IU/ml/minutes) and the production declined by reaching the minimum on the 5th day (Starch 1.84 IU/ml/minutes, Rice powder 1.65 IU/ml/minutes, Wheat powder 1.19 IU/ml/minutes, Ragi powder 0.88 IU/ml/minutes). Of the four substrates used, starch gave the highest production of the enzyme on all the days.

The production of the α-amylase from the two *B. licheniformis* strains used, MTCC strain 2618 showed the highest production on all the four substrates used as carbon source, i.e., on the 3rd day, the maximum production of the enzyme was seen and of the substrates used the medium containing starch gave the highest production of enzyme.

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BIOAUGMENTATION – APPLICATIONS AND LIMITATIONS

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Bioaugmentation or Biological augmentation is the addition of selected strains or mixed cultures (Archaea or bacterial cultures) required to speed up the rate of degradation of a contaminant. Enhancing the microbiota of a contaminated site will not only enhance the elimination of the pollutants from the particular site but also at the same time increases the genetic capacity of the desired site. Therefore, bioaugmentation corresponds to an increase in the gene pool and thus, the genetic diversity of the site. In many cases, cultured microorganisms used for bioaugmentation are "specialists" in degrading specific target contaminants. For practical purposes, different types of microorganisms can be used for bioaugmentation. Common inocula used for bioaugmentation include: Mixed cultures (collection of indigenous bacteria that have been highly enriched on the contaminant of interest), pure cultures (Enrichment of single strain capable of degrading the contaminant. Examples include *Dehalococcoides ethenogenes* or *Desulfomoni letiedjei*), genetic elements (Introduction of naturally occurring gene vectors e.g., plasmids, which are extra-chromosomal DNA molecules separate from the chromosomal DNA that are capable of replicating independently and transferring catabolic capacity from one strain to another instead of bacteria), Genetically modified microorganisms [GMOs] (to introduce specific traits into microbial communities via genetic manipulation, Examples of the use of GMOs to enhance biodegradation include the degradation of phenol, toluene, chlorobenzene, indole.

Applications of Bioaugmentation

In wastewater treatment plants, the bioaugmentation is one of the most important strategies to remove organic contaminants from wastewater. Wastewater activated sludge contains naturally occurring microorganisms that biodegrade a wide range of pollutants, but some pollutants are resistant to biodegradation. Several factors account for this resistance: high toxicity, low water solubility, low bioavailability, high stability and low biodegradability in many cases, recalcitrant compounds may be new, and as a result, microorganisms may not have yet adapted to use them as a substrate. Bioaugmentation can overcome these challenges, as one of its main advantages is that treatment can be tailored to a specific pollutant that is dominant in the environment. Over the last decade, many investigations have been dedicated to testing bioaugmentation strategies to clean wastewater, and most have focused on recalcitrant molecules. At sites where soil and groundwater are contaminated with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene, bioaugmentation can be used to ensure that the *in-situ* microorganisms can completely degrade these contaminants to ethylene and chloride, which are non-toxic.

Limitations of Bioaugmentation

There have been many instances where bioaugmentation had deficiencies in its process. The implementation of bioaugmentation on the environment can pose problems of predation, nutritional competition between indigenous and inoculated bacteria, insufficient inoculations, and disturbing the ecological balance due to large inoculations. Each problem can be solved using different techniques to limit the possibilities of these problems occurring.

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ANTIMICROBIAL PROPERTIES OF EARTHWORM EXTRACTS

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Earthworms are a group of annelid worms found all over the world ranging in varying lengths and dimensions. These have a tube within a tube body plan with external and internal segmentation. Approximately over 3000 species of earthworms have been identified till date, which fall into different groups based on the habitats they live in and the way they collect their food.

Earthworms are known to have many anti – inflammatory, analgesic, antipyretic and antibacterial properties. The excreta of earthworm are an excellent antimicrobial product. They mainly feed on dead and decaying matter so they are always exposed to microbes and microbial infections. Excellent humoral and cellular mechanisms most of which are components of coelomic fluid, help them fight various microbial infections. This is because the coelomic fluid of earthworms contain biologically active molecules and leucocytes that play roles in phagocytosis and encapsulation of the pathogenic cells. Therefore, coelomic fluid of earthworms are of great value and had been subjected to a series of studies.

Tissue extracts of earthworm was studied and was found effective against several strains of bacteria and fungi. It was experimentally concluded that when tissue extracts of earthworm or powdered form of the same was introduced into an animal it showed some antimicrobial properties that aided to the immunity of that animal. It is also efficient against human pathogenic microbes like *Escherichia coli* which causes human

urinary tract infections. This fact implies that consuming earthworm extract decreases the chances of urinary infections in human. Also earthworm powder can be given orally to people as it has a thrombolytic activity which gives an anti-coagulatory effect and relaxes the vascular system hence is also effective in the treatment of thrombosis.

The first antimicrobial peptide was identified from the earthworm *Lumbricus rubellus*. Lumbrician I showed antimicrobial property against microbes without homolytic activity. Later on, two more antimicrobial peptides where identified from two different species of earthworms namely *Eisenia foetida* and *Pheretima tschiliensis* respectively which were also found to show some antimicrobial functions.

Researches have observed that the antimicrobial properties of earthworm is mainly confined to its ceolomic fluid and that property does not undergo any change even after the worm feeds on soil containing harmful microbes. So to have a clear cut picture of this, they partly sterilized the gut of the worm with streptomycin and confirmed that antimicrobial activity remains the same in earthworms fluid at all conditions. Also the gut extracts of earthworm has an antifungal activity in addition to the antimicrobial property. These studies may formulate the introduction of a new antimicrobial drug in the near future. These extracts contain bioactive substances and enzymes that prevent the growth of harmful pathogenic microbes and inhibit the growth of bacteria and fungi. Earthworm extracts can be used in its powdered forms or as paste or even they can be included in one's daily diet after proper cooking.

In the current scenario, earthworm extracts having antimicrobial properties is gaining tremendous importance. The healing process in one's body could be fastened when earthworm extracts are being supplied in right amounts. All these properties of earthworm are confined mainly to its coelomic fluid which is capable of defending the microbial attacks. The main and the sole reason behind the importance of earthworm extract is that, in its coelomic fluid it supports the growth of various bioactive agents and enzymes that prevent and kill off harmful microbes. Thus, in every sense earthworm extract are potentially capable of developing into a new powerful drug.

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EFFECT OF COPPER SULPHATE ON GROWTH OF *Rhizobium* AND NODULATION OF *Trigonella foenum-graecum*

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Introduction

Heavy metal polluted soils tend to leach heavy metals in water bodies leading to bioaccumulation and subsequent Biomagnification. Heavy metals are required for growth at lower concentration but at excess concentrations they lead to inhibition of cellular metabolism and produce pathogenic effect on life forms. Copper at higher concentration damage brain and kidney, cause liver cirrhosis, anaemia, irritation in digestive tract. Heavy metals cause damage oxidative stress by excessive production of reactive oxygen species. It has been observed that at lower concentrations Cu promotes but at higher concentrations inhibits bacterial growth.

Bioremediation especially phytoremediation is being studied as an alternative to costly and environmentally damaging physical and chemical methods. Use of microorganisms is becoming popular due to its ease, efficacy and cost effectiveness. In many studies it has been observed that rhizosphere bacteria augment phytoremediation potential of plants. Plants such as *Glycine max, Medicago lupina, cicer, Trigonella foenum graceum, Hemp, Aschynomen* in association with nodulating rhizobia which help plant growth by nitrogen fixation are being used in phytoremediation. This study aimed to understand how *Rhizobium* help plant to remediate heavy metal contaminated soil specifically copper by using fenugreek as plant. This project aims to understand role of

Rhizobium inoculation in phytoremediation activity of plant using Fenugreek-*Rhizobium* symbiosis.

Materials and Methods

1. ***Plantation*:** Surface sterilized fenugreek seeds were germinated using paper towel method and 5 day old seedlings were planted in each nursery bag in a sterilized and fungicide treated mixture of soil, coco peat and sand in ratio 2:1:1 (5 seedlings/bag). Bags with seedlings were divided in two groups - To both groups $CuSO_4$ concentration was given as aqueous solution to get final concentration of 0, 50, 100, 200, 300 and 400 mg/kg. Five days post sampling bags form inoculated with *Rhizobium* isolate capable of growing in highest $CuSO_4$ concentration. These plants were harvested when growth is sufficient or by one and half month.

2. **Selection of $CuSO_4$ tolerant *Rhizobium*:** $CuSO_4$ tolerating *Rhizobium* was selected on basis of MIC to $CuSO_4$, and used to inoculate the bags containing fenugreek plants.

3. **Growth rate of Fenugreek plants after one month:** Height of each plant was measured after 1 month of growth and was compare by ANOVA.

4. **Determination of chlorophyll content in leaves**: Chlorophyll from 0.1 g of the leaves was extracted in 2 ml of 80 % chilled acetone and absorbance of $1/10^{th}$ dilution of this extract in acetone was determined at 663 and 645 nm. Chl a and Chl b and Total Chlorophyll was determined using following formulae

Chl a = (0.999 * OD 663 nm) – (0.989 * OD 645 nm).

Chl b = - (0.328* OD 663 nm) + (1.77 * OD 645 nm).

Total Chlorophyll= Chl a + chl b.

Results and Discussion

Rhizobium with the MIC of 6×10^{-4} and growth on $CuSO_4$ containing YEMA proved that the culture is resistant to Cu concentration of 0.0006 M. Growth of *Rhizobium* inoculated and uninoculated plants with different Cu levels was compared as height of plants. It was seen that in growth in uninoculated plants is less than those of inoculated plants at different copper concentrations suggesting that copper concentration is affecting the growth of plants by inducing stress($p<0.001$, ANOVA, Figure - 1).

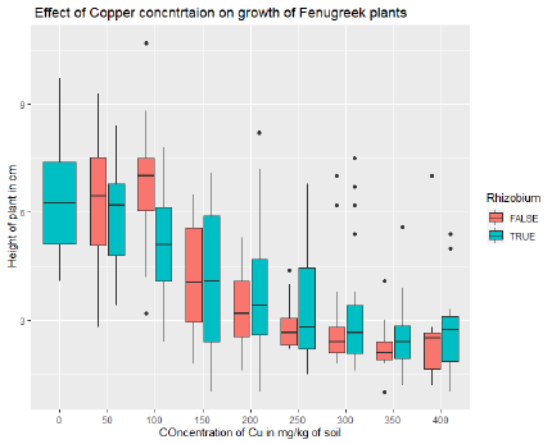

Figure - 1: Effect of Cu concentration on growth of fenugreek plants inoculated or uninoculated with *Rhizobium*

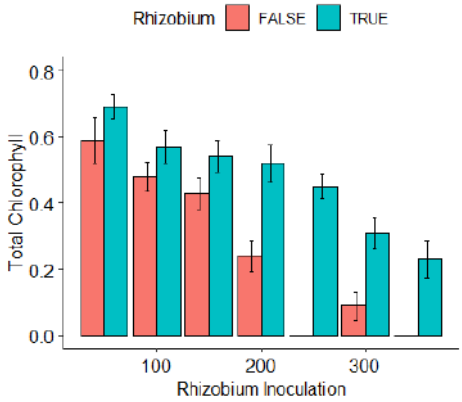

Figure - 2: Effect of Cu concentration on Chlorophyll content of fenugreek plants inoculated or uninoculated with *Rhizobium*

There difference in growth of with and without *Rhizobium* is more pronounced at Cu 150, 200, 250, 300 (p=0.00799, ANOVA) than that at lower Cu (p = 0.52, ANOVA) suggesting that *Rhizobial* inoculation is protecting plants from copper stress. This suggest that copper and *Rhizobium* are interacting with each other.

In case of the inoculated plants as the Cu level increases from 50 to 150, it was found that the length of primary root is found to be more in inoculated group with more number of secondary roots, root hairs and root nodules than in uninoculated group. At Cu levels from 200 to 300 it was observed that the primary root had less length and were thick, pinkish and had more secondary roots and root hair. The only difference is seen in the nodulation pattern. Now as concentration of copper increases the size of nodule is bigger, the root had developed intermediate nodules which were more pinkish

suggesting that more nitrogen has been fixed under stress. After the concentration of copper reaches 350 plant has little shrunken primary root however due to production of intermediate nodules (less in number) the plant can survive. After 350 mg/kg concentration the plant gets affected due to stress starts to show very less number of nodules, with poor root development.

After plotting the graph of Copper content verses total chlorophyll content in leaves (harvested after 2 months of growth) it was seen that as the concentration of copper increases the chlorophyll content is decreasing. However, after the inoculation of Rhizobium inoculation the amount of chlorophyll content in the test plant had increased suggesting better growth than the uninoculated plants (Figure - 2).

Discussion

The *Rhizobium* isolated from Fenugreek till date was only screened for its ability to combat salt stress. It was the first time that Fenugreek is being screened for its ability to produce Chalkophores. This has also helped to understand that even Fenugreek can be used for phytoremediation as till date cover crops were noted for this ability. The *Rhizobium* isolated from Fenugreek did not die (cidal) even after increase in the copper which is not seen in the case of *Medicago* Plant or *Zea mays* plant in the earlier studies. Thus, Fenugreek continued to grow but at a slower rate. Although the chalkophore detection, amount of copper remediated from soil could not be studied due to lockdown still study conducted so far suggest that Rhizobium can be used for bioremediation of copper. Using Fenugreek is advantageous because it is easily available, produces various antimicrobial peptides.

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Helicobacter pylori: A BACTERIAL CARCINOGEN

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A carcinogen is any agent capable of causing cancer by inducing mutation in the genome or by disrupting the cell's normal metabolism. *Helicobacter pylori* is the only known bacterium considered as a class I carcinogen by the International Agency for Research on Cancer (IARC, 1994). It is a helical shaped Gram-negative, fastidious microaerophile with lophotrichous flagella and shows positive for urease, catalase, and oxidase biochemical tests. *H. pylori* was first identified by two Australian physicians Barry Marshall and Robin Warren (1982) and they were awarded the Nobel Prize (2005) for their seminal discovery of *H. pylori,* and its role in peptic ulcer disease. Over the last 30 years, this bacterium has proven to be a key etiological factor in several common gastro-duodenal diseases such as chronic gastritis, peptic ulceration, duodenal ulcer, and Gastric cancer (GC). Among different cancers, GC ranks third in mortality and fifth in morbidity rate (WHO, 2018). The infections are acquired in childhood via the fecal-oral or oral-oral route. About 50 % of the world population is at risk of this infection and approximately 10 % develop peptic ulcer disease, 1 – 3 % develop gastric adenocarcinoma, and 0.1 % develop MALT lymphoma.

H. pylori pathogenesis in causing intestinal-type gastric adenocarcinoma (95 % of gastric cancer type) has been studied *in vitro* as well as *in vivo* by using C57/BL6 mice, INS-GAS mice, and Mongolian gerbils. The pathogenesis of *H. pylori* is complex and involves multiple stages such as chronic gastritis, atrophic gastritis, intestinal metaplasia,

and dysplasia. In the stomach, for adaptation in the inhospitable acidic environment, *H. pylori* burrow inside the mucous layer to reach the epithelial cells where it is less acidic. *H. pylori* has evolved the ability to metabolize urea to ammonia *via* urease, which neutralizes the environment enveloping the bacterium. Further, *H. pylori* possess virulence factors such as CagA, VacA, etc. In response to *H. pylori* infection, the host epithelial cells were stimulated to produce pro-inflammatory cytokine IL-8, causing the infiltration of innate immune cells like neutrophils, and macrophages at the site of infection. Activated macrophages release reactive oxygen and nitrogen species resulting in increased oxidative stress which causes the mutation in major tumor suppression gene, p53, leading to a tumor mass. Lymphocytes are also activated, resulting in the induction of Th1-predominant response by the secretion of pro-inflammatory cytokines such as IL-β, TNF-α, and IFN-γ through NF-κB mediated pathway. The COX-2, a regulatory enzyme which converts arachidonic acid to prostaglandins is also upregulated by the cytokine TNF-α. The expressed PGE-2 is directly involved in the overexpression of Ki-67, MMP-9, and VEGF responsible for the increased proliferation, invasion, and angiogenesis activity respectively.

The diagnosis of *H. pylori* infection and gastric cancer is done by endoscopy, urea breath test, biopsy, imaging, and CT scans. The most recommended treatment for *H. pylori* infection is the combination of antibiotics (amoxicillin, clarithromycin, metronidazole, etc) and proton pump inhibitors (omeprazole, pantoprazole, rabeprazole, etc) for 14 days. The chance of cancer initiation can be inhibited by the eradication of *H. pylori* from the gastric environment. The effectiveness of the present treatment is demarcated due to emerging antibiotic resistance and untreated *H. pylori* infection leads to tumorigenesis. The initial diagnosis is often delayed because up to 80 % of patients are asymptomatic during the early stages. Accurate staging of gastric wall invasion and lymph node involvement is important for determining prognosis and appropriate treatment. Surgical excision of the stomach (partial or total gastrectomy) combined with palliative therapy has shown some promising approaches in treating GC. The adverse effects of gastrectomy include the inability to have larger meals, vitamin B-12 deficiency, dumping syndrome, malnutrition, etc. The extensive recommendation of drugs and radiation can adversely affect the patient's compliance and life quality.

The research has been continued to uncover the oncological mechanism of *H. pylori* which is not understood properly. For further advancement, studies need to be done for development of safer, non-toxic, and cost-effective drugs that can overcome the problems posed by present-day treatment to improve the therapeutic standards.

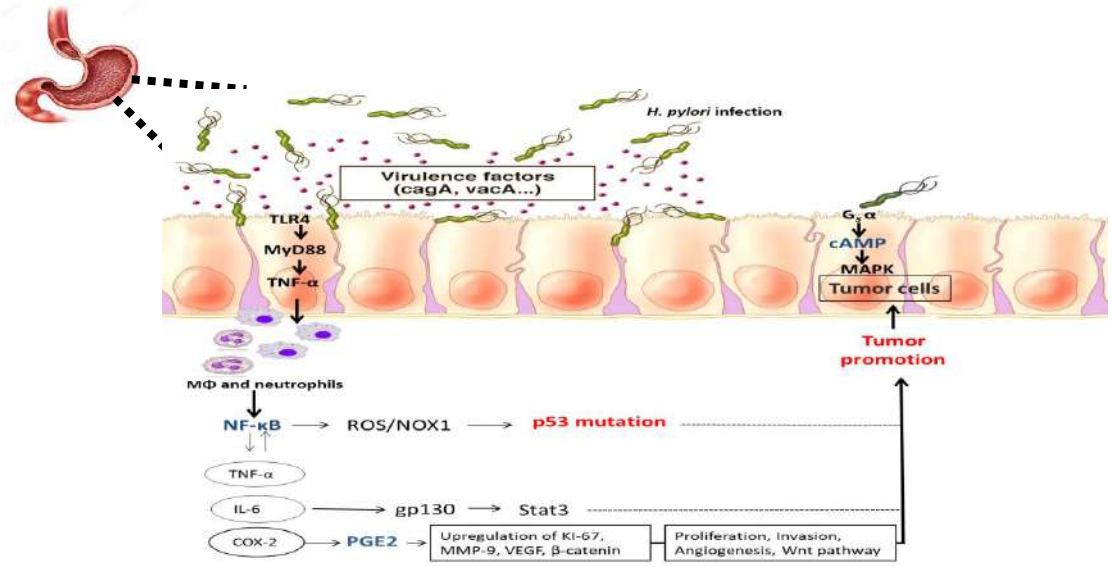

Figure - 1: *H. pylori*-induced chronic inflammation leading to gastric carcinogenesis.

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BOVINE COLOSTRUM

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Colostrum (commonly known as first milk, beestings, bisnings) is the first milk produced by the mammary glands of mammals (including humans) immediately following delivery of the newborn. It is a rich natural source of antibodies, nutrients and growth factors for the newborn.

Bovine colostrum is also known as cow milk colostrum, bovine colostrum immune milk, lactobin, LC2N, BCC (Bovine Colostrum Concentrate), hyperimmune milk, early milk, and lactoferrin. Bovine colostrum is collected from dairy cows shortly after calving. Dairy cattle are naturally exposed to different pathogens and produce immunoglobulins against them. These antibodies are present in the cow's blood stream and in the colostrum. These immunoglobulins are specific to many human pathogens, including *Escherichia coli, Cryptosporidium parvum, Shigella flexneri, Salmonella* species, *Staphylococcus* species, and rotavirus (which causes diarrhea in infants). Before the development of antibiotics, colostrum was the main source of immunoglobulins used to fight bacteria. In fact, when Albert Sabin made his first oral vaccine against polio, the immunoglobulin he used came from bovine colostrum. When antibiotics began to appear, interest in colostrum waned, but, now that antibiotic-resistant strains of pathogens have developed, interest is once again returning to natural alternatives to antibiotics, namely, colostrum.

Bovine colostrum is extremely nutritious and contains more nutrients than regular milk. In particular, it's higher in protein, fat, carbs, magnesium, B vitamins, and vitamins A, C, and E than cow's milk. While colostrum is rich in macronutrients,

vitamins, and minerals, its claimed health benefits are mostly linked to specific protein compounds, which include:

- ***Lactoferrin*:** Lactoferrin is a protein involved in your body's immune response to infections, including those caused by bacteria and viruses (4Trusted Source, 5Trusted Source, 6Trusted Source).

- ***Growth factors*:** Growth factors are hormones that stimulate growth. Bovine colostrum is especially high in two protein-based hormones, insulin-like growth factors 1 and 2, or IGF-1 and IGF-2 (1).

- ***Antibodies*:** Antibodies are proteins, also known as immunoglobulins, used by your immune system to fight bacteria and viruses. Bovine colostrum is rich in the antibodies IgA, IgG, and IgM

The Isle of man had a local delicacy called "Groosniuys", a pudding made with colostrum. In Finland, a baked cheese called Leipäjuusto is traditionally made with either cow colostrum or reindeer milk. A sweet cheese-like delicacy called 'Junnu' is made with colostrum in the south Indian states of Andhra Pradesh and Telangana. It is made with both cow and buffalo milk; in both cases it is the milk produced on the second day after giving birth which is considered best for making this pudding-like delicacy. Colostrum is in very high demand in these states, resulting in product adulteration.

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THE ROLE OF BACTERIOCIN IN FOOD SAFETY

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For the past years bacteriocin have attracted considerable interest for their use as safe food preservatives as they are easily digested by human gastrointestinal tract.

Now we all have to know what the name bacteriocin mean?

Bacteriocin was first observed by Gratia, but the term bacteriocin was coined by Jacobet al. Bacteriocins are antibiotic like agents produced by some bacteria which are encoded in the plasmids with the purpose of killing or inhibiting closely related species or even different strains of the same species.

Bacteriocin in different food system

Nisin producers, strain of Lactic Acid Bacteria (LAB) was approved by Food and Drug Administration FDA to be used as food additive in some foods.

Lacticin and pediocin producers have been used as protective cultures in food system.

Bacteriocin together with different preservation methods

Bacteriocin produced by some LAB have shown wide antimicrobial activity against food related pathogenic species such *Bacillus, Listeria, Staphylococcus* and *Clostridium.* In recent years bacteriocin having specifically narrow spectrum antimicrobial activity has been introduced.

Bacteriocins are used either directly in food systems or by addition of producer strains. By this way it is possible to prevent pathogenic microorganisms in various fermented food products. The effectiveness of the LAB bacteriocin may reduced due to their adsorption on hydrophobic surfaces and degradation with proteases. therefore combinational usage of bacteriocin with other preservation methods known as hurdle technology such as high hydrostatic pressure, pulse electrical field or essential oil ,were reported successful at inhibiting pathogens including gram negatives.

The use of bacteriocins as natural food preservatives fulfills consumer demands for high-quality and safe food without the use of chemical preservatives. Although bacteriocin as food additives can be limited for effectiveness of pathogen elimination or its high price research interest in bacteriocin continuing to search for new and more effective bacteriocin to address both biologic and economic concerns.

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PREBIOTICS

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Definition

Prebiotic was described as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health". The prebiotics concept was introduced for the first time in 1995 by Glenn Gibson and Marcel Roberfroid. According to this definition, only a few compounds of the carbohydrate group, such as short and long chain β-fructans [FOS and inulin], lactulose, and GOS, can be classified as prebiotics. In 2008, the 6th Meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) defined "dietary prebiotics" as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health. Prebiotics are present in fiber-rich foods, such as fruits, vegetables, and whole grains.

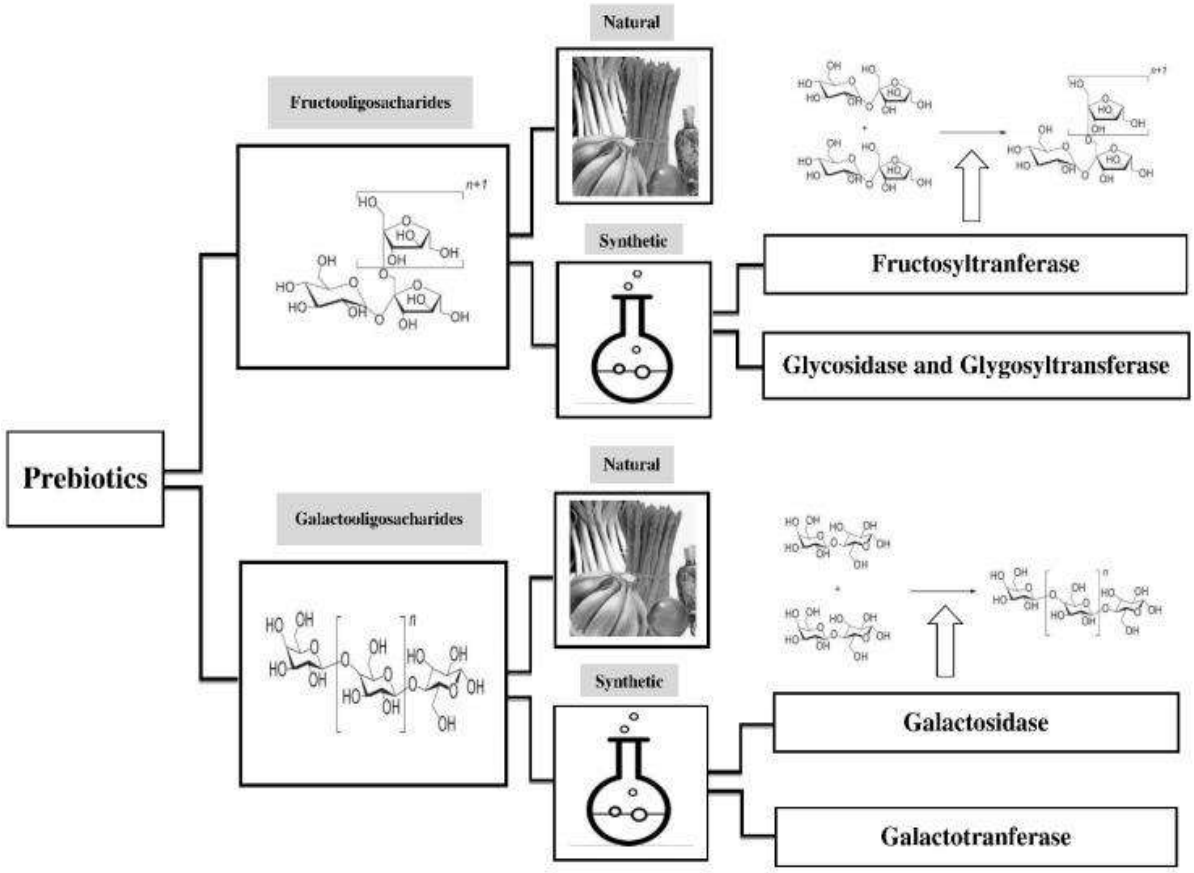
The following criteria are used to classify a compound as a prebiotic: (i) it should be resistant to acidic pH of stomach, cannot be hydrolyzed by mammalian enzymes, and also should not be absorbed in the gastrointestinal tract, (ii) it can be fermented by intestinal microbiota, and (iii) the growth and/or activity of the intestinal bacteria can be selectively stimulated by this compound and this process improves host's health.

Types of Prebiotics

There are many types of prebiotics. The majorities of them are a subset of carbohydrate groups and are mostly oligosaccharide carbohydrates (OSCs) namely Fructans, Galacto oligosaccharides, starch and glucose derived oligosaccharides.

Production of Prebiotics

Prebiotics play an important role in human health. They naturally exist in different dietary food products, including asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana, barley, tomato, rye, soybean, human's and cow's milk, peas, beans, etc., and recently, seaweeds and microalgae. Because of their low concentration in foods, they are manufactured on industrial large scales. Some of the prebiotics are produced by using lactose, sucrose, and starch as raw material. Since most prebiotics are classified as GOS and FOS regarding industrial scale, there are many relevant studies on their production.



Prebiotics and the Immune System

Consuming prebiotics can improve immunity functions by increasing the population of protective microorganisms. Animal and human studies have shown that prebiotics can decrease the population of harmful bacteria by *Lactobacilli* and *Bifidobacteria*. For example, mannose can reduce colonization of pathogens by promoting mannose adhesion to *Salmonella*. Mannose binds to *Salmonella* via type 1 fimbriae (finger-like projections). In addition, pathogens are not able to bind to the epithelium in the presence of OSCs. Prebiotics can also induce the expression of immunity molecules, especially cytokines. Interestingly, maternal prebiotics metabolites are able to cross the placenta and can affect the development of the fetal immune system. In 2010, Fugiwara and his co-workers reported that FOS administration in a pregnant mouse model modified offspring microbiota, and consequently, their skin inflammation was attenuated. In contrast, Shadid and his co-workers in a placebo-controlled, randomized, and double-blinded study demonstrated that bifidogenic effects of prebiotics supplementation in humans could not be transferred to the next generation.

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SOLID WASTE MANAGEMENT (BIOMINING) TO THE POLLUTION FREE ENVIRONMENT

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India has been doing combating the war against squander, be it through sound reusing rehearses or proficient removal of trash. In spite of the city being named the cleanest in India, in Swachh Survekshan study (2017), the dumping yard, outfitted by loads and stacks of rancid trash for quite a long time together, stayed a worry for authorities and residents the same. As presentation, the Rising earnings, quickly developing yet impromptu urbanization, and changing ways of life have brought about expanded volumes and changing piece which is expanding utilization of paper, plastic and other inorganic materials of city strong waste in India. The volume of waste is anticipated to increment from 65-75 million tons at present to 130 million tons by 2035.

Solid Waste Management Process is a composed by the procedure of assortment, transportation, capacity, handling and removal of solid refuse residuals in a built sterile landfill. It is a coordinated procedure involving a few assortment techniques, changed transportation gear, stockpiling, recuperation components for recyclable material, decrease of waste volume, and amount by strategies, for example, treating the soil; refuse derived fuel (RDF), waste-to-vitality, and removal in an assigned designed sterile landfill.

Bio-mining is the procedure which makes it conceivable to remove assets from the settled waste and reuse the equivalent profitably, while simultaneously delivering space for different utilizations at the landfill locales. It works both towards money related and ecological maintainability. The untreated waste, which implies a blend of biodegradable or wet waste and non-biodegradable waste from Indian urban communities lies for a considerable length of time and years at dumpsites where land was initially distributed for creating landfills for safe removal of just the leftover waste.

Most current biomining activities utilize normally happening microbial networks. Since these kinds of living beings are as of now normal in the earth, the dangers from the arrival of the organisms themselves into the neighborhood condition are viewed as generally little. The best natural dangers are identified with spillage and treatment of the acidic, metal-rich arrangement made by the organisms, which is like the corrosive mine waste from some deserted mines. This hazard can be overseen by guaranteeing that biomining is directed under controlled conditions with legitimate fixing and waste administration conventions.

The choice of a reasonable Solid Waste Management process is driven by the source and nature of waste created. Solid waste is produced from various sources which incorporate family units (kitchen and yard), business zones (eateries, inns and shops), enterprises (bundling and crude material), foundations (workplaces, schools and emergency clinics), development and destruction destinations, wild and trained creatures (excrement, bodies of dead creatures), parks which implies fallen leaves, branches from trees, and In boulevards, the recourses are black-top, blocks, sand, earth, solid, sediment, buildups from air statement, and residue.

Figure – 1: Experiment on Solid Waste Management in KSAH, KARE by J. Sivanantha (Left) & M. G. Arun Kumar (Right)

A successful technique for overseeing waste needs to begin with isolation of strong waste at the wellspring of age and the treatment of various parts of the loss in properly various manners, in this way diminishing the leftover waste that may some way or another go to landfills. While the standards of strong waste administration are as a rule better comprehended and more examined in open area, no Indian city has accomplished an all-encompassing answer for the difficulties of strong waste administration. The consideration with respect to city authorities to assortment of isolated waste and its transportation, treatment/preparing, reusing and safe removal is still in a beginning stage. Shopper personal conduct standards in Indian urban areas have likewise not adjusted to encourage the procedure of the board of this loss by isolating natural or biodegradable waste from other waste at the wellspring of age.

The Risks on bio-mining, there are a few potential ecological dangers related with bio-mining ventures and in this manner an arrangement tending to these potential dangers ought to be kept prepared. The greater part of the conditions present at the landfill and its environmental factors will be one of a kind to the particular landfill, and explicit to the age of the waste being exhumed. Significantly the dangers would be related with legitimate administration of perilous waste that may be revealed during the tasks of recovery, dealing with the arrivals of gases, smells, its related dangers to human wellbeing and controlling any fire, subsidence or breakdown. Ecological dangers can be overseen well whenever considered ahead of time of the tasks and suitable moderation measures have been structured by the executing office.

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Francisella tularensis: THE BACTERIUM OF BIOTERRORISM

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In the year of 1911 the bacterium *Francisella tularensis* was first discovered by Dr. Edward Francis in the Tulare county of California, hence the generic epithet of the bacterium was named after the scientist and the specific epithet was named after the place where it was discovered. It is Gram negative bacteria, Coccobacilli in shape and 0.2 - 0.7 mm × 0.2 mm in size. The size of the genome of this aerobic and non-spore forming bacteria is 1892 kb of DNA. This organism lacks any organ of motility, hence it is non motile. *F. tularensis* is not closely phylogenetically related to any pathogen or commensal of the human flora. It belongs to a group of intracellular bacteria, which includes *Mycobacteria, Listeria, Legionella, Brucella, Coxiella and Rickettsia*. *Francisella tularensis* is one of two species in the genus *Francisella*, which is the only genus of the family Francisellaceae, a member of the gamma-subclass of Proteobacteria. The family is distinguished by a unique set of phenotypic characteristics including a coccoidal morphology, gram-negativity, a capability to degrade only a limited number of carbohydrates resulting in acid but no gas production, a growth requirement for cysteine, and a unique fatty acid composition. *F. tularensis* is a fastidious bacteria it essentially needs cysteine enriched media for its growth. Sheep Blood Agar (SBA), Cysteine Heart Agar with Blood (CHAB), Martin Lewis, Thayer-Martin, Buffer Charcoal Yeast Extract Agar (BCYE), Cysteine Enriched Chocolate Agar, etc. are the suitable media to culture this organism. At 48 to 72 hours of incubation it forms colonies of 1 to 3 mm size and non-hemolytic, grey white in color.

The Japanese began germ warfare research using *F. tularensis* as early as 1932 and conducted biological warfare research with it on prisoners of war. Both of the former Soviet Union and the United States had bio-weaponized *F. tularensis* by the mid-20th, and both countries experimented with streptomycin-resistant strains of *F. tularensis*. In fact, a former Soviet Union biological weapons scientist has indicated that tularemia outbreaks on the Eastern European front during World War II were the result of intentional use of it. He also disclosed that the Soviets continued their biological weapons research into the 1990s, engineering strains of *F. tularensis* to be resistant to vaccines as well as antibiotics. In the event of a bioterrorist attack, tularemia would likely be released in an aerosolized form in a densely populated urban setting. In 1970, the World Health Organization (WHO) estimated that the release of 50 kg of *F. tularensis* by aircraft over a population of 500,000 people would result in 30,000 deaths and 125,000 incapacitated. They also noted that vaccinated individuals would only be partially protected from an aerosol exposure. In 1997, the CDC estimated that exposing 100,000 persons to an aerosol of *F. tularensis* would result in 82,500 cases of tularemia (82.5 % attack rate) and 6,188 deaths (6.2 % death rate), and cost between $456 million and $561.8 million.

After the 1972 convention on the prohibition of the development, production, and stockpiling of Bacteriological and Toxin Weapons, all United States stockpiles were destroyed. Since that time, the United States has continued defensive research in the areas of immunology, genomics, and vaccine research. We Indians should also uplift ourselves in the arena of such researches so that we can fight against any bioterrorists if biological war fare occurs in future.

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PROBIOTIC-DERIVED ANTIMICROBIAL PEPTIDES

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Pathogenic microorganisms have always been deliberated as a major reason for morbidity and mortality in humans. The world-wide arrival of multi-drug resistant (MDR) microorganisms including bacteria, fungus, virus etc. is progressively causing treatment failure. Several life-threatening infections triggered by numerous kinds of food-borne pathogens showing resistance to the conventionally used antibiotics may be caused by over-expression of MDR efflux pumps, remains the key concerns of clinicians as well as the microbiologists. To fight the MDR pathogenic infections, currently, many alternative regimens have been emerging, such as phytomedicines, probiotics and other natural products like honey. Probiotic proteins are precursors of various biologically active peptides including antimicrobial ones, and these have already been selected for application both as dietary supplements and as medicines.

Antimicrobial action of probiotic *Lactobacilli* may be demonstrated by one or combination of the following activities including competition for nutrients, adhesion and fabrication of different antimicrobial metabolites such as organic acids, hydrogen peroxide, bacteriocins, etc. Lactic acid bacteria (LAB) produce lactic acid and other organic acids consequently lower the pH of the environment and subsequently prevent the growth of the pathogenic microorganisms. The term 'bacteriocins' was firstly coined in 1953 by Jacob, these are ribosomally synthesized proteinaceous antimicrobial peptides (AMPs) produced by bacteria. Bacteriocins are most abundant and diverse

classes of antibacterial molecules having been identified in all major lineages of Eubacteria. Especially two types of bacteriocins (Lantibiotics and Non-lantibiotics with molecular weight <5 kDa and <10 kDa – >30 kDa, respectively) are common, which have been classified depending upon their characteristics and nature of activities. Different AMPs derived from whey proteins (from several sequences of α-lactalbumin and β-lactoglobulin) and caseins (related to α_{s1}-Casein, α_{s2}-Casein and β-Casein family peptides).

Now AMPs are redirected as model candidates for the improvement of new classes of antimicrobials effective against resistant microorganisms. Although, after formation of biofilms bacterial cells become thousand-folds more resistant to antibiotic treatments than the planktonic forms of the same bacterial strains, and biofilm-associated toxicities are a major health concern. Several types of antimicrobial peptides are being recognized in food protein hydrolysates and fermented food products. Bioactive peptides can be free from the parent protein by enzymatic hydrolysis during gastrointestinal digestion, fermentation or maturation during food processing or proteolysis by food-grade enzymes derivative from different origins like animal, plants and microorganisms. Generally chemical nature of these peptides is cationic as well as amphipathic (5–100 amino acids long) with secondary structures, typically α-helices and β-sheet, which can selectively interact with anionic membranes of microorganisms using electrostatic interactions. In addition to their antimicrobial action, it modulate the activity of inflammatory and epithelial cells, stimulating various processes such as mitosis, chemotaxis, protease-antiprotease stability, cytokine release, redox homeostasis and wound healing. Most of the current studies on probiotic-derived AMPs with molecular weight of diverse range from 0.2 to 1000 kDa and their activities against microorganisms portrayed in Figure - 1.

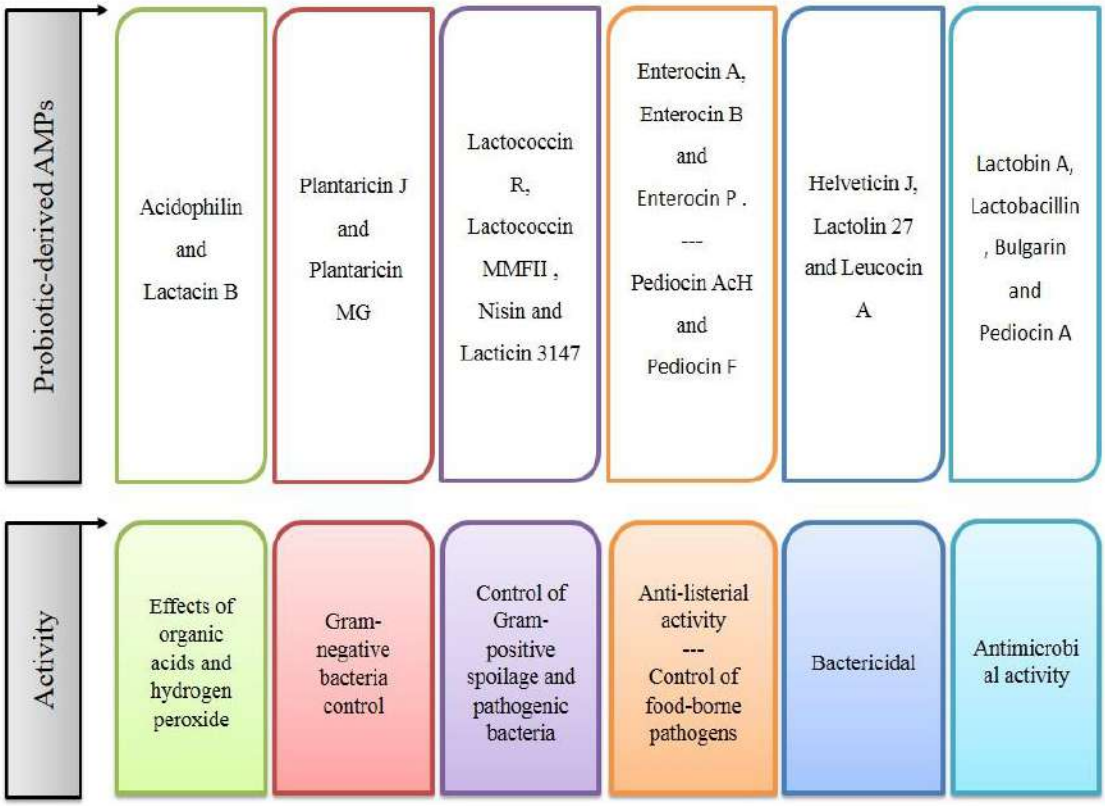

Figure - 1: Different type of probiotic-derived AMPs and their activities (Diagrammatic figure constructed by the author. Data collected from Mandal *et al*., 2014; DOI:10.1016/j.drudis.2014.05.019); AMPs: Antimicrobial peptides.

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EFFECT OF PARAQUAT ON SOIL MICROBIOTA

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Herbicides are the chemical substances that are used to control and destroy the unwanted vegetations, i.e. weed. Herbicides have its advantages over manual and mechanical control methods of weeding. Mechanical and manual weed control methods are much slower in wet soil but herbicides can be used successfully in these conditions. Use of herbicides as a chemical method for weed control has its economic advantages too. Weed control method by the herbicides are much cheaper than manual weed control. Due to these certain benefits in the use of herbicides over other weed control methods; farmers use it in higher quantities.

After herbicide application it may evaporate, washed out through surface run off, drained into deep soil, may become inactive due to the action of plants or maybe adsorbed in soil. Accumulation of these herbicides to toxic levels in the soil may leads to soil contamination and may become harmful to non target organisms e.g. beneficial microorganisms, crop plants, wildlife, etc. Accumulation rate may depend upon the physicochemical characteristics of the herbicides i.e. chemical stability, sorption in soil, solubility etc. The effect of herbicides on soil microorganisms depends on kind of microorganisms present as well as the chemical composition, dosage and shelf lives of herbicides. Herbicides may interfere to microorganisms mediated processes such as decomposition of organic matter, maintenance of soil texture, etc that are important in agricultural practices.

Soil microorganisms can help in degradation of particular herbicides as herbicides can be their source of nutrition. After the herbicide application if changes occur in growth and activities of a specific microbial strain, then this specific strain may act as a biological indicator of the applied herbicide. Therefore it is essential to study the effect of herbicides on soil microbiota.

Glyphosate is the most extensively used herbicides in the world, and its impacts on soil microorganisms when applied in different formulations of Roundup have been more comprehensively studied than any other herbicide. Paraquat is a non-selective bipyridilium herbicide that is used to control broad leaf weeds and grasses. The consumption of this product in tea of Assam and West Bengal is very high.

Research has shown that Paraquat has some transient negative effects on soil microorganisms more specifically bacterial and fungal communities, but actinomycetes population remains unaffected. There were no significant effects on soil enzymatic activity by Paraquat. Phosphatase activity can be analyzed by the method of Tabatabai *et al*. in 1969. When untreated soil was used as reference soil, significant decrease in phosphatase activity was found after 5th week in pure Paraquat dichloride, Gramoxone W and formulation additives treated soil, before there were no significant effects of these treatments were observed. Increment in the phosphatase activity was detected for both Alachlor and Paraquat herbicides in peat soil but the magnitude of the phosphatase activity was considerably decreased after incubation of 12 days. Initially Paraquat inhibited the phosphatase and dehydrogenase activity in rhizospheric soil of tea but the inhibition was significantly reduced in later observations. No significant changes found in beta glucosidase activity, and FDA analysis in Paraquat treated clay loam soil. There is no sufficient evidence that can prove the effect of different doses of Paraquat on soil enzymatic activities for sure so more work is needed in this field.

Studies have shown a stimulatory effect on CO_2 evolution by Paraquat but presence of organic matter may decrease rate of CO_2 evolution. Paraquat treatments may have some effects on biochemical processes of soil, either for short or long period, but these effects can be changed by addition of some metabolites or organic matter in soil.

Recently a Gram negative, symbiotic, nitrogen fixing bacterium *Ensifer meliloti* isolated from two locations of rice fields in Indonesia were found to be resistant to the 3.500 ppm of Glyphosate and 1.400 ppm of Paraquat. *Bacillus* sp. and *Pseudomonas* sp. were the most commonly isolated bacteria from herbicide polluted soils. Different Bacterial species *Bacillus, Kocuria, Enterobacter, Acinetobacter, Corynebacterium, Paenibacillus, Pseudomonas, Lerclercia, Proteus;* Actinomyces such as *Actinomyces naeslundi,*

Actinomyces isrealii, Actinomyces meyeri , Actinomyces viscosus; Yeast isolates *Candida krusei, Candida stellatoidea* and *Saccharomyces cerevisiae* while moulds *Cladosporium carionii, Talaromyces* and *Curvularia* sp. were identified as Paraquat and Carbofuran degrading microorganisms isolated from Delta State, Nigeria. So the researches are going on regarding the isolation and identification of herbicide degrading microflora. The isolated microbial species can also play a vital role as biomarkers for detection of herbicidal effects on soil. Culture independent methods can be used for the identification and selection of specific gene sequences from microbes for the development of biomarkers.

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BIOFILM SUPPRESSION OF PATHOGENIC BACTERIA BY PLANT EXTRACT OF *Azadirachta indica* L.

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A biofilm comprises any syntrophic consortium of microorganisms in which cells stick to each other and after also to a surface. These adherent cells become embedded. Biofilm may form on living or non-living surfaces & can be prevalent in natural. A biofilm serves to promote bacteria persistence by resisting antibiotic treatment and host immune responses. Biofilm have been reported to show increased resistance to antimicrobial agents including antibiotics compared to free-floating cells. Biofilm producing organisms like *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Frequently colonize catheters and medical devices and may cause foreign body related infections.

Biofilm can be composed of a single or multiple organism on various biotic and abiotic surface. There is association between biofilm production with persistent infection and antibiotic. The differentiation with respect to its biofilm phenotype might help to modify the antibiotics therapy and to prevent infection related to biomedical devices. These antibiotics principals are actually the defensive mechanism of the plant against different pathogens.

Biofilms have great significance for public health, because biofilm-associated microorganisms exhibit dramatically decreased susceptibility to antimicrobial agents. Biofilms are multi-cellular communities formed by bacteria, and they consist of bacteria encased within a non-crystalline extracellular matrix of proteins, polysaccharides, and small molecules. Biofilm formation provides increased protection of bacteria from antibiotics and host defenses.

Plants extracts (Neem) is used in traditional medicine as a source of many therapeutic agents in the Indian culture. Natural products are known to play an important role in human life. Various parts of the plants like root, bark, seed and leaves have been an important source of medicine since thousands of years. In recent years a predominant interest has been observed in evaluating different plant extracts for their antimicrobial properties against bacteria causing dental caries and periradicular pathology.

The extracts of neem when used as a medical value could be useful for the growth inhibition of the carcinogenic bacterium. *Azadirachta Indica* leaves possessed good anti-bacterial activity, confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care. Increasing resistance among pathogens to conventional antibiotics and undesirable side effects of existing therapies have made traditional medicinal plants an attractive source to screen for antimicrobial agents. *Azadirachta indica* (Neem) is perhaps the most useful traditional medicinal plant. Every part of the tree has been used as traditional medicine for household remedy against various human ailments.

The tree is still regarded as "Village dispensary" in India. *Azadirachta indica* has complex of various constituents including nimbin, nimbidin, nimbolide, and limonoids and such types of ingredients play role in diseases management through modulation of various genetic pathways and other activities. Quercetin and ß-sitosterol were first polyphenolic flavonoids purified from fresh leaves of neem and were known to have antifungal and antibacterial activities. Numerous biological and pharmacological activities have been reported including antibacterial, antifungal, and anti-inflammatory. Earlier investigators have confirmed their role as anti-inflammatory, antiarthritic, antipyretic, hypoglycemic, antigastric ulcer, antifungal, antibacterial and antitumour activities.

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MELANIN PRODUCTION BY ACTINOMYCETES

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Actinomycetes are generally Gram-positive and anaerobic and have mycelium in a filamentous and branching growth pattern. A group of Gram-positive bacteria that produce various bioactive agents including antibiotics, enzymes, and vitamins. The Actinomycetes have shown their importance Biotechnologically and Industrially. Naturally they are colourful organisms, which produce variety of intra and extracellular pigment including melanin with different biological functions. Approximately 7,000 metabolites derived from Actinomycetes were Reported in the Dictionary of Natural Products. Actinomycetes are produces dark- brown pigment in the culture media, generally referred to as melanin or melanoid pigments

Melanin are negatively charged compound composed of multi-function polymers and polyphenolic compounds that are produced by various microorganisms by fermentation oxidation. It is a pigment which is ubiquitous in nature and can be made using L-tryosine as precursor through the action of tyrosine. Biosynthesis of melanin is as tyrosine is converted into L-DOPA (3, 4- dihydroxy phenyl- L-alanine) in presence of tryosinase and further converted into dopachrome and indol-5, 6-quinone. Melanin is the main pigment responsible for the various pigmentations found in animal and human skin, hair, and eyes.

There are three basic types of melanin: Eumelanin, Pheomelanin, and Neuromelanin. The most common type is Eumelanin, of which there are two types—brown eumelanin and black eumelanin. Pheomelanin is a cysteine-derivative that contains polybenzothiazine portions that are largely responsible for the color of red hair, among other pigmentation. Neuromelanin is found in the brain. Melanin is an effective absorbent of light; the pigment is able to dissipate over 99.9 % of absorbed UV radiation. Because of this property, melanin is thought to protect skin cells from UV radiation damage, reducing the risk of folate depletion and dermal degradation, and it is considered that exposure to UV radiation is associated with increased risk of malignant melanoma, a cancer of melanocytes (melanin cells). Melanin may play a number of possible pharmacological effects such as protective, stimulatory, diagnostic and curative roles in human health.

Apart from the scavenging activity, melanin exhibits other biological activities, including thermoregulatory, radio- and photoprotective, antimicrobial, antiviral, cytotoxic, antiinflammatory, and immunomodulatory. Melanin from natural sources has reported to possess a broad spectrum of biological activities, which include protection against UV radiation, enzymatic lysis, damage by oxidants, resistance to drugs by pathogens, protection of insects against bacteria and antiviral protection. Melanin's have the ability to undergo polymerization is interesting in industry for its nanotechnology uses in bioplastic and biopolymers. Melanin compounds are irregular, dark brown polymers. In biological life, they have broad spectrum properties including antioxidant, antimicrobial activity, Antitumor activity, antivenin activity, anti-virus, hepatoprotective activity and Radio protective.

Melanin synthesis has been reported in various bacteria, including *Actinomycetes,* recombinant *Escherichia coli, Bacillus thuringiensis, Bacillus cereus Klebsiella* sp. GSK, *Pseudomonas stutizer* and recently by *Azotobacter chroococcum*. Some fungal species are also able to synthesize melanin, including *Aspergillus fumigates*, *Aspergillus Bridgeri*, *Pneumocystis carinii.* Future prospects for the purpose of the study may include various Potential Applications in Dermatology and cosmetics including specific zones of abnormally high pigmentation. Natural melanins and synthetic melanin-like nanomaterials have been suggested as novel nano-bio platforms in biomedical applications.

Recently, melanin production by microorganisms has attracted attention as an environmentally friendly and economic alternative to chemical production. Actinomycetes are the biotechnologically valuable bacteria which are well exploited for secondary metabolites. Naturally they are able to synthesize and excrete dark soluble pigments, the melanins or melanoid pigments.

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ANTIGEN - ANTIBODY RECATION

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Antigen is defined as any substance which when introduced parenterally into the living animal body evoke specific immune response either by producing specific antibody with which it reacts specifically and in observable manner or by producing specifically sensitized T-cell or Both. It is not necessarily microbes or their products but any substance foreign to the body may act as an antigen and stimulate specific immune Response.

Antibody are group of glycoprotein present in serum and tissue fluids of all mammals Antibody also called as immunoglobulin. The detailed structure of antibody was studied by Proter *et al*. (1962) by cleaving Immunoglobulin molecule. Based on finding porteral put forward a basic four chain model for immunoglobins containing two distinct type of polypeptide two heavy chains and two light chain linked together by di-sulphide bond and non-covalent linkage.

When an antigen solution is mixed in a correct proportion with a potent antiserum an antigen-antibody complex is formed. This reaction occurs in three phase. There are different type of reaction. This reaction play importance role *in vivo* as well as *in vivo* in body they play importance role in Ab-mediated immunity against infection disease, tissue injury against infection diseases. Tissue injury in hypersensitivity and autoimmune disease. *In vitro* antigen Antibody Reaction are useful in diagnosis of infection and epidemiological survey as well as identification of infections agents and identification of non-infectious agent such as enzymes.

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WST MICROPLATE ASSAY AS RAPID METHOD FOR DETECTION OF ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL PATHOGENS

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Antimicrobial susceptibility test

Antimicrobial susceptibility testing (AST) is a laboratory procedure performed by medical technologist to identify which antimicrobial regimen is specifically effective for individual patients.

Methods for antimicrobial susceptibility testing

a) Indirect method - Cultured plate from pure culture
b) Direct method - Pathological specimen (e.g., Urine, a positive blood culture or a swab of pus)

Gram positive bacteria are important nosocomial pathogens. The objective of this study is to estimate the frequencies and resistance rate of Gram positive pathogens that will be isolates from blood, wound, urine. Gram positive bacterial pathogens: *Staphylococcus aureus, Micrococcus* and *Staphylococcus epidermidis.*

Gram negative bacteria cause infection including pneumonia, blood stream infection wound or surgical site infections and meningitis in healthcare settings. Gram negative bacteria are resistant to multiple drugs and are increasingly resistant to most available antibiotics. Gram negative bacterial pathogens: *Escherichia coli* and *Pseudomonas aeruginosa.*

For antimicrobial susceptibility CLSI method at an incubation time 24 hrs give lower MICs. Extension of time is necessary to obtain consistent MIC. MICs can determine in 24 hrs using WST (Water soluble tetrazolium) – Colorimetric method. MIC can obtain after 48 - 96 hrs using broth micro dilution method. The WST- 8 assay will be useful method for rapid determination of consistent MICs for drug- resistant bacteria.

In addition, the Minimum Inhibitory Concentrations (MICs) against a variety of different pathogens were determined by susceptibility testing using the proposed method and compared with those obtained using the conventional broth micro dilution method. There was an excellent agreement between the results obtained using the WST-8 colorimetric method and those obtained using the conventional Clinical and Laboratory Standard Institute method. The WST-8 colorimetric assay is a useful method for rapid determination of accurate MICs for a variety of different microbes. Urinary tract infections are common infections that can be caused by many bacterial pathogens. the present study was conducted to isolate and identify bacteria culture from urine sample and compare the result of direct sensitivity test against Kirby-Bauer's disk diffusion antimicrobial sensitivity (AST) with respect to reliability time and cost.

MIC - Minimum Inhibitory Concentrations

MBC - Minimum Bactericidal Concentration

The majority of infectious diseases are bacteria in origin with the discovery of laboratory methods to grow these microorganisms using appropriate growth medium known as culture, determining the sensitivity and resistance of specific pathogens to a wide range of antimicrobial agents becomes necessary to the Health care providers can immediately Institute proper treatment regimens to their patients.

Newly Formulated Broth Microdilution test (WST)

Dispense 100 µl of medium into all wells

Add different concentrations of selected antibiotics (100 µl) solution in column 1 of well

Add prepared standard 0.5 MacFarland suspension of pure bacterial culture

Incubate at 37 ^{0}C for 4 - 8 hrs

Add prepared solution of Tetrazolium dye (WST-8)

Observe for color change

MIC can be taken as the lowest concentration of drug that do not show the change in colour at that concentration.

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EMINENCE OF PROBIOTICS

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The word probiotic comes from the Greek word pro means "promoting" and biotic means "life". Probiotics are live microorganisms which intends numerous health benefits while consumed. The probiotics are supplied through food, beverages and diet supplements. Many of the microorganisms in probiotics are similar to the normal flora of the body. Probiotics includes a variety of microorganisms. The most common probiotic bacteria are from the group *Lactobacillu*s and *Bifidobacterium* and probiotic yeast from the group *Saccharomyces*.

The probiotics benefits human with various health conditions. It treats acute watery diarrhea, diarrhea caused by Rotavirus and gastroenteritis. The researchers also concluded that probiotics can reduce the bad cholesterol. It can even reduce high blood pressure. Certain strains of probiotics are involved in treating Irritable Bowel Syndrome (IBS). Probiotics have been found from birth, new born acquires bacteria such as *Bacteroides*, *Bifidobacterium, Lactobacillus* from the mother. *Lactobacillus* are usually present in human gut and they are also found in the fermented foods like yogurt. The main role of it is aiding digestion. The *Lactobacilli* species mostly found in probiotic food are *Lactobacillus acidophilus, L. bulgaricus, L. rahamnosus, L. plantarium, L. reuteri, L. salivarius, L. casei* and *L. gasseri. Lactobacillus* are implicated to treat yeast infections, vaginosis, IBS, antibiotic related diarrhea. It also treats lactose intolerance, skin disorders such as canker sore, eczema and acne. It can even prevent respiratory infections. The other important group of bacteria, is bacteroides which play a major role in intestinal health. The bacteroides involved are *Bifidobacterium bifidum, B. lactis, B. longum, B. infantis, B. thermophilum* and *B. pseudolongum.* The benefit of *Bifidobacterium* includes improving

blood lipids and glucose tolerance. The other probiotic bacteria such as *Streptococcus thermophilus* prevents lactose intolerance and yeast probiotic *Saccharomyces boulardii* treats traveller's diarrhea, antibiotic associated diarrhea and acne.

The chief probiotic foods are kefir, yogurt and kimchi. Kefir is a thick and creamy fermented milk product. It is considered as an optimal probiotic food because it contains bacteria such as *Lactobacillus lactis, Lactobacillus kefir, Lactococcus cremoris, Lactobacillus casei* and also yeast. As kefir has lactic acid bacteria it treats diarrhea and boosts immunity. Then kimchi is a fermented vegetable made from Chinese cabbage, radish, green onion, garlic and ginger. It consists of any these bacteria, *Leuconostoc* species include *L. mesenteroides*, *L. citreum* and *Lactobacilli* species include *L. brevis, L. curvatus, L. plantarum, L. sakei* and *L. lactis.* The benefits of kimchi as probiotic are antiobeseity, anticonstipation, health promotion in colon, cholesterol reduction, immune promotion, skin health promotion, antioxidative and antiaging properties. The other probiotic, yogurt contains *Streptococcus thermophilus, Lactobacillus bulgaricus, L. acidophilus* and *Bifidobacterium bifidum*. Yogurt helps in the improvement of lactose intolerance and also improvement of dental and bone health. Other probiotic foods include natto, buttermilk, tempeh, miso, kombucha and sauerkraut. There are also plant probiotic bacteria which promotes plant growth. It includes *Bacillus, Paraburkholderia, Pseudomonas, Acinetobacter, Alcaligenes* and *Serratia* which constitutes applications such as increasing fruit yield, enhancing leaf length, leaf width, leaf number and also increases root and shoot lengths.

Probiotics are also used in animal feeding. Animal probiotic species includes *Bacillus Saccharomyces, Enterococcus and Lactobacillus*. The beneficial effects of probiotics include greater resistance to infections, improved digestion, better absorption of nutrients and improved milk quality in farm animals. Probiotic also has an enormous application in aquaculture. Fish probiotics include species of *Lactobacillus, Lactococcus, Bacillus* and *Enterococcus*. These probiotics reduces pathogenic bacteria, improves gut health, and efficiency in production of aquatic species.

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HOW DO BACTERIA FIGHT AGAINST VIRUSES?

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Bacteria vs viruses is one of the oldest fights on Earth. Certain viruses need to infect bacteria in order to reproduce but the bacteria do not want to be infected. How do bacteria that survive viral infection make sure that it does not happen again? Many kinds of bacteria have developed a process called CRISPR that helps them remember viruses they have seen before. CRISPR also allows bacteria to keep the virus from destroying them.

How does a virus reproduce? virus lands on and attaches to the outside of the bacterium and injects its DNA into the bacterium. If the bacterium does not realize that the viral DNA is not its own, it will follow the instructions in the viral DNA and make more viruses. The bacterium will make copies of viral DNA and lots of virus proteins and will allow the new viruses to assemble inside the bacterium. Finally, the new viruses burst open the bacterium and go out to infect more bacteria. Most bacteria that get infected by a virus they have never seen will die. Every so often, though, a bacterium does not die from viral infection. This might happen because of a mutation in that bacterium's DNA. Mutations are changes to the DNA sequence of a gene, like little mistakes, and they happen all the time in bacteria when they are copying their DNA for the next generation. Some of those mistakes kill the bacterium, so it does not get the chance to pass the mutation on to the next generation. Other mutations, however, might just slide by unnoticed, until the bacterium gets invaded by a virus! Suddenly it turns out

that the mutation actually helps the bacterium fight off the virus. The lucky few bacteria that have this helpful mutation are the ones that survive to reproduce, and they pass on those helpful mutations to their offspring. Those offspring in turn reproduce, and eventually the helpful mutation is present in most of the bacteria in the population.

Now you may be wondering what resistance to viruses looks like in bacteria, and this is where CRISPR comes in. CRISPR stands for Clustered Regularly Inter Spaced Palindromic Repeats. It is basically a description of some special regions of bacterium DNA. At these regions, there are two kinds of DNA sequences that alternate: repeats and spacers. Repeats are the same collection of letters repeated over and over, but the spacers in between them are all different. When scientists first found these special regions of DNA, they were not sure what their purpose was. But soon they realized that the spacers were often very similar to viral DNA.

In 2007, Rodolphe Barrangou and his lab decided to explore this idea. When Barrangou compared the CRISPR region of one non-virus-resistant type of bacteria to that of a virus-resistant version of the same species, they found out that the only difference between them was that the virus-resistant version had some extra spacers. They decided to do an experiment to figure out where these extra spacers came from. First, they exposed non-virus-resistant bacteria to viruses until the bacteria became virus-resistant. When they compared the CRISPR regions of the newly-resistant and non-resistant bacteria, they found that there were usually one to four new spacers in the resistant bacteria, and that those new spacers were similar to the DNA of the viruses the bacteria had been exposed to. This made the researchers think that the spacers might have been made from the viral DNA. Barrangou and his lab also deleted and inserted several spacers that matched different viruses. They found that when they deleted a spacer from a virus-resistant bacterium, that bacterium lost its resistance to the matching virus, and when they added spacers, the bacterium would be resistant to the matching virus, even if it had never seen that virus before. Barrangou and his lab concluded that the spacers in CRISPR regions provide resistance to viruses by saving some of the viral DNA, which allows the bacterium to "remember" them.

The resistance to the virus (immunity) arises in response to infection. Normally, immunity is not passed to offspring, but with CRISPR, it can be, because the immunity is actually encoded in the DNA, which is passed on through the generations. The bacterium clips a bit of the viral DNA and adds it into a CRISPR region of its own DNA. If the virus comes back, the bacterium makes RNA from the region of CRISPR specific for that virus. These RNA copies pair up with some CAS (CRISPR-associated) proteins. The RNA guides the CAS protein to the invading viral DNA, so the protein

can destroy it. No more viral DNA, no new viruses. These RNA copies pair up with some CAS (CRISPR-associated) proteins, which are made from CAS genes.

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CELL BASED THERAPY TO TREAT COVID-19

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The way of thinking of cell-based treatment has changed the field of regenerative medication towards the normal favourable to Physiological process. At present, a few cell-based treatment approaches are undergoing clinical preliminaries to arrive at an affirmed remedial of COVID-19. So far, stem cells, Natural Killer cells (NK), Dendritic cells (DN), and exosomes are the main applicants of possible therapeutics for COVID-19 treatment. Mesenchymal Stem Cells (MSCs) because of their pro-inflammatory and immune modulatory conduct, Natural Killer (NK) cells owing to their capacity of lysing virus-infected cells and direct the resulting immune reaction, Dendritic cells on account of immunotherapy and immunization, and MSC-inferred exosomes because of cell-based therapy and advantageous manufacturing angles, uphold the cell-based treatment application for treatment of COVID-19 and similar viral contaminations. Additionally ALVR109 (AlloVir) as an allogenic, virus-explicit T-cell treatment has been presented that this product is derived from healthy donors who have recuperated from COVID-19. As of late, AlloVir announced the start of phase-1 clinical trials for this cell-based treatment. Also, an imaginative way to deal with COVID-19 based on designed human MSC has been presented recently, which is consistently cleared and corrupted by the body's immune system during the antigen acknowledgement measure. Chinese analysts have reported that the counter acting agent reaction of this antibody is compelling and quick. This methodology has opened a new viewpoint for COVID-19 antibody research.

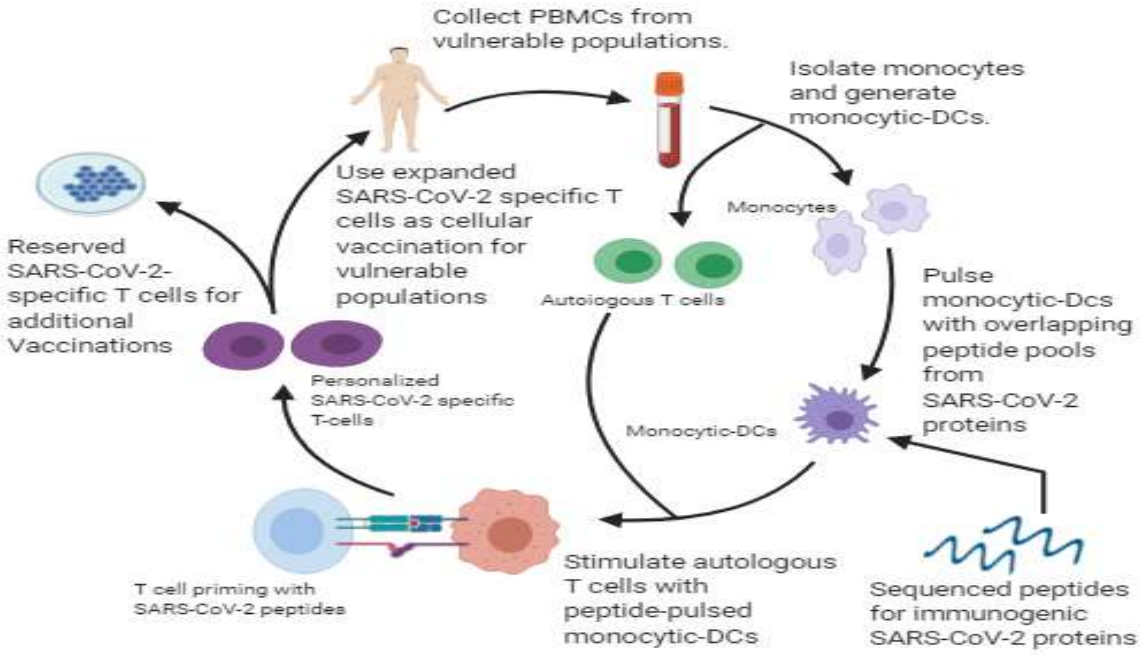

Figure - 1: Synopsis Schematic of DC and monocyte-based treatment that can be utilized in COVID-19 treatment

Generally, Cell based treatments can be arranged into two leading gatherings, including autologous and allogenic. However, both of these groups can be utilized in clinical applications by insignificant controls. The autologous source is produced at a little scope, in a dedicated set, with localized manufacturing facilities near the patients. One of the main challenges of autologous cell treatment is its deficiency to take advantage of the objective scale and cost-adequacy in providing treatment for a total treatment measure. The preparation of autologous cell therapeutics is very hand-worked and labor-intensive. The requirement for an excessive number of creation workers, the lengthy culture times, and pollutions increment the danger of disappointment of the analogous cell-based treatments.

Then again, creation measures for allogenic cell based therapies are like different biologics. This well spring of cells can be expanded a lot utilizing the current scalable technologies and banked through cryopreservation for future applications. Thus, allogenic cell treatments are utilized to produce bigger cell volumes at a diminished treatment cost. However, cell steadiness can be an obstacle with a long shelf-time. One of the primary worries in cell-based treatments costs. Not at all like regular therapeutics, ought cellular treatments to be conveyed on-request. Using a proper dynamic cost model

can push makers to assess the usage of developing elective innovations and determine the expenses related with the expanded market for the item and future development in demand.

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WORLD LARGEST FUNGI - HUMONGOUS FUNGUS

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The Humongous Fungus is the world largest and oldest living organism. The scientific name of humongous Fungus is *Armillaria ostoyae*. *Armillaria ostoyae* synonym is *Armillaria solidipes* is a species of plant-pathogenic fungus mushroom in the family of Physalacriaceae. It is the largest known terrestrial organism on the planet, according to the United States Forest Service. It is a deadly forest pathogen. The Humongous Fungus was discovered by taking samples from all the known infected trees in the park and comparing their fungal DNA. "Humongous fungus", sometimes called is by some counts still the largest living organism ever found. In the late 1980s, researchers discovered the biggest organism on record, "humongous fungus" on Michigan's Upper Peninsula that covered 37 hectares, about the same size as the Mall of America in Bloomington, Minnesotan. The Humungous Fungus Fest is held in August every year Aug 8-10, 2008. It covers 38 acres beneath an Iron County forest near the Wisconsin border. It is believed to be 1,500 to 10,000 years old and weigh about 100 tons. It's larger than the 110-foot long, 200-ton blue whale and occupies about 2,385 acres deep in Oregon's Blue Mountains. It's not only the world's largest mushroom; it is also the world's largest known organism. *Armillaria ostoyae* or, nickname is the Humongous Fungus.

It is an organism that covers 2,385 acres of the Malheur National Forest in Oregon. *Armillaria ostoyae* is common on both hardwood and conifer wood in forests west of the Cascade Range in Oregon, United States. The *Amarillia* grows by feeding off of tree roots, leaching off of them and actually killing them, causing them to decay. So, in a forest, it has a good shot of growing to a massive size. The fungus has a huge network of roots, called mycelia, it permeates below the ground of the forest. They usually pop up around the base of infected or newly-killed trees. The large clumps of yellow-brown mushrooms that appear above ground are the fruiting bodies of much larger organisms. They consist mainly of black bootlace-like rhizomorphs that spread out below surface in search of new hosts, and underground networks of tubular filaments called mycelia. This particular fungus it will produce mycelial cords the shoestrings also known as rhizomorphs. The bulk of this rhizomorphs behemoth resides underground in a stringy network of roots called rhizomorphs. There are two mating types for spores not male and female but similar in effect. Spores can be dispersed by environmental factors such as wind, or they can be redeposited by an animal.

Armillaria ostoyae in particular grows a wide and thin sheet-like plates radiating from the stem which is known as its gills. The gills hold the spores of a mature mushroom. This is stained white when seen as a spore print. Once spore formation is complete, this signifies a mature mushroom and now is able to spread its spores to start a new generation. *Armillaria ostoyae* can create such a toxic chemical environment. These fungi cause root rot disease in plants in forests, parks, orchards and vineyards across North America, Europe and Asia. *Armillaria* develop their unusual rhizomorphs, grow big and get good at killing host plants. The pathogenicity of *Armillaria ostoyae* appears to be more common in interior stands, but its virulence is seen to be greater in coastal conifers.

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BIOPLASTIC

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Plastic have varied application and have become an essential part of our daily lives. Plastic is used in several companies for a packaging of products. The production, use and disposal of the plastic emerged as a persistent and potential environmental nuisance. So, the improper disposal of plastic in our environment, resulting deaths of million animals annually and also the reduction in on fertility of soil. Therefore, the plastic products are manufactured to be biodegradable with similar functionality to that of conventional plastics. Which has reduced the dependence on petrochemical based plastics and released environmental problems. The bioplastic innovation would be a key to the long-term solution for the plastic pollution. However, a widespread public awareness is also essential in effecting longer term change against plastic pollution. Bioplastic are plastic materials produced from renewable biomass sources such as vegetable fats and oils, corn starch, straw, woodchips, saw dust, recycled food waste etc. bioplastics are used for disposable items such as packaging, crockery, cutlery, pots, bowls and straws. Biopolymer are available as coatings for paper rather than the more common petrochemical coatings. Bioplastics are made from agricultural by products and also from used plastic by using microorganisms. The bioplastic are biodegradable plastics and biobased origin of plastics which are derived from plant and microorganisms instead of fossil fuels. Similar to conventional plastic, the bioplastics also can be used in several ways under ordinary conditions sugar, disaccharides and fatty acid are also used as the basic raw materials in the production of bioplastics where the renewable resources are modified and processed into biobased plastics.

Bioplastics are differentiated into various types – starch, cellulose, polylactic acid, polyhydroxy alkanoate, fossil fuel-based bioplastics. The first bioplastic was invented with maize starch substituted plastics and sold under names such as ever corn and nature works. These plastics were manufactured by the blending of petrochemical plastic polymer compounds. Currently the starch-based polymer can be produced from potato, corn, wheat, tapioca. Cellulose is polymer of glucose in which the glucose units are linked by β- 1,4 glucoside bonds. The cellulose is found in the cell wall of all plants, green algae and fungi. The most predominant source of cellulosic plastic is cotton fiber and wood pulp. The organic cellulose esters and regenerated cellulose are the two types of cellulose plastics polylactic acid or polylactide plastic are most important bioplastics on the market. Polylactic Acid (PLA) is based on lactic acid and is mainly produced by the process of microbial fermentation of starch obtained from maize, cassava, potato, sugarcane and sugar beet.

Polyhydroxyalkanoates based plastic are produced from the plant-based starch by the process of microbial fermentation. Polyhydroxyalkanoates are having the physical and chemical properties very similar to polyester, polyethylene and polypropylene. The different group of Polyhydroxyalkanoates normally using for the manufacturing of plastics are Polyhydroxybutyric acid (PHB) and Polyhydroxybutylate polyhydroxy valerate (PHV), poly 3- hydroxybutyrate co -valerate (PHBV). They are used for large production of boioplastic. Fossil fuel-based bioplastic are biodegradable plastic can be manufactured not only from bio-based feed stock but also from the raw materials.

The bioplastic is suitable for a wide range of end of life options reuse, mechanical recycling, chemical recycling, organic recycling and energy recovery. The bioplastic wastes also act as potential substrates for composting since it is biodegradable in nature where the valuable organic materials are recovered as an end product. The main advantage of bioplastic product is that they are produced from renewable resources rather than fossil resources. Compared petrochemical plastics the bioplastic production can emit about 80 % less than carbon dioxide. The production of bioplastic also consumes 65 % less energy than the production pf petrochemical plastics. The bioplastics can avoid some of environmental problems like uncontrolled dumping on land and disposal at sea, and related emission of toxic substances. Disadvantage is the higher manufacturing cost of bioplastic is also limiting the use of these plastics.

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COVID-19: PATHOPHYSIOLOGY AND TRANSMISSION

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The Coronavirus illness 2019 (COVID-19) pandemic has caused an unexpected critical expansion in hospitalizations for pneumoniae with multiorgan sickness. Coronavirus is brought about by the novel extreme intense respiratory condition COVID 2 (SARS-CoV-2). SARS-CoV-2 disease might be asymptomatic or it might cause a wide range of side effects, for example, mild symptoms of upper respiratory tract infection and dangerous life-threatening sepsis. Coronavirus first arose in December 2019, when a group of patients with pneumonia of unknown reason was perceived in Wuhan, China. Starting at July 1, 2020, SARS-CoV-2 has influenced in excess of 200 nations, bringing about in excess of 10 million recognized cases with 688,000 affirmed deaths. This article sums up the current proof with respect to Pathophysiology, and the transmission of COVID-19.

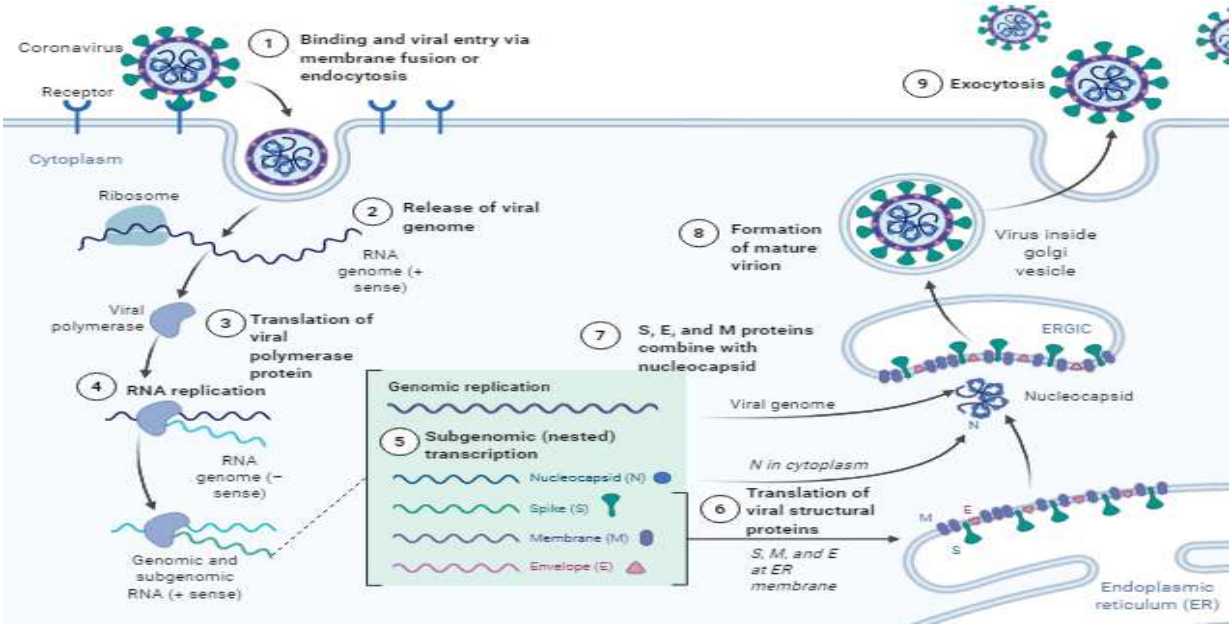
Pathophysiology

COVID-19 are huge, enveloped single-stranded RNA virus found in people and different warm blooded animals, for example, chicken, cows, pigs and winged creatures. COVID-19 cause respiratory, gastrointestinal, and neurological sickness. The most widely recognized COVID-19 in clinical practice are 229E, OC43, NL63, and HKU1, which regularly cause normal cold manifestations in Immunocompetent people. SARS-

CoV-2 is the third coronavirus that has made serious infection in people spread internationally in the previous twenty years. The first coronavirus that cause serious infection was extreme intense respiratory disorder (SARS), which was thought to start in Foshan, China, and brought about the 2002-2003 SARS-CoV pandemic. The second was the coronavirus caused Middle East Respiratory Condition (MERS), which began from the Arabian landmass in 2012.

SARS-CoV-2 has a breadth of 60 nm to 140 nm and distinctive spikes going from 9 nm to 12 nm, giving the virions the presence of a solar based crown. Through genetic recombination and variety, coronavirus can adjust to and taint new host. Bats are believed to be characteristic supply for SARS-CoV-2, however it has been proposed that people got trained with SARS-CoV-2 through a transitional host, for example, the pangolin.

Current comprehension of the severe acute respiratory condition COVID-19 (SARS-CoV-2) incited have resistant reaction. SARS-CoV-2 targets cells through the viral auxiliary spikes (S) protein that ties to the Angiotensisn Converting Enzyme-2 (ACE-2) receptor. The serine protease Type 2 Transmembrane Serine Protease (TMPRSS2) in the host cell further advances viral take-up by separating ACE-2 and initiating the SARS-CoV-2 protein. In the beginning phase, viral duplicate number can be high in the lower respiratory tract. Inflammatory signalling molecules are delivered by contaminated cells and alveolar macrophages in addition to enlisted T-lymphocytes, monocytes, and neutrophils. In the late stage, neumonic edema can occupy the alveolar spaces with hyaline layer arrangement, viable with early stage acute respiratory distress syndrome.



Transmission

Epidemiologic information proposes that droplets expelled during person-to-person contact during talking, coughing or sneezing is the most well known method of transmission. Prolonged exposure to a infected individual (being inside 6 feet for at any rate 15 minutes) and briefer exposure to people who are indicative (eg, coughing) are related with higher danger for transmission, while brief presentations to asymptomatic contacts are more adverse to result in transmission. Contact surface spread (contacting a surface with infection on it) is another conceivable method of transmission. Transmission may likewise happen through aerosols (small droplets that stay suspended in air), yet it is unclear if this is a huge wellspring of disease in people outside of a lab setting. The presence of mist concentrates in physiological states or the detection of nucleic acid noticeable in the air doesn't imply that little airborne particles are infectious. Maternal COVID-19 is right now accepted to be related with generally safe for vertical transmission. In most detailed arrangement, the mom's contamination with SARS-CoV-2 happened in the third trimester of pregnancy, with no maternal passings and a great clinical course in the youngsters.

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FORMULATION OF ALTERNATIVE CULTURE MEDIA BY USING WHEY

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Microbiological studies depend on the ability to cultivate and maintain microorganisms under laboratory conditions by providing suitable culture media that offer favourable environmental conditions. A nutrient material prepared for the growth of microorganisms in a laboratory is called culture media. Microorganisms can obtain energy directly from sunlight while carbon can be made available in organic forms such as carbohydrates, or inorganic form such as carbon dioxide and water. Nutrient agar medium is commonly used as general purpose medium for the cultivation of broad range of bacteria. It is a basic media composed of peptic digest of animal tissue, beef extract, yeast extract, sodium chloride and agar. PDA is commonly used for the growth of fungi in laboratories and composition is well defined. PDA contain potato infusion, dextrose and agar. The readymade media PDA, NA and commercially available media such Cetrimide agar, MacConkey agar etc. are used for the growth of microorganisms but these are very expensive to be used in schools and colleges. Microbiological researchers are carried out at high cost and scarcity of culture media. It is one of the serious problems in a developing country. Microbial research is hindered by high cost of culture media. Therefore, we have to try to use various alternative media and agar to reduce the cost involved.

In today's large quantities of whey are produced in cheese making industry and the whey can be harmful to the environment if disposed on to farmland or into water ways. Elimination of millions of tons of whey is the major challenging problem among the world. In other hand the whey is widely used as valuable ingredient in the food industry. So lot many researchers are carried so as to use whey for production of cheap media. Because the whey contain high nutritional value and it can influence on the growth of microorganisms. Different types of media for the growth and isolation of organism have been reported from this whey. We can cultivate both fungi and bacteria by using this alternative culture media. It is like easily available and low cost material for the culture media preparation. In the present study an innovative way was attempted to use the sweet whey for the production of general microbiological growth media. The study was based on the fact that the whey product is rich in nutrients which support growth of bacteria and fungi.

Utilisation of whey product as a culture medium for *rhizobium meliloti* is very effective. Mannitol agar medium is the standard medium for the cultivation of *Rhizobium* species because of the mannitol are rich in carbon source. It is the routine laboratory medium. The standard medium contains source of nitrogen, growth factors and different minerals such as potassium, phosphorus, magnesium sulfate and sodium chloride. The whey medium also contain carbon source (lactose), nitrogen, potassium, sodium, calcium and magnesium. The present study shows that the whey is a suitable inexpensive medium for the massive production of *Rhizobium meliloti*. Pepsinized sweet whey medium is suitable for the cultivation of *Lactobacillus acidophilus.* Three pepsinized whey based media are used for the cultivation. The concentration of whey in each media are 2.5, 5, 7.5 % respectively. The culture reached its maximum population in media which contain 2.5 and 5 % whey only after 18 hours. The population in 7.5 % is lower than maximum in 2.5 and 5 % medium. In 7.5 % whey contain medium the organisms appeared to enter a death phase after 12 hours incubation.

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THE COLONY OF METAL DEGRADING HALOPHILIC BACTERIUM FOUND ON R.M.S. TITANIC: LEADING TO DESTRUCTION & SUNKEN IT'S HULL

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Halomonas titanicae is a Gram negative, halophilic species of proteobacteria, aerobic, non-endospore-forming, peritrichously flagellated and motile bacterial strain, which was discovered on rusticles recovered from the wreck of the RMS Titanic. Cristina Sánchez-Porro researcher at Dalhousie University in Halifax, Canada, gathered samples and first separated the bacterium in 2010 from rusticle acquired from the RMS Titanic in 1991. One of the specialists, Henrietta Mann has assessed that the activity of organisms like *Halomonas titanicae* that may achieve the absolute destruction of the Titanic by 2030. While the microorganisms have been recognized as a possible risk to oil rigs and other man-made items in the remote ocean, it likewise can possibly be utilized in bioremediation to quicken the decay of wrecks littering the sea depths. In summer 2016, utilizing neutron diffraction, the offices of the Institut Laue Langevin exhibit that an atom called ectoine is utilized by these microbes to endure the osmotic weight that salt water causes on their films. Systematically, the sort *Halomonas* is heterogeneous and to date incorporates in excess of 60 species. The most as of late depicted species, at the

hour of composing, are *Halomonas ilicicola*, *Halomonas fontilapidosi* and *Halomonas zhanjiangensis*.

Despite the fact that the arrangements were collaborating with life, no one had distinguished the particular microorganisms on the boat, rather gathering them into general classes, for example, microbes or growths. The one they picked ended up being another species, which the pair named *Halomonas titanicae*. The microbes are important for a family that had never been found in waters as profound as those in which the Titanic sits, about 2.4 miles (3.8 kilometers) underneath the surface. These bacterial cells are rod shaped, 0.5 – 0.86 millimeter, happen separately are motile by peritrichous flagella. Their settlements are smooth, raised, white–cream and 1.0 – 1.2 mm in measurement following 3 days of brooding on 10 % Mueller Hinton Agar medium at 37 degree celsius. In fluid medium, cells develop on the outside of the medium. Decently halophilic and fit for filling in salt fixations somewhere in the range of 0.5 and 25 % weight by volume Sodium Chloride; no development without Sodium Chloride. Ideal development is at 2 – 8 % weight by volume of Sodium Chloride. The temperature range for development is 4 – 42 degree celsius, with ideal development at 30 – 37 degree celsius. Development happens at pH 5.5 – 9.5 and is ideal at pH 7.0 – 7.5, Chemo-organotrophic. Metabolism is respiratory with Catalase and Oxidase is delivered. Corrosive nature is developed from D-galactose, D-glucose and D-fructose yet not from D-arabinose, maltose, D-mannose, glycerol, D-mannitol, trehalose or D-xylose. All individuals from the Halomonadaceae are aerobes; some *Halomonas* species have been appeared to develop anaerobically with nitrate, nitrite or fumarate as an electron acceptor and with glucose on strong media in fixed containers. They have been secluded from various water and soil conditions and mostly from saline, hypersaline or soluble natural surroundings. They are viewed as non-pathogenic; nonetheless, a human contamination brought about by *Halomonas venusta*, following a fish chomp, has been accounted for. As of late some different *Halomonas* strains have additionally been perceived as human microbes, causing diseases and defilement in a dialysis place. The comprehensive ordered portrayal of these segregates has allowed them to be depicted as three novel types of *Halomonas*: *Halomonas stevensii*, *Halomonas hamiltonii* and *Halomonas johnsoniae*.

The Titanic sank 98 years ago and sat largely undisturbed on the seafloor until its rediscovery in 1985. Since then researchers have learned that microorganisms, seafloor currents, and the explorers themselves have been hastening the destruction of the ship. A few specialists want to protect the disaster area by executing the metal-eating microbes and protecting the boat from flows, permitting sightseers and narrative producers to visit Titanic for quite a long time to come.

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GREEN TEA ACT AS WOUNDERFUL HERB

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Green tea has been used as a medicine for thousands of years, originating in china but widely used through Asia. It's consumption has great health benefits like prevents cavities, lower cholesterol, protects against heart disease, prevent bad breathe, etc. Green tea solution use as hand disinfectant. By drinking and gargling could be useful against to prevent transmission of flu virus. It has great antimicrobial effects against a variety of Gram positive and Gram negative bacteria (e.g., *Escherichia coli, Salmonella* spp*., Staphylococcus aureus, Enterococcus* spp.), some fungi (e.g., *Candida albicans*), and a variety of viruses (e.g., HIV, Herpes simplex, Influenza). Studies have been done that show the ability of green tea is act synergistically with gentamicin and amikacin against *Escherichia coli*. In ancient times green tea used for treating diarrhea and typhoid.

Green tea contains the natural element called catechin. There are four types of catechins: epicatechin, epigallocatechin, epicatechin-3-gallate, and EGCG. Its amount is higher than other tea. The amount of catechins depends on where on the plant the leaves are harvested, leaf processing, geographical location, growing conditions, and tea preparation. It is act as natural anti-oxidants and prevents cell damage. It can reduce the formation of free radicals in the body, protecting cells and molecules from damage. Green tea also useful in prevent of food poisoning. As it can kill bacteria, drinking green tea with meals may reduce the risk of bacterial food poisoning. It prevents the growth of dangerous intestinal bacteria like *Clostridia* and *Escherichia coli* and promotes the growth of friendly *Bifidobacteria*. Catechins of green tea have inhibitory effects against *Helicobacter pylori* infection as well as influenza virus, Herpes simplex virus and

adenovirus infection. By daily drinking 3-5 cups of green tea give good health benefit. There are flavonoids which is present in green tea helpful in insulin-enhancing activity. Green tea can inhibit digestive enzymes and absorb fat, which leads to decreased body waist circumference, intra-abdominal fat, plasma total and LDL cholesterol, triglycerides, and blood pressure. Studied has been shown that green tea catechins have the ability to interfere with DNA replication by interacting with, and thereby inhibiting the function of DNA gyrase.

There are many health benefits by consuming green tea like including the prevention of cancer and the anti-inflammatory, anti-arthritic, antibacterial, anti-angiogenic, anti-oxidative, anti-viral, neuroprotective, cholesterol-lowering effects and is beneficial in Cardiovascular Disease (CVD), diabetes and obesity, and neurologic and oral health of green tea and isolated green tea constituents are under investigation. Green tea linked to the prevention of different kind of cancer, like lung, colon, esophagus, mouth, stomach, small intestine, kidney, pancreas and mammary gland. Its components possess anti-oxidant, anti-mutagenic, anti-carcinogenic effects. Due to this it gives protection against environmental agents which can causes cancer. Research with HIV-1 has shown that green tea EGCG binds to the CD4 T-cell receptor, blocking the binding of the virus. This ability to block viral cell binding has been proposed for use in therapy for HIV-1 infection.

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DINOSAURS AND MICROORGANISMS: COMMUNITY AND HISTORICAL ASPECTS

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For a very long time after their discovery, and their succeeding acknowledgement as a formal group by Sir Richard Owen in 1842, dinosaurs were considered as heavy and clumsy big lizards, with an aggressive or solitary attitude. However, since the early 1970s, a revolution in the field of paleontology has completely changed the usual iconography of these extinct vertebrates, providing a rather different and more exciting framework for restructuring their paleobiology. During successive decades, within the framework of a another "golden age" of dinosaur studies, various new species have been described from both Laurasia and Gondwana, and a complex, multi-dimensional picture of the physiology, evolution and behavior of dinosaurs has arose.

Surprisingly, many of the possible unintended inferences about the biology and complex behavior of various groups of dinosaurs came from a discipline that had long lingered in the shadows, and had been considered incidental compared with research on body fossils: ichnology, i.e. the study of the life traces of an organism. For dinosaurs, this involves study of footprints and track ways, burrows, coprolites (fossil dung), eggs and nesting sites. In recent decades a new multidisciplinary approach to paleoichnology has shown the huge inferential power of fossil traces, throwing light on biological and behavioral aspects.

Fossils were assumed to lack original organic molecules due to decaying process, but chemical analyses show that some can survive. Dinosaur bones has been suggested to preserve collagen, osteocytes, and blood vessels. During the fossilization process, biological tissues degrade over millions of years, with some types of molecules breaking down more rapidly than others. However, few traces of biological material have been found inside some fossils. While some researchers believe these could be the remains of primordial proteins, blood vessels, and cells, conventionally believed to be among the least stable components of bone. One hypothesis is that they are in fact biofilms formed by bacteria.

An international team of researchers led by Evan Saitta, Field Museum of Natural History, Illinois, U.S.A proposes another source of the biological material found in these fossils. The proteins, they claims, are not dinosaur in origin, but microbial. Around 76 million years ago, a massive herd of the horned dinosaur *Centrosaurus (Centrosaurus apertus)* died in what is now Alberta, Canada. While they were still alive, these creatures, like all other animals have had trillions of microbes living inside their bodies and on their skin. And even now, long after their demise, their remains still harbor life with a distinct microbiome all of its own. Biological and chemical analyses of Late Cretaceous dinosaur bones and sediment matrix also showed that dinosaur bone hosts a diverse microbiome. Fossils and matrix were analyzed using High-performance liquid chromatography (HPLC), Qubitfluorometer, Energy dispersive X-ray spectroscopy (EDS), Light microscopy, Attenuated Total Reflectance Fourier - Transform Infrared Spectroscopy (ATR FTIR), Variable Pressure Scanning Electron Microscopy (VPSEM), Pyrolysis - Gas Chromatography-Mass Spectrometry (Py-GC-MS), Radiocarbon Accelerator Mass Spectrometry (AMS), Epifluorescence microscopy, Propidium iodide (PI) and SYTO 9 staining and 16SrRNA gene amplicon sequencing. The fossil organics differ from modern bone collagen chemically and structurally. A key finding is that 16S rRNA amplicon sequencing shown that the subterranean fossil bones host a unique, living microbiome different from that of the surrounding sediment. The team found 50 times more microbial DNA within the bone fragments than in the mudstone closely surrounding them. It could even see some of the cells under a microscope. This microbial community is dominated by a few bacterial species that are very different than those in the surrounding sediment. Their exact identity is still uncertain, but around a bit of the DNA is a close by match for *Euzebya*, a bacterium that's been found in Etruscan tombs and in the skins of Japanese sea cucumbers. Even in the subsurface, dinosaur bone were biologically active and behaved as an open system, attracting microbes that might alter original organics or obscure the identification of original organics. These results propose caution regarding claims of dinosaur bone 'soft

tissue' preservation and demonstrate a potential role for microbial communities in post-burial taphonomy.

Also, microbiota sharing as a trigger for sociality in herbivorous dinosaursin large nesting sites, excrement is sure to be deposited, and it may even be used as a building material in the construction of nests, as in many birds today. Therefore, large nesting sites obviously provide numerous opportunities for the gut-associated microbiota to be transmitted from one generation to the next for transmitting symbiotic microbial communities would be one of the most important factors for the continual, independent appearance of social behavior among vertebrates. In recent decades, the symbiotic microbiome found in nearly all groups of hosts, has increasingly been shown to be crucial for the host's fitness, in some cases proving to be essential for host survival. Among the various beneficial effects for the host, gut microbiota prevents the invasion by pathogens by stimulating the immune system, are able to extract nutrients and thus the energy necessary for the host, and can also promote the tissue differentiation.

It is sobering to think that microscopic beneficial bacteria, invisible to the human eye, may have acted as internal pilots driving even the social evolution of titans weighing as much as 80 tons.

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ENDOPHYTIC FUNGI: A SUSTAINABLE DEVELOPMENT OF PHARMACEUTICAL DRUGS

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Communicable diseases are causing most dangerous health problems which increase the morbidity and mortality in developing countries. The increase in resistance toward multiple drugs is a major problem in the treatment of infectious diseases caused by pathogenic microorganisms. This multidrug resistance is presently an urgent focus of research and new bioactive compounds are necessary to battle these multidrug resistance pathogens. Therefore, there is an urge to investigate new bioactive compounds. The current trend is about opting the natural products from microbial, plant and animal origin. Over the years, natural products have been playing a key role in the search for novel drugs. Natural products are naturally derived metabolites and byproducts from microorganisms, fungi, plants or animals. The naturally derived products are harmless and of low cost in the prevention of diseases. Therefore, in the present scenario a huge work is being carried out in the field of endophytic fungi for the isolation of novel bioactive compounds. The endophytes are the microorganisms which live inside the plants without causing any symptoms and have been known from thousands of years.Endophytic fungi are considered as a promising way to overcome the problem of drug-resistant microbes of humans and plant pathogens, such as an endophytic fungi *Pastelotiopsismicrospora*of *Fagraeabodenii* yields highly functionalized cyclohexenone called as ambuic acid responsible for the inhibition of biosynthesis of cyclic pepetidequormones of *Staphylococcous aureus* and *Listeria innocua*. In another investigation the metabolites

rubrofusarin B, fonsecinone A, asperpyrone B and aurasperone A from an endophtic fungi *Aspergillus niger* IFB-E003 in *Cyndondactylon* were isolated which displayed growth inhibitions against pathogenic microorganisms having Minimal Inhibitory Concentrations (MICs) range between 1.9 - 31.2 μg/ml. The two recently recorded antibiotics pyrrocidines A (C31H37NO4) and B (C31H39NO4) obtained from *Acremonium zeae* endophytic in maize represents notable antibacterial activity against the most gram-positive bacteria and also to drug resistant strain and antifungal activity against *Aspergillus flavus* and *Fusarium verticollioides*. Similarly, the endophytic fungus *Cryptosporiopsis cf. quercina* was reported producing cryptocandin a novel antifungal compound that holds the capacity to inhibit the activity of *Candida albicans*, and *Trichophyton rubrum*. Also displayed the inhibitory effect against many plant-pathogenic fungi including *Sclerotinia sclerotiorum* and *Botrytis cinerea*. Recently, some endophytic fungi are reported effective against the causal organisms of tuberculosis. Tenuazonic acid was separated via bioassay-guided fractionation of dichloromethane extract of *Alternaria alternate* that displayed activity against *Mycobacterium tuberculosis*. *Fusarium solani* from the medicinal plant *Glycyrrhiza glabra* found in Kashmir Himalayas was reported to be highly active against mycobacteria and non-mycobacteria pathogenic strains. The discovery of potent antiviral compound from endophytic fungi is yet in its infancy. Limited endophytic fungi are known to have antiviral activity. However, some endophytic fungi showed promising results against viruses. For example, a unique quinine related metabolites xanthoviridicatins E and F obtained from endophytic *Penicillium chrysogenum* possess the ability to inhibit the cleavage reaction of HIV-1 integrase. In the last few decades, endophytic fungi have become the center of attraction for many researchers. Some endophytic fungi are identified as a sustainable source that yields potent anti-inflammatory drugs. To illustrate, *Talaromyces wortmannii* an endophytic fungus display activity against *Propionibacterium acnes* and also shows anti-inflammatory properties by inhibiting TNF-a-induced ICAM-1 expression and *Propionibacterium acnes* - induced IL-8 release. Endophytic fungi produce a plethora of bioactive metabolites which has shown promising potential and usefulness in safety and human health concerns. It is a wide field of research that is almost completely open for new finding. Isolation of endophytic fungi from medicinal and other plants results to produce bioactive compound which has greater activity against various pathogenic microbes. Therefore, the use of endophytic fungi can unlock the novel vicinity of biotechnological exploitation which may lead to discovery of novel bioactive compounds and can help the human being against a number of pathogenic diseases.

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IMPLICATION OF *Escherichia coli* STRAINSIN FOOD BORNE DISEASES IN TOGO

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Foodborne infections are a global public health issue, especially in African countries which estimation involved food borne infections to be responsible of 137000 deaths by year with more than 91 million cases. Togo is a country located at West Africa, limited by Ghana and Benin respectively at west and east, Burkina Faso at the north and is opened to sea by his south. His capital is Lomé and his population is estimated to be 84,28,439 habitants.

Studies conducts in Togo about food borne infections designed Total coliforms, thermotolerant coliforms, *Escherichia coli, Staphylococcus aureus* and *Salmonella* to be the main bacteria responsible. *Escherichia coli* are mostly harmless bacteria that live in the intestines of people and animals and contribute to intestinal health. Some types of pathogenic *Escherichia coli*, such as Shiga toxin-producing *Escherichia coli* (STEC), can be life-threatening. *Escherichia coli* is a coliform bacterium, one of the 30 members of the bacterial family of Enterobacteriaceae. It is a Gram negative, non-spore-forming, facultative, anaerobic, rod shaped, mesophilic bacterium that grows in 37 – 45 °C. Presence of coliform group in food is indicative of fecal contamination and poor hygienic conditions.

In Lomé, 68.89 % of the samples of ready-to-eat (RTE) of out of home food analyzed were unsafe and between them, *Escherichia coli* induced 33.33 % non-conformities. In the north of the Togo, an evaluation conducted on the food safety of meals sold around the streets of Kara city isolated *Escherichia coli* to be responsible of 6,70 % non-conformities.

Symptoms, Sources and Transmission

Usually it is abdominal cramps and diarrhea which can progress to bloody diarrhea, as well as fever and vomiting. The incubation period ranges from 3 to 8 days with a median duration of 3 to 4 days. Most patients recover within 10 days, but for some (especially young children and the elderly) the infection can progress to a life-threatening form, such as hemolytic uremic syndrome, which is characterized by acute kidney failure, hemolytic anemia and thrombocytopenia. The most documented form of *Escherichia coli* producing Shiga toxins relates to serotype O157: H7. The reservoir for this pathogen appears to be primarily cattle. *Escherichia coli* O157: H7 is transmitted to humans primarily through contaminated foods, such as raw or undercooked ground meat and raw milk. Contamination is also possible with the consumption of fruits and vegetables; cases of water transmission have also been reported. Human-to-human transmission *via* the faecal - oral route is also important.

Prevention

Some tips can help to stop the transmission of *Escherichia coli*. It can be resumed as :

- Wash hands with warm water and soap for at least 20 seconds and wash the inside walls and shelves of the refrigerator, and utensils.
- In industry, it is essential to apply a bactericidal treatment to food, such as heating (cooking or pasteurization for example) or irradiation, which is the only effective method to eliminate *Escherichia coli* which produces Shiga toxins; Fruit and vegetable producers should protect fields from contamination by animal faeces, use treated fecal waste, and monitor and manage the risks associated with irrigation water. They must also ensure that harvesting and storage equipment is clean and dry.

Strains of pathogenic *Escherichia coli* reported in TOGO

- A presence of Extended Spectrum β-lactamases (ESBLs) producing *E. coli* in Togo was previously declared. Extended Spectrum β-lactamases (ESBLs) are enzymes produced by some bacteria that inactivate oxyimino-cephalosporins, a class of β-lactam antibiotics family. Their profile is TEM/SHV, TEM/CTX-M1and TEM/SHV/CTX-M1.
- Multidrug resistant Uropathogenic *Escherichia coli* were also isolates from Center of Regional Hospital of Lomé (Togo) from September 2017 to March 2018.
- *Escherichia coli* and *Klebsiella* spp. were found harboring at least one *qnr* gene consisting of 47.74 % *qnrB*, 47.10 % *qnrS* and 2.58 % *qnrA*. Strains encoding *qnr* genes, carried *CTX-M1*, *TEM* and *SHV* type ESBL. The presence of *qnr* genes induces fluoroquinolone resistance.

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GENOTYPE OF *Vibrio cholerae* STRAINS CIRCULATING IN AFRICA

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Cholera is an acute diarrheal infection caused by ingestion of food or water contaminated with *Vibrio cholerae*. Every year, cholera cases are reported to be about 1.3 to 4.0 million cases and 21000 to 143000 deaths in worldwide. This disease has a significant socio-economic impact and represented a global threat to public health. Cholera pandemics origin is the Ganges Delta in India and during the 19th century, the disease killed millions of people in all continents. The seventh and final cholera pandemic began in South Asia in 1961 and reached Africa in 1971 and the Americas in 1991. According to World Health Organization report, 54% of all reported cholera cases were from Africa in 2016. The epidemiology of cholera in Africa in recent years shows that cholera is endemic in many countries and disproportionately affects the poorest and most vulnerable populations.

Prevalence of Cholera in Africa

West and Central Africa region recorded 34957 choleras cases (suspected and confirmed cases) with 629 deaths, in 2019. Compared with 2018, cholera cases decrease and this is mostly due to the decrease in the number of cases in Nigeria, with 29239 cases in 2018 compared to 3513 in 2019. In 2020, the cumulative number of cholera cases until week 13 is 7803 cases. The Democratic Republic of Congo is the most affected country by cholera pandemic with 95.7 % and 87.9 % of reported cases in the

region respectively in 2019 and 2020, following by Cameroon and Nigeria (OMS, 2019; Platform cholera).

In the Eastern and Southern Africa Region, cholera outbreaks begin since 2019 with more than 12102 cholera cases, including 51 deaths reported in 10 countries. The most affected countries are Mozambique (58.1 %, corresponding to 7034 cases) and Kenya (23 %, with 2789 cases); But Zambia have recorded the highest mortality rates in 2019 (2.4 %) (OMS, 2019; Platform cholera).

Strains of *Vibrio cholerae* circulating in Africa

Vibrio cholera biotypes classification is based on chicken erythrocytes clumping, Voges-Proskauer (VP) test results, to polymyxin B susceptibility and biotype-specific phages, variants of the gene encoding cholera toxin sub-unit B (ctxB) and the transcriptional regulator gene of the repeated sequence R (rstR) and the pilus gene co-regulated by major toxin(tcpA) in conventional and El Tor-specific alleles, while repetition in the toxin gene (rtxC) is present in El Tor but absent in conventional biotype isolates.

*S*everal sero groups of *Vibriocholerae*, such as *Vibriocholerae* O1, *Vibriocholerae* O139and non-*Vibrio cholerae* O1, nonO139 and their variant have been detected in Africa. Analyses show that several authors detected the biotypeEl Tor *Vibriocholerae*O1 Ogawa in all regions of Africa and *Vibrio cholerae* O1 remains the most circulating variant in Africa. In sub-Saharan Africa, after reviewing, none of the studies reported the O139 serogroup from any country in the region and some studies detected or confirmed cholera toxin and toxin-co-regulated pilus genes (*ctxB*, *ctxA*and *tcpA*); this review also underlined that the biotype atypical El Tor is existing in sub-Saharan African region and the serotypes Ogawa and Inaba were found (Figure - 1).

Note of Authors

There is a lack of data about cholera in African countries, especially sub-Saharan countries; it is necessary to conduct more studies to make the genotype of strains of *Vibrio cholera* which are circulating in every country in Africa. It will help to choose the vaccine which fits for every country and prevent future outbreak of Cholera. Only access to safe water, combined with sanitation and hygiene, treatment and oral cholera vaccines could break down cholera pandemic in Africa. But all of these points remain a big challenge for African population.

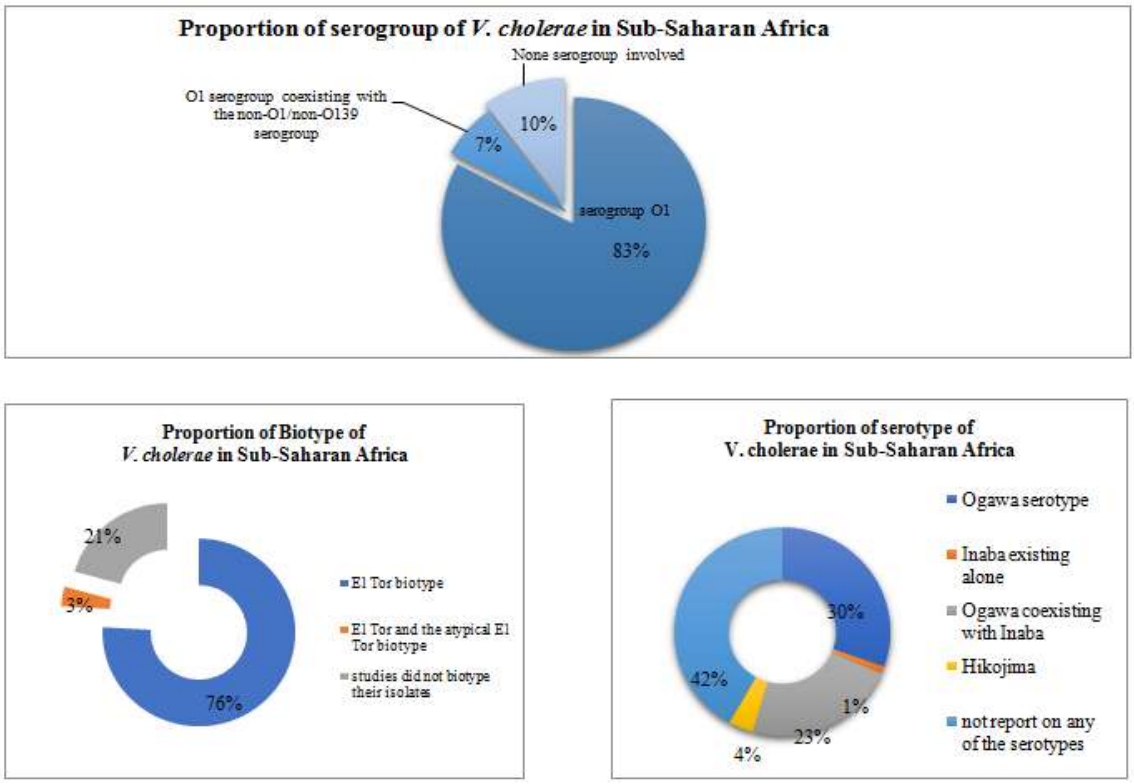

Figure - 1: *Vibrio cholera* in sub-Saharan Africa

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PREBIOTICS

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We all know what 'Probiotics' is, but only a few of us know about 'Prebiotics'. Prebiotics are compounds in foods that induce the growth or activity of beneficial microorganisms such as bacteria and fungi most importantly in the gastrointestinal tract, where probiotics can alter the composition of organisms in the gut microbiome. The human gut microbiota is involved in a cascade of activities essential for body health. Their imbalance can lead to significant metabolic abnormalities and a plethora of diseases. Prebiotics have emerged as an effective non pharmacological approach to reestablish gut symbiosis and promote wellbeing. Prebiotics are basically non digestible fiber compound in foods and are composed of oligosaccharides. Common examples of prebiotics include fructo-oligosaccharides (FOS), inulin, arabinogalactan, polydextrose, lactulose, lactitol. They are subjected to selective fermentation by beneficial microbiota of the intestine.

Prebiotics generally stimulate the growth of *Bifidobacterium* and *Lactobacillus,* which confers several beneficial effects like improving digestion and strengthening the immune system. *Lactobacilli,* prefers inulin and fructo-oligosaccharides while *Bifidobacterium* shows specificity for inulin, fructo-oligosaccharides, xylooligsaccharides and galactooligosaccharides. Prebiotics can also inhibit the growth of detrimental and potentially pathogenic microbes in the gut. The health benefits of prebiotics are mainly attributed to the increased production of short chain fatty acids. They are involved in regulating the immune system and inflammatory response. They also aid in the absorption of several minerals and help in the prevention of colorectal cancer, inflammatory bowel diseases, obesity and relieving constipation.

The endogenous source of prebiotics in humans is human breast milk, which contains oligosaccharides structurally similar to GOS referred to as Human Milk Oligosaccharides (HMOs). These HMOs increases the *Bifidiobacteria* bacterial population in breast fed infants and to strengthen the infant immune system. Prebiotics can be found in variety of foods like apples, asparagus, artichokes, bananas, barley, berries, chicory, cocoa, dandelion greens, flaxseeds, garlic, green vegetables, legumes, oats, onions, tomatoes, soy beans, wheat, leeks, etc. Fermentable carbohydrates derived from fructans and xylans are the most well documented examples of prebiotics. Some products are fortified with prebiotics. These include baby formula, bread, cereal, cookies, yoghurt. Prebiotics have positive influence on human health and they are necessary in our daily diet in required proportions for maintaining the health of our gut microbes and in turn give us many beneficial effects.

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SYNBIOTICS

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Synbiotics is similar to the term symbiots which means the mutual relationship of plant and organisms. In food microbiology, Synbiotics refer to food ingredients or dietry supplements, which contains both probiotics and prebiotics in a form of Synergism. The basic concept of Synbiosis is that the mixture of probiotics and prebiotics that is beneficial to host by improving the survival and metaboilc activities of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and by activating the metabolism of one or a limited number of health-promoting bacteria, thus improving host welfare". As of 2018, the research on this concept is preliminary, with no high-quality evidence from clinical research that such benefits exist.

Synbiotics is of two types:

- ***Complementary synbiotics***: Each component is independently chosen for its potential effect on host health
- ***Synergistic synbiotics***: Prebiotic component is chosen to support the activity of the chosen probiotic.
- ***Optibiotics***: Research is evaluating if synbiotics can be optimized, which are purported to enhance the growth and health benefits of existing probiotics.

Probiotics are live bacteria which are present in the large intestine. A prebiotic is a food or dietary supplement product that may induce the growth or activity of beneficial microorganisms (probiotics). A prebiotic may be a fiber, but a fiber is not necessary to be a prebiotic.

Using prebiotics and probiotics in combination may be described as synbiotic, but the United Nations Food & Agriculture Organization recommends that the term "synbiotic" be used only if the net health benefit is synergistic. Synbiotic formulations in combination with pasteurized breast milk are under preliminary clinical research for their potential to ameliorate necrotizing enterocolitis in infants, although there was insufficient evidence to warrant recommending synbiotics for this use as of research in 2016.

Some the major examples of probiotics are:

a) *Bifidobacteria* and Fructooligosaccharides (FOS)
b) *Lactobacillus rhamnosus* GG and Inulins
c) *Bifidobacteria* or *Lactobacilli* with FOS or Inulins or Galactooligosaccharides (GOS)
d) Polyphenol

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PROBIOTICS

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Probiotics are live bacteria and yeasts that are good for us, especially our digestive system. We usually think of these as germs that causes diseases. But our body is full of bacteria, both good and bad. The root of the word *probiotic* comes from the Greek word *pro* meaning "promoting", and *biotic,* meaning "life". Probiotics are often called "good" or "helpful" bacteria because they help keep our gut healthy. For a microbe to be called a probiotic, it must have several characteristics. These include being able to be isolated from a human, survive in our intestine after ingestion, have a proven benefit to us, be safely consumed. According to the currently adopted definition by The Food and Agriculture Organization (FAO)/The World Health Organization (WHO), probiotics are "Live microorganisms which when administered in adequate amounts confer a health benefit on the host".

The main function of probiotic, or good bacteria, is to maintain a healthy balance in our body. Good bacteria work to fights off the bad bacteria and restore the balance within our body. Certain type of good bacteria can also help to digest food, keep bad bacteria from getting out of control, create vitamins, breakdown and absorb medications. Though, there are many types of bacteria that can be considered probiotics, there are two specific types of bacteria that are common probiotics found in stores. These include *Lactobacillus, Bifidobacterium.* Probiotics are also made up of good yeast. The most common type of yeast found in probiotics is *Saccharomyces boulardii*. There are several ways to take a probiotic supplement. They come in a variety of forms, including in foods, drinks, capsules or pills, powders and liquids. Probiotic supplements may be combined with a prebiotic. Basically, prebiotics are the "food source" for the good

bacteria. Food that contain natural probiotics include some types of yogurt, kefir, buttermilk, soft cheese, soy-based products, such as miso, tempeh, and some soy beverages, kimchi, unpasteurized sauerkraut, nutrition bars, juice and cereal.

Probiotics are likely to be safe for most people, but there are some points to consider before using them and increasing the intake. The National Center for Complementary and Integrative Health note that while probiotics are likely safe for those in good health, these bacteria may pose a risk to people with weakened immune system or other health issues. These people may face a risk of infection, harmful substances being released in the body and antibiotic resistance. It is always best to check with a doctor before taking supplements or making significant dietary changes, especially for people with existing health issues. In ancient Indian society, it became common place to enjoy a before-dinner yogurt drink called a lassi. These Indian traditions were based on the principle of using milk as a probiotic delivery system to the body.

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BIOPROSPECTING OF RED SEA COASTAL ECOSYSTEM FOR CULTURABLE BACTERIA

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Antibiotics are called miracle drugs because they kill bacteria, and thereby cure people of potentially fatal infectious diseases. Marine microorganisms may represent an efficient and successful source for natural products research. Marine bacteria and fungi are of considerable importance as new promising sources of a huge number of biologically active products. Some of these marine species live in a stressful habitat, under cold, lightless and high-pressure conditions. Surprisingly, a large number of species with high diversity survive under such conditions and produce fascinating and structurally complex natural products.

Microorganisms that inhabit unchartered unique soil such as in the highly saline and hot Red Sea lagoons on the Saudi Arabian coastline, represent untapped sources of potentially new bioactive compounds. The increasing need for new antimicrobial agents able to control emerging diseases or resistant strains of microorganisms inspired a growing number of research groups to explore the oceans for new bioactive compounds. Moreover, the Red Sea is found to have geochemical and physical parameters which make it a unique marine environment in comparison on to the marine ecosystem. In this research, a culture-dependent approach was applied to three types of sediments: mangrove mud (MN), microbial mat (MM), and barren soil (BS), collected from Rabighharbor lagoon (RHL) and Al-Kharrar lagoon (AKL). A culture-dependent approach was applied to these sediment samples for the isolation and identification of microorganisms using 16S rRNA gene sequencing. We have also reported their capacity

to inhibit the growth of three laboratory pathogens, *Staphylococcus aureus* ATCC 25923, *Pseudomonas syringae* pv. tomato dc 3000, and *Salmonella typhimurium* dT2, as well as their genomic potential to produce secondary metabolites with antimicrobial activity. Only cultivated strains have been studied in detail, yet they represent less than 5 % of the total microbial biomass in the soil. Thus, in an attempt to broaden the diversity of the isolated strains, we employed several culture media: Difco Marine Agar 2216 (MA), 10 % Difco Marine Agar 2216 (10 % MA), Difco Marine agar 2216 with 1 g/L Streptomycin (Anti-MA), Actinomycetes Isolation Agar (AIA) and Difco Marine Broth 2216 Gellan gum (MB-GM). Bacterial colonies grew fast on marine agar, exhibiting a more diverse colony morphology and density than on other media.

Bacteria from the Actinobacteria phylum were isolated from barren soil at Rabighharbor lagoon, and from Mangrove mud collected from Rabighharbor lagoon and Al-Kharrar lagoon, while Bacteroidetes were only isolated from BS collected from both locations. Planctomycetes were cultured from various sites such as Microbial mat and Barren soil at Rabighharbor lagoon, and Mangrove mud at Al-Kharrar lagoon. The Firmicutes genus *Bacillus* was the predominant taxon isolated from all samples, especially from barren soil. The *Bacillus* species, recognized as an important source of natural bioactive products, are commonly found in nutrient-poor soils.

We found that the most abundant cultured and widely distributed species in Rabighharbor lagoon and Al-Kharrar lagoon were: *Bacillus subtilis, Bacillus simplex, Blastopirellula cremea, Pseudoalteromonas flavipulchra, Bacillus sonorensis and Bacillus licheniformis,* etc. In addition, *Virgibacillus pantothenticus, Microbulbi fermaritimus, Bacillus foraminis, Vibrio alginolyticus* and *Vibrio furnissii* strains were isolated three times or more from Mangrove mud only. We evaluated all 251 isolates for their antimicrobial activity against three pathogens: *Staphylococcus aureus ATCC25923, Pseudomonas syringae dc3000, and Salmonella typhimurium dT2*. The reason behind the selection of these pathogens was to cover the different spectrum of antimicrobial use. *S. aureus* causes several human infections such as skin and wound infections, septicemia, endocarditis, food poisoning, toxic shock syndrome, meningitis, pneumonia and osteomyelitis. Of all isolated strains, we found that 49 exhibit potential antimicrobial activity. From these, 25 have at least one type of biosynthetic gene sequence, indicating that these isolates are a valuable resource for the potential discovery of bioactive compounds. Moreover, 75 % of putative novel species and 67 % of strains that exhibited antimicrobial activity were isolated from Rabigh harbor lagoon.

The isolated bacteria were evaluated for their potential to produce bioactive compounds. The phylogenetic characterization of 251 bacterial isolates based on the 16S rRNA gene sequencing, supported their assignment to five different phyla: Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Planctomycetes. 15 putative novel species were identified based on a 16S rRNA gene sequence similarity to other strain sequences in the NCBI database, being ≤98 %. We report the previously unknown antimicrobial activity of *B. borstelensis, P. dendritiformis* and *M. salipaludis* against all three indicator pathogens. The study demonstrates the evidence of diverse cultured microbes associated with the Red Sea harbor/lagoon environments and their potential to produce antimicrobial compounds.

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Bifidobacterium sp.

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Bifidobacterium represents a genus within the phylum Actinobacteria which is one of the major phyla in the healthy intestinal tract of humans. *Bifidobacterium* is one of the most abundant genera in adults, but its predominance is even more pronounced in infants, especially during lactation, when they can constitute the majority of total bacterial population. Infant feeding is critical factor for bifidobacterial establishment in the gut and breast-fed infants have been shown to possess higher levels of *Bifidobacteria* than formula-fed infants. *Bifidobacteria* are Gram positive, rod shaped, often branched, anaerobic bacteria. They are commensals that colonize orogastrointestinal tract of humans and are considered as major components in human microbiota. Their colonization and cell number in human intestine varies with aging.

Bifidobacterium longum is widely detected from new borns to centenarians with highest detection rate. *Bifidobacterium breveis* found in approximately 70 % children under 3 years of age. *Bifidobacterium adolescentis* and *Bifidobacterium catenulatum* are found predominantly after weaning. *Bifidobacterium dentium* is found in elderly people while *Bifidobacterium bifidium* is found in people of almost all ages.

Bifidobacteria are helpful in digestion. They are known to play important roles in the metabolism of dietary components. These beneficial bacteria digest dietary fiber, help prevent infection and produce vitamins and other important chemicals. Low counts of *Bifidobacteria* have been linked to many diseases and *Bifidobacteria* supplements may help in treating symptoms of certain diseases. Hence, *Bifidobacteria* are also used as probiotics to improve digestion. *Bifidobacteria* also play role in the maturation of immune

system. Early life Bifidobacterial establishment have role in programming the future health. They have been shown to interact with human immune cells and to modulate specific pathways, involving innate and adaptive immune responses. *Bifidobacteria* exert their beneficial effects on host health through the immunomodulatory action of some of their surface-associated molecules. *Bifidobacterial* proteins are one of the targets of human immunoglobulins, notably IgA, which is secreted into the gut lumen in order to control the commensal microbiota populations. Best known example of immunomodulatory protein is the extracellular Serpin (Serine protein inhibitor) secreted by *Bifidobacterium longum.* Several studies have investigated the potential of *Bifidobacteria* to prevent and treat colorectal cancer. *Bifidobacterium bifidium* was found effective in combating cancer cells and was associated with a substantial improvement in gastrointestinal cancer.

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IMPACTS OF MULTIDRUG RESISTANCE AND SOME NOVEL APPROACHES TO COMBAT MDR INFECTIONS

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Introduction

The emergence of multidrug-resistant (MDR) bacteria could be a growing threat to public health. Natural phage lysins are one amongst the simplest alternatives within the treatment of infections caused by gram-positive pathogens, however excluding gram-negative ones, like *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, because of the barriers posed by their outer membrane. Due to the range of resistance mechanisms that will cause MDR or perhaps pandrug resistance, *Acinetobacter baumannii* and *pseudomonas aeruginosa* are among the progressively reportable MDR or perhaps PDR nosocomial pathogens. they are accountable for several hospital-acquired infections, delicate skin wounds and urinary tract infections and severe life-threatening infections like respiratory disease. Simultaneously, the progress in developing new antibiotics against these strains is very slow and tedious. So, we are in need to develop ways to manage these MDR pathogens. A great interest in the medical field is the application of nanoscience and nanotechnology for management of infectious diseases. Nanotechnological applications are giving auspicious resolution for this drawback. Eminent usage of technology in our life have created new strategies for the development of effective antimicrobial compounds or nano-antimicrobials, capable of overcoming this drawback of drug

resistance. The development and safe manipulation of those nano-antimicrobials can build a brand new promise to cure these health issues. Frequent use of antibiotics has led to the evolution of multi-drug resistant bacterium that are termed as "superbugs". Inherent mutations and lateral factor transfer among bacterium are primarily accountable for the emergence of Superbugs.

Innovative approaches to tackle MDR Infections

Broad spectrum antimicrobial resistance in MDR and XDR isolates have restricted promising therapeutic choices. Natural medicines, like ancient Chinese medicine (TCM) and Ayurveda, were shaped and developed within the standard of living of ancient folks and within the method of their battle against diseases over thousands of years. Herbal product are the first sources for brand spanking new drug development since times of yore. From the Nineteen Forties to the end of 2014, nearly half the FDA approved chemical medication for treating human diseases were derived from natural products. The lab-intensive, long extraction and isolation method has been the bottle neck of the application of natural product in drug development. We are in urgent need to develop effective strategies for the extraction of natural products. The Nanotechnological application provides a promising strategy to manage infections caused by MDROs. In this respect, Nanoparticles have shown therapeutic promise attributable to their distinctive physical and chemical attributes Nanoparticles exhibiting bactericide activities will target multiple biomolecules and have the potential to cut back or eliminate the evolution of MDROs. Design and progress of herbal nanoparticles has become a pioneer analysis in nanoscience. Biosynthesis of metallic nanoparticles has acquired explicit attention thanks to its economic practicability, low toxicity, and ease of the method. Polymeric nanoparticles like those manufactured from polyose, starch, gelatin and chitosan have potent antimicrobial activities attributable to their varied therapeutic properties. Several nanoparticle-based drug delivery systems are beneath investigation, or have already been approved, for clinical use.

Conclusion

Nanoparticles possess antimicrobial activity which will overcome common resistant mechanisms, together with enzyme inactivation, attenuate cell permeableness and modification of target sites/enzymes to flee from the bactericidal activity of antimicrobial agents. Moreover, NPs conjugated with antibiotics show synergistic effects against bacteria, forbid biofilm formation, and are used to combat MDROs. Several characteristics of NPs make them alternatives to ancient antibiotics. Despite many advantages of nanoparticles in combating MDR pathogens, their toxicity and safety issues opposed their potency. The presence of nanoparticles within the atmosphere that

may be internalized *via* digestive system or direct entry through broken skin, are the main reasons limiting their manipulation.

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NON-*SACCHAROMYCES* YEAST: A PROMISING TOOL FOR IMPROVING WINE AROMA

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In the past, the non-*Saccharomyces* yeasts have been treated as spoilage microbes in the wine industry, but now they are regarded as crucial microbes for improving the organoleptic tastes of wine. Over decades, *Saccharomyces* yeasts i.e., *Saccharomyces cerevisiae* are largely used in wine fermentation, for its availability and are cheap as well and it is considered as the main starter in Oenology. But recently, the evaluation of non-*Saccharomyces* yeasts made it possible to ferment wine in a very sequential and indigenous way. These 'non-*Saccharomyces*' yeasts referred to the wide variety of yeast genera including in both the Ascomycota and Basidiomycota phyla present in the grape must. Although, having great strain discrimination and genetic diversion the distinct features i.e., phenotypic similarities of non-*Saccharomyces* with *Saccharomyces* yeast made it an easier and effective way of replacing each other in wine making. The non-*Saccharomyces* yeasts may contribute to the wine's aroma by producing a wide range of secondary metabolites and extracellular enzymes. Some species can reduce alcohol levels in wine or have the superior fermentative capability in harsh conditions.

The co-existence of *Saccharomyces* and non-*Saccharomyces* yeast in grape must

The fermenting grape musts co-existed a wide diversity of microorganisms, including diverse forms of yeasts. In the early stages, the dominance of non-*Saccharomyces* yeasts is seen and in the late stages, the dominance of *Saccharomyces* yeast is noted in several studies.

Impact of non-*Saccharomyces* yeasts on wine aroma

Despite having the advantages of using pure cultures of *Saccharomyces cerevisiae* regarding the ease of control and homogeneity of fermentation, the wine produced with this monoculture yeast lacks the complexity of flavor, stylistic discrepancy and vintage variability produced by native yeasts. However, it is worthwhile to note that the world's best quality wines are produced, wherein a greater or lesser extent several species of non-*Saccharomyces* yeasts have played an important role in the wine fermentation process and leave an imprint on the final composition of the wine.

Wine aroma can be divided into three groups i.e., the varietal or primary aroma which depends on the grape variety; the secondary aroma formed during the fermentation and the bouquet or tertiary aroma related to the aging of the wine.

Primary Aroma

The primary or varietal aroma is formed during the ripening of grapes; its quality depends on the state of ripeness and the agronomic and oenological practices. The primary aroma belongs to several chemical families including methoxy pyrazines have been associated with vegetal, green, herbaceous aromas and C_{13}-norisoprenoids have considered impact volatiles of non-floral grapes and some volatile sulfur compounds, terpenes are also formed.

Secondary Aroma

The secondary aroma is formed during the fermentation process. Their quality is mainly dependent on the yeasts and fermentation conditions. After fermentation, several secondary aromas are formed, among those the most abundant compounds are ethanol, glycerol, CO_2 and moderate are volatile fatty acids, esters and lesser is aldehydes respectively.

The role of some widely used non-*Saccharomyces* yeasts in winemaking is described in the below given Table.

Species name/Synonym (Anamorph)	Contribution in Wine making
Torulaspora delbrueckii (Candida colliculosa)	Volatile acidity reduction.
Hanseniaspora uvarum (Kloeckera apiculate)	Aroma production.
Hanseniaspora guillermondii (Kloeckera apis)	Acetate ester production.
Starmerella bacillaris (Candida zemplinina)	Fructophily; reduced ethanol production; glycerol production.
Candida stellata / Torulopsis stellate	Fructophily; glycerol production.
Lachancea thermotolerans / Kluyveromyces thermotolerans	Glycerol overproduction; acetate ester production; reduction of volatile acidity
Debaryomyces hansenii / Pichia hansenii (Candida famata)	Enzymatic activities

The detailed study of some specific strains of *Saccharomyces cerevisiae* and non-*Saccharomyces* yeast has shown that this mixture of strain can be used as an excellent starter for winemaking which could give the wine an extraordinary organoleptic taste and it might be a promising tool in winemaking.

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APPLICATIONS AND CHALLENGES OF CRISPR/CAS9 GENOME EDITING TECHNOLOGY

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Introduction

It has been since last decade a new approach named 'genome editing' emerged and widely used in the studies of functional genomics, transgenic organisms and gene therapy. The foundation of genome editing is based on engineered, programmable and highly specific nucleases. This nuclease can induce site specific changes in the genomes of cellular organisms through a sequence-specific DNA-binding domain and a nonspecific DNA cleavage domain. Subsequent cellular DNA repair process generates desired insertions, deletions or substitutions at the loci of interest. Researchers have developed multiple artificial nuclease systems for genome editing of which zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR/Cas 9 are the most widely applied engineered nucleases.

CRISPR (clustered regularly interspaced short palindromic repeats) together with the Cas (CRISPR-associated) proteins is an adaptive immune system found naturally in 40 % of bacteria and 90 % of archaea. They were first discovered in *E. coli* by Ishino *et al*. (1987) and in archaea by Mojica *et al*. (1993). CRISPR/Cas generate a cellular memory by incorporating short sequences from past invaders into the host's CRISPR locus. This

cellular memory destroys integrated DNA sequences which is identical to past invaders and provides resistance against foreign nucleic acids. Bacterial and archaeal organisms have three categories Type I, Type II, and Type III of the CRISPR/Cas system. Type II systems are the simplest, comprising a nonspecific Cas nuclease (Cas9) and a set of programmable sequence-specific CRISPR RNA (crRNA), which can guide Cas9 to cleave DNA and generate double-strand breaks at target sites. Hence, Type II systems are the most commonly used and popular CRISPR-based gene-editing approach.

Advantages of CRISPR/Cas9 Genome Editing

Genome-editing technologies can be used for genome-wide screens to identify genes and mutations responsible for complex biological processes. Compared with nuclease systems ZFNs and TALENs, CRISPR/Cas9 is a simple and more efficient tool for genome editing. ZFNs and TALENs are built on protein guided DNA cleavage, which needs complex and time-consuming protein engineering, selection and validation. In contrast, CRISPR/Cas9 only needs a short programmable gRNA to generate sequence specificity, which is relatively cheap and easy to design and produce. CRISPR/Cas9 allows for specific gene repair or permanent gene knockout. CRISPR/Cas9 can simultaneously induce genomic modifications at multiple independent sites. Owing to which, CRISPR/Cas9 has been used widely to develop human disease models in laboratory animals and to investigate gene function and epistatic relationships.

Challenges of CRISPR/Cas9 Genome Editing

Despite CRISPR/Cas9 has the great therapeutic potential in next-generation therapeutics, it raises various ethical concerns that need to be addressed before being translated for clinical use. Here we have summarized three major challenges to use CRISPR/Cas9 technology in clinical trials. The first challenge is Off-target effects. Large genomes may consist of DNA sequences that are identical or closely resembling the target sequence. Cas nuclease may cut at non targeted sites and it may lead to off-target mutations or cell death or adverse transformation. The second challenge is to increase homology-directed repair efficiency of CRISPR/Cas9 to decrease non-specific insertion or deletion of bases in a DNA sequence. Third challenge is to design sgRNA which is not restricted to only gene targets containing a Protospacer Adjacent Motif (PAM) sequence.

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PRIONS: AN OVERVIEW

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Prions are unprecedented infectious pathogens that cause a collection of invariably fatal neurodegenerative disorders by an entirely new mechanism. The prions have a characteristic feature of neuro-degeneration and lethality. They are also sometimes referred as "Transmissible Spongiform Encephalopathies (TSEs)". They bring about a variety of genetic, infectious, or sporadic disorders, which entails modification of the Prion Protein (PrP). Bovine Spongiform Encephalopathy (BSE), Scrapie of sheep and Creutzfeldt – Jakob Disease (CJD) of humans are among the most noteworthy of prions disease.

Prions are transmissible particles which have seen to be lacking nucleic acid and are seem to be constituted to exclusively of a modified protein (PrP^{Sc}). The normal, cellular, PrP, is symbolised by PrP^{c}, is converted into PrP^{Sc} through procedure by which a portion of its α-helical and coli structure is again refolded into β-sheet. This structural, change is accompanied by subtle changes in the physico-chemical properties of PrP. In many cases, the cellular prion protein is converted in to the defective form through a post-translational process that comprises a change of conformation. Often, it has been seen that, the human prion diseases are transmissible to experimental animals.

The only organ system in which histopathological damage along with its resulting clinical consequences, are apparent as a result of infection is the nervous system. However, it is apparent that prions can establish their presence in organs other than the nervous systems, and may be shown in extracerebral compartments. The TSEs are characterized by vaculation, gliosis and spongiform degeneration. It is believed that

most natural infections are acquired by the alimentary route, so that the first barrier that the infectious material comes in contact with is the gastrointestinal epithelium. Once this barrier is surpassed, TSEs agents could theoretically reach the brain by the same two pathways as any other infectious agents.

Prion diseases lead to brain damage when prion proteins cause the abnormal clumping in the brain. This abnormal aggregation of protein in the brain can cause memory impairment, personality changes, and problem with locomotion. In Fatal Familial Insomnia (FFI), mutated prions get accumulated in the thalamus, with the result that the patients are not able to sleep. The efficiency of so called "species' barrier", at least in part depends on the interaction between the intruding PrP^{sc} and the infected species, that is the degree of sequence homology between Prnp of the donor and the recipient in prion disease. The experimental conformation that a novel form of human prion disease, variant CJD, occurs by same strain as those occurring in cattle's, has brought forward the emergent requisite to understand the molecular basis of propagation of prion disease and the transmission hurdle that prevents their movement between mammalian species. It is reassuring that none of the human prion diseases are contagious by natural routes. The natural contact between healthy and infected individuals does not possess a risk of transmission. However, unnaturally close contact e.g., skin contact with the brain or spinal cord tissues in association with injury when performing an autopsy may possess a risk of contracting the disease.

The diseases caused by prions are currently only with diagnosed with certainty post death of organism, unless it is possible to get a brain biopsy, which is seldomly done. An important aspect of prions is that neither during incubation period nor during the clinical manifestations of the disease, there is possibility of observing an inflammatory or immunological reaction.

Diseases caused by Prions

a) ***Creutzfeldt - Jakob disease (CJD):*** Classic CJD is a human prion disease. It is a rapidly progressive and fatal disease. Those who are infected with CJD usually die within one year of from the beginning of disease. Cases of CJD occurs throughout the globe and the annual incidence in many countries, including united states of America, has been reported to be about one cases per million of population.
b) ***Gerstmann – Straussler - Schienkler Disease (GSS):*** It is also an extremely rare neuro-degenerative brain disorder. The disease is hereditary and is found only in a handful of families on the planet. The common age of the onset of disease is normally between ages of 35-55.

c) ***Fatal Familial Insomnia:*** The disease is characterized by the abnormal alterations in the sleep cycle and causes an inability to sleep (insomnia). Insomnia can begin suddenly and can rapidly take a turn for worse over the next few months. The disease is fatal.

d) ***Kuru:*** It is a rare and fatal disease that first discovered among the people of the Fore people in the Highlands of New Guinea that took the form of an epidemic during the 1950s.It was the result of the cannibalism that was performed by the people of the Fore.

Few of the prions diseases that primarily infect the animals are Bovine Spongiform Encephalopathy (BSE), Chronic Wasting Disease (CWD), Scrapie of sheep and goats, Transmissible Mink Encephalopathy, Spongiform Encephalopathy of Primates. The mode of infection of the animals is basically through the acquisition of infection from the feed or through exposure to the contaminated surroundings.

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MICROBIOME OF HUMAN GUT

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The microbiome of human gastrointestinal tract or gut is as vast and mysterious as the Milky Way. An amazing fact of microbiome is that about 100 trillion bacteria, both good and bad, live inside our digestive system. In fact, our human body is composed of 10 trillion human cells, but is host to around 100 trillion bacterial cells. So, there are more bacterial cells in our human body than human cells. Although many different types of microbes live inside human body, the most studied are bacteria. The diversity of the human microbiome was first observed by Antonie Van Leewenhoek. Joshua Lederberg first coined the term "Microbiome" in 2001.

A community of microorganism (such as bacteria, fungi and virus) that inhabit a particular environment and especially the collection of microbes living in or on the human body is collectively known as microbiome of human body. The gastrointestinal tract or gut is the tract from mouth to the anus which includes all the organs of the digestive system in humans and other animals. Human gut microbiome are the microbes live in the human gut. *Escherichia coli, Helicobacter pylori, Staphylococcus aureus, Candida albicans* etc. are the representative species of gut microbiome.

Family genes, environment, medication and diet plays a large role in determining what kinds of microbiome live in our gut. All these factors create a unique microbiome from person to person. Colonize of the gut by the human microbiome was thought to begin at birth, but some scientists have reported that a mother-to-child transfer of commensal bacteria through the placental barrier may occur. The maternal oral microbiome has been suggested as the source of the first bacteria that colonize infants.

The gut microbiome plays a very important role in our health by helping control digestion and benefiting our immune system and many other aspects of health. An imbalance of healthy and unhealthy microbes in the gut may contribute to weight gain, high blood sugar, high cholesterol and other disorders. The microbiome helps to produce vitamins including vitamin B12, thiamine, riboflavin and vitamin K, which is needed for blood coagulation. Imbalance of the normal gut microbiome have been linked with gut conditions such as Inflammatory Bowel Disease (IBD), Irritable Bowel Syndrome (IBS) and wider systemic manifestations of disease such as obesity, type 2 diabetes and atopy. Vegetables and probiotics keep our gut healthy whereas fried and spicy foods, citrus fruits, artificial sugar etc. are bad for our gut microbiome. Upset stomach, sleep disturbances, skin irritation, autoimmune conditions are the signs of unhealthy gut.

Recent studies have described the structure and functional capacity of the bacterial microbiome in the healthy state and in a variety of disease states. Now, some scientists are trying to decode the alphabet that composes the language by which we and our internal microbes communicate in our health. In the future, this language may be discovered and open a new horizon.

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EXTENSIVELY DRUG RESISTANT (XDR) BACTERIAL STRAIN

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Antimicrobial resistance prevalence poses a major challenge for clinicians in treating patients with infections. The evolving trends of resistance towards the effective antibiotics in the current scenario poses a major concern in treating even common infectious diseases. This may lead to increased mortality rate as well as increased medical cost and prolonged stay in hospital.

Extensively drug resistant (XDR) are different form of MDR Strain. Specifies the pathogens which are resistant to all available effective antibiotics. In XDR strains Gram negative bacilli are the most threatening compared to Gram positive cocci. The resistance mainly attributed by acquisition of Carbepenemase which makes Carbepenem redundant β-Lactam resistant.

This type of bacterial strain is difficult to treat and is associated with a high mortality rate, and it is generally more severe to have XDR-GNB Blood Stream Infection (BSI) and can cause many serious infections, such as pneumonia, peritonitis (inflammation of the membrane that lines the abdominal cavity), urinary tract infections, bloodstream infections, wound or surgical site infections, and meningitis.

In Gram negative bacilli *Escherichia coli, Klebsiella pneumoniae* is more predominant microorganism follow by *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are causative agent XDR. In Gram positive organism, Coagulase Negative *Staphylococcus* (Cons)*, Enterococci, Streptococcus* spp. and *Staphylococcus aureus.* It appears likely that there will be increasing reports of XDR Gram negative bacilli. We would define such strains as lacking susceptibility to all of the β-lactam and quinolone plus ticarcillin-clavulanate, ampicillin-sulbactam, all aminoglycosides (including amikacin), tigecycline, and the polymyxins (colistin and polymyxin B). XDR drug-resistant infections can be prevented by immunization, safe food preparation, handwashing, and using antibiotics as directed and only when necessary. In addition, preventing infections also prevents the spread of resistant bacteria.

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ADVANCEMENT IN ANTIBIOTICS

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Antibiotics are medications used in the treatment of bacterial infections. The emerging problem antibiotic resistance is a threat to people's health. It is crucial to control current and future infectious diseases by creating some advancement in antibiotics. A study published in Biofilms and Microbiomes showed Scientists at George Mason University in the US found Komodo dragon "The largest living lizard" blood contains an important compound that could offer a new treatment for infected wounds. The reptile's saliva harbours many different types of bacteria, which somehow do not affect the dragon. They created a synthetic compound based on a molecule in dragon blood that had antimicrobial activity. They found it promoted the healing of infected wounds in mice. The study suggests that the protein could potentially be developed into an antibiotic in the future.

They believe that this could be a scientific advancement in the quest to find new antibiotics that are needed to fight multidrug-resistant pathogens. The dragons that are found on five islands in Indonesia have more than 80 bacterial strains in their mouths, including some that cause blood poisoning or sepsis. The reptiles are not harmed by the bacteria - suggesting they are immune. Monique van Hoek *et al.* 2017, in Virginia found DRGN-1(a peptide) worked well on infected wounds in mice against two bacterial strains, the "superbugs" *Pseudomonas aeruginosa* and *Staphylococcus aureus*, also known as MRSA. These two bacteria are particularly hard to treat as they have bacteria that stick together to form biofilms that are much more resistant to antibiotics than a single bacterium. They suggest that DRGN-1 assists wound-healing both through antimicrobial activity and also by promoting the migration of skin cells to close the wound. Although it has only been tested on mice and on only two bacteria strains, they

believe DRGN-1 is a good candidate for additional studies and possible development as a topical therapeutic agent for infected wounds.

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BACTERIOCIN AND LACTIC ACID BACTERIA

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Microorganisms are one of the greatest blessings to humanity as they produce plethora of organic substances that are beneficial to the humankind in numerous ways. One such blessing is Bacteriocins which are produced by the Lactic Acid Bacteria. Lactic Acid Bacteria (LAB), are Gram positive, non-aerobic microbes which ferment carbohydrates for energy and lactic acid production. They produce aromatic organic substance. LAB is designated as GRAS (Generally recognized as safe) by the FDA due to their omnipresence in food and their contribution to the healthy microflora of the human mucosal surface. These microbes are present in milk, meat, fermented vegetables, and beverages that hinder the growth of other deteriorating microorganisms. They help to preserve the consistency of the products, expand their shelf life and also play a vital role in the development of texture and taste of the food products.

Many LAB produce a wide range of bacteriocins. Nisin is the only approved bacteriocin and has commercial applications. Probiotics are defined as live microorganisms, which when given in required amounts are seen to be beneficial to the host by improving or restoring their intestinal flora. There are currently several well-characterized strains of *Lactobacilli* and *Bifidobacterium* available for human use that reduces the risk of gastrointestinal infections. LAB are the most popular probiotics.

Bacteriocins are basically complex proteins that are biologically active with antimicrobial properties against other microorganisms, specifically to closely associated species. They too are produced by the bacteria but they should not be confused with

therapeutic antibiotics, as bacteriocins are synthesized by ribosome and are produced in the initial growth phase, whereas antibiotics are secondary metabolites.

LAB is considered as the most potent sources of gram-positive bacteria to produce bacteriocins, and their compounds contains a proteinaceous primary functional motif whose destruction unequivocally eliminates their ability to cause cell death. Bacteriocins are broadly divided into different classes based on various parameters like its molecular size, physical properties, chemical structures, mode of action, source organisms. Bacteriocins are classified into Class I, Class II, Class III, and Class IV respectively.

Antimicrobial peptides (AMPs) or proteins that are produced by the bacteria are known as bacteriocins. Bacteriocins being abundant, their ribosome synthesized AMPS or proteins are encoded by the genes, which targets to kill other related (narrow spectrum) or non-related (broad spectrum) pathogenic microorganisms. Most of the commercially accessible preservatives and antibiotics being produced by chemical synthesis, their long-term use can have an adverse effect on human health as they minimize the gut microbial flora. The inhibitory substances bacteriocin generates antagonistic environment against foodborne pathogens and thus it prevents food spoilage.

The very first step for bacteriocin isolation entails a screening of LAB from various sources, which might also differ from plant material and food products to human and animal isolates. Moreno *et al.* (1999) have removed countless traces of LAB from food products which includes milk and cheese. General bacteriocin extraction strategies are generally based on their affinity to organic solvents. The presence of hydrophobic regions in bacteriocin molecules is invaluable for their work against susceptible bacteria, because the deactivation of microorganisms by bacteriocin depends primarily on the hydrophobic association between bacterial cells and bacterial molecules. The purification steps are different for all groups of bacteriocins.

Besides being commonly used in the food industry as a bio preservative, bacteriocins has many other applications as well. Several laboratory findings have indicated the therapeutic ability of bacteriocins against various types of cancer cell lines as anti-cancer agents. Bacteriocins have been proved to be effective against respiratory infections as bacteriocins showed *in vitro* inhibitory activity against *Streptococcus pneumoniae*. Nisin F inhibits the growth of *Staphylococcus aureus* in the nasal cavities of immunosuppressed rats. Studies have shown that bacteriocins are potentially effective against skin and urogenital tract infections as well.

Bacteriocin research has flourished in the last decade. There is a vast potential for the use of purified bacteriocin in various food applications such as, in food processing, bacteriocin can be used in food packaging substances so that processed food can be kept uncontaminated to avoid the development of harmful bacteria. Since there are varieties of mutating viruses found in nature, the exclusive application of a single bacteriocin cannot get rid of all bacterial infections. Consortium of bacteriocins can be used as cocktail drug against human or animal pathogens. Hence, bacteriocins can unfold the most challenging problems of some multi-drug resistant pathogens.

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WORLD OF ALKALIPHILES

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Most of the life forms present on the earth cannot grow in the extreme environment but special ones can! They are none other but known as extremophiles. They can potentially grow or survive in the extreme values of life essential parameters such as physical (water, temperature, pressure, radiation) or chemical conditions (pH, salts, metals). Alkaliphiles is one of the important groups of extremophiles, distributed widely in all the domains of life (Prokaryotes, Archaea and Eukaryotes). In 1999, professor Horikoshi defined alkaliphiles as a group of microorganisms that show optimum growth at or above pH 9 but cannot grow or grow slowly at neutral pH. Physiologically, they are divided into two broad groups as follows.

Figure - 1: Physiological classification of alkaliphiles

Unlike alkaliphiles, haloalkaliphiles require high salt concentration along with high pH for optimum growth. Alkaliphiles can be found in neutral to alkaline environment like soil samples, marine water samples, alkaline effluents from dye or leather industries etc whereas halo alkaliphiles are found only in extreme environment. Soda lakes, alkaline hydrothermal vents, Yellowstone national park etc are some of the reported sources of halo alkaliphiles. Another very interesting source of alkaliphiles is

the hindgut of insects. These sources indicate that the alkaliphiles are distributed in diverse ecological niches.

Despite the high external pH, alkaliphiles maintain internal pH in the near neutral range of 7.5 - 8.5. This is possible because of their cell surface modification that took place with the help of different biomolecules than found in neutrophils. Acidic polymers such as teichuronic acid, teichurono-peptide, polyglutamic acids, galactonic acid, phosphoric acid etc are present in the cell wall. They give negative charge to the cell wall that attracts H^+ and repels OH^- ions. This mechanism helps them to overcome the H^+ shortage and make it easier for them to drive ATP synthesis in a fuel less (H^+) environment. Other than this Na^+ and K^+ antiportersystems help H^+ ions to enter inside the cell and thereby reduce the internal pH. These adaptive features make them efficient ATP producers. Acidic proteins also help to retain the water cage around them in a highly salty environment. Halo-alkaliphiles actively accumulate ions and organic osmolytes such as glycine betaine, ectoine etc to maintain turgor pressure across the cell membrane. Though alkaliphiles have been evolved with remarkable structural and physiological adaptations, genetic modifications responsible for it are still unexplored. Alkaliphiles have diverse applications in different fields represented in the following diagram

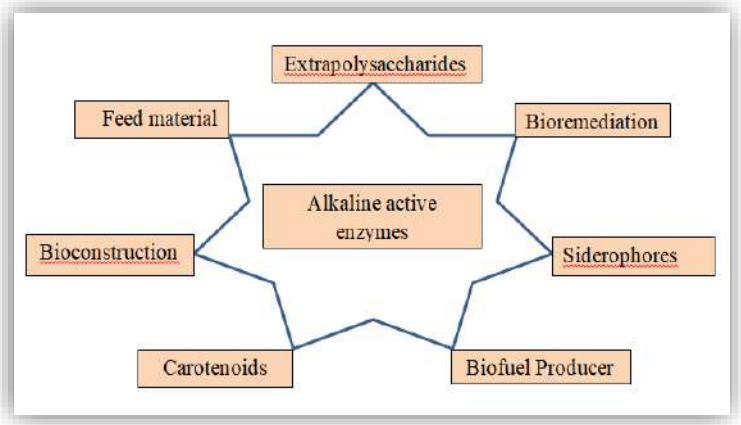

Figure - 2: Applications of alkaliphiles

In the recent time, as it is proved that extremozymes from alkaliphiles are golden assets for various industries, they have gained a lot of attention in research communities. Laundry industry is one of the major beneficiaries of alkali and halo stable enzymes from alkaliphiles. One of the US studies showed that CO_2 emissions can be reduced by 3 %, if people adopt environment friendly low temperature washing with detergents

having alkaline active enzymes. Following table represent some applications of enzymes from alkaliphiles:

Applications	Alkaline active enzymes and their roles
Detergent formulation	1) Proteases and lipases remove proteinaceous and lipids containing stains respectively, from cloths. 2) Cellulases remove the fine cellulosic fibers and give firm shine to the cloth.
Textile and fiber industry	1) Pectinases- for cotton scouring and fiber degumming processes. 2) Cellulases- for biostoning of denims.
Biofuel production	1) Lignocellulosic enzymes are used for direct hydrolyzation of alkaline treated lignocellulosic biomass.
Synthesis processes	1) Cyclomalto dextrin glucanotransferas- for cyclomaltodextrin synthesis from starch. 2) Esterase- synthesis of intermediate molecules for the production levofloxacin (an antibiotic) and paroxetine hydrochloride (antidepressant). 3) Proteases- for in vitro long peptide synthesis under alkaline and non-aqueous conditions.
Oil recovery process	1) Mannanses- for lowering the viscosity of polymers used for fracturing the bedrock.

Though alkaliphilic bacteria and archaea have led to many different biotechnological innovations, there is still a lot left to research about. In general, alkaliphiles have enormous potential but they are relatively less studied, and therefore not widely used in industries. This may be partly due the alkaliphiles cultivation is not well-established compared to the industrially important acidophilic and neutrophilic model microorganisms. Therefore, in order to use full potential of alkaliphiles there is a need to study and develop alkaliphilic model microbes.

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WATER BIOREMEDIATION

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Environmental pollution and its remediation are one of the major problems around the globe. Broad spectrum of pollutants *viz.*, heavy metals, pesticides, Hydrocarbons, Pharmaceuticals pollute the surface water and groundwater as well. Bioremediation is one of the most efficient technique for removal of environmental pollutants that recovers the contaminated site back to the actual form.

Bioremediation or we can call it as biology to the rescue is the utilisation of organisms are derivatives from the organisms to degrade the pollutants which are present in the contaminated site. Several different types of pollutants can be present at a time in the water which is supposed to be treated so a biomass or consortium of microorganisms are required for the effective by remediation. Microorganisms can decompose the hazardous substances into less toxic metabolites and use them as its energy source. are Due to the different types of pollutants there is no 'silver bullet' technique has been made for specific pollution site. There are wide variety of sources that contaminate the wastewater resources include septic walls, agricultural drainage, pharmaceuticals, geological formation and mines. Different methods are used to remove them they are bio accumulation, biosorption, biotransformation, biominerilization. The organisms used are *Aspergillus versicolour, Aspergillus fumigatus, Penicillium canescens* from the fungi; *Arthrobacter* spp*., Pseudomonas veronii, Bacillus cereus, Bacillus subtilis* from bacteria and some form algae *Sporosarcina ginsengisoli* and *Spirogyra* spp.

Conventional methods for removing pollutants may appear insufficient because of expensive costs that's why bio remediation comes forward with lower cost and higher efficiency. The efficiency of microorganisms can be increased by genetic engineering that is by making GEMs (Genetically Engineered Microorganisms). The small number of organisms and its slower rate in cleaning up the human made pollutants tends to modify their characteristics to accelerate the growth rate and give right chemical appetite. So far, this scientists are experimenting with bio enhancers and other techniques to increase their effectiveness.

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ALLERGENS

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Allergen from various sources such as pollen, fungi, animal dander, insects, dust mites, drugs and foods act as sensitizing agent inducing type I hypersensitivities. Most of the allergens characterized are proteins or their modified forms e.g., glycoprotein, lipoprotein, or proteins conjugated with chemical or drug haptens. Based on the route of exposure, allergens are classified into four categories i.e. inhalants (aeroallergens), ingestants (food), injectants (insect bite, stings etc) and contactants (cosmetics).

Inhalants

The suspended biogenic material from pollens, spores, mites, insects and animals belong to inhalant allergens responsible for allergic triggers.

Ingestants

Ingestants include foods or drugs that can cause nausea, vomiting, stomach upset, diarrhea as well as rhinitis, asthma or skin rashes.

Contactants

Contactants can result in rashes, itching, weeping blisters or other skin allergies on contact. The examples are cosmetics, metal, chemicals etc.

Injectants

Injectants group includes injectible drugs, insect sting/bites, bee/fire ant venom etc. Exposure to them can cause severe systemic reactions (anaphylaxis) and could be fatal. Based on the prevalence of an allergen in a given subset of population, they are

classified as major and minor allergens. An allergen is considered 'major' when it elicits a reaction in 50 % or more of an allergic population and 'minor' when less than 50 % of the allergic population responds to the allergen. Lipid Transfer Protein (LTP) of hazelnut is classified as a minor allergen in northern and middle Europe, while the PR-10 protein is acknowledged to be major allergen in this region. LTP is suspected to cause more severe reactions than the PR-10 protein, which usually induces milder reactions.

Purified allergens

A large number of native proteins from different sources are purified and characterized in recent years. Despite the knowledge available, the native proteins could not become popular for clinical use due to certain disadvantages. The cost of protein purification is high and the yield is low with most of the methods. The purified samples from different chromatographic techniques show variation from one batch to another. Partially purified allergens get degraded during long time storage due to inherent proteases.

Recombinant allergens

Conventionally whole allergen extracts of the source materials are used as diagnostic agents or therapy. The majority of such extracts are complex mixtures of proteins, and the actual allergen content is usually a small fraction of all proteins. The diagnosis of type I allergic disorders using crude allergen extracts allows only the identification of source but not the disease-eliciting molecules. Recombinant allergens can be produced as defined molecules in consistent quality and large amounts. Furthermore, they can be modified to reduce their allergenic activity and to foster advantageous immunologic properties.

Food allergens

IgE-mediated food allergy is a significant health problem affecting 6 - 8 % children and 3 - 4 % adults in general population. Various food allergens have been produced in recombinant form and have improved the diagnostics of food allergy.

Fungal allergens

Airborne fungal particles have been implicated as causative factors in respiratory allergy, particularly asthma. Fungi produce complex repertoire of allergens due to which cross-reactivity occurs between different fungal species. At present, more than 70 fungal allergens have been cloned, sequenced and some of the proteins are commercially available. Many allergic proteins from fungi namely *Aspergillus*, *Alternaria*, *Cladosporium* and *Penicillium* were characterized.

House Dust Mite Allergens

They are major source of indoor allergens with high frequency of IgE-mediated sensitization in many countries. House dust mites namely *Dermatophagoides farinae*, *D. pteronyssinus* and *Blomia tropicalis* play crucial role in triggering allergic disease.

Animal allergens

According to the American Academy of Allergy Asthma and Immunology, approximately 3 million children in United States have asthma triggered by allergens e.g., animal saliva, dander, dust mites and pollen.

Insect allergens

IgE-mediated hypersensitivity has been reported to insects such as, cockroaches, hornets, bumblebees, ants, mosquitoes, flies or kissing bugs. However, a majority of allergic reactions to insects are due to bites and stings. Stings from bees, wasps and ants produce a variety of clinical manifestations.

Pollen allergens

Pollen grains of weeds, trees and grasses are important sources of inhalant allergens. The advent of molecular biology techniques has led to isolation and cloning of genes encoding major pollen allergens.

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BIODIVERSITY OF MANGROVES IN CUDDALORE DISTRICT

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Introduction

Cuddalore is the southern part of the India. It is considered as historic moment. It is the city of district headquarters. "Nisha" cyclone in 2008 and "Thane" in December 2011 were caused heavy damage to this district in many respect, in recent years. This district experienced a worst impacts of Tsunami during the year 2004. The devastation caused by this Tsunami was billions of dollars worth infrastructure, economic assets and materials, more than this, the proportion of devastated precious human loss was more. Now in 2020, "Nivar" cyclone leads to worst impacts in all human life which includes all the organisms.

(Picture in google images)

The control human activities in these ecologically fragile areas, study co-author (Supriyo Chakraborty of IITM) Pune said that the mangroves carried out by MSSRF and the forest department at Pichavaram. It is the one way to enhance the carbon sequestration of the ecosystem. A 2011 study by Selvam *et al.* documents a decrease in the total area of mangroves from 1930 to 1996 and then an increase from 1996 to 2011. Then that area was reduced to about 411 hectares from 1165 hectares within a period of 60 years that is from 1930 to 1994. Even though, the mangrove forest cover increased from 411 h to 941 h in about 18 years from 1994 to 2011, according to the study. They restored huge amounts of mangroves and its Nationally Determined Contribution (NDC), India has pledged to sequester an additional 2.5 to 3 billion tonnes of carbon-di-oxide (CO_2) equivalent by 2030 under the Paris Climate Agreement. Nearly, 25 % of India's total land area is now under the forest and tree. India has a target of having 33 % of its total area under the forest and tree. The Tamil Nadu State Action Plan on Climate Change 2.0 (2021 to 2030) which is aligned with the NDC and Sustainable Development Goals, states the state targets 5,000 h (56 sq. km) area under mangrove cover by 2030 as against the current mangrove cover of 4,500 h (45 sq.km).

Pichavaram productivity

Pichavaram mangrove flora is split into two zones: *Rhizophora* in the fringes and *Avicennia* in the interior are the Avicennian zone comprises nearly 75 % of the total area under mangroves and these mangroves are shorter so that the wetland receives less rainfall and freshwater flow, impacting their productivity. "At Pichavaram, the mangroves are shorter since the wetland receives less rainfall and freshwater flow. Only limited water is being let out inside the mangroves, allowed during the monsoon. The soil is also sandy. According to the studies the Net Ecosystem Productivity (NEP) value of Pichavaram is only slightly less compared to the denser Sundarban mangroves, but considerably lower than those reported from other mangrove ecosystems across the world such as the Florida Everglades mangrove forest. Pichavaram is semi-arid and dams upstream of the Cauvery delta have interfered with the freshwater flow. Only during floods a good amount of freshwater flow into mangroves. In the remaining period, freshwater flow into mangroves is very less.

Phytoplankton

Asterionella galcialis
Biddulphia mobiliensis
Biddulphia sinensis
Ceratium furca
Cocconeisdis culoidus

Coscinodiscus centralis
Coscinodiscus gigas v. pretexta
Coscinodiscus lineatus
Coscinodiscus oculus-iridis
Coscinodiscus thorii
Hemidiscus hardmanianus
Leptocylindrus danicus
Navicula salinarum
Nitzschia closterium
Pleurosigma angulatum
Pleurosigma elongatum
Pleurosigma normanni
Rhizosolenia alata
Rhizosolenia alata f. gracillina
Rhizosolenia imbricata
Rhizosolenia setigera
Rhizosolenia stolterfothii
Rhizosolenia styliformis
Skeletonema costatum
Stephanopyxis palmeriana
Thalassionema nitzschioides
Thalassiothrix frauenfeldii

Shell Fishes

Modiolus metcalfei
Melampus ceylonicus
Cassidula nucleus
Pythia plicata
Perna viridis
Cerithidea cingulata
C. obtusa
Telescopium telescopium
Meretrix meretrix
Crassostrea madrasensis

Sacostrea cucculata
Littorina scabra
Assiminea nitida
Nerita crepidularia

Zooplankton

Acrocalanus chilkaensis
Acrocalanus gracilis
Brachionus angularis
Brachionus calcuflorus var. annuraeiformis
Brachionus calyciflorus
Brachionus forficula
Brachionus patulus
Brachionus plicatilis
Brachionus quadridentatus
Brachionus rubens
Cephalodella gibba
Colurellabicus pidata
Encentrum felis
Eucalanus crassus
Euchlaris oropha
Euterpina acutifrons
Keratella cochlearis
Keratella procurva
Keratella tropica
Lacenecur vicornis
Lacen eluna
Macrosetella gracilis
Mytilina ventralis
Oithona brevicornis
Oithona rigida
Paracalanus parvus
Platyias quadricornis
Ploesoma lenticulare
Pontellopsis scolti

Pseudodiaptomus serricaudatus
Temora turbinata
Testudinella patina
Trichocera tortuosa
Trichotria tetractis

Conclusion

I here with concluding by saying that mangroves are very useful for many organisms to survive in this world. Biodiversity is more important in all organisms to know about that particular organisms. So, we should protect our mangroves and marine sources also for our future generation also.

Stay safe! **Stay healthy!**

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ANTIMICROBIAL PROPERTIES OF *Cocos nucifera*

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Cocos nucifera belongs to Monocotyledon group of Palmae family. It is an economically important plant. It has many uses such as ornamental purpose, food crop etc. It consists of a root system which has thousands of roots which grows throughout the palm's life span. *Cocos nucifera* is straight and consist almost 12 leaves annually. It has a single seed. The cross section of the fruit shows a smooth, waxy, colorful epidermis, a fibrous mesocarp or husk, and a very hard endocarp A brown coating surrounds the seed which encloses a white layer of about 1-2 cm thickness. From this part, we get the copra oil and coconut water.

Cocos nucifera has many antimicrobial properties. Coconut water contains micro minerals and nutrients which are essential to human health. We use coconut as food. Antibacterial effect of coconut endocarp extract gives a strong activity against *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Micrococcus luteus* whereas antimicrobial properties of coconut shell showed strong antibacterial effect on *Escherichia coli* and *Salmonella typhi*. Tender coconut water can be used to treat urinary tract infection and diarrhoea. They can also cure oral diseases. The oil we obtained from heating the coconut shell is very useful in treating ringworm infection. Alcoholic extract of ripe dried coconut shell has antifungal activity against *Microsporum canis*, *Microsporum gypseum* and *Trichophyton rubrum*. Coconut oil is also effective against a variety of lipid coated viruses. The medium chain fatty acids in coconut oil destroy the viruses by disturbing their membrane, interfering virus assembly and maturation.

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ROLE OF GUT MICROFLORA

Hritam Barman

It is estimated that the adult human body is composed of trillions of cells. The human also serves as the natural habitat for the 10 times the number of microbial cells. The term normal flora implies that these microbes are harmless, and for the most cases they do not cause diseases and are even beneficial. Most are commensals: they benefit from the association with the host. Although some are also opportunistic pathogens; they may cause infections if tissue injury occurs at specific sites or if the resistance of the body to infection is decreased. This is especially important because in recent years there have been a rising incidence of infections from these microorganisms.

The microbiome has received great attention over the last 10 to15 years. Although gut microbes have been explored for several decades, scientists of the role of microorganisms that reside in the human gut has attracted much attention beyond classical infectious diseases. For example, various studies have reported changes in the gut microbiota during not only obesity, diabetes, and liver diseases but also cancer and even other neurodegenerative diseases.

Microbes that reside in the human gut are key contributors to host metabolism and are considered potential sources of novel therapeutics. After last 15 years we are now able to characterize the composition and function of microbiomes. They are related health, including the early life3–5 but also specific diseases, such as cardiometabolic disorders, inflammatory bowel diseases, neuropsychiatric diseases and cancer. The gut microbiota is now considered an important partner of human cells, interacting with all the human cells.

In addition to bacteria, other key microorganisms, such as archeae, viruses, phages, yeast and fungi, are also present in the gut. These microorganisms, which likely control the activity of the host and, most importantly, of the gut microbes, may be as important as bacteria. Therefore, the archaea, the virome, the phageome and the mycobiome offer an additional dimension to the investigation of host–microorganism interactions. Major mechanisms involved in the crosstalk between microbes and host: impact of metabolism. The balance between healthy and pathological situations (eg, metabolic disorders) is crucial. This is under the tight influence of several factors including the genes, food and drugs.

The composition of the gut microbiome is associated with a higher mucus layer thickness, the production of antimicrobial signals and different short-chain fatty acids such as butyrate and propionate. Both butyrate and propionate bind to G protein coupled receptors (GPR)-43 and GPR-41 expressed on the enteroendocrine L-cells thereby stimulating the secretion of gut peptides such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). This effect contributes to reduce food intake and to improve glucose metabolism. Propionate can also bind to GPR-43 expressed on lymphocytes in order to maintain appropriate immune defence. Butyrate activates peroxisome proliferator-activated receptor-γ (PPAR-γ) leading to beta-oxidation and oxygen consumption, a phenomenon contributing to maintain anaerobic condition in the gut lumen. During metabolic disorders, changes in the gut microbiome are linked with a lower mucus thickness, decreased antimicrobial defense and butyrate and propionate production. As a consequence, L-cells secrete less gut peptides. The lack of PPAR-γ activation lead to higher oxygen available for the microbiota at the proximity of the mucosa and increases the proliferation of Enterobacteriaceae. The decrease in propionate also contribute to the lower abundance of specific T cells (mucosal-associated invariant T cells (MAIT) and Treg) in the lamina propria of the gut. Altogether, such changes in the microbial environment and metabolites induce a leakage of pathogen associated molecular patterns (PAMPs) such as the lipopolysaccharide (LPS) that are increased in the blood, and trigger low-grade inflammation.

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ECONOMIC IMPORTANCE OF *Agaricus bisporus* MUSHROOM

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Agaricus bisporus, better known as white button and edible mushroom, establishing over 90 percent of mushrooms consumed in America. It is a part of the human diet for over 200 years and is produced in atleast 70 countries around the world. It has increased due to their high nutritional and therapeutic value, pleasant taste. *Agaricus bisporus* are white mushroom, it's not only low in calories, but also packed with essential nutrients, including fiber, protein and vitamin D, with the latter promote in bone health, decreasing the risk of fall and fracture causing osteoporosis. It contains multiple antioxidant compounds that are fighting properties of cancer, while also conflict the harmful effects of oxidative stress, which stimulates aging and high risk of heart disease and cancers. The phenolic compounds present in white mushrooms, flavonoids and phenolic acids act as antioxidants and pro-oxidants, both to help in improving cell survival and to prevent tumor growth, lead to its death. It also contains bioactive compounds. The present in glutathione helps destroy your body of potentially harmful foreign substances, acting both as an antioxidant and a detoxification agent. The polysaccharides in white mushrooms improving in insulin resistance and control in blood sugar levels. Its content also doubles as prebiotics that help improve gut health.

Healthy lever

Agaricus bisporus present inβ-glucans and proteins it's prevents liver steatosis and protects the liver in hepatic injury.

Lowering the cholesterol level

It contains dietary fibers and antioxidants, vitamin C, D and B12, folates and polyphenols. These compounds help in decreasing blood cholesterol, low density lipoprotein cholesterol, triglycerides and increase plasma high density lipoprotein.

Prevent cancer

Ergothioneine, lectin and beta-glucan found in white button mushrooms act as an antioxidant for breast cancer. It inhibits the cell proliferation and promotes apoptosis thus prevent prostate cancer in men and also reducing risk of heart disease.

Control diabetes

It contains polysaccharides which is natural antidiabetic may improve impaired glucose tolerance and reduce hyperglycemia and α-amylase activity.

Anti-aging properties

It is an excellent antioxidant which remits and improves the aging properties by neutralizing free radicals and prevents age related diseases.

Boost brain health

The antioxidants such as ergosterol can be converted to vitamin D2 by treatment with UV light and helpful in the development of the central nervous system.

Healthy strong bones

The presence of ergosterol in mushroom is a natural source of vitamin D2 is essential for calcium absorption and bone health and a deficiency can lead to softening of the bones in children and adults as well as osteoporosis in adults, thus intake of mushroom improve bone mineralization and prevent osteoporosis.

Improve sperm quality

Selenium present in white button mushroom helps in the improvement of sperm production and sperm quality and fertility in person.

Anti-inflammatory properties

Polysaccharides, terpenoids, phenolic compounds and many bioactive compounds present in the white button mushroom act as potent anti-inflammatory agents.

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ADVANCES IN PLANT HEALTH MANAGEMENT IN THE TWENTIETH CENTURY

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Plant health management is vital, the science and practice of understanding and best the succession of biotic and abiotic factors. An important science based approach is essential to address the risks of plant health and problem that affect productivity. Healthy plants are needed to sustainable and beneficial crop production and to the quality and expenditure of the nation's supply of fuel, food and fiber. Health is also critical to plants used for ornamentals, natural resources, and animal feed. Plant Health Management is important for the viable agriculture, food safety, food security, agro based industries. That limits plants from attain their full genetic ability as crops, timber trees, ornamentals. Although performed as long as agriculture, Plant health management used in Integrated Pest Management (IPM). It is an ecosystem-based strategy that observes on long-term prevention of pests and controls their damage. It's used in biological control, modification of cultural practices, habitat manipulation and use of resistant varieties. The vast scientific and technical approaches for plant health management are diagnosed and control plant disease pathogen level and planting crops. This management improves soil fertility used in bio fertilizers, bio pesticides and crop rotation and clean tillage. Incorporation of biofertilizers, especially mycorrhizae play a vital role in agriculture, it's promoted of soil health and utilize the important macro and micro nutrients by the crops. Biological control through predators, parasitoids, and

microbes establish a significant basic in holistic management. The greater significant improvement in the twentieth century of produced high yield crops used in new gene production and induced plants. Research aimed at the management of plant affect pathogens is also the basis for modern theory on epidemiology, plant pathology, population biology, disease prediction and aerobiology. The role of microbes in enhancing soil fertility and crop protection & high yield crops production, the role of rhizosphere microbes and facility for rhizosphere manipulation, promote plant health, Isolation techniques for beneficial nonpathogenic micro-organisms, fungal bio-inoculants for soil and *Trichoderma* sp., *Pseudomonas* sp., endomorphogenic fungi and endomorphogenic nematodes are used to control plant management. Bacterial and fungi endophytes are phytohormones that help in growth and development of the plant. Endophytic microbes have antibacterial, antifungal insecticidal properties, it's inhibits the growth of organisms including phytopathogens. IPM begin mainly in respond to the crops protect from pests. In the twenty-first century and compact through modern biotechnology use the biological control includes the plant induced or genetically modified gene production.

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ANTICANCER ACTIVITY OF VINCAMINE

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A major alkaloid of *Vinca minor* L., Apocynaceae. It has been used therapeutically as a vasodilator and antihypertensive agent, particularly in cerebrovascular disorders. Vincamine is an Vinca alkaloid, an alkaloid ester,an organic heteropentacyclic compound a methyl ester and an hemiaminal. It has a role as an antihypertensive agent, a vasodilator agent and a metabolite. It derives from an Eburanamonine.

Vincamine is a monoterpenoid indole alkaloid obtained from the leaves of vinca minor with a vasodilatory property. Vincamine, Vincopectine and Eburanamonine are alkaloids known for their neuroprotective attributes enhancement of cerebrovascular blood flow and antitumour effect of their derivatives. However, the relative metabolic stability of these alkaloids and their extrusion by the drug efflux transporters expressed at the Blood-Brain Barrier (BBB) are not clear till now.

Structure of Vincamine:

Cerebrovascular diseases

Vincopectine (ethyl-apovincamine-22-oate vp) is a derivative of the alkaloid vincamine (Commercial name Cavinton) used in the treatment of ischemic stroke and other cerebrovascular diseases also was found that Vinopepsin was encapsulated shown positive influence in the aggregate blood and plasms and also changes in viscosity of the whole blood.

Peri wrinkle is used for circulatory disorders, cerebral circulatory impairment, support for the metabolism of the brain and its improved oxygen supply, prophylaxis of memory and concentration impairment, improvement of memory and thinking capacity, mental productivity, prevention of premature aging of brain cells, for geriatric support, as a sedative and as a blood pressure-lowering remedy , for catarrhs, feebleness, and for improvement of the immune function for diarrhea, vaginal flux, throat aliments, tonsillitis and angina, sore throat, intestinal inflammation, toothache, dropsy, as a diuretic and blood-purifying remedy, for promotion of wound healing, as a hemostatic remedy, and a bitter principle. Vincamine is widely used in human medicine to increase global and regional blood flow in patients suffering from acute or sub-chronic cerebral ischemia. It is mostly used in combination with heptaminol (a central nervous system stimulant) and papaverine (a vasodilator). Vincamine is sold in Europe as a prescription medicine for the treatment of primary degeneration and vascular dementia. In the United states it is permitted to be sold as dietary supplement when labeled for use in adults for six month or less. Most common preparation are in the sustained tablet forms.

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A COMPREHENSIVE STUDY ON STIGMASTEROL

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Stigmasterol, also known as Stigmasterin or Wulzen anti-stiffness factor (Figure 1), an unsaturated plant sterol present in various medicinal plants. Stigmasterol is utilized in a number of chemical processes which are designed to yield numerous synthetic and semi-synthetic compounds for pharmaceutical industry. It acts as a precursor in the synthesis of progesterone and acts as an intermediate in the biosynthesis of androgens, estrogens, corticoids 1 and in the synthesis of vitamin D3

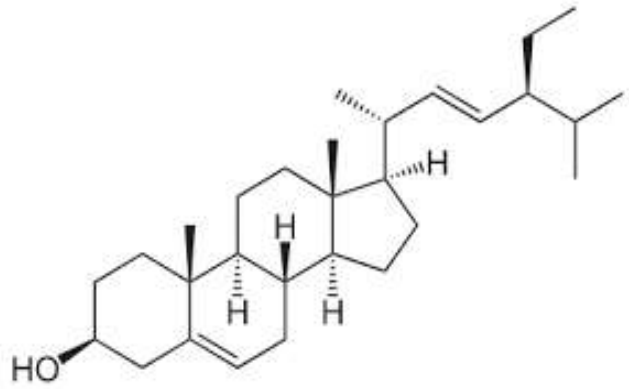

Figure - 1: Structure of Stigmasterol

Stigmasterol has been investigated for its pharmacological prospects such as Antiosteoarthritic, Antihypercholestrolemic, Cytotoxicity, Antitumor, Hypoglycaemic, Antimutagenic, Antioxidant, Antiinflammatory and CNS effects. *Aloe* is known for its topical use for treating wounds and burns. Many previous studies reported the healing effects of *Aloe vera*. However, there are few clinical studies on the effect of orally administered *Aloe vera* gel on the skin. Aloe sterols are a type of plant sterols that have the capability to regulate the metabolism of glucose and lipids. In a recent study, we confirmed that ingested Aloe sterols reached the peripheral tissues through the bloodstream.

Recently, it was confirmed that the daily oral intake of plant sterols of *Aloe vera* gel (*Aloe* sterol) significantly increases the skin barrier function, moisture, and elasticity in photo protected skin. As one of the major phytosterols, Stigmasterol is included among sterol compounds in the diet having potential to reduce the risk of cardiovascular diseases. Consumption of 2 grams per day of plant sterols is associated with a reduction in blood LDL cholesterol of 8 – 10 %, possibly lowering cardiovascular disease risk. As a factor in cellular processes of plants, stigmasterol may have roles in plant stress responses, metabolism, and enzymes involved in biosynthesis of plant cell membranes. Stigmasterol has also been shown to exert anti-angiogenic and anti-cancer effects *via* the down regulation of TNF-alpha and VEGFR-2.

Stigmasterol is a food additive in manufactured food products in the United Kingdom and European Union. It was introduced as a precursor by Percy Lavon Julian for industrial large-scale manufacture of Semi-synthetic Progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens estrogens, and corticoids. It is also used as the precursor of Vitamin D3.

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SCP: A POTENTIAL FOOD SOURCE FOR ASTRONAUTS

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The consumption of several microbially processed foods like alcoholic beverages, cheese, yogurt, etc. Either intentionally or unintentionally has been practiced by the human for thousands of years but the idea of consuming whole microbial cell as food is started in few decades' past. The protein obtained from several single-celled microbes like *Spirulina, Cellulomonas, Chlorella and* yeast like *Saccharomyces* is known as Single Cell Protein (SCP), with the advancement of technology now this SCP is modified in such a way that can fulfil a human's nutritional requirements.

Sources of SCP

The SCPs are generally classified according to their source organism. Major sources of SCP are bacteria (*Methylophilus methylotrophus*), cyanobacteria (*Spirulina maxima*), algae (*Chlorella* spp.), fungi (*Fusarium graminearum*) and yeast *Saccharomyces cerevisiae*. Being high in nutritional content SCP is often the chief choice as a food supplement.

Traditional protein sources vs SCP

Proteins are made up of about 22 amino acids, of which 8 are essential for human life. These essential amino acids cannot be synthesized by our bodies and must be ingested. SCP is a massive straightforward in simplifying and improving the efficiency of

the protein food chain. Grains and cereals fed to animals convert to meat on the table at extremely low efficiencies but SCP converts at greater magnitudes.

Conventional Space foods

Nowadays space foods are similar to those eaten every day on earth. Nutritionists ensure the food that is being consumed provides them with a balanced supply of vitamins and minerals. Although the calorific requirements might differ for astronauts from person to person. Considering that a space mission can last for months together, foods in space must be designed and packaged to prevent spoilage. The conventional space foods usually fall into the eight categories are described in Table - 1.

Table - 1: Conventional Space Foods for Astronauts

Categories	Characteristics of foods	Examples
Rehydratable Food	Dehydrated before packaging and rehydrated before eating.	Hot cereal such as oatmeal etc.
Thermostabilized Food	Heat-processed foods so they can be stored at room temperature in cans.	Processed meat or fish etc.
Intermediate Moisture Food	Contains enough water to maintain its soft texture.	Dried peaches, pears, apricots, etc.
Natural Form Food	These are ready to eat foods are packaged in flexible pouches.	Nuts and cookies etc.
Irradiated Food	These are cooked, packaged in flexible foil pouches and sterilized by ionizing radiation so they can be kept at room temperature.	Beefsteak, Smoked Turkey, etc.
Frozen Food	Quick-frozen to prevent a build-up of large ice crystals that retain the original texture and taste of the food.	Quiches, Chicken pot pie, etc.
Fresh Food	Unprocessed foods.	Fresh fruits.
Refrigerated Food	Require cold or cool temperatures to prevent spoilage.	Cream cheese and sour cream etc.

SCP as Astronauts' food

The goal is to provide astronauts with foods that are rich in nutrients, vitamins, and minerals. *Spirulina* spp. are rich sources of beta-carotene, which helps to improve eyesight and has very high natural protein content with high antioxidant and fiber content. This protein has 8 of the 22 essential amino acids that the body needs and is easily digestible. This ensures proper utilization as well as assimilation of food.

Spirulina's rich content of natural iron and folic acid also helps to enhance the hemoglobin levels in the blood.

Advantages of SCP over Conventional food

Conventional space foods consumed by astronauts needed a large specialized refrigerated container to store and some of those may also require complex processing before storage. In contrast to conventional space foods, SCP plays a vital role in terms of storage as these are readily available in dried or tablet form so there is no requirement for a special storage container and can be kept in normal condition for a longer period. This SCP is widely accepted by both types of people whether vegetarian or non-vegetarian. Moreover, SCP does not mean that it only contains protein supplements but also its nutritional components can be altered as requirements. So, this SCP is might be a good alternative to conventional protein supplement food for the astronauts that keep them energetic for a longer period.

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ANTIBIOTIC RESISTANCE AND ITS IMPACT ON HUMAN HEALTH

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Key facts

- In 1928, Alexander Fleming discovered the first natural antibiotic called Penicillin.
- Antibiotics cannot fight against viral infections.
- An increasing number of diseases like pneumonia, tuberculosis, gonorrhea is becoming less effective with the antibiotics used to treat them, making them more difficult to treat.
- Antibiotics do not work against bacterium if they are taken too often because they are adapted property to survive Antimicrobial resistance

Introduction

Antibiotics is nothing but it's group of chemotherapeutic agents or produced by microorganisms that are used to treat infection of bacteria, fungi, and protozoa. Antibiotics are powerful medicines that fight certain infections and can save lives when used properly to treat infectious microbes such as bacteria, fungi and protozoa. Recently, these bacteria have become so prevalent that even the most effective antibiotics. Antibiotic resistance is a public health concern around the world. It is important to be careful when using antibiotics to prevent this. All classes of microbes are develop

resistance such as bacteria develop antibiotic resistance, fungi develop antifungal resistance, protozoa develop antiprotozoal resistance and viruses developed antiviral resistance. Antibiotic resistance and multidrug resistance nothing but it's simple mechanism when you're taking specific designed drug for cure infection than drug cannot respond kill the infection instead it survives.

Background and History

The term antibiotics is first used in medical science by the Ukrainian - born Jewish-American inventor, biochemist and microbiologist Selman Waxman who discovered Streptomycin antibiotic. In 1943, the first antibiotic Penicillin was invented by a Scottish Physiologist Alexander Fleming in 1928. He was working in his laboratory on *Staphylococcus aureus* but accidently bacterial culture was contaminated by blue mold *Penicillium notatum*. Eventually he noticed the bacteria around the mold were killed by antibacterial substance. He awarded Nobel prize in physiology and medicine in 1945, after that antibiotics were widely used as a largely effective remedy. But, improper use gives bacteria a chance to identify the mechanism of action of antibiotics and then Mutations in them to give them a boost. And they are becoming more and more effective and powerful. They have got the property that they develop antibiotic resistance property not respond to any of the drugs at all.

Classification of Antibiotics

(i) On the basis on Mechanism of action

Classification on the basis of mechanism of action divided in two groups: Bactericidal and Bacteriostatic. Bactericidal antibiotic can kill bacteria and inhibit the cell wall synthesis of bacteria. Example include Beta lactam e.g., Penicillin, Trimethoprim and Tetracycline. Bacteriostatic antibiotics can limit or stops the growth and reproduction of bacteria.

(ii) Classification of Antibiotics on the basis on Chemical structure

On the basis of chemical structure there are 3 classes

a) Beta lactam antibiotics (inhibit Cell wall synthesis) e.g., Penicillin.
b) Non-beta lactam antibiotics (inhibitors of Protein synthesis) e.g., Tetracycline and Streptomycin.
c) Nucleic acid synthesis inhibitors (Target the Nucleic acid) e.g., Rifamycin

(iii) Classification on the basis of Spectrum of Antimicrobial activity

a) Narrow spectrum - e.g., Bacitracin and Penicillin.
b) Broad spectrum - e.g., Chloramphenicol and Streptomycin.

Scope of problems

Usually antibiotics are prescribed only for more serious bacterial infections, as many infections go away on their own. Proper use of antibiotics is absolutely essential to help reduce antibiotic resistance. Over time, microorganisms become resistant to antibiotics, making them less effective. Resistance to tuberculosis, pneumonia, gonorrhea can become more and more difficult if infectious bacteria become strong, it can lead to increased disease and stress on our health system.

Mutation

Bacteria develops resistance to antibiotics through the modification of genetic material means mutation. Most of bacterium are adapt the property to survive quickly to environmental conditions so bacteria can develop more resistant to antibiotics. During reproduction in bacterial mutation is arise and this mutation make resistant to antibiotics.

Gene transfer

Bacteria are having property to gene transfer from each other and most of microbes get genes from each other so this property make drug resistant DNA this DNA may transfer a copy of drug resistant gene in other bacteria. This gene transfer process is also known as horizontal gene transfer process.

Inappropriate use

Antibiotics should be taken only on the advice of a doctor. Antibiotics should not be prescribed to a doctor or pharmacist for minor ailments. Most people are do self-medication.

Impact on Human health

Some health experts fear that people will overuse antibiotics, he believes that this overuse increase the growing number of bacterial infections that are becoming more resistant to drugs that inhibit the growth of bacteria. There is no need to use antibiotics for any viral disease, no antibiotics are prescribed for it, but many patients take antibiotics for common cold and viral fever, which makes antibiotics still more effective and resistant. In dairy, Fisheries, Poultry Farms use a lot of antibiotics. Some antibiotics are stored in the body of these animals. The same antibiotics come back into your body

in the form of meat and milk. But scientists have not confirmed this officially. It is very important to create awareness in the society about the adverse effects. Most of the time, allergies cause temporary illnesses such as rashes, coughs, and colds. We use a lot of antibiotics for treated such allergy, we take drugs from a drug store without a doctor's permission, one of the most harmful effect is taking antibiotics too much until your body immune to antibiotics and course of antibiotics can weaken your immune system. That's why doctors not recommend like daily medicine. Also, some antibiotics have an effect on organs like kidney and liver so it would be beneficial to take them from a proper professional doctor. We can't say for sure what will happen due to antibiotic resistance, so it is very important to take precautions now.

WHO response

Resolving antibiotic resistance is a high priority for the WHO. The global action plan on Antibiotic resistance, including Antibiotic resistance, was endorsed by the World Health Assembly in May 2015. The goal of the Global Action Plan is to ensure the prevention and treatment of infectious diseases with safe and effective drugs.

The Global Plan on Antibiotics Resistance has 5 objectives

a) To improve antibiotic resistance awareness and perception.
b) Surveillance and strengthening research.
c) To avoid the incidence of infection.
d) To avoiding the misuse of Antibiotics.
e) Ensuring sustainable investment in antibiotic resistance.

World Antimicrobial Awareness Week

Held annually since 2015, WAAW is a global campaign aimed at raising awareness around the world about immunization and promoting best practices among the general public, health workers and policymakers to prevent the emergence and spread of antibiotics resistance infections. WAAW from 18 to 24 November each year. Earlier it was the slogan, "Antibiotics: be careful with handling" but was changed to "Antimicrobials: Handle with Care" in 2020 to reflect the wide range of drug resistant infections.

Preventing Antibiotics Resistance

Individual precautions

- Antibiotic resistance has caused a stir in the medical field. It is a matter of time before we have to face another terrible situation.
- Doctor's use of so many antibiotics on patients, use of more powerful antibiotics even when not needed, strict enforcement of drug laws by the administration.

- In order to reduce the use of antibiotics, it is necessary to try to prevent infection. For this, it is necessary to implement various measures at various levels such as washing hands with soap, water, personal and social hygiene, vaccination.
- Use antibiotics only if prescribed by a certified health professional.
- Never ask for antibiotics if your healthcare provider says you do not need them.
- Always follow the advice of your healthcare provider when using antibiotics.
- Never share or use residual antibiotics.

Health Professionals

- When the diagnosis is not made exactly which disease is caused by a virus or a bacterium, then doctor prescribes a course of antibiotics as a precautionary measure, after that patient stops the dose of antibiotics if he recovers in two days.
- Due to self-medication and selling without a doctor's prescription antibiotics are being over controlled, its main reason of maximum misuse of antibiotics. a course of antibiotics needs to be taken regularly for a certain period of time to completely cure and reverse the infection.

Health Industry

- The health care industry can
  - ✓ Investment of new research
  - ✓ Improving new strategy against drug
  - ✓ Development of new Antibiotics
  - ✓ Improving new diagnostic methods and other techniques
  - ✓ Vaccination

Agriculture Sector

- To prevent and control the spread of antibiotic resistance, the agricultural sector can: Promote and apply best practices at all stages of food production and processing from animal and plant sources.
- Improve biological safety in the field and prevent infections through improved hygiene and animal welfare.

Veterinary Uses

- Since in 1940's antibiotics given to farm food producing animal such as meat and milk producing animal in order to cure infection and prevent an illness.
- Mostly antibiotics are used in low doses in animal feed for promoting growth, that means increasing production of meat and milk in short period.
- The misuse of antibiotics in meat and milk producing animal increase resistant in bacteria, also known as ``Superbugs".

Conclusion

We take antibiotics without any training, we have no idea what are the consequences of antibiotics resistance. This will place a greater financial burden on our health care system. According to WHO antibiotics can be purchased for human or animal without any prescription. This reason increase in resistance and spread is made worse. In some countries that do not have standard treatment guidelines, antibiotics are more commonly prescribed by health workers and veterinarians and more commonly used by people. In short, use antibiotics only when needed. It is an effective weapon, only if it is taken in the right amount and for the right period with an accurate diagnosis.

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GUT BACTERIA AND HEALTH

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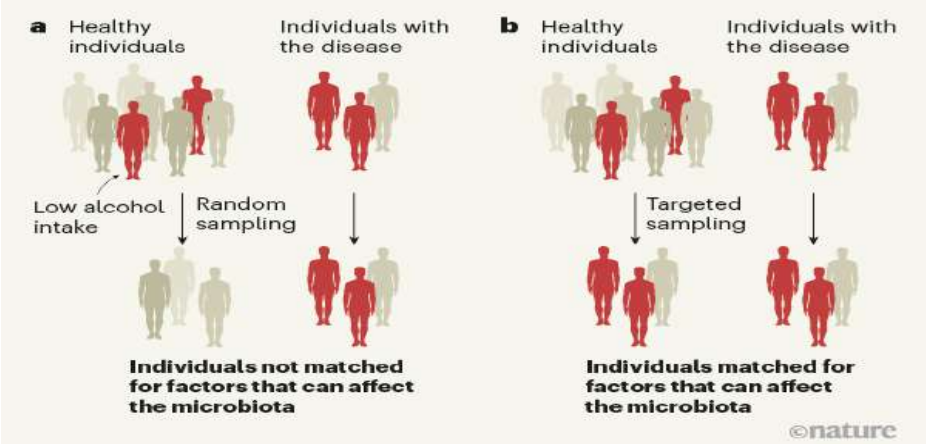
The human gut microbiota may be viewed upon as an organ, and contributes to the digestion of food and the breakdown of toxins and drugs, regulates lipid and glucose metabolism, plays a fundamental role in the induction, training and function of the host immune system, modulates gene expression, and reduces inflammation**.** Accumulating evidence suggests that gut bacteria play critical roles in maintaining human health in many aspects. For example, gut bacteria could train the immune system, prevent the growth of pathogenic bacteria, regulate the gut development, maintain epithelial integrity, and shape the neuronal development. The particular composition and diversity of the gut microbiota are associated with many health conditions. However, it is usually not known whether such associations are just correlative or a consequence of the health condition, or whether they might cause, or contribute to, the illness. Addressing this problem is highly challenging because of the many physiological and lifestyle differences that can exist between individuals who are healthy and those who have the illness of interest.



Comparing populations to assess connections between gut microbes and human disease

Vujkovic-Cvijin reported that gender, age, bowel-movement quality (categorized as stools that are solid, normal or loose), body mass index and level of alcohol consumption are among the strongest potential confounders that could hinder efforts to identify true associations between disease and gut-microbiota composition. This is because these characteristics are strongly associated both with microbiota composition and with disease status. When examining the differences between individuals with a condition such as type 2 diabetes and people who do not have this condition (but who might have other diseases), there seem to be many statistically significant associations between disease status and the abundances of different gut bacteria. By contrast, if individuals who have or do not have the disease are matched using some of the confounder criteria mentioned, many of these associations cease to be statistically significant. This implies that some gut-microbiota changes previously attributed to certain diseases might instead stem from other underlying causes related to these confounders. It has been proposed that gut bacteria are required to maintain epithelial integrity by regulating tight junction permeability. *Lactobacillus plantarum*, for example, was reported to regulate tight-junction proteins to protect against chemical-induced disruption of the epithelial barrier. Loss of gut epithelial integrity will allow gut bacteria, bacterial toxins, incompletely digested fats and proteins, and wastes to pass the epithelium into the blood stream, triggering inflammatory responses and leading to gastrointestinal problems, such as abdominal bloating, excessive gas and cramps, and food sensitivities. These symptoms are characteristic of leaky gut syndrome, which exhibits intestinal hyper permeability. Two major types of IBD are ulcerative colitis (UC) and Crohn's disease (CD), both of which have been shown to be associated with dysbiosis of the gut microbiota It is known that colonising gut bacteria are critical to the normal development of host defence. Strikingly, fully functional development of the GALT (gut-associated lymphatic tissue), a sophisticated set of immune tissues, critically

depends on the interactions with an intact bacterial gut flora. Recent studies suggest that the gut microbiota influences CNS development and function, and that gut dysbiosis is associated with significant neurological problems. However, most of these results have been collected in experimental animals and cannot be transferred to humans immediately. In summary, commensal bacteria play numerous important roles in maintaining human health, and they also affect a variety of complex behaviours, including social, emotional and anxiety-like behaviours, and contribute to brain development and function. Large-scale efforts have described in great detail the constituents of the healthy human microbiome and its functional capacity, and these studies are followed by similar characterizations of the microbiome in particular diseased states.

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Spirulina: A NUTRITIOUS CYANOBACTERIA

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Spirulina is a common term for a large number of cyanobacteria in the Spirulinaceae family. It is a genus of the phylum cyanobacteria which are classified as bacteria. The name originates from Latin word "spiral" due to its physical characteristics. They are single celled photosynthetic organisms also known as blue green algae that have 0.2mm to 0.5mm long spiral shape generally found in alkaline lakes, fresh waters, and natural springs in subtropical and tropical climates. *Spirulina* was first used in Mexico by Aztec people in the 16th century. They harvested spirulina from Lake Texcoco and made dry cakes called "tecuitlatl". Later, they were dried in the form of patties and were eaten together with cereal grains. There are few regions worldwide that have ideal climate for the growth of *spirulina* including Greece, Japan, India, United States and Spain. In India they are widely cultivated in Auroville, Tamil Nadu. The most common used species are *Spirulina maxima* and *Spirulina platensis* because of their high nutritional as well as potential therapeutic values.

Spirulina contains high concentration of macronutrients, vitamins, minerals. It contains 60 % protein content, 5 % lipid content by weight, 13.6 % carbohydrates and 18 amino acids including essential as well as non-essential amino acids. They grow in high temperature up to 60 ℃ at pH 9 to pH 11 with high salt concentration (>30 g/l). It is also known as "superfood" due to its richness in protein content. *Spirulina* can be easily digested as it lacks cellulose cell wall. It requires 10 times less water than vegetables to grow. It is generally mixed with juices, yogurts to lessen its bitter taste. *Spirulina* became

famous as a food supplement after astronauts from NASA used them for their space missions.

Benefits of *Spirulina*

a) **Antiantioxidant and Anti-inflammatory properties**: It has main active antioxidant component phycocyanin which gives the *spirulina* blue green colour as well as inhibits the production of inflammatory signals leading to Anti-inflammatory effects.
b) **Used as Dietary supplements**: It is used in the form of tablets, health drinks, powder due its high protein value. Improves muscle strength and endurance. Additionally, used as feed supplement in aquaculture, aquarium and poultry industries.
c) **Helps build Strong immunity**: enhances the function of immune cells like B cells and T cells to improve immunity. It also aids to overcome malnutrition in growing children.
d) **Improves digestion as well as lowers blood cholesterol levels**: It increases *Lactobacillus* which facilitates digestion. It reduces LDL (bad cholesterol). Therefore, leads to reduce the risk of heart disease.
e) **Treatment against allergies**: helps in treatment of allergic rhinitis, ulcers. Provides relief by reducing symptoms of nasal discharge, congestion, sneezing and itching.
f) **Promotes hair regrowth**: *spirulina* contains vitamin A, vitamin B5, vitamin B8, magnesium and zinc which enhances growth of hair and restore damaged hair.
g) **Effective in weight loss treatment**: helps to boost body metabolism as well as can be helpful in reducing weight without compromising the nutritional value.

To conclude, *Spirulina* is considered as a boon for the human body which can be consumed regardless the age of the individual. In certain conditions like pregnancy or in any other medical conditions the dosage of *Spirulina* should be taken under medical supervision. It is considered one of the natural sources of proteins and can be used as a sustainable food source.

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MONOCLONAL ANTIBODIES

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Monoclonal antibodies are developed by cloning a unique B-cell which characteristically binds to only the respective epitope. All the cloned antibodies trace back to one specific antibody-producing parent B-cell. This technique was first described by Milstein and Kohler in 1975.

Development process

- **Immunization of Mice:** The lab mouse is immunized by exposing it to the specific antigen with the aid of adjuvants which cause an improvement in the immune reaction. After the limited antibody level is attained in the serum of the mouse, it is executed and the spleen is removed to isolate the cells by filtration followed by centrifugation.
- **Hybridoma cell preparation:** The prepared myeloma cells are fused with the isolated spleen cells by centrifugation in the presence of polyethylene glycol that is responsible for the protoplast fusion. Thus, the hybridoma cells are prepared.
- **Hybridoma cell screening:** The prepared hybrid cells are grown in a selective medium called HAT medium which contains Hypoxanthine, aminopterin and thymidine. Hypoxanthine is used up as a nucleotide source for DNA synthesis only by the cells containing Hypoxanthine-guanine phosphoribosyl transferase (HGPRTase) by salvage pathway, i.e., B cells and hybridoma cells. Aminopterin, a drug responsible for inhibiting the activity of dihydrofolate reductase which in turn stops folate metabolism, thus halting the de Novo pathway of DNA synthesis in the hybridoma and myeloma cells. Thymidine acts as a general

nucleotide supply for nucleic acid synthesis. Thus, this medium selects and lets only the hybridoma cells to grow due to its property of uncontrolled division (immortality from myeloma cells) and nucleic acid synthesis by Salvage pathway (by HGPRTase from B cells).

- **Cloning the hybridoma cells:** The fused cells are diluted in such a way that mostly only one cell remains per test solution and is grown to clone. These cells are subjected to immunoassays in order to know its binding capacity and specificity to the particular antigen.
- **Expansion and Stabilization:** The respective hybridoma cells are grown in a larger quantity, stable in its properties and the antibodies are made to be produced under stressed conditions. Those secreted antibodies are purified from the mixture and let to undergo further processes.

Application

- The paratopes of the purified monoclonal antibodies are modified based on our interests so that the antibodies are made suitable for humans.
- These monoclonal antibodies can be used to purify and precipitate particular proteins by immuno-precipitation.
- It can be used to detect particular antigen or protein in the body.
- It can be used to modify certain metabolisms by targeting the cells or proteins that are responsible.
- It can be used to track the cancer cells through fluorescence or radioactivity by modifying these antibodies to target the cancer cells.
- These antibodies can be used to deliver drugs or any protein into the body to a specific cell or region.
- Their ability to bind with one particular antigen and precipitate out of the solution makes it an ideal tool to track or test antigens or toxins or any hormones and proteins in the body. For example, monoclonal antibody coated strips for detecting human chorionic gonadotropin hormone (HCG) in urine for pregnancy test.

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QUORUM SENSING: ITS ROLE IN MICROBIAL SOCIAL NETWORKING

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Quorum sensing is a process of cell-cell communication that allows bacteria to share the information about cell density and adjust genes expression according to the need. This process enables bacteria to express their expensive processes as a collective only when the impact of those processes on environment or on a host will be maximized. Quorum sensing is a bacterial cell communication process that involves the production, detection, and response to extracellular signaling molecules called Autoinducers (AIs). AIs accumulate in the environment as the bacterial population density increases, and bacteria monitor this information to track changes in their cell numbers and collectively alter gene expression. QS controls genes that direct activities that are beneficial when performed by groups of bacteria acting in synchrony. Processes controlled by QS include bioluminescence, sporulation, competence, antibiotic production, biofilm formation, and virulence factor secretion.

QS mechanism activities demand high energy, which are undertaken only when the community size is big enough to meet the mandate such as biofilm production, motility, antibiotic resistance and so on. In certain environments, QS is indeed social, and at the precise population level, QS regulates the production of extracellular products for the whole community. Despite differences in regulatory components and molecular mechanisms, all known QS systems depend on three basic principles. First, the members of the community produce AIs, which are the signalling molecules. At low cell density

(LCD), AIs diffuse away, and, therefore, are present at concentrations below the threshold required for detection. At high cell density (HCD), the cumulative production of AIs leads to a local high concentration, enabling detection and responses. Second, AIs are detected by receptors that exist in the cytoplasm or in the membrane. Third, in addition to activating expression of genes necessary for cooperative behaviours, detection of AIs results in activation of AI production.

Gram positive and Gram negative bacteria use different types of QS systems. Gram-positive bacteria use peptides, called autoinducing peptides (AIPs), as signalling molecules. Once produced in the cell, AIPs are processed and secreted. When the extracellular concentration of the AIP is high, which occurs at HCD, it binds to a cognate membrane-bound two-component histidine kinase receptor. Usually, binding activates the receptor's kinase activity, it autophosphorylates, and passes phosphate to a cognate cytoplasmic response regulator. The phosphorylated response regulator activates transcription of the genes in the QS regulon. In some cases of Gram-positive bacterial QS, AIPs are transported back into the cell cytoplasm where they interact with transcription factors to modulate the transcription factor's activity and, in turn, modulate gene expression changes. Gram negative bacteria communicate using small molecules as AIs. These are either acyl-homoserine lactones (AHLs) or other molecules whose production depends on *S*-adenosylmethionine (SAM) as a substrate. AIs are produced in the cell and freely diffuse across the inner and outer membranes. When the concentration of AIs is sufficiently high, which occurs at HCD, they bind cytoplasmic receptors that are transcription factors. The AI-bound receptors regulate expression of the genes in the QS regulon. In some cases of Gram negative bacterial QS, AIs are detected by two-component histidine kinase receptors that function analogously to those described in the preceding paragraph for Gram positive QS bacteria.

The infamous Gram negative pathogenic bacteria *Vibrio cholerae* uses quorum sensing for virulence during a cholera infection. *Vibrio cholerae* builds biofilms to help transport nutrients between colonies while simultaneously protecting them. The ability to form biofilms within its host ensures the success of the bacteria's reproduction cycle and eventual secretion of cholera toxin, 1 of 2 virulence factors that contribute to 21,000 to 143,000 cholera deaths worldwide each year. Quorum sensing between different bacterial species occurs as well. For example, some species cannot produce their own autoinducers, but have receptors for the autoinducer molecules of other species, allowing them to sense and respond to others in their environment. Like humans, bacteria operate on a continuum of individualism and collectivism. This quality can breed conflict, but also collaboration and interspecies quorum sensing can take both forms.

The study of QS in natural populations in environmentally complex settings is often complicated. This is because there are a variety of cell-intrinsic and cell-extrinsic factors that modulate the kinetics of QS signal production, detection and response. Intrinsic factors include the roles of other regulators outside of the direct QS circuit that respond to environmental stimuli, such as phosphate availability, metabolic pools that may influence signal synthesis and genetic differences between strains that alter the dynamics of QS activation. Extrinsic factors include QS interference or 'quorum quenching', in which QSMs are inactivated or degraded, environmental factors that influence the stability and diffusion of QSM, diffusion kinetics and population size.

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MICROBIAL PIGMENTS AS A NATURAL COLOR

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Developing new colours for food industry is challenging, as colorants need to be compatible with the food flavours, safety, and nutritional value. Synthetic colours have been widely used in various industries including food, textile, cosmetic and pharmaceuticals. However, toxicity problems caused by synthetic pigments have triggered intense research in natural colours and dyes. In addition, food colorants should preferably be natural rather than synthetic compounds. In present market natural pigments possess a huge potential compared to the synthetic pigment. Among the various classes of natural pigment, microbial pigment is more suitable because of its manufacturing and commercial advantages. The natural pigments extracted from microorganism are termed as "microbial pigments". The major pigments found in micro-organisms which are used as food colorants are canthaxanthin, astaxanthin, prodigiosin, phycocyanin, violacein, riboflavin, beta-carotene, melanin, and lycopene. Microbial pigments can be either inorganic or organic, although organic pigments tend to be more useful as food colorants. These microbial pigments have numerous beneficial properties like anti-cancerous, immunosuppressive, antibiotic, anti-proliferative and bio-degradability. Further, they have broad area of application mainly in food, dairy, printing, textile and pharmaceutical industries etc.

The various microorganisms such as *Micrococcus, Bacillus, Rhodotorula, Monascus, Phaffia, Sarcina* and *Achromobacter* have the capability to produce different pigments. Some of the pigment producing microorganisms are:

- ***Monascus purpureus*** **(Red Mold species):** The cultivation of red mold can be done on substrates rich in starch. Due to unique structure of Monascorubrin and Rubropunctatin they have high affinity towards primary amino groups (Aminophiles) and produce water soluble pigments with improved functional properties in the color range of orange-red to violet-red through *Monascus* fermentations.
- ***Chromobacter violaceum:*** *Chromobacterium violaceum* is a Gram negative bacteria belonging to the Rhizobiaceae family. The pigment produced from the violet colony is violacein which have a strong bactericidal, trypanocidal, tumoricidal, mycobactericidal and antioxidant activity.
- ***Sarcina*****:** These are gram-positive, non-motile, and spherical in shape and produce yellow colour pigment namely carotenoid. This pigment has antioxidant, anticancer and photoprotectant activity. *Sarcina* flavour carotenoid has four fractions which are hydrocarbons, mono and dihydroxylated compounds and polar fractions.

Advancements in fermentation techniques have led to the easy production and isolation of colour pigments. Microbial pigments can be produced either by solid substrate fermentation or by submerged fermentation. There are numerous new advances in the speedy and simple identification of microbial shades. Probably the best model is the consolidated handheld Raman spectrometer, utilized for distinguishing shades with the assistance of a 532 nm excitation laser. Mass spectrometry with electrospray ionization can also be used for faster identification of pigment produced by fungal strains and for grouping them in classes and subclasses.

The continuous use of synthetic colours not only causes considerable environmental pollution but also many health related problems in human beings i.e. carcinogenic effects etc. Hence, natural products produced by microorganism as pigments are safer and better than synthetic products. To be commercially viable these microbial pigments has to be stable against various environmental factors like light, pH, temperature, etc., and food matrices. Many microbial pigments are not commercially successful because of their instability against ambient conditions and shorter shelf life. Various techniques available to produce stable microbial pigment are Microencapsulation, Nanoformulations like Nanoemulsions etc. These techniques will increase the shelf life and enhance the market value. Further, various marine microbes and unexplored endophytic organism can be utilized for forthcoming study.

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MICROBIOMES

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Introduction

We human are mostly microbes, over 100 trillion of them. Microbes out number our human cells ten to one. The majority lives in our gut, particularly in the large intestine. The microbiome is the genetic material of all the microbes – bacteria, fungi, protozoa and viruses that lives on and inside the human body.

Dynamic character of Microbiomes

The microbiome is defined as a characteristic microbial community occupying a reasonably well-defined habitat which has distinct physio-chemical properties. The microbiome not only refers to the microorganisms involved but also encompasses their theatre of activity, which results in the formation of specific ecological niches. The microbiome, which forms a dynamic and interactive micro-ecosystem prone to change in time and scale, is integrated in macro-ecosystems including eukaryotic hosts, and here crucial for their functioning and health.

The Microbiome to do with Health

The microbiome is essential for human development, immunity and nutrition. The bacteria living in and on us are not invaders but beneficial colonizers. Autoimmune diseases such as diabetes, rheumatoid, arthritis, muscular dystrophy, multiple sclerosis and fibromyalgia are associated with dysfunction in the microbiome. Autoimmune diseases appear to be passed in families not by DNA inheritance but by inheriting the family's microbiome.

Human Microbiome Project

To understand the range of human genetic and physiological diversity, the microbiome and the factors that influence the distribution and evolution of the constituent microorganisms must be characterized. This is one of the main goals of the Human Microbiome Project (HMP). The outcome might also provide perspective on contemporary human evolution: that is, on whether and how rapidly advancing technology, and the resultant transformation of human lifestyles and the biosphere, influences the 'micro-evolution' of humans and thereby health and predisposition to various diseases.

Microbial Genome Project Catalog

The HMP has assembled a key reference data set of microbial genome sequences collected from the major body regions of the human microbiome, primarily bacterial, although it also includes archaea, viruses, bacteriophages, and eukaryotic microorganisms. The project's target catalog of 3000 microbial genome sequences is intended as a reference for the interpretation of the 16S ribosomal RNA gene sequences, as well as a scaffold for rapid assembly of metagenomic sequences determined from the microbial communities.

Achievements

Development of new database systems allowing efficient organization, storage, access, search and annotation of massive amounts of data. Development of tools for comparative analysis that facilitate the recognition of common patterns, major themes and trends in complex data sets. These include RAPSearch2, a fast and memory-efficient protein similarity search tool for next-generation sequencing data. Establishment of the Data Analysis and Coordination Center (DACC), which serves as the central repository for all HMP data. Various studies exploring legal and ethical issues associated with whole genome sequencing research.

Development by Human Genome Project

New predictive methods for identifying active transcription factor binding sites. Identification, on the basis of bioinformatics evidence, of a widely distributed, ribosomally produced electron carrier precursor. Identification of unique adaptations adopted by segmented filamentous bacteria in their role as gut commensals. Segmented filamentous bacteria are medically important because they stimulate T helper 17 cells, thought to play a key role in autoimmune disease. Demonstration that pathogenic species of *Neisseria* involved in meningitis, sepsis, and sexually transmitted disease exchange virulence factors with commensals species.

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AN INFLAMMATORY DISEASE : PNEUMONIA

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Pneumonia is a type of Acute respiratory tract infection of lungs (i.e. Alveoli and Bronchi). It is caused by inhaled bacteria (*Streptococcus pneumoniae, aka pneumococcus*). When person is infected with pneumonia, the alveoli are filled with pus and fluid which cause difficulty in breathing. In developing world, *Streptococcus pneumoniae, Haemophilus influenzae* Type B (Hib) and Respiratory Syncytial Virus (RSV). *Streptococcus pneumoniae* are the most common pathogen which causes pneumonia in children under five years. At any age pneumonia can caused, person in contact with smoke and tobacco have higher risk of lung infection and pneumonia.

Pneumonia is classified in three types as follows:

1) **Community-Acquired Pneumonia (CAP) -** Pneumonia which gotten outside the hospital is knows as community acquired pneumonia
    - **Causative agent -** *Streptococcus pneumoniae, Haemophilus influenzae.*
    - **Signs and symptoms -** Chills, rigor, fever, cough, dyspnea, and chest pain.

2) **Ventilator Associated Pneumonia (VAP)** – Person who is on ventilator having lungs infection develops ventilator associated pneumonia.
    - **Causative agent -** *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae.*

- **Signs and symptoms -** Fever, Pus in lungs, Changes in breathing and Low oxygen.

3) **Hospital Acquired Pneumonia (HAP) -** Infection of lungs caused during hospital stay is known as Hospital acquired pneumonia.

- **Causative agent:** Gram negative bacilli - *Escherichia coli*, *Pseudomonas aeruginosa* and Acinetobacter; Gram positive cocci - *Staphylococcus aureus.*
- **Signs and symptoms:** Fever and chills**,** Loss of appetite, Nausea and Vomiting.

Treatment

Treatment include prevention are control of pneumonia. On the basis of severity of pneumonia, cardiopulmonary condition, age, treatment is decided. By considering this factors patient should be treated in outpatient or inpatient setting. Patient need to be hospitalized when you are older than 65, rapid breathing, kidney problem. Out patienting is managed mostly in pneumonia. Checking medical history of person by the doctor is the first step to cure the disease. Many antibiotics are effective against pneumonia in primary level like fever reducer, cough medicine etc., then the second step is to do X-Ray (Chest), taking sample culture (Blood, Sputum). After conformation of the disease proper antibiotic treatment, respiratory therapy, oxygen therapy under doctor is the last step.

Prevention

- Vaccination- Prevnar 13 and Pneumovax 23
- Flu vaccine.
- Regular hand wash with soap and water.
- Keep distance from smoke and tobacco.
- Maintain good diet and healthy lifestyle.

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VULNERABILITY OF INDIA TO COVID-19

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The vulnerability of India to COVID-19 in five main areas can be observed in: socioeconomic, demographic, housing and hygiene, availability of health care, and epidemiological conditions. The examination of the five dimensions and 15 indicators of vulnerability for all the districts and states of India separately, which can contribute to mitigating the ongoing pandemic. However, the study has some shortcomings

The proportion belonging to a scheduled caste or tribe might be a weak indicator of socioeconomic vulnerability because, in the northeastern states, most of the population are part of scheduled tribes and their socioeconomic status is entirely different from the rest of India. Further, the categorisation of households as non-poor on the basis of having a bicycle is very weak because about 16% of households in India have only a bicycle out of the full list of household commodities, of which about 90% are in the lowest two wealth quintiles. The residents of Bihar are less socioeconomically vulnerable than those in Madhya Pradesh, Rajasthan, and Tripura and are equally vulnerable as the residents of Dadra and Nagar Haveli, but Bihar is in fact less socioeconomically developed than these states and Union Territory. Therefore, demographic vulnerability indicators should have been derived using the 2011 census for the year 2020 via an appropriate projection method or using an available population projection report.Most of the indicators in availability of health-care domain are derived using perception-based questions in the National Family Health Survey 2015–16 (NFHS-4),2 and these should have been given lower weighting in the vulnerability index than other indicators of health care (eg, the availability of public health hospitals and beds). Also, the variable related to health insurance underestimates the current scenario,

because most of the health insurance schemes such as Ayushman Bharat Yojana were launched after the survey. However, the onset of most chronic morbidities in both men and women starts after the age of 40 years,3 hence the results are not aligned with the part of the lifespan in which these diseases manifest. Furthermore, the burdens of diabetes, cancer, and cardiovascular diseases might not truly estimate the district prevalence in the total adult population because this information was collected to provide state-level estimates in NFHS-4. So, considering other surveys such as the National Sample Survey and Longitudinal Ageing Study in India in the computation of epidemiological vulnerability might provide better results.

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ANTIBACTERIAL ACTIVITY OF *Azadirachta indica* AND *Ocimum sanctum* OF AGAINST FISH BACTERIAL PATHOGENS

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Fish are susceptible to several bacterial infections, mainly when reared in high densities conditions. The bacterial fish diseases constitute major challenges for the sustainable aquaculture production. Particularly, the fin rot diseases, tail rot diseases, skin ulcer, haemorrhagic septicaemia and gall bladder, enteric septicaemia, furunculosis, ulcerative disease etc., have been caused by bacteria. The non-indigenous pathogens include, *Clostridium botulinum*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* sp., *Shigella* sp. and *Escherichia coli* which contaminate the fishes and its habitats. The indigenous bacterial pathogens *viz*., *Vibrio* sp. and *Aeromonas* sp. are naturally living in the fish habitat. Nevertheless, the exploration of antimicrobial therapeutics from marine resources for disease free fish culture. In this connection, the present study has made an attempt to find out the potential leads for the development of antimicrobial therapeutics for disease free fish culture.

Medicinal plants have been used in human medicine as immune boosters for millennia. Furthermore, they are alternatives to antibiotics in aquaculture. A large number of medicinal plants with antiviral, antibacterial, and antiparasitic properties are used in the treatment of fish diseases. Several parts of these medicinal plants are used to extract the active substances. Leaves are mostly used as well as the rhizome, fruits, roots,

seeds, bark, and bulbs. Medicinal plants are rich in various secondary metabolites and phytochemical compounds, such as tannins, alkaloids, and flavonoids, which affect various diseases in fish. These active substances are mostly extracted with methanol, ethanol, ethyl acetate, chloroform, and water while garlic can be used raw and squeezed. *Azadirachta indica* is a tree from the mahogany family indigenous to India and southeast Asia. Commonly called the neem tree, it contains vast number of bioactive compounds that are chemically diverse and is an important alternative herbal therapy. Neem extract suppresses several species of pathogenic bacteria. *Ocimum sanctum* (tulsi), of the Lamiaceae (mint) family, grows in India and Suriname. Its leaf extract has been used to treat variety of conditions including catarrhal bronchitis, dysentery, and skin diseases. Its herbal extract produces hypoglycemic effects in rats. Aqueous and solvent extracts of tulsi stimulate humoral and cellular immunity in rats and aids in ulcer healing, which involves a combination of wound retraction and re-epithelialization, and promotes anti-ulcer activity.

Antimicrobial active principles are widely distributed among the plants through many possible sources of extraction and synthesis of antibiotics has been elaborately worked out. These antimicrobial compounds produced by plants are active agents' plant and human pathogenic microorganisms. A major issue in intensive fish culture is bacterial disease. In an attempt to keep production undisrupted, synthetic antibiotics are often relied upon for disease control. Thus, treatment with plants and/or plant parts that have antibacterial activity is a potentially beneficial alternative. While organic agriculture has developed alternative methods and treatments to combat plant diseases, information on use of natural medicines to treat fish diseases as had been limited. Antibiotics derived from phytochemicals may be toxic, but since these are natural, their decomposition would be faster plants have been used to treat infectious diseases throughout the history of mankind Secondary metabolites play a role in the medicinal properties of plants.

The paper disc method was used to determine the growth inhibition of bacteria by the plant extracts of *Acalypha indica* and *Anisochilus carnosus*. Petri plates were prepared by pouring 10.0 ml of Muller-Hinton agar for bacteria and allowed to solidify and were seeded with 24 hrs old culture of selected bacterial strains (10^6 cells ml^{-1}) sterile Whatman No.1 filter paper discs (6 mm diameter) containing 1 percent. Streptomycin was used as positive control. The Muller - Hinton agar assay plates used for testing bacterial susceptibility were incubated at 37 °C for 24 hrs. Assessment of antibacterial activity was based on the measurement of diameter of inhibition zone formed around the disc. Antibacterial activity was tested using disc diffusion method.

In our study was the antimicrobial activity of leaves extraction of *Azadirachta indica* and *Ocimum sanctum* inhibit the growth of *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Klebsiella pneumoniae.* The study can be performed further with *Azadirachta indica* against fish pathogens which could yield fruitful results. The study can be performed the fish bacteria pathogens *viz*., *Escherichia coli, Pseudomonas aeruginosa,* also the inhibitory activity against the medicinal plants.

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INHIBITORY EFFECTS OF *Acalypha indica* AND *Anisochilus carnosus* AGAINST SELECTED FISH PATHOGENS

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Fish are susceptible to several bacterial infections, mainly when reared in high densities conditions. Medicinal plants constitute an effective source of both traditional and modern medicines. About 80 % of rural population depends upon the herbal medicine for their primary health care. The world health organization complied more than 20,000 species of medicinal plants. Indian medicinal plants and their products are used to control the various types of diseases such as bronchitis, pneumonia, diarrhoea and ulcer. Medicinal plants are containing inherent active ingredients used to cure disease.

Medicinal plants produce bioactive compounds used mainly for medicinal purposes. *Acalypha indica* it is a common annual herb, found mostly in the backyards of houses and waste places throughout the plains of India. Plants are emetic, expectorant, laxative and diuretic, useful in bronchitis, pneumonia, asthma and pulmonary tuberculosis. Leaves are laxative and antiparasiticide ground with common salt or quicklime or lime juice applied externally in scabies. *Anisochilus carnosus* wall, an annual herbaceous plant which is a member of the family Lamiaceae, breeds on high elevation a mid small rocks. *Anisochilus carnosus* for the treatment of ulcer, stomach ache, cough, and eczema. This plant's phytochemical study has revealed it to be rich in active compounds

such as saponins, tannins, flavonoids, phytosterols, triterpenoids, and essential oil components (carvacrol, β-selinene, camphor, α-cis-bergamotene, and caryophyllene).

Fish are susceptible to numerous diseases, which could lead to partial or complete loss of produce. They are exposed to numerous pathogenic microorganisms inhabiting the water environment. The high density of breeding, poor hydrodynamic conditions, and feeding lead to physiological changes in the organisms, stress, and suppression of the immune system, thus increasing the fishes' sensitivity to infections. The lack of sanitary barriers facilitates the spread of pathogenic microorganisms, leading to high mortality levels. However, the bacterial fish diseases constitute Major challenges for the sustainable aquaculture production. Particularly, the find out diseases, tail rot diseases, skin ulcer, hemorrhagic septicemia and gall bladder, enteric septicemia, furunculosis, ulcerative disease etc., have been caused by bacteria. The non-indigenous pathogens include, *Clostridium botulinum, Listeria, monocytogenes, Staphylococcus aureus, Salmonella* sp., *Shigella* sp., *Escherichia coli*, etc. which contaminate the fishes and its habitats. The indigenous bacterial pathogens *viz., Vibrio* sp. and *Aeromonas* sp. etc are naturally living in the fish habitat.

Antimicrobial active principles are widely distributed among the plants through many possible sources of extraction and synthesis of antibiotics has been elaborately worked out. Although, reported of antibacterial activity of indigenous have been systematically contacted. A major issue in intensive fish culture is bacterial disease. In an attempt to keep production undisrupted, synthetic antibiotics are often relied upon for disease control. Thus, treatment with plants and plant parts that have antibacterial activity is a potentially beneficial alternative. While organic agriculture has developed alternative methods and treatments to combat plant diseases, information on use of natural medicines to treat fish diseases as had been limited. Antibiotics derived from phytochemicals may be toxic, but since these are natural, their decomposition would be faster plants have been used to treat infectious diseases throughout the history of mankind Secondary metabolites play a role in the medicinal properties of plants

The paper disc method was used to determine the growth inhibition of bacteria by the plant extracts of*Acalypha indica and Anisochilus carnosus*. Petri plates were prepared by pouring 10.0 ml of Muller-Hinton agar for bacteria and allowed to solidify and were seeded with 24 hr old culture of selected bacterial strains (10^6 cells ml^{-1}) sterile Whatman No.1 filter paper discs (6 mm diameter) containing 1 percent. Streptomycin was used as positive control. The Muller - Hinton agar assay plates used for testing bacterial susceptibility were incubated at 37 ºC for 24 hrs. Assessment of antibacterial activity was based on the measurement of diameter of inhibition zone formed around the disc. Antibacterial activity was tested using disc diffusion method.

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MICROBIAL ANALYSIS OF WASTEWATERS FROM THIRUVANNAMALAI DISTRICT OF TAMILNADU

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Fecal pollution or surface waters constitutes a serious environmental and public health threat. Coliforms such as *Escherichia coli* are strong indicators of waste water contamination and its pathogenic serotypes may lead to severe infections. Though antibiotic treatment is a common way to treat bacterial infections, antibiotic sensitivities of different strains of *Escherichia coli* vary widely. Also, *Escherichia coli* often carries multi-drug resistant plasmids and readily transfers these plasmids to other species of bacteria under stressful conditions. Twelve waste water samples from sewage, industrial and veterinary sources from different areas were collected, *Escherichia coli* were isolated and their susceptibility was assessed against twelve different antimicrobial agents. BOD values of the waste water samples varied from 78 - 130 mg/L and COD values varied from 90 - 220 mg/L indicating high level of pollution. Industrial waste water showed the presence for highest content of biodegradable and non-biodegradable pollutants as compared to other samples. On the basis of NCCLS 2000 guidelines for interpretation, 80 % isolates were found to be resistant to at least one of the 12 antimicrobial tested Resistance levels varied from 79.8 % for tetracycline, 69.3 % for sulphonamide to 12.1 % for gentamycin. Also, nearly 90 % *Escherichia coli* isolates exhibited muti-resistance to the tested drugs with most being isolated from veterinary clinic waste water samples. It is hence concluded that veterinary clinics wastes are the high selection sites for maximum

transfer of resistance genes among its constituent elements thus resulting in high morbidity and mortality rates. Efforts should be made in hospitals to avoid contamination of patients with such muti-drug bacterial strains which aggravate the clinical picture of infections.

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GUT BACTERIA TRIGGER IMMUNE DEFENCE MECHANISM TO PROTECT AGAINST VIRAL INFECTIONS

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The human body, like that of other mammals, is colonized by trillions of microbes, which include bacteria, viruses, and fungi, which are collectively referred to as the commensal microbiota, the current estimates suggest that there are roughly as many bacterial cells as human cells in the body, the vast majority of which reside in the lower gastrointestinal (GI) tract, which is colonized by an estimated 10^{14} bacteria. And although scientists know relatively little about the molecular mechanisms by which commensal microbes interact with the host, research does suggest that they play an important role in modulating key physiological systems in the body like the digestive system. Recent studies show that these microbial communities also influence the nervous system too, but this article will focus on the immune system and the mechanisms these microbes employ while an infection.

One mechanism by which microbes modulate the host immune system is through regulating cytokine signalling, Type 1 interferons (IFN-Is) are a family of structurally similar cytokines, which are involved in the body's response to viral infections these IFN-Is play a critical role in the response to the majority of virus infections through induction of a restrictive anti-viral state in cells, induction of apoptosis in infected cells, and regulation of immune cell subsets crucial to the antiviral

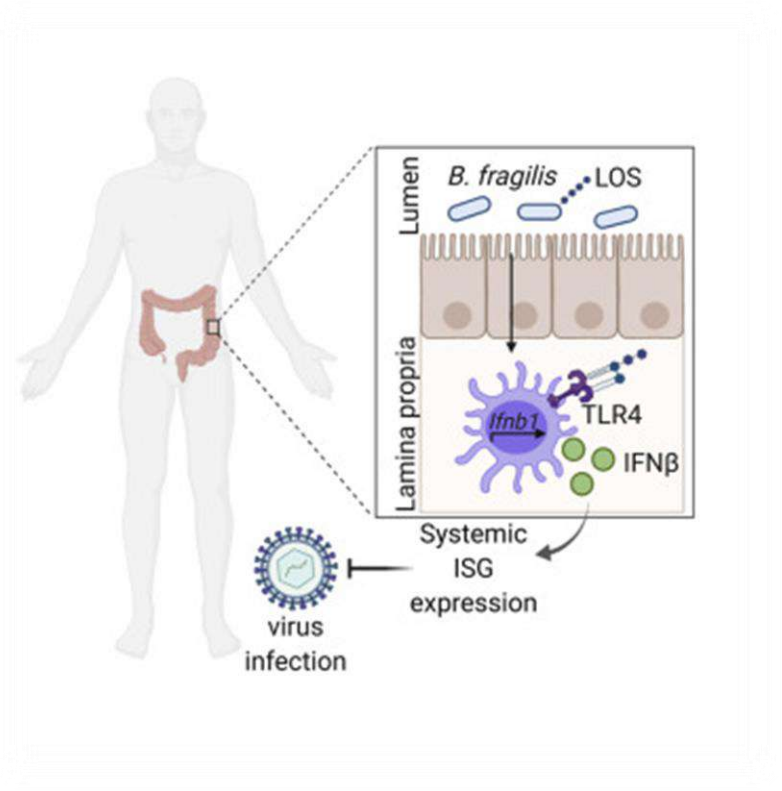
response. This protective response arises from immune cells that reside in the walls of the colon. These dendritic cells release protective interferons when stimulated by the Prostrate-Specific Antigen (PSA) molecule residing on the outer surface membrane of the *Bacteroides fragilis* gut bacterium.

The *Bacteroides fragilis*, which is present in the majority of human GI tracts, effectively initiates a signalling cascade that induces the immune cells in the colon to release interferon-ß (IFN-ß), an important immune chemical that confers antiviral protection both by inducing virus-infected cells to self-destruct, and by stimulating other classes of immune cells to attack the virus. The bacterial molecule stimulates the immune-signalling pathway initiated by one of the nine Toll-Like Receptors (TLR) that are part of the innate immune system. Specifically, *Bacteroides fragilis* unlocks the signalling pathway when its surface molecule communicates with immune cells of the colon through their TLR-4 TRIF receptors. Induction of IFN-ß through TLR4 signalling is not limited to *Bacteroides fragilis* but is a shared function of an entire class of commensal microbial molecules.

To determine whether *Bacteroides fragilis* protect mammals from infection, the researchers at Havard Medical School tested two groups of mice. One group was treated with antibiotics to deplete their gut microbiota and the other retained an intact gut microbiota. Both groups of mice were exposed to Vesicular Stomatitis Virus (VSV), an organism that infects nearly all mammals, but which leads to largely asymptomatic infections in humans. Compared with mice that did not receive antibiotics and had intact gut microbiota, antibiotic-treated animals with depleted gut microbiota were more likely to develop active infections after exposure to VSV, and to have worse disease when they did get infected. The results show the role of gut microbes in inducing protective IFN-ß signalling and in boosting natural resistance to viral infection. Interestingly, there were no differences among mice that lacked receptors for IFN-ß regardless of whether their gut microbiota was depleted. The observations confirmed that it was via IFN-ß signalling that the commensal microbiota exerted their protective effects.

Later, to investigate whether the *Bacteroides fragilis* surface molecule that triggers interferon signalling in cells could also modulate how animals respond to viral infection, researchers gave animals with depleted microbiotas a purified form of the molecule in their drinking water. When the animals were a few days later exposed to VSV, those pretreated with the molecule had markedly milder infections and identical survival to mice with intact gut microbiota and intact immune defenses.

These findings demonstrate that not only is commensal induction of IFN-ß necessary for protection against VSV, but treatment with a single IFN-ß-inducing commensal microbial molecule is sufficient to restore the protective effects of the whole microbiota in this model. Delivery of an IFN-ß-inducing microbial molecule thus represents a novel IFN-I-based therapeutic approach, which could enhance the IFN-I response while still being subjected to homeostatic regulatory mechanisms, reducing the potential for undesired side effects.



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CHANGES DURING ISOMERIZATION AND Σ-SUBUNIT OF BACTERIAL RNA POLYMERASE

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Bacterial RNA polymerase catalyze De-novo RNA synthesis. RNA polymerase is a holoenzyme consist of 2 copies of α-subunit and 1 each of **β,**β^1 and W subunit (2α **β**β^1W) they are capable of melting the DNA or leading to initiate transcription specificity factor σ, which plays a vital role in recognizing and directing the core to transcribe specific genes *Escherichia coli* has the σ^{70} factors of holoenzymes to transcribe various housekeeping genes, σA in *Thermus thermophilus*. Bacterial RNA-pol resembles the 'crab claw'. It consist of 2 flangs like structure **β,**β^1 called princers. The N-terminal domain of σ dimer called (αNTD) join these princers and provide a pulprit for RNA-pol to catalyze and organize. αCTD are connected αNTD by a flexible linker. The αCTD up-element interaction is responsible for a 30 - 70 folds increase *in vivo* promoter activity at *rnn* BP1 (most studied promoter). In the active centre cleft, the two β andβ^1 princer connected by a long bridge of α-helices, the two flexible α-helices of trigger loop and F-loop (extended loop), catalytic loop. W-subunit present near β^1 c-terminus to the downward part of β princer. The σ^{70} factor is divided into 4 regions called region - 1 through region - 4. The region that recognizes - 10 elements and -35 elements of promoter are region 2 and 4 respectively. Within -10 elements DNA meeting/isomerization is initiated in transition from a closed to open complex. Several essential aromatic amino acids interacts with base in non-template strand in a manner that stabilize the DNA after isomerization.

Typical isomerisation does not require energy derive from ATP hydrolysis. Within -10 element base A_{11} and T_4 flip out from their base stacking interactions and inserted into pockets of σ-protein, also by stabilizing the single stranded from of -10 element these interaction drive separation or isomerization of promoter region.

Binding to core relives region 1.1 autoinhibition of DNA binding in free σ. In holoenzyme region 1.1 is removed from its location in σ and placed in active site channel of polymerase possibly because its high negative charge allows it to act as a downstream DNA mimic. The mechanistic for this σ (1.1) function has left a point to ponder.

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SECONDARY METABOLITES OF MICROORGANISMS

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Microbial Secondary metabolites are organic compounds mainly synthesized by bacteria, fungi and also by some plants. SM are also known as secondary products, specialised metabolites, toxins or natural products. These compounds are sophisticated and vary with their chemical structures, produced by strains of certain microbial species. A characteristic of secondary metabolism is that the metabolites are usually not produced during the phase of rapid growth (Trophophase), but are synthesized during a late in growth cycle in Stationary phase (Idiophase). Production of secondary metabolites starts when growth is limited by the exhaustion of one key nutrient source like carbon, nitrogen or phosphate. There are enormous range of other fields biological activities mainly like: cosmetics and pharmaceutical, food, agriculture and farming. Microbial secondary metabolites are low molecular mass products with unusual structures. The structurally diverse low molecular mass molecules and not involved directly in the development, growth and reproduction of organism. A metabolite shows variety of biological activities like antimicrobial agents, inhibitors of enzymes, suppressive, antiparasitic agents, anti-inflammatory, hypotensive, antitumour, anticholesterolemic activities, herbicides and also insecticides, plant growth regulators and important ecological function. These compounds are usually produced by liquid submerged fermentation, but some of these metabolites could be advantageously produced by solid-state fermentation.

Secondary metabolites consist of natural products that (a) are restricted in taxonomic distribution, (b) are synthesized for a finite period by cells that have stopped dividing, and (c) most probably function as convenient disposal packages of excess primary substances. A minority of secondary substances also can affect growth, health or behaviour of non-producer cells. And, the microorganisms producing the compounds, bacteria, including actinobacteria, and fungi produce a diverse array of bioactive small molecules with significant potential to be used in medicine. The main synthetic pathways of secondary metabolite production in bacteria are; b-lactam, oligosaccharide, polyketide and non-ribosomal pathways. The importance of secondary metabolites from various sources like plants, microorganisms including bacteria, actinobacteria, and fungi and its classification, production and applications in various fields. The microbial secondary metabolites like antibiotics, alkaloids, pigments, growth hormones, antitumor agents, and others, are not essential for the growth and development of microorganism, but they have shown a great potential for human and animal health.

Many of the identified secondary metabolites have a role in ecological function, including defence mechanisms by serving as antibiotics. Although SM are best known antibiotics the discovery of penicillin initiated the researchers for the exploitation of microorganisms for secondary metabolite production, which revolutionized the field of microbiology. Examples of secondary metabolites are atropine and antibiotics like bacitracin, erythromycin. Atropine derived from various plants and competitive antagonist for acetylcholine receptors, specifically those of the muscarinic type, which can be used in treatment of bradycardia. Antibiotics like erythromycin and bacitracin are also considered to be secondary metabolites. Erythromycin derived from *Saccharopolyspora erythraea*, is a commonly used antibiotic with a wide antimicrobial spectrum. Bacitracin, derived from *Bacillus subtilis*, is an antibiotic commonly used a topical drug, it is used in the clinic as an antibiotic. Many bacterial secondary metabolites are toxic to mammals. An example of a bacterial secondary metabolite with a positive and negative effect on humans is botulinum toxin synthesised by *Clostridium botulinum*. However, botulinum toxin also has multiple medical uses such as treatment of muscle spasticity, migraine and cosmetics use.

The three main classes of fungal secondary metabolites are polyketides, non-ribosomal peptides and terpenes. Although, fungal SMs are not required for growth they play an essential role in survival of fungi in their ecological niche. Fungal secondary metabolites can also be dangerous to humans. *Claviceps purpurea*, a member of the ergot group of fungi typically growing on rye, results in death when ingested. The build-up of poisonous alkaloids found in *Claviceps purpurea* lead to symptoms such as seizures and spasms, diarrhoea, psychosis, itching and gangrene. Since there is a constant and crucial requirement for new pharmaceutical agents to fight cancers, cardiac disorders,

pests, cytotoxic, mosquitoes, infectious diseases, and autoimmune disorders of both animals and plants as climate changes provide conditions favourable to repeated outbreaks of these events. Secondary metabolites are one of their essential means of growth and defence, and these metabolites are readily available for discovery. Secondary metabolites with noteworthy biological activity are considered as an alternative to most of the synthetic drugs and other commercially valuable compounds.

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DIVERSITY, ISOLATION, CHARACTERIZATION OF MICROORGANISM FROM DOMESTIC AND INDUSTRIALLY OBTAINED SOLID WASTE AND DEGRADATION OF SOLID WASTE BY USING *Pseudomonas aeruginosa*

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Microbial diversity as the rays of different kinds of unicellular organisms, bacteria, archaea, protists and fungi various different microbes thrive throughout the biosphere, defining the limits of life and creating conditions, conductive for the survival and evolution of other living beings.

For isolation and characterization total of 10 samples were collected from waste dumpsite and industrially obtained solid waste such as, Bagasse, press mud and Sugar molasses from Doiwala Sugar Industry and in and around Suddowala, Dehradun, Uttarakhand. Isolation of bacteria were isolated using Standard Count Plate (SPC) by using Tryptone Glucose Yeast Extract Agar and another selective medium such as,

Nutrient Agar - NAM, *Pseudomonas* Agar, Eosin-Methylene Blue Agar, Mannitol Salt Agar, MacConkey Agar, etc.

The optimal cultural conditions, microbiological characteristics, biochemical and antagonistic with-in the strain tolerance to 5 heavy metals (arsenic, zinc, lead, mercury and cadmium) their sensitivity towards four different antibiotics (Gentamycin, Oxytetracycline, Penicillin, Streptomycin) and production of extracellular enzyme of the bacterial strain were documented. These results have increased the scope of finding industrially important bacteria from municipal and industrial solid waste. These isolates could be vital source for the discovery of industrially useful enzyme/molecules such as, Bioactive components.

Biodegradation or composting in a natural process that stems through microbial succession marking the degradation and stabilization of organic matters present in waste. In this study *Pseudomonas aeruginosa* were used to degrading the waste under controlled consortia. However, looking at the environment implication of these method, undergo by different level of temperature and number of days sample took to degrade or to compost using bacteria, these particular bacteria *Pseudomonas aeruginosa* took around 60 days to degrade industrial waste and 45 days for municipal waste. Further compost used in the agricultural field to improvement the betterment of yield by using different percentage of compost that derived by biodegradation using selective microbes.

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BIODEGRADATION OF LOW-DENSITY POLYETHYLENE BY USING MARINE BACTERIA FROM IN AND AROUND PORT BLAIR, NORTHEASTERN INDIAN OCEAN, ANDAMAN AND NICOBAR ISLANDS

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Low-Density Polyethylene (LDPE) is an environmental problem because it is discarded randomly in dumpsites which is near to shore line, it does not readily degrade. This study report evidence of biodegradation by bacterial species that will isolate from the dumpsites, further these bacteria were then identify and characterise using Standard Plate Count (SPC) microbiological procedure. Biodegradation study will do experiment using selective bacteria that has been isolate from the dumpsite, single and in consortia to degrade heat sterilise ground LDPE in media devoid of carbon source. Biodegradation will monitor using gravimetric methods and Fourier Transform Infrared Spectroscopy. This study reveals some bacterium, that biodegradation of linear low-density PE particle will evidence clearly by morphological change of the polymer's mixture by SEM (Scanning Electron Microscope). Hence, it is of scientific interest to explore plastic degrading microorganisms in the ocean for further understanding of the plastic biodegradation, the future development of effective bioremediation processes.

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ECO-DIVERSITY OF AQUATIC BACTERIA FROM VELLAR ESTUARY, PORTO NOVO

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Studies suggested that bacterial count for most of the diversity of life on our planet, due to their presence of almost all ecosystem. The study, bacterial community compositions in various area of Vellar estuary, bacterial richness and diversity were positively regulated by sediments and mangroves sediments around the shore by sediments inorganic phosphorus, and nitrate nitrogen were found to be important drives for bacterial community composition, *Proteobacteria*, *Planctomycetes* and *Actinobacteria* were the major components of bacterial communities several were non-identical.

The microbial world compasses most of the phylogenetic diversity and from varied climatic extremities. There are so many recent approaches in several research focused on isolate and study of novel microbes and extracting the valuable chemicals and products out of it. Although, it was estimate that less than one present of all microbial species have been identified so far, and most of the research believe that deeper study of microbes will reveal novel biochemicals and by-products useful for human in all the possible way; Enzyme, Proteins, Drugs and Biofuel etc.

Even though we still struggle to get vaccine which is most needed one in day-todays life due to the Novel Corona Virus. '2019-nCov' or 'Covid-19'. Thus, now there is an urge to undergo study of possible antimicrobial and adaptation to the clinical study of all the possible organism during the study period. The study includes life cycle in varied environment in the estuary like before cyclone and after cyclone (Nivar Cyclone

estimated on 23.11.2020-27-11-2020 in Porto Novo) while the freshwater combines with the sea water, there is a chance to change in action of microbial diversity as compared with previous research study undergone. The organism niche, which will give us some clear ecological status to understand global microbial diversity and their evolution in and around the world.

In the present study, have tried to compile few recent researches on the aquatic biodiversity especially bacteria and the valuable source, i.e., enzyme that contribution in the industrial sector and work market. The scope of by-products of the bacteria, with enzyme is currently in demand at global scale and there are ample possibilities for exploration of bacterial wealth in the near future with the fastest techniques in several other upcoming source of study, its still just commencement, but it has huge potential for exploration and application with industrial medicinal benefits and gives a comprehensive insight into the structural of bacteria community of the estuary, indicates that the environmental factors played a key role in influencing the bacterial community composition in the estuarian diversity or ecosystem.

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THE WALKING DEAD

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The topic on which I'm going to talk about is a fact which always fascinates us. "THE ZOMBIES". People walking in a group to kill other people. They are eating other people even snatching their eyes, but the point is does it really has an existence? Will there be any situation occurring that we are running from ZOMBIES to save our lives? Is there any fungus or a virus present that can create such vulnerable situation? So, yes mother-earth have this possibility too. A day can definitely come where we are running away from ZOMBIES to save our life. Let's know the science behind this fact if to be precise then ZOMBIE ATTACK isn't impossible. If there is an existence of a parasite which can transform a creature into a ZOMBIE then YES we are only few steps far from ZOMBIE APOCALYPSE. According to scientific study researchers have found some Fungus which can transform a living healthy creature into a "A Half Dead" Slave, then the organism comes under the control of that particular Fungus. From the studies obtained the most dangerous Fungus existing in the world is *Ophiocordyceps unilateralis* and commonly it's known as ZOMBIE FUNGUS. When ants render here and there in a search of food and if they carries a part of Zombie Fungus then that fungus penetrate inside the ant's body and start multiplying themselves slowly and gradually not only the body but also they started infecting the brain of ants too and finally they started controlling the brain then started controlling even the body of the infected ants that fungus controls the ant in such a random way which end up as a fatal condition for them, and when the ant dies then the Fungus excretes themselves out from the ant's body and started infecting other ants too and in this way the Fungus starts increasing their population and existence in the environment. By looking at this situation we get an idea behind the depth of the mother nature also we get an idea about the fact that there

is definitely an existence of Fungi, Virus or Pathogen which can control the brains and body of a particular group of an organism. Due to evolution the Zombie fungus have evolved the ability to control the brain and body of their host. Even at this moment also somewhere at a particular place they might be reproducing and increasing their population but the safest thing is that till now they didn't have the ability to infect humans. The brain and body of human is more complicated as compared to the brain and body of the Ants that is why till now the Zombie Fungus are not able to infect humans. But the question is if something happens and this Fungus can develop the ability to leave the ant's body and to infect the body of other organism or even the body of humans? Do you remember Ebola Virus that virus also use to belong to Bats only but these virus started infecting other organisms too, for example Chimpanzee, pigs, & with the interval of time they even started infecting humans too. With the interval of time, virus develops ability to infect other group of organisms too because they want their population to keep growing. So, the major fear of scientists is that what will happen if with passing time this Zombie Virus or any other biological community develop the ability to infect other organisms. If this will happen and started spreading then the result will be unimaginable. If any virus penetrates in the body and if it's prevented by the immune system then the effect can be controlled but in case if immune system fails in preventing the body then the virus will start infecting the whole body and even the virus will make the host cells their slave and then the cell will start making copies of that virus. In this manner only the virus starts infecting any organisms their major key role is to control that particular organism. With the interval of time viruses have become more expert in controlling. But in real life there is existence of Zombie like disease in which the person doesn't actually become a complete Zombie but yes they will start acting like Zombie. Catatonic Schizophrenia is a condition in which that infected person at its last stage is unable to differentiate between real and unreal.

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EFFECTS OF SOUND ON MICROORGANISMS

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A number of different sounds have been experienced, may it be sounds of nature or the different varieties of music composed, each of them have different effects on human beings. To experience calmness or heal music is always the best way preferred. So, it won't be surprising to think that how sound affects the growth of microorganisms.

Microbes, as one of the primary producers of the biosphere, play an important role in ecosystems. Exploring the mechanism of adaptation and resistance of microbial population to various environmental factors has come into focus in the fields of Modern Microbial Ecology and Molecular Ecology. However, facing the increasingly serious problem of acoustic pollution, very few efforts have been put forth into studying the relation of single cell organisms and sound field exposure. Herein, the biological effects of sound exposure on the growth of *Escherichia coli* K-12 with different acoustic parameters were studied. The effects of sound exposure on the intracellular macromolecular synthesis and cellular morphology of *Escherichia coli* K-12 were also analyzed and discussed. Experimental results indicated that *Escherichia coli* K-12 exposed to sound waves owned a higher biomass and a faster specific growth rate compared to the control group. Also, the average length of *Escherichia coli* K-12 cells increased more than 27.26 %. The maximum biomass and maximum specific growth rate of the stimulation group by 8000 Hz, 80dB sound wave was about 1.7 times and 2.5 times that of the control group, respectively. Moreover, it was observed that *Escherichia coli* K-12 can respond rapidly to sound stress at both the transcriptional and posttranscriptional levels by promoting the synthesis of intracellular RNA and total protein. Some potential mechanisms may be involved in the responses of bacterial cells to sound stress.

Effect of a type of Indian classical music (Raag Kirwani) comprised of the sound corresponding to a frequency range of 38 – 689 Hz, on microbial growth, production of certain important metabolites, and antibiotic susceptibility was investigated. All the bacteria and yeasts used as test organisms were found to register better (3.15 – 40.37 % higher) growth under the influence of music, except *Serratia marcescens*. Music treatment was also found to affect production of bacterial pigments (prodigiosin and violacein) whose production is normally linked with quorum-sensing in the producing bacteria. All the test organisms exhibited an increased antibiotic susceptibility (increase ranging from 3.81–18.69 %) under the influence of music. *Chromobacterium violaceum* and *Serratia marcescens* were found to degrade cephazolin at a faster rate when incubated with music. Membrane permeability of the test organisms seemed to get altered owing to music treatment. Intracellular concentration of cations (calcium and potassium) and protein content of the music treated cultures was also significantly different than the untreated control. The audible sound in form of music employed in this study was able to affect growth, metabolism, and antibiotic susceptibility of prokaryotic as well as eukaryotic microbes.

Effect of two different audible sound (music) patterns on six different microorganisms was investigated. Both the sound patterns namely Ahir Bhairav (172-581 Hz) and Piloo (86-839 Hz) were able to significantly affect microbial growth and production of certain key metabolites by the test microbes. Faster uptake of glucose from the growth medium by *Brevibacillus parabrevis* and *Saccharomyces cerevisiae* was observed under the influence of sound. Production of quorum sensing-regulated pigments, prodigiosin and violacein, respectively by *Serratia marcescens* and *Chromobacterium violaceum* was also notably affected by sound treatment. Various experiments definitely prove that the microorganisms show effects due to the various variety of sounds. The intramolecular substances, pigments, size, shape, transcription and post transcription processes, growth rate, etc. all are affected.

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CLASSES OF CARBEPENEM RESISTANT MDR GRAM NEGATIVE BACILLI (CRMDR)

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Carbapenems are highly stable antibiotics commonly used for the treatment of severe or high-risk bacterial infections. These class of antibiotics is usually reserved for known or suspected (MDR) bacterial infections. Compare like Penicillins and cephalosporin, carbapenems are members of the beta lactam antibiotics, which kill bacteria by binding to penicillin, thus inhibiting bacterial cell wall synthesis. Carbapenem resistance, mainly among Gram negative pathogens is an ongoing public-health problem of global dimensions. This type of Antimicrobial resistance, especially when mediated by transferable Carbapenemase-encoding genes, is spreading rapidly causing serious outbreaks and dramatically limiting treatment options. These types of resistant organism occur due to a recent stay in the hospital. This is especially true if you were in a hospital where CRE has been found and in an intensive care unit). A stay in a long-term care facility. This is especially true if it is a facility where CRE has been found, Exposure to antibiotics, Recent organ or stem-cell transplantation, Use of mechanical ventilation, Use of medical devices inside the body such as urinary catheters Other factors may raise the risk for death from a CRE infection. Carbepenem Resistant based on gene encoding the May three classes, class A, class B, Class D

Carbepenem Resistant Class A

Chromosomally-encoded class A Carbapenemases have been identified in rare Gram negative species that appeared sporadically in clinical or environmental samples. KPC gene are major Gene in Class A. The may causes pneumonia, UTI, Blood stream infection, Sepsis, meningitis.

Class B Carbapenemases

Class B Carbapenemases, also known as Metallo-β-lactamases (MBL), have one or more zinc atoms in their active site. They are resistant to most β-lactamase inhibitors, lack hydrolytic activity against aztreonam, and are inhibited by metal chelating agents. Metallo-β-lactamases were initially described in chromosomes of environmental bacteria and opportunistic pathogens. IMP-1 gene, VIM Gene in class B. The may causes pneumonia, UTI, Blood stream infection, Sepsis, meningitis.

Class D Carbapenemases

Class D β-lactamases, also known as OXA-type enzymes or oxacillinases, are represented by more than 350 genetically diverse enzymes that are widely disseminated in Gram-negative bacteria. The vast majority of these enzymes have been identified in Acinetobacter spp. This may occur due to Hospital acquired infection (HAI).

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QUORUM QUENCHING - A POTENTIAL STRATEGY TO REDUCE BIOFILM FORMATION IN *Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa is a Gram negative opportunistic pathogen that causes several acute and chronic infections in patients such as cystic fibrosis leading to a cause of mortality and due to the emergence of multidrug resistant strain. So, there is an absolute requirement of certain alternative therapy and novel approaches that can prevent the action of pathogenic bacteria. *Pseudomonas aeruginosa* exhibit their pathogenicity by the formation of biofilm associated matrix and upregulation of genes responsible for the synthesis of virulence factors by Quorum sensing (QS) *via* the secretion of some diffusible molecules termed Autoinducer (AI) e.g N-Acyl Homoseriene Lactone (AHL). Efforts to disrupt the bacterial biofilm formation to reduce the expression of virulence genes have provided the recognition of molecules that impede the functionality of QS signal, Quorum Sensing inhibitors (QSI) by the process of Quorum Quenching (QQ).

Quorum sensing system of *Pseudomonas aeruginosa*

In the *las* system, N-3(oxododecanoyl)-homoserine lactone (3OC12-HSl) is produced by the enzyme encoded by the *lasI* gene. When a threshold cell density is

achieved, 3OC12-HSL binds to it transcriptional activator LasR. LasR, in turn, dimerizes and binds to target promoter elements immediately proceeding the genes encoding a number of selected virulence factors that are responsible for host tissue destruction during infection. Similarly, in the *rhl* system, the *rhlI* gene encodes the enzyme involved in the production of N-butyryl-homoserine lactone (C4-HSL) and binds to its transcriptional regulator, RhlR, to control the activity of target genes involved in Pyocyanin synthesis. Besides this *Pseudomonas aeruginosa* encodes an orphan receptor protein, QscR, unlike LasR and RhlR, QscR does not have a paired signal synthase; instead it binds to 3OC12-HSL and to several other long-chai AHLs by an unknown mechanism.

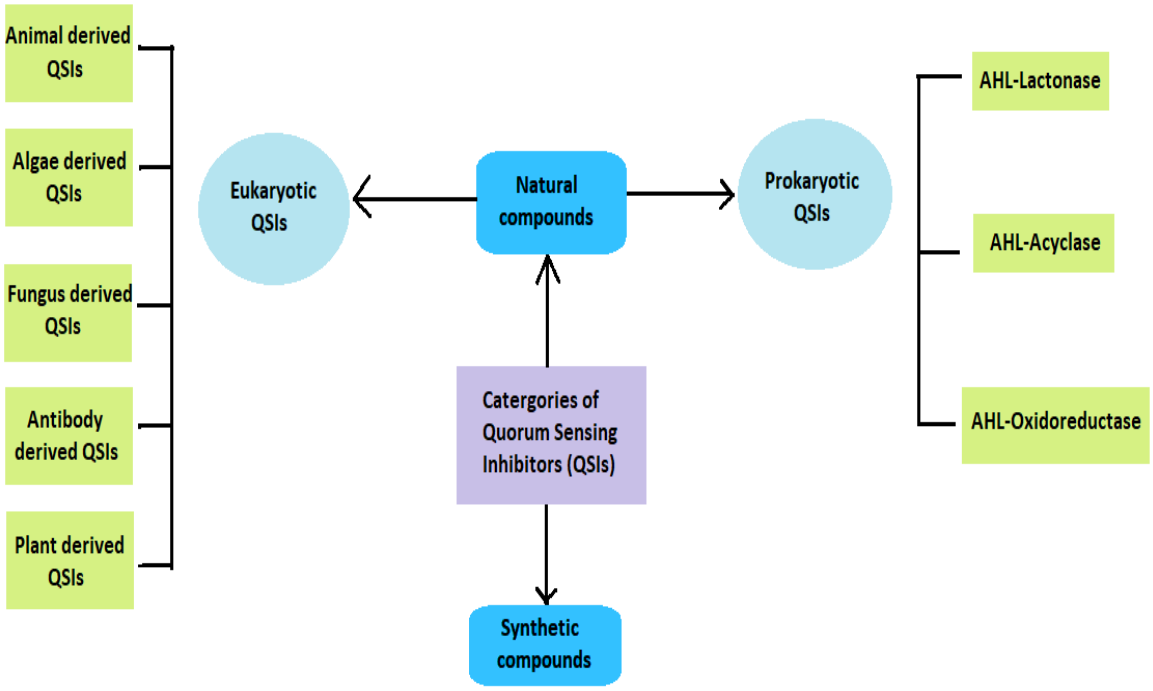
Inhibition of QS: Quorum quenching

Difficulty in removing biofilms and increasing antibiotic resistance necessitates to find out alternative and sustainable novel approaches to combat bacterial infection. The best possible way to trigger this phenomenon, a promising strategy is to disintegrate the bacterial quorum sensing system without altering their growth i.e. Quorum quenching (QQ).

Mechanism of Quorum sensing inhibition – QQ

- Inhibition of signal molecule synthesis (e.g., blocking of Las operon proteins).
- Inactivation or enzymatic degradation of signal molecules by lactonase, acyclase and oxidoreduxtase.
- Competing with signal molecules-receptor analogues.
- Blocking the signal transduction cascades (e.g., by blocking AI receptor complex formation).

Categories of Quorum Sensing Inhibitors (QSI)



Conclusion

The emerging threat of antibiotic resistant pathogens has called for alternative strategies that could replace the usage of antibiotics and minimize the development of resistance mechanism and the best possible way to target is QS signaling. Development of such QQ mechanisms has led to a revolutionary aspect not only in medical application but also in other biotechnological approaches and agricultural field. Additionally, QS inhibition alone cannot affect the antibiotic susceptibility of bacteria. Thus more studies are required to design more potent combinatorial therapies with optimal mechanism of action and safer application.

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ANTIMICROBIAL RESISTANCE (AMR) AND EFFECT ON ENVIRONMENT

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Introduction

Antibiotic resistance is the ability of microorganisms to survive and grow in the presence of antimicrobial agents. Drug resistance is a serious problem as it poses a challenge to treatment strategies as well as the environment. Some microbes develop resistance to multiple drugs which are known as 'Superbugs'. Antimicrobial Resistance (AMR) has emerged as major concern for the environment especially in the developing countries due to the indiscriminate use of antibiotics for multiple purposes without implementing proper control over the usage. Public hospitals and release of untreated waste in the environment further aggravates the problem. India is an agriculture-based country and antibiotics are used in agricultural fields and fish farms to protect the crops from microorganism. Most farmers use antibiotics in uncontrolled manner which drains into the surrounding water bodies and accelerates the development of antibiotic resistance in the wild species due to horizontal transfer and bioaccumulation. It may also trigger the selection of pathogenic species which will pose a serious threat to health care system of the countries. Covid-19 pandemic has also accelerated the issue as the use of biocides and disinfectants has increased drastically within a span of few months which may induce selection of multidrug resistant superbugs over the natural flora, causing a major misbalance in the ecosystem. There is found to be an increase in pan-emergence of multidrug resistant bacterial strains all over the globe. Some of the major super-bugs include methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant

Enterococcus (VRE), and extended spectrum β-lactamase (ESBL) producing *Klebsiella pneumoniae* and *Escherichia coli* and pan-drug resistant Gram negative bacteria like *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Our present study has showed the presence of MRSA and VRE along with resistant *Bacillus* spp. in Ganga water system. 58 samples were collected from 5 different stations along the banks of Ganges with outlet from city during 2018-2020. Pre Covid samples showed higher Heterotrophic Plate Count than the Lockdown period. About 68 % of the representative microbes were found resistant during 2018 and 2019. Microbes from intermediate and resistant zones were also higher during the end of 2019. During the lockdown, significant decrease $p \leq 0.01$ in THC was found and resistant species count also decreased. This can be contributed to lesser anthropogenic pollution and regulation on traffic and industries.

Factors contributing to development of Multidrug resistance

Multi drug resistance can be triggered in a species by natural causes or can be acquired by a species due to changes in its environment. Resistance can develop naturally by random mutations in the genomic material or by horizontal gene transfer from the environment. Selection pressure can also result in the dominance of drug resistant variant. Use of antibiotics in food or release of untreated hospital wastes directly in the environment is a major cause. Biodiversity index, pH, BOD, COD, concentration of heavy metals and minerals present in water are also major factors to be considered.

Conclusion

Multi drug resistance can be triggered in a species due to several factors. Our study shows similar results to other researchers where they have shown an increase in AMR worldwide. Use of antibiotics in food or release of untreated hospital wastes directly in the environment is the major cause of growing AMR in microbes. Discharge of water from fish culture farms and agricultural fields also adds to the concern. Our study also corroborates with the work of others, who have showed increase resistance due to human activities and effluents along with hospital wastes. About 20% decrease in resistance of the identified species was found during the country wide stop of human activities and closure of industries during April to July, 2020. Public awareness and controlled use of drugs along with the availability of only prescription medicines is the need of the hour, to keep a check on the rising concern of AMR worldwide.

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MICROBE-PLANT INTERACTION: INSIGHT TO A FASCINATING AND RARE PHENOMENON

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Microbial interaction with plants has been considered under negative aspects and often described as "biotic stresses leading damage to the plant". But only a few are aware of their symbiotic and associative interaction which results in plant productivity, disease resistance, and stress tolerance. Microbes utilize nutrients from plants and in turn induce production of the certain stress-mediated chemical compound, assist growth and overall immunity. The researchers of the Noble Research Institute, attempted to find the variables involved in understanding the outcomes of these plant-microbe interactions. In this context, a plant-microbe combination can be utilized to fix negative environmental factors, productivity limitation, and symbiotic relationships to produce plants for obtaining desired products to compensate human needs.

There is a saying that a simple trace of a scent can evoke a wonderful memory. We believe that perfumes are not more than a source of refurbishing our freshness, but it is more than that. However, perfume contains a wide range of oils and aromatic compounds, are major highlights of commercial aroma and cosmetic industry. Oils and aroma compounds which are mostly plant-based have long been used in perfumery. These compounds are usually secondary metabolites which are produced in response to threat or stress by the plants. These chemical compounds can be derived from various parts viz. bark, flower, leaves, fruits, resins, roots, bulbs, woods etc. Interestingly a single

plant from its different parts can offer varieties of compounds with different odors. One example that justifies the above statement are the species of the genus Aquilaria. Agarwood, which is the result of plant-microbe interaction is reported to be the most expensive wood in the world because of its fragrance, medicinal value, composite materials, and occurrence. Besides, low yield from the source material (20 kg of wood yields only 12 ml oil), labour-intensive extraction process, few resins producing species (*Aquilaria agollocha, Aquilaria malaccensis, Aquilaria crassna* and *Aquilaria malaccensis*), adds on to its value. Resins formed by these plant species which are rarely available nowadays, are converted to produce incense and other perfumery products and further distilled to form ingredient in branded perfumes. The oil derived from agarwood has cultural and religious significance around the world and is described as a fragrant product. Agarwood is plant specific, only formed when certain species of fungus and bacteria naturally infect certain species of plant of genus *Aquilaria* belonging to the Thymelaeaceae family. With advances in research and development followed by repeated experiment, revealed microbial infection to be essential for fragrant resin formation and certain bacterial genus (*Bacillus*, *Lactobacillus*, *Vibrio*, *Pantoea*, etc.) and fungal genus (*Fusarium*, *Lasiodiplodia*, *Phaeoaccremonium*, *Melanotus*, *Penicillium* etc.) are responsible for up regulation of some plant elicitors (Jasmonic acid and Methyl jasmonate) and thereby inducing production of secondary metabolites and fragrant volatile compounds in agarwood.

Sesquiterpenes and phenylethyl chromones are the main compounds in agarwood which contribute to the fragrance and quality of wood and oil. Interestingly chemical variation of the compounds of different Aquilaria species was observed and reported which may serve as a source for production of the new and competing to the existing fragrance in near future. However, the down-market of perfumes produced from purely plant-based products led to the development of modern perfumery with the commercial synthesis of aroma compounds such as coumarin or vanillin. Perfumery products derived from such chemically synthesized aroma compounds were made available with high market value. This felt the need for substitution of chemically synthesized products.

Thus, microbes can be utilized for the production of plant metabolite derived compounds which would be more potent, competent and promising not only in the field of aroma, cosmetics, and medicine but also in other sectors of research and development. However, selection of strains has always remained as a limitation in artificial host-plants interaction as in nature seasonal fluctuation of the endophytes takes place and hence possibility that some other microbes may make difference in the output product is high. Apart from this, the type of soil the plant is growing can also influence

the endophytic microbial diversity. It opens a scope for further research on both agarwood producing Aquilaria species and its associated microbes. Limitation in the existing research is in understanding the complex regulation of metabolic pathways during the interaction process. It is very essential to gain understanding on the biotic interaction, gene expression, transcriptional regulation and important key enzymes involving in major metabolic pathways which would eventually through light and strengthen research and finding of potent biogenic fragrance.

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BIODIESEL: ONE OF THE BIOFUEL OF THE FUTURE

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What is Biofuels?

A biofuel is an alternative to Green fuels. A biofuel is a fuel that is produced from biomass, instead of a fuel produced by the very slow geological processes. Biofuel is derived from biomass like plant or algae material or animal waste. Biofuel is known as a source of renewable energy, unlike fossil fuels such as coal, petroleum and natural gas.

Why the world needs biofuels?

- The role of Biofuels is not meant to replace fossil fuel but to help to extend the use of longevity of diesel. The primary goal of biofuel is to provide security and benefit to societies.
- Traffic is one of the largest sources of greenhouse gas. So that substituting fossil fuels with renewable alternatives such as biofuels is an efficient way to reduce carbon emissions. Biofuel will reduce emissions of carcinogenic compounds as high as 85%.
- Biofuel can be used in today's engines, infrastructures and vehicles without the need to make changes.
- Biofuel provides energy security that is available and affordable for consumers as well as the industry.

- Biofuel is proven to be less harmful to the environment and cost less damage. Biofuel is found to be safer to handle than petroleum fuel because of its low volatility.
- Using waste and residue as raw materials for biofuels is an excellent example of the circular economy. Reducing the amount of waste and making the most of our valuable natural resources like biofuels.

The two types of Biofuel are Bioethanol and Biodiesel

a) Bioethanol is an alcohol made by fermentation from carbohydrates produced in sugar or starch crops such as corn, sugarcane, or sweet sorghum. Cellulosic biomass, derived from trees and grasses, is also being developed as a feedstock for ethanol production. Ethanol can be used as a fuel for vehicles in its pure form (E100) to increase octane and improve vehicle emissions. Bioethanol is used in the United States and in Brazil.
b) Biodiesel is produced from oils or fats using transesterification and is the most common biofuel in Europe. It can be used as a fuel for vehicles in its pure form (B100), but it is usually used as a diesel additive to reduce levels of carbon monoxide and hydrocarbons from diesel-powered vehicles.

History of Biodiesel

- In the 1890s, Rudolf Diesel invented the diesel engine which runs on a variety of fuels, including vegetable oil. In 1900, diesel engines featured at the Paris Exposition was powered by peanut oil. In the 1930s, vegetable oils convert into compounds called fatty acid methyl esters from palm oil (which today we would call biodiesel). During World War 2, after petroleum fuel supplies vegetable oil was used as fuel by several countries like China, Brazil, Japan, and India. When the war ended, petroleum supplies were again cheap and vegetable oil fuel was forgotten.
- After the death of Dr. Diesel in 1913 petroleum became huge available in a variety of forms in more class, in today we know as "Diesel fuel".
- Martin Mittelbach is a researcher. His Pioneering work to develop the biodiesel fuel industry in the early 1990s. One of the first biodiesel plants in the United States became Pacific Biodiesel in 1996, establishing a biodiesel production operation to recycle used cooking oil into biodiesel on the island Maui in Hawaii.
- In lab-scale production, the first biodiesel manufacturing plant specifically designed to produce fuel was started in 1985 at an agricultural college in Austria. Since 1992, biodiesel has been commercially manufactured across Europe and Germany being the largest producer in the world.

How Biodiesel is produced?

- Biodiesel production is the process of producing biodiesel by chemical reactions of Transesterification and Esterification. This involves vegetable oil or animal fats reacted with methanol or ethanol. Because of its low cost, Ethanol is the most used whenever greater conversions into biodiesel can be reached using methanol. Although the Transesterification reaction can be catalyzed by sulphuric acids. The process requires a catalyst to increase the rate of the chemical reaction between the alcohol and vegetable oil. This can be Potassium Hydroxide or Sodium Hydroxide used in the creation of biodiesel is an alkaline.

Vegetable oil/Animal fats + Alcohol + KOH → Biodiesel + Glycerine

- The process leaves behind two products (1) Methyl esters (the chemical name for biodiesel) and (2) Glycerol. The density of glycerol is greater than that of biodiesel, do that these products are easily separated from the mixture. Residual alcohol is typically recovered by distillation and reused in this process. then after glycerol is converted into glycerin (a valuable by-product used in soaps and other products). Water is also removed from the fuel.

Advantages of Biodiesel

- Biodiesel is made from animal and vegetable fat that's why it is a domestic and renewable energy source.
- Experts believe that using biodiesel instead of petroleum diesel can reduce greenhouse gases up to 78 %.
- Biodiesel release less toxic chemicals and it is Biodegradable (four-time faster than diesel)
- Flashpoint of biodiesel is about 150 °C, whereas petroleum diesel has 50 °C. That's why biodiesel is safe to handle, store and transport.
- Biodiesel improves the engine efficiency of vehicles.

Disadvantage of Biodiesel

- Biodiesel is not suitable for use in low temperatures because of oil and the fat to make it.
- Biofuel crops are not the same amount of vegetable oil so there is variation in quality of biodiesel
- Biodiesel is more expensive than other conventional fuels. currently, it is 1.5 times more expensive than petroleum.

- Biodiesel has about 10 % higher Nitrogen oxide than other petroleum products which is responsible for acid rain and the formation of smog and ozone.
- According to some scientists, to produce one gallon of biofuel we need several gallons of energy from petroleum fuel.

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PHYTOCHEMICAL ANALYSIS, ANTIBACTERIAL, ANTIFUNGAL & ANTIOXIDANT PROPERTIES BERKELEY'S POLYPORE MUSHROOM

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Macrofungi have long been used as valuable food source and as traditional medicines around the world. The fungus *Bondarzewia berkeleyi* (*Polyporus berkeleyi)* of the family Bondarzewiaceae (Basidiomycota) grows at the base or roots of Abies and other conifers of the family Fagaceae. It is edible when young and gets tough and unappetizing with age. This species lives as a network of cells (mycelium) within living trees as a parasite, and dead trees as a saprobe, digesting and decomposing the wood. When ready to reproduce the mycelium develops the mushroom, or "fruiting body" that emerge from near the base of the tree; this is the reproductive structure. Spores are produced in pores on the undersides of the caps and are released to begin new mycelia elsewhere. The aim of present study is to evaluate the phytochemicals and investigate the antibacterial, antioxidant activity in Ethyl acetate, Chloroform and Aqueous extracts of *Bondarzewia berkeleyi (Berkeley's polypore)*. As a possible source for antimicrobial activity against *Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Staphylococcus aureus, Streptococcus mutans* and *Bacillus subtilis.* Antimicrobial assay was done by Agar well diffusion method and Zone of inhibition on microorganisms was measured in mm. The

antioxidant activity of these extracts was estimated by various methods such as DPPH and Metal Iron Chelating assay. The Qualitative phytochemical reveals the presence of Carbohydrates, Proteins, Glycoside, Terpenoid and Steroid. Most of the phytocompounds were eluted in chloroform extract. *Bondarzewia berkeleyi* also shows better antibacterial activity against pathogens like *Bacillus subtilis* and *Klebsiella pneumoniae.* The results of antioxidant activity showed that the aqueous had the highest inhibition of free radical DPPH, Metal Iron Chelating assay. From the study it is clearly seen that the *Bondarzewia berkeleyi* can be and medicinal fungi with more number and activity and rich in antioxidants. *Bondarzewia berkeleyi* also rich in carbohydrates and proteins. Furthermore screening and therapeutic characterization will be carried out for the extracts of *Bondarzewia berkeleyi. Berkeleyi* is edible, although nutritional information is not available for the species, edible mushrooms are usually quite healthy, though most people do not eat them in nutritionally significant quantities. The species has been adequately studied for its medicinal purposes. That doesn't mean *Bondarzewia berkeleyi* lacks medicinal value, only that its value isn't known. *Bondarzewia berkeleyi* has not been formally assessed, it seems prudent not to eat it raw. Not only are many mushrooms undigestable, but, like some closely related russellas, some individuals of this species are spicy when raw but not when cooked and those russellas are toxic when raw. It seems likely that the spicy principle in both cases is a toxin that breaks down when heated.

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ANIMAL VIRUSES

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Animal viruses are viruses that infect animals. Humans cannot be infected by plant or insect Viruses, but they are susceptible to infections with viruses from other vertebrates. These are called viral zoonoses or zoonotic infections. Examples include, rabies, yellow fever and pappataci fever. Jersey and Guernsey breeds of cattle are particularly susceptible to pox viruses, with symptoms characterised by widespread, unsightly skin lesions. And most people have heard of myxomatosis, which is a fatal pox virus infection of rabbits: once infected they die within twelve days. Companion animals such as cats, dogs, and horses, if not vaccinated, can catch serious viral infections. Canine parvovirus 2 is caused by a small DNA virus, and infections are often fatal in pups. Marine mammals are susceptible to viral infections. In 1988 and 2002, thousands of harbor seals were killed in Europe by the measles-like phocine distemper virus. Many other viruses, including caliciviruses, herpesviruses, adenoviruses and parvoviruses, circulate in marine mammal populations. Fish too have their viruses. They are particularly prone to infections with rhabdoviruses, which are distinct from, but related to rabies virus.

Animal virus capsids come in many shapes. One of the craziest-looking (to me, at least) is the Ebola virus, which has a long, thread-like structure that loops back on itself. Chikungunya looks like a sphere, but is actually a 202020-sided icosahedron. Animal viruses can be divided into DNA and RNA viruses, depending on the nature of their genomes. DNA and RNA viruses are described in Part II and Part III, respectively. In Part IV, two virus families, which replicate their genome *via* Reverse Transcription (RT), will be described. Although, these viruses have either an RNA or DNA genome, they

are classified as reverse transcribing viruses or RT viruses. Retroviruses1 have an RNA genome, while Hepadnaviruses have a DNA genome. Largest animal virus is the parrot fever virus. The smallest animal viruses belong to the families Parvoviridae and Picornaviridae and measure about 20 nm and about 30 nm in diameter, respectively. Animal virus reproduction stages: adsorption, penetration and uncoating, replication of virus nucleic acids, synthesis and assembly of capsids, and virus release. In conclusion, humans frequently sustain bites that often result in infection caused by the biter's oral flora as well as environmental bacteria. It is important for providers to be familiar with the evaluation and treatment of bite wounds, recognize which are most likely to become infected and, if indicated, which antibiotics are most effective.

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NANOTUBES MANIFESTATION OF BACTERIA

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The endospore forming in the extreme condition same as a Nanotubes (NTs) formation, however, NTs research said as the same Bacteria having linked between of the intraspecies (both Gram positive bacteria and Gram negative bacteria) contact all so known as the cell to the cell communication system. Extreme condition or dying situation will be the formation of NTs from that cytoplasmic content exchange between Bacterium live cell; tubular formation of the intercellular tube and extending tube, frequently surrounding such as 'mycelium' structure. Their extremely rapid (with a min) transferring to nutrition, protein, nucleic acid all those things will be one cell to neighboring cell be exchange on their own self in front of cannibalization. The discovered scientists are Gyanenddra P. Dubey in 2011 using of the *Bacillus subtilis* wildly as a tools study Bacteriology form "membrane nanotubes" that allow to intercellular exchanges. Onlook of membrane nanotubes of membranous segment a hold on to the following lumen, Found that low cell density of NTs. Those processes reveal over a non-conjugative plasmid transferring to the cell into cell. Furthermore, Quorum Sensing (QS) detect single is offer by the bacteria for cannibalization of cell that is a major function on sensing. NTs necessary genesis of Ymdb, flagellin as flagellar body protein, Phosphodiesterase hydrolysis cAMP. The flagellar body proteins needed for the flagellar trade contraption, called CORE, work both in flagella and in NT get together. Those nanotubes are classified as Extending nanotubes (adhesion to a single cell) are did to increase into surface area nutrition contribution and intercellular nanotube (linking to two cells) did to function for transferring molecules alike metabolites proteins and non-

conjugative plasmids. The intercellular tube can be the formation of between two single cells of Bacterium or two different types of Bacterium species or a relationship with a eukaryotic host, *Escherichia coli* formation of NTs to extract nutrition from the host cell. The living bacteria cells having approximately 70 percent of nanotubes production microorganisms lived. NTs as activated by the synergistic activity of intercellular communication in the middle of bacterial community members, allow the implementation of the complicated processes such as antibiotic production, secretion of virulence factors, and bioluminescence. Besides, essentially all microorganisms are equipped for framing a strong multicellular structure, named biofilm, containing cells with various functionalities. Characteristic biofilms are normally made out of a few bacterial animal categories and consequently request a planned quality articulation of the different occupants.

The visualization of nanotubes in the Scanning Electron Microscope (SEM) and Structured Illumination Microscope (SIM) of two types of filamentous structures: i). Thicker filamentous as 30 nm diameter and ii) Thinner filamentous approximately 70 nm. All things considered, the NT-bearing cells showed sketchy staining with Nile Red, which may demonstrate non-ideal cell conditions (Fig.1a). Fluorescence Microscope using the visualization of the Green Fluorescence Gradient (*gfg*) with aid of SYTOX as cytoplasmic molecular and nanotubes (Fig. 1b). The recently find nanotube formation Bacteria are *Bacillus subtilis, Bacillus megaterium, Escherichia coli, Deinococcus radiodurans, Agrobacterium tumefaciens,* and more. Besides, *Shewanella oneidensis* into NTs or nanowires synthesis of Arsenic-Sulfide and Cadmium-Sulfide element for electrochemical synthesis and device fabrication a next-generation on super semiconductors, Nanopods in *Delfiitia* sp.

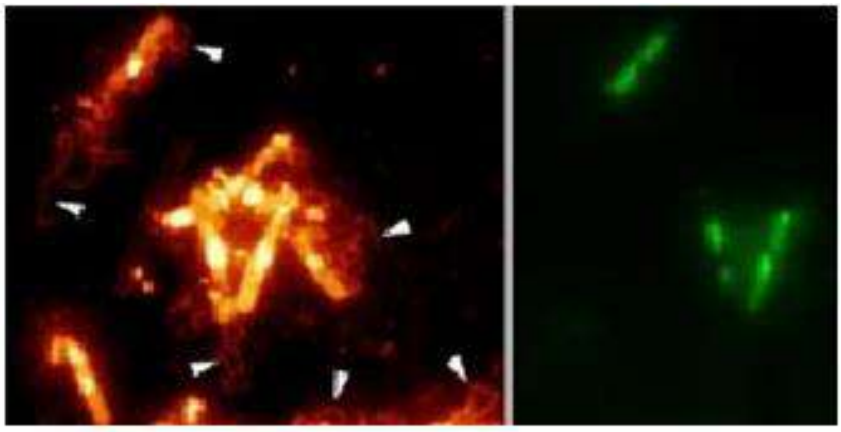

Figure 1a **Figure 1b**

Figure 1a: Nile red staining view cytoplasmic with nanotube
Figure 1b: Sytox as on nanotubes

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MILK BORNE DISEASE

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Milk is arguably one of the most versatile ingredients among cooks and a staple in most households. Milk borne disease is a transmissible disease transmitted from inflected or carrier individuals. The spoilage and pathogenic micro-organisms causing illness could come from the animals, handler, Environment and due to the poor sanitation practices.

Milk is an efficient carrier for a variety of disease producing microbial agents. The disease control, however, can be maintain only by the constant supervision of the heath of dairy animals and by the adequate controls at all point from the time the milk leaves the udder until it reaches the consumer. When the slightest relaxation of attention at any crucial links in the milk chain from the farm to consumer invites problem. A second factor that is common to advanced and the developing countries, is the disease causing microbes. Such microbial agent can be conveniently classified as Communicable diseases causing microbes such as viruses and bacteria, specific and non-specific sensitizing agent and toxic chemicals such as drugs pesticides and other substances.

Milk contains all a sort of factors like nutrients, can serve as an excellent media for microbes, including pathogens. While growing at the expenses of milk constituents these microbes release certain metabolites like lactic and other organic acid, gases, etc. Which may be useful and for harmful, and thus, affects the quality of milk. Generally, these metabolites lead different spoilage conditions in milk products and make these unfit for consumption. Pasteurization has reduced milk-borne illnesses from milk.

Difference Source of Pathogens

- ***Animals:*** The health of dairy animals is very important parameters because a number of diseases including brucellosis, Q-fever etc. This infections of foot and mouth disease virus may be transmitted to man through milk.
- ***Handlers:*** The diseased persons may transmit diseases like Typhoid fever, Scarlet fever, Diphtheria etc. By coughing, sneezing and talking during milking or subsequent handling of milk at farm level.
- ***Environment:*** Dairy farm environment may also introduce pathogens into milk products at different stages of pathogens are like group A streptococci, *Mycobacterium tuberculosis* and some viruses of respiratory origin. Contaminated water, fodder and unclean vessels and containers used for handling milk and other hygienic condition at farm and plant may significantly contribute to pathogen and spoilage causing micro-organisms in milk.

Diseases from Milk to Man

- ***Brucellosis:*** Brucella is a bacterial microbe that if found in unpasteurized dairy products. Brucella infection or brucellosis as also been called "undulant fever" because of the regular recurrence of fever associated with the disease. It is one of the possible causes of a prolonged fever of unknown origin in children. Symptoms include profuse sweating and joint and muscle pain.
- ***Q-fever:*** It infects a variety of animals, including livestock and pets. The microbe of can be found in cow's milk and is resistant to heat and drying. Infection by resistant to heat and drying. Infection by *Coxiella burnetii* results in Q-fever, a high fever that may last up to two weeks. Like *Brucella*, it may be a cause of an unknown prolonged fever in children.
- ***Salmonella infections:*** *Salmonella* contamination of raw milk and milk products has been the source of several out breaks in recent years. Symptoms include diarrhoea and high fever.

Preventions of Milk Borne Diseases

- Don't drink raw milk. Drink only pasteurized milk and other dairy products.
- Think twice and read labels when you shop "organic" many organic food stores sell unpasteurized dairy products.
- Keep dairy products refrigerated within the expiration date marked on the package.

- Milk and unpasteurized dairy products are not the only sources of food poisoning is likely much more common most people think, considering most cases of "stomach flu" in adults are really food poisoning.
- Proper vaccination of individuals against disease is efficient prophylactic measure.

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STRATEGIES OF FOOD PRESERVATION

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Food preservation is the process of treating and handling food to stop or slow down food spoilage, loss of quality, edibility, or nutritional value and thus allow for longer food storage. Preservation usually involves preventing the growth of bacteria, fungi (such as yeasts), and other microorganisms, as well as retarding the oxidation of fats which cause rancidity.

Methods of Food Preservation

A number of methods of prevention can be used that can either totally prevent, delay, or otherwise reduce food spoilage. Preservatives can expand the shelf life of food and can lengthen the time long enough for it to be harvested, processed, sold, and kept in the consumer's home for a reasonable length of time. Maintaining or creating nutritional value, texture and flavor is an important aspect of food preservation, although, historically, some methods drastically altered the character of the food being preserved. In many cases these changes have now come to be seen as desirable qualities, as with cheese, yogurt, and pickled onions.

Drying is one of the most ancient food preservation techniques, which reduces water activity sufficiently to prevent bacterial growth. Vacuum-packing stores food in a vacuum environment, usually in an air-tight bag or bottle. The vacuum environment strips bacteria of oxygen needed for survival, thereby slowing spoiling. Vacuum-packing is commonly used for storing nuts to reduce the loss of flavor from oxidation.

Sugar is used to preserve fruits, either in syrup with fruit such as apples, pears, peaches, apricots, plums, or in crystallized form where the preserved material is cooked in sugar to the point of crystallisation and the resultant product is then stored dry. This method is used for the skins of citrus fruit (candied peel), angelica, and ginger. A modification of this process produces glacé fruit such as glacé cherries where the fruit is preserved in sugar but is then extracted from the syrup and sold, the preservation being maintained by the sugar content of the fruit and the superficial coating of syrup. Preservative food additives can be antimicrobial. These inhibit the growth of bacteria or fungi, including mold, or antioxidant, such as oxygen absorbers, which inhibit the oxidation of food constituents. Common antimicrobial preservatives include calcium propionate, sodium nitrate, sodium nitrite, sulfites (sulfur dioxide, sodium bisulfite, potassium hydrogen sulfite, etc.), and disodium EDTA. Antioxidants include BHA and BHT.

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LOW DENSITY POLYETHYLENE DEGRADING MICROFLORA

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Plastics have been widely used as a packing material in the form of Low density polyethylene. Continuous accumulation of plastic in the environment can cause threat to humidity and environment. Low-density polyethylene (LDPE) is a thermoplastic made from the monomer ethylene. It was the first grade of polyethylene, produced in 1933 by Imperial Chemical Industries (ICI) using a high pressure process *via* free radical polymerization. They are found to be considered non-degradable, once it enters the environment it has been found to remain there indefinitely. Plastics are widely used in the global economy, and each year, at least 350 to 400 million tons are being produced. Due to poor recycling and low circular use, millions of tons accumulate annually in terrestrial or marine environments. However, significant attention has been placed on biodegradable polymer, identification of microbes with degradative potential on plastic material. Biodegradation can be defined as the decomposition of substances through microbial activity.

The present work indicates that naturally growing soil microbes like bacteria and fungi show great efficacy in degrading polyethylene. Generally, fungi have a higher degrading effectiveness compared to bacteria. However, both fungi and bacteria showed capacity to degrade virgin polyethylene under laboratory conditions. Recently, microorganisms have become the focus of interest for environmental friendly disposal of plastic and polymer-based waste. *Pseudomonas* species isolated from environmental matrices have been identified to degrade polyethylene, polypropylene, polyvinyl

chloride, polystyrene, polyurethane, polyethylene terephthalate, polyethylene succinate, polyethylene glycol and polyvinyl alcohol at varying degrees of efficiency. Today it has become clear that plastic causes adverse effects in all ecosystems and that microplastics are of particular concern to our health. There are four mechanisms by which plastics degrade in the environment: Photodegradation, Thermooxidative degradation, Hydrolytic degradation and Biodegradation by microorganisms.

The initial step of the microbial degradation process is to secrete depolymerases to break down the long-chain polymers into low molecular weight oligomers or monomers, which can be further assimilated into microbial cells or metabolized into CO_2. Degradation of polyethylene can be classified as abiotic or biotic, the former being defined as deterioration caused by environmental factors such as temperature, UV irradiation, while the latter is defined as biodegradation caused by the action of microorganisms that modify and consume the polymer leading to changes. Most conventional plastics such as polyethylene, polypropylene, polystyrene, polyvinyl chloride and polyethylene terephthalate are non-biodegradable, and their increasing accumulation in the environment has been a threat to the planet. Fungi can be used to break down waste plastic and create sustainable building materials, according to scientists from Kew Gardens in London. *Aspergillus tubingensis* can grow on the surface of plastics, where it secretes enzymes that break the chemical bonds between plastic molecules

It's certainly not realistic to remove all plastic from your life. But, taking a look at some steps down below may encourage you to reduce your single-use plastic footprint. This can be done by ditching straws, switching to reusable water bottles, and bringing cloth bags to the grocery store. Plastic waste has a very negative impact on our planet and its biodiversity and we decided that we must take a leading role and start using plastic-free alternatives. It is the right thing to do and meets the increasing expectations of our customers.

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RECENT OUTBREAKS OF FOOD BORNE DISEASES OF BACTERIAL ORIGIN AND THEIR IMPACT ON PUBLIC HEALTH

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Food borne diseases of bacterial origin can be classified into two types: food borne bacterial infections and food borne bacterial intoxications. The bacterial infections are generally caused by *Salmonella enterolytica, Bacillus cereus, Escherichia coli* and *Listeria monocytogenes* in which they colonize the part of body and produce infections by release of certain toxins. The food borne intoxications are caused by *Clostridium divicine, Clostridium perfringens* (*Clostridium perfringens* enterotoxin), *Vibrio cholerae* (Cholera toxin), *Staphylococcus aureus* (*Staphylococcal* Enterotoxin B), *Yersinia enterocolitica, Shigella dysenteries* (Shiga toxin) neurotoxin of *Clostridium botulinum*. These bacteria produce the toxins in food, which on consumption result in poisoning. Further the toxins are of two types: exalux's that are released extracellularly by the bacteria, which include: enterotoxins which act on the intestinal mucosa generally causing diarrhea cytotoxins which kill host cells; neurotoxins which interfere with normal nervous transmission. The second is endotoxins which are pyrogenic; lipopolysaccharides released from the outer membrane o the Gram-negative cell envelope by bacterial lysis, Bacterial pathogens have been the source of recent toabou illness cases and outbreaks. *Staphylococcus aureus* Rolfs Patisserie Desserts outbreak in Illinois, U.S on November 2019 was caused by consuming Dessert, Tiramisu, Cream cake and Frosting; a total of 100 cases of in reported. *Shigella* Subway Restaurants Outbreak affected 116 individuals in Chicago

between February 24 and May 2020: symptoms included any gastro-intestinal illness. *Salmonella* Infections occurred in Heidelberg between 27 and August 9, 2020, were caused due to consumption of Turkey affecting around11 individuals, causing fever, and abdominal cramps within 12 to 12 hours after infection. Another outbreak resulted due to SS 52 producing *Escherichia coli* Ol04:H4 (STEC O104:H4) between June 10 to July 5, 2011; affecting morcindividuals in Germany with Hemolytic Uremic Syndrome (HUS) Symptoms included stomach envies diarrhea and vomiting caused by consuming contaminated raw sprouts. The food pathogen disease and food safety agencies like CDC, USDA, FDA, HACCP offer various control measures Against the pathogen's contamination, invasion and outbreak's

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EXTENDED- SPECTRUM BETA-LACTAMASE (ESBL)

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Introduction

Extended-spectrum Beta-lactamases (ESBL's) are the enzymes capable of conferring resistance to Penicillin's 1st, 2nd and 3rd generation Cephalosporin's and Aztreonam but not Cephamycin and Carbapenemes. These are transmissible Beta-lactamases which are inhibited by Clavulanic acid, Tazobactam or Sulbactum. Beta-lactamases were discovered in Germany that were capable of hydrolyzing Extended-spectrum Cephalosporins hence named as Extended-spectrum Beta-lactamases (ESBL's). Beta -lactamses are enzymes capable of hydrolyzing Beta-lactam ring of Beta-lactam antibiotics there by deactivating their antibacterial properties there are plasmid mediated inducible enzymes. The Extended- spectrum Beta-lactamases (ESBL's) are mutants form of TEM1, TEM2 (named after the 1st plasmid Beta- lactamase patient named Temoniera) and SHV-1. Extended-spectrum Beta- lactamases are the products of point mutations (change in their amino acid sequences at the active site of TEM, SHV and CTX-M). ESBL belongs to the Ambler molecular class A and Bush and Jacoby

functional group 2. A large number of outbreaks of infections due to ESBL producing organisms have been described worldwide, this may lead to increase patient mortality when the given antibiotics to the patient does not work on ESBL producers.

Infection associated with ESBL producing bacteria

- Urinary tract infections
- Bacteremia
- Respiratory tract infection
- Gastrointestinal infection
- Skin and soft tissue infection
- Theatre or device related infection
- Neurosurgical meningitis

Risk factors for infection or Colonization with ESBL producing organisms

a) Device Related

- Urinary tract catheters
- Arterial catheters
- Umbilical catheters

b) Surgical Related

c) Antibiotic Exposure

- Third generation Cephalosporin
- Flouroquinolones
- Prolonged duration of hospital or ICU stay can lead to more severe diseases

Enterobacteriaceae is the main bacterial family associated to ESBL production. Some of the common organism is *Escherichia coli* and *Klebsiella pneumoniae* however *Proteus mirabilis*, *Salmonella*, *Pseudomonas* also produce Extended- spectrum beta-lactamases to acquire resistance. Extended- spectrum Beta-lactamases strains are multidrug resistant because they can become resistant to available to antibiotics as they

can pass the gene from one clinical strain to another clinical strain (Plasmid mediated resistance).

Specimens used for ESBL detection

- Urine
- Pus/Wound swab
- Sputum
- Tracheal aspirate
- Blood
- Ascetic fluid

Screening test for ESBL's

a) **Disk diffusion methods**: According to CLSI guidelines, Ceftazidime with inhibition zone of <22 mm, Ceftriaxone with <25 mm and Cefotaxime <27 mm were identified as ESBL producers and selected for further confirmatory procedures.
b) **Screening by dilution antimicrobial susceptibility tests**
    - Phenotypic confirmatory tests with combination disks
    - E-test ESBL strips
    - Vitek ESBL cards.

Antibiotics used for treatment of ESBL are Cephamycins (Cefoxitin and Cefotetan) and Carbapenems (Imipenem, Ertapenem and Meropenem) and Fosfomycin is also given. Some of the Beta–lactamase inhibitors.

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DIVERSITY OF ENDOPHYTIC BACTERIA

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Endophytic bacterial diversity has been reported for a number of plant species. Endophytes are microorganisms that do not visibly harm the host plant but can be isolated from surface-disinfected plant tissue or the inner parts of plants. Furthermore, they colonize an ecological niche similar to that of phytopathogens. Endophytes are microorganisms (bacteria or fungi or actinomycetes) that dwell within robust plant tissues by having a symbiotic association. They protect plants from herbivory by producing certain compounds which will prevent animals from further grazing on the same plant and sometimes act as biocontrol agents. They produce a wide range of compounds useful for plants for their growth, protection to environmental conditions, and sustainability, in favour of a good dwelling place within the hosts. The most common genera of endophytic bacterial community isolated from forest trees include *Pseudomonas, Bacillus, Acinetobacter, Actinobacteria, Sphingomonas* and some genera belong to the Enterobacteriaceae family.

Endophytic bacteria can promote plant growth and yield and can act as biocontrol agents. Endophytes can also be beneficial to their host by producing a range of natural products that could be harnessed for potential use in Medicine, Agriculture or Industry. Because of the effective functions of endophytic microbes may significantly reduce use of agrochemicals (fertilizers, fungicides, insecticides, and herbicides) in the cultivation of crop plants. Bacterial endophytes have been found in every plant species that has been studied. Thus, an endophyte-free plant is a rare exception in the natural environment. In fact, a plant without the associated beneficial bacteria would be less fit to deal with phytopathogens and more susceptible to the stress conditions.

The type of endophytic diversity present in a plant can depend on several factors Endophytic bacteria can benefit their host both directly, by improving nutrient uptake and by modulating growth/stress related phytohormones and indirectly, by targeting pest/pathogens and antagonizing competing plants (Grey box). To confer benefits, these bacteria have to competitively colonize the plant interior, achieved using a battery of colonization traits. The colonization is a sequential process, starting from the plant rhizosphere (bacteria respond to plant root exudates), followed by rhizoplane (root surfaces) and root interior colonization. Once inside, the competent endophytes can move to the aerial parts of the plants (stem and leaves). The diversity of endophytic colonizers is affected by various bacteria, plant and environment related factors (Blue box). The type of method used can also affect the bacterial diversity analysis.

The most dominant among reported genera in most of the leguminous and non-leguminous plants are *Bacillus, Pseudomonas, Fusarium, Burkholderia, Rhizobium* and *Klebsiella*. In future, endophytic microbes have a wide range of potential for maintaining health of plant as well as environmental conditions for agricultural sustainability. Many of these novel compounds produced by endophytes have been shown to have important medical applications such as antimicrobial, antiparasitic, cytotoxic, neuroprotective, antioxidant, insulin mimetic and immunosuppresant properties.

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DO BABIES BORN BY C-SECTION HAVE DIFFERENT GUT BACTERIA TO VAGINALLY BORN BABIES?

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The gut microbiome is a complex eco-system of millions of microbes and is important for the development of the immune system. As the amniotic sac is considered to be sterile, the early colony that takes up residence in a baby's gut forms a crucial starting point for the microbiota, that will develop over the next few months and setting the trajectory for several years, which is determined by mode of birth. Babies born vaginally have a colony of microbes similar to the mother's birth canal. The lactic acid bacteria present in the canal helps to convert lactose into galactose and glucose which are absorbed by small intestine into blood and then converting it into energy for the baby. As C-section babies are directly exposed to hospital environment leading to antimicrobial resistance, making them more susceptible to diseases and also due to lack of exposure to healthy microbes, they are more prone to autoimmune disorders like asthma and allergies. Their gut is generally dominated by opportunistic bacteria like *Enterococcus* and *Klebsiella*. To mimic the vaginal birth microbe processes like vaginal swabbing is carried out in C-section infants, but it is ruled out as it is risky to introduce undefined microbes to immunologically underdeveloped infant. But caesarean birth cannot be considered as the only cause for early stage disorders as they may be also caused due to poor nutrition and unnecessary use of antibiotics. By the age seven there is barely any discernible difference between the gut flora of children born naturally and

those by C-section. The study of gut microflora at prior development stages is important, as it can help in reducing the susceptibility to diseases and development of therapeutics to create a healthy microbiome.

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A REVIEW ON *Acalypha indica*

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Acalypha indica (Kuppaimeni) (Euphorbiaceae family) is an erect, often simple-stemmed annual herb distributed widely throughout the world. It can grow average height of one meter; leaves broadly ovate, base rounded to attenuate fairly rapidly. The monoecious flower of *Acalypha indica* gives it the characteristics features due to its cup-shaped involucre that surrounds the small flowers in the catkin-like inflorescence. Acalypha indica has been acknowledged by local people as useful source of medicine for several therapeutic treatments. They consume parts of the plant for many medicinal purposes and its potent pharmacological activities enhance its use in traditional medicine such as inflammation, anti-bacterial, anti-cancer, anti-diabetes, anti-hyperlipidemic, diuretic, anti-helminthic herbal plant. is known to be good cure for respiratory problems, rheumatoid arthritis, scabies, treatment of insect bites, and it also promotes wound healing. The decoction made from the whole plant is used for treatment of bronchitis, epilepsy, emmenagogue, mouth ulcers, and as an expectorant to treat asthma and pneumonia. The leaf of *Acalypha indica* is used to treat ganglions, diarrhoea, leprosy, laxative, diuretic gonorrhoea, rheumatism ulcers, ring worms, eczema, intestinal worms, bolts, swelling, post-partum, pains, scabies, venereal disease. The decortion made from a mixture of the leaf powder and garlic is administered to treat intestinal worms. The leaf decoction is dropped into the ears to treat earache and infections. Crushed leaf poultice is tropically applied to treat boils and skin infections. The leaf sap or ground leaves in water is used as eyedrops to treat eye infections. The crushed aerial parts are also applied to the skin parasites and an infusion is taken as purgative and vermifuge. The paste made for the treatment of scabies. A leaf infusion is also taken as purgative. The active ingredients of the plant include Steriods,

Triterpenoids, Glycosides, Carbohydrates, Alkaloids, Flavonoids and Tannins. Cyanogenic glycosides acalyphin and tri-O-methyl ellagic acid.The presence of these bioactive compounds may explain the anthelminitic ,anti-inflammatory and analgesic effects of this plant and hence justify its potent use in traditional medicine for the treatment of myarid disease and disorders.

Figure – 1: ***Acalypha indica***

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METHEMOGLOBINEMIA DISEASES

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Methemoglobinemia disease may arise from a variety of etiologies including genetic, dietary, idiopathic, and toxicologic sources. Hemoglobin molecules contain iron within a Porphyrinheme structure. The iron in hemoglobin is normally found in the Fe^{2+} state. The iron moiety of hemoglobin can be oxidized to the Fe^{3+} state to form methemoglobinemia. However, this is not the case, because allosteric changes to the hemoglobin molecule cause oxygen to bind more tightly in the partially oxidized hemoglobin molecules. This increased affinity causes a left shift in the hemoglobin-oxygen saturation curve. To combat this process, there are several enzyme systems in the RBC that inhibit oxidation or reduce MHb back to hemoglobin. The cytochrome-b5–MHb reductase system is the predominant system and accounts for approximately 99 % of daily MHb reduction. Ascorbic acid and glutathione account for small amounts of reduction. Under normal circumstances, these enzymes/molecules play a minor role in reduction. However, patients with methemoglobinemia as a result of complete congenital cytochrome-b5 reductase deficiency are able to maintain MHb levels below 50 %.

Oxygen transport, the major function of hemoglobin, is dependent upon reduced heme iron. In the red cell, the heme iron is maintained in the reduced form by the methemoglobin reduction system. When the balance between oxidation and reduction of heme-iron is perturbed due to the presence of excessive oxidants, decreased reducing capacity or the presence of abnormal hemoglobin, methemoglobinemia ensues. In most cases methemoglobinemia is transitory and of no major clinical consequence. Occasionally, however, it can be life threatening and must be rapidly diagnosed and

treated. When methemoglobinemia is of hereditary nature, either due to deficiency of red cell NADH-methemoglobin reductase or due to the presence of M hemoglobin, it is a lifelong problem. Since most of these patients do not have major disabling symptoms, the treatment is aimed at correction of cyanosis

Another enzyme that reduces MHb is reduced nicotinamide adenine dinucleotide phosphate (NADPH)–MHb reductase. Also known as NADPH flavinreductase and NADPH-MHb–diaphorase, under normal conditions this enzyme plays a negligible role in reducing MHb. It is actually a generalized reductase with an affinity for dyes, such as methylene blue, Nile blue, and divicine.12-14 In the presence of the cofactor NADPH, this enzyme will reduce these dyes, which in turn reduce MHb. The primary function of this reductase is probably to metabolize oxidant xenobiotics and not MHb. It is somewhat fortuitous that the reduced form of methylene blue has a high affinity for MHb. NADPH-MHb reductase deficiency has also been described, but does not lead to methemoglobinemia.10 This enzyme is not responsible for endogenous MHb reduction, and only reduces MHb in the presence of an exogenous catalyzing agent such as methylene blue. When patients with this disorder develop chemically induced methemoglobin, they do not respond to conventional methylene blue therapy, because methylene blue is dependent on this enzyme to reduce MHb.

Protective mechanisms against oxidative stress include sulfation enzymes, ascorbic acid, and glutathione. These enzymes and peptides serve to detoxify oxidative exogenous chemicals and thereby indirectly prevent methemoglobinemia. Reduced glutathione is quantitatively the most important cellular antioxidant, and is of key importance in all cells for the preservation of protein sulfhydryl groups and to prevent oxidative damage in general. Glutathione is a minor pathway in the reduction of MHb. Glutathione may also metabolize potentially toxic xenobiotics to nontoxic intermediates by forming mercapturic acid conjugates. Oxidized glutathione is cytotoxic and will diffuse out of cells if it is not reduced back to reduced glutathione. Hence, oxidative stress in the presence of glucose-6-phosphate dehydrogenase (G6PD) deficiency leads to intracellular depletion of total glutathione. Other enzymes involved in free radical metabolism include superoxide dismutase, catalase, and glutathione peroxidase. Despite the role these enzymes play in detoxifying agents that cause methemoglobinemia, congenital deficiencies of virtually all these proteins have been described, and none are associated with methemoglobinemia. This is likely due to the extraordinary efficiency of the cytochrome b5/cytochrome-b5 reductase system in reducing MHb.

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URGENT NEED FOR DEVELOPMENT OF NEW BETALACTAMASES STABLE ANTIBIOTIC TO SAVE HUMAN LIVES

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Beta-lactam antibiotics are the backbone of chemotherapy for many life-threatening infections over the past 70 years because of their excellent antibacterial activity and selectivity, accounting for over 65 % of injectable antibiotics in the clinical settings worldwide. B-lactam antibiotics, the biggest family have a core structure consisting of a 4-member "beta-lactam" ring including Penicillin, Cephalosporins, Carbapenems and Monobactams, which account for over half of all antibacterial drugs approved, are facing the severe threats of Resistance. One of the primary reasons for bacteria generating antibiotic resistance is the presence of b-lactamases which can hydrolyze b-lactam antibiotics. B-lactamases are distributed into four classes (A, B, C, and D) based on their primary structure. Classes A, C and D are serine hydrolyses while class B enzymes are zinc metallo β-lactamases. The most clinically important β-lactamases include extended spectrum β-lactamases, AmpC enzymes and carbapenemases. ESBL enzymes (e.g. TEM, SHV, and CTX-M) are classified in Ambler class A, are generally inhibited *in vitro* by β-lactamase inhibitors (clavulanic acid, tazobactam, and sulbactam), are plasmid mediated and are mainly present in the Enterobacteriaceae family. AmpC β-lactamases (Ambler class C) are usually chromosomally mediated but can also be plasmid encoded. Carbapenemases are β-lactamase enzymes that hydrolyze carbapenems and are present in three different Ambler classes (A, B and D). They are characterized by the ability to hydrolyze all

classes of β-lactam antimicrobials (with the exception of monobactam for class B enzymes) and are generally not inhibited by currently used β-lactamase inhibitors. The World Health Organization (WHO) categorized the Carbapenemase producing *Acinetobacter*, *Pseudomonas* and *Enterobacteriaceae* as Priority1 (critical) in the list to help in prioritizing the research and development of new and effective antibiotic treatments. Centers for disease control (CDC) listed Carbapenem-resistant *Acinetobacter* as serious threat in 2013 and then shifted it to urgent threat in 2019 because of the emergence of resistance in *Acinetobacter* and lack of antibiotics, in the development and treatment of these infections. Antibiotic stewardship programmes, including education should be implemented at global level and target stakeholders in hospital and community settings which should be combined with public awareness campaigns. Innovative tools to support appropriate use of antibiotics and adequate long-term planning are urgently required to combat this resistance.

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BACTERIAL CULTURE MEDIA

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Culture media is classified into six major types. The principle of culture media, in preparing a culture medium for any microorganisms, the primary goal is to provide a balanced mixture of the required nutrients, at a concentration that will permit good growth. A culture medium or growth medium is a liquid or gel designed to support the growth of microorganisms. Here we will discuss microbiological culture media used for growing microbes, such as bacteria or yeast. There are Basal media, Enriched media, Selective media, Indicator media, Transport media, and Storage media. The Basal media is maybe used for the growth (culture) of bacteria that do not need enrichment of the media. *Staphylococcus* and Enterobacteriaceae grow in this media. Enriched media are enriched usually by adding blood, serum or egg. Example: Enriched media are blood agar and Lowenstein-Jensen media. Streptococci grow in blood agar media.

Selective media favour the growth of a particular bacterium. Example: MacConkey agar, Lowenstein-Jensen media, Tellurite media. Antibiotics may be added to a medium for inhibition. Indicator or different media in this media, an indicator is included in the medium, (Example the Blood, Neutral red, Tellurite. Example: Blood agar and MacConkey agar are indicator media. Transport media is used when speciemen cannot be cultured soon after collection. Example: Blair medium, Amies medium, Stuart medium. Storage media: Media used for storing the bacteria for a long period of time. Example: Egg saline medium, Chalk cooked meat broth. MacConkey agar is an indicator, a selective and differential culture medium for bacteria designed to selectively isolate Gram-negative and enteric (normally found in the intestinal tract) bacilli and

differentiate them based on lactose fermentation. Blood agar is an enriched, bacterial growth medium. Fastidious organisms, such as *Streptococci*, do not grow well on ordinary growth media.

Agar-Agar is used in culture media. Agar is a gelatinous polymer substance derived from red algae and commonly used in a biological laboratory setting as a substrate. Agar plates are Petri dishes containing agar in combination with a growth medium to culture microorganisms such as bacteria. Present review focused basic knowledge of culture media and their types, this review may be help to basic learner, research and development center especially microbiology lab technicians.

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ROLE OF MARINE ALGAE IN SKIN WHITENING EFFECT

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Nowadays, due to more beauty care and focus, skincare products are in high demand. Cosmeceutical industries developing more products by using synthetic chemicals or ingredients. These synthetic chemicals or chemical constituents, accumulate in various skin layers and creates toxic effects such as genotoxicity, reproductive toxicity, mutagenicity, carcinogenicity, etc. As an alternative, many natural sources such as plants, algae, and its products, are useful as ingredients. Instead, cosmeceutical industries developed new attention in the use of natural ingredients for skin benefits and reducing harmful effects on human health. The aim of this chapter is to highlight the applicability of seaweeds in the skin whitening effect by inhibiting melanin, tyrosinase, and other means. Seaweeds are one of the major resources with a wide variety of applications. Marine macroalgae also are known as Seaweeds, eukaryotic, multicellular, and oxygenic photosynthetic organisms belong to the Plantae kingdom that is mainly found in aquatic environments. Based on the presence of photosynthetic pigments, it can be classified into three types: the green algae, the brown algae, and the red algae. The green algae are included in phylum "Chlorophyta". It contains mainly chlorophyll a, b, and carotenoid pigments. The brown algae are the large and most complex type of algae include in phylum "Phaeophyta" and it contains diversified pigments such as chlorophyll a, c, and fucoxanthin. Third, the red algae contain a variety of pigments including chlorophyll a, red phycoerythrin, blue phycocyanin, β carotene, lutein, zeaxanthin, and phycobiliproteins, etc. It is rich in bioactive

compounds that can be exploited as a key source of many natural Phycocompounds with potential biological activities for cosmetic applications. There are various diversified compounds such as polysaccharides, pigments, phenolic compounds, fatty acids, vitamins, proteins, amino acids, and peptides are exhibited a wide range of bioactivity, and this bioactivity direct relevant in cosmetics. It also reduces the effect of toxic chemicals of cosmetic products and beneficial for different skin benefits such as skin whitening effects, photoprotection effects, skin moisturization effects, skin aging, antioxidant effects, wound healing effects, antimicrobial effects, and skin wrinkle activity. This noted skin beneficiary applications due to the presence of Phycocompounds and its potential effects on inhibition of Matrix Metalloprotein complex (MMP), effects on inhibition of tyrosinase enzyme, inhibition of melanin pigment, radical scavenging activity, reduced UV induced damage, Anti-collagenase, decolorizing, anti-allergic and anti-elastase activity, etc.

Among all, skin lightening and whitening is a basic requirement for people to foe their beauty concern. Melanogenesis is the process that produces melanin pigment, which contributes to skin and hair color. Melanin is the key pigment that is responsible for skin color in mammals. Melanin pigment is produced from its precursor, tyrosinase enzyme through a series of various enzymatic and chemical reactions. The inhibition of the tyrosinase enzyme plays a vital role in the skin whitening effect. Tyrosinase is the key enzyme responsible for the synthesis of melanin.

The first step of melanin biosynthesis is the inhibition of the tyrosinase enzyme. Nowadays researchers are searching for natural tyrosinase inhibitors for preventing skin darkening and browning reactions by controlling melanin synthesis. In recent years, marine algae have attracted great attention as a natural tyrosinase as well as melanin inhibitors. Tyrosinase inhibitors are also useful for the treatment of some dermatological diseases associated with melanin hyperpigmentation and important in cosmetics for depigmentation. Seaweed metabolite is used in cosmetic industries to prevent or treat the overproduction of melanin. Mainly, algal polysaccharides, fatty acids, and phenolic compounds exhibited anti-melanogenesis activity. Many researchers carried out work on skin whitening activity by inhibiting tyrosinase and melanin inhibition, its mechanism of action by in vitro and in vivo studies. Fucoxanthin isolated from seaweed *Laminaria japonica* has been reported to inhibit tyrosinase enzyme activity in UVB irradiated guinea pig and melanogenesis in UVB irradiated mice. Brown algal constituent phloroglucinol possesses tyrosinase inhibition activity due to their ability to chelate copper in this enzyme. The aqueous extracts of brown algae exhibited potent mushroom tyrosinase inhibition activity. Besides, screening new anti-browning and whitening agents from *Ecklonia cava* and *Sargassum silquastrum* contain inhibitors reduced tyrosinase activity and

melanin synthesis in zebrafish. Many researchers found that *Padina boergesinii* exhibited the most in vivo anti-tyrosinase activity that inhibited zebrafish tyrosinase more potent than kojic acid. Here, this chapter contributes a basic review on the use of marine algae in skin whitening activity. This study further needful to study its mechanism of action at the molecular level which can be applied in further development for cosmeceutical evaluation and enhancement.

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IDENTIFICATION OF BACTERIAL STRAINS USING REAL TIME PCR AND MICROARRAYS

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Introduction

Accurate and definitive microorganism identification, including bacterial identification and pathogen detection, is essential for correct disease diagnosis, treatment of infection and trace-back of disease outbreaks associated with microbial infections. Bacterial identification is used in a wide variety of applications including microbial forensics, criminal investigations, bio-terrorism threats and environmental studies.

Challenges in Bacterial Identification

Traditional methods of bacterial identification rely on phenotypic identification of the causative organism using gram staining, culture and biochemical methods. However, these methods of bacterial identification suffer from two major drawbacks. First, they can be used only for organisms that can be cultivated in vitro. Second, some strains exhibit unique biochemical characteristics that do not fit into patterns that have been used as a characteristic of any known genus and species.

In the past decade or so, molecular techniques have proven beneficial in overcoming some limitations of traditional phenotypic procedures for the detection and characterization of bacterial phenotypes. Several non-culture based methods have emerged in the past 15 years.

Real time PCR and microarrays are currently the most commonly employed molecular techniques. Real time PCR is highly sensitive and allows quantitation of bacteria at a species level. Microarray based bacterial identification relies on the hybridization of preamplified bacterial DNA sequences to arrayed species-specific oligonucleotides. Each probe is tagged with a different colored dye which fluoresces upon hybridization.

Real Time PCR-based Bacterial Identification

Using a DNA based assay, one can easily detect bacterial strains directly from clinical samples or from small amounts of cultured bacterial cells, thus improving the sensitivity and decreasing the time required for bacterial identification. PCR has been particularly useful in this regard, which relies on primer sequences designed to facilitate bacterial identification at any level of specificity: strain, species or genus.

In recent years, real-time PCR methods have been developed and described for the rapid detection and identification of several bacterial strains. Real-time PCR is a promising tool for distinguishing specific sequences from a complex mixture of DNA and therefore is useful for determining the presence and quantity of pathogen-specific or other unique sequences within a sample. Real-time PCR facilitates a rapid detection of low amounts of bacterial DNA accelerating therapeutic decisions and enabling an earlier adequate antibiotic treatment.

Microarray Based Bacterial Identification

Microarrays combines the potential of simultaneous bacterial identification and speciation. This method is versatile and makes it possible to detect and discriminate different bacterial samples on a single slide. The rapid identification of the bacteria in clinical samples is important for patient management and antimicrobial therapy. DNA microarray-based approach is used for the quick detection and identification of bacteria using species-specific oligonucleotide probes designed for specific regions of various targeted genes.

Software for Bacterial Identification

AlleleID® automatically designs oligos for microarray and real time PCR assays for pathogen detection, bacterial identification, taxa discrimination and cross species research by combining ClustalW alignments with probe design.

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WATER HYACINTH: A POTENTIAL SUBSTRATE FOR PRODUCTION OF BIO-ETHANOL

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Bioethanol is considered as a potential liquid fuel due to the limited amount of natural resources, cellulosic biomass is also being investigated as a potential substrate for bioethanol production. The water hyacinth (*Eichhornia crassips*) is native plant of Brazil but has been naturalized in many countries. In India water hyacinth, an aquatic weed, was first observed in West Bengal in the beginning of 1890. Water hyacinth is fresh floating, aquatic plant distributed in out of the world. It has extremely high growth rate, a single plant can reproduce 140 million daughter plants. It is recognized as fastest growing extent plant. Recently, research has focused on using non-edible bio-mass as a raw material. The utilization of water hyacinth as feedstock for bioethanol production has a number of advantages.

Water hyacinth is low lignin content with high amount of cellulose and hemicelluloses. These lignocelluloses can more easily be bio-converted by enzymatic means to fermentable sugar, thus resulting large amount of utilizable biomass for bioethanol production. In addition, being an aquatic plant, it does not compete with food crop for arable lands. It has very high growth rate, 60-100 ton/year, is also favorable for its commercial cultivation. Studies have shown that fresh water hyacinth plant consist of 95 % moisture, 0.04 % Nitrogen, 1.0 % Ash, 0.06 % P_2O_4, 0.20 % K_2O and 3.5 % organic matter. On zero moisture basis, it has 75.8 % organic matter, 1.5 %

Nitrogen and 24.2 % Ash contains 28.7 % K_2O, 1.8 % Na_2O, 12.8 % CaO, 21.0 % Cl, and 7.0 % P_2O_5. The crude protein using Kjeldahl method contains, 100 g, 0.72 g methionine, 4.72 g phenylalanine, 4.32 g threonine, 5.34 g lysine, 4.32 g isoleucine, 0.27 g valine and 7.2 g leucine. Water hyacinth consist of high percentage of water, fibrous tissue, high energy and protein content can be useful as potential substrate for a variety of useful applications. A number of possible uses of the plant include in the field of bio-fuel production.

Water hyacinth is highly growing plant causing hazardous effect on environment. This topical plant takes large area of water resources and consequently leads to reduction of biodiversity, blockage of river, and drainage system, reduction of dissolved oxygen, alternation of water chemistry, and involvement in environmental pollution and destruction of eco system, irrigation problems and also as a mosquito breeding place leading to increase in mosquito population. It is considered as most productive plant on earth and now considered as a serious threat to biodiversity. This negative effect of water hyacinth leads to several research and development activities for the control of this notorious weed. To control this weed high cost and labor required. Several biological, physical and chemical methods have been tried to control of water hyacinth. The use of plant like water hyacinth is really necessary in the sense that the waste is of no economical value and if not well deposited it could turn into a form of hazards and a threat to the environment hence it would be beneficial if this waste material could be processed into a source of useful energy. Thus, keeping in view all the research on utilization of the water hyacinth to produce ethanol by harnessing the cellulose degrading capability of microorganisms helpful in many ways. Bioethanol is being considered as a potential liquid fuel due to limiting amount of natural resources. Water hyacinth is also investigated as a potential substrate for bioethanol production. The water hyacinth (*Eichhornia crassips*) could be useful as a source of biomass because it is abundant and easy to cultivate. The Bioethanol is colourless liquid. It is biodegredable, low in toxicity and causes little environmental pollution. It burns to produce carbon dioxide and water. Ethanol is a high octane fuel and has replaced lead as an octane enhancer in petrol. It will be promising biofuel in future. Thus, the use of water hyacinth will definitely open up as new potential substrate for production of bioethanol. This will helpful for saving environment from pollution as well as non-renewable sources of energy.

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GUT MICROBIOTA AND BRAIN FUNCTION

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Gut flora or gut microbiota are the microorganism including bacteria, archaea and fungi that live in the digestive tracts of humans and other animals including insects. These human digestive- tract associated microbes are referred to as the gut microbiome. The gastrointestinal metagenome is the aggregate of all the genomes of gut microbiota. The gut is the main location of human microbiota. Its specific function in host nutrient metabolism, xenobiotic and drug metabolism, maintenance of structural integrity of the gut mucosal barrier, immunomodulation, and protection against pathogens. The human gut microbiome and its role in both health and disease has been the subject of extensive research, establishing its involvement in human metabolism, nutrition.

The gut-brain axis (GBA) consists of bidirectional communication between the central and the enteric nervous system, linking emotional and cognitive centers of the brain with peripheral intestinal functions. The Enteric nervous system is a massive web spread over your entire digestive tract, it's made up of more than 500 million neurons that control your guts. The vagus nerve represents the main component of the parasympathetic nervous system, which oversees a vast array of crucial bodily functions, including control of mood, immune response, digestion, and heart rate. The microbiota has the potential to affect neuronal function directly or indirectly through vitamins, neurotransmitters, and neuro active microbial metabolites such as short-chain fatty acids. Experimental data suggest that the microbiota may send signals to the brain by activating afferent sensory neurons of the vagus nerve via neuro immune and neuroendocrine pathways. The vagus nerve is the direct communication observed between the bacteria and the brain. The cross talk between gut microbiota, the immune

system and the brain-gut axis plays an important role in the modulation of the stress responses of the gut in the context of the development of different gut disorders as microbiota communicate with the gut-brain axis through different mechanisms *viz*., direct interaction with mucosal cells (endocrine message), *via* immune cells (immune message), *via* contact to neural endings (neuronal message). Preclinical/animal studies demonstrate that probiotic effects on brain are dependent on vagal afferent signals. *Lactobacillus rhamnosus* directly activates vagal neurons which, induces region-dependent alterations in GABA receptor expression in the brain and reduced stress-induced corticosterone and anxiety- and depression-like symptoms *via* vagus nerve signaling in mice. Early research starting in the 1970s showed that stress could affect the kinds of microbes found in the guts of mice. Some research from the early 2010s found that, while the germ free mice were more affected by certain kinds of acute stress (like restraint). They were less anxious about other kinds of stress, like being placed in a new environment. In a study from 2011, one group of scientists found that exposing germfree mice to the microbiomes of other mice could influence their behavior. A shy germfree mouse might do more exploring if it was implanted with microbes from a more adventurous mouse. Microbes interact with the immune cells in the gut, prompting the cells to make cytokines that circulate from the blood to the brain. These cytokines release from the mucosal immune cells, via the release of gut hormones such as 5-HT from endocrine cells, or via afferent neural pathways, including the vagus nerve. Stress and emotions can also influence the microbial composition of gut through the release of stress hormones or sympathetic neurotransmitters (GABA, 5-HT precursors etc.) that influence gut physiology and alter the habitat of the microbiota and also these catecholamine alter the growth, motility and virulence of pathogenic and commensally bacteria. Alternatively, host stress hormones such as noradrenalin might influence bacterial gene expression or signaling between bacteria, and this might change the microbial composition and activity of the microbiota.

The gut also connects with the brain through chemicals like hormones and neurotransmitters that send messages, like butyrate. They circulate in the brain, where some of them are small enough to cross the blood-brain barrier, and others alters cell activity of the barrier itself. Seretonin modulates many brain functions including emotions, cognitions, motor function, pain as well as neuroendocrine function. While 5-HT is an important signaling molecule in the brain-gut axis and the 5-HT released from eneterochromaffin cells modulates peristaltic, secretory, vasodilatory, vagal and nonciceptive reflexes. A disruption in the microbial composition of the gut has been associated with many metabolic, inflammatory, neurodevelopmental, and neurodegenerative disorders. Nutrition is one of the several key factors that shape the

microbial composition during infancy and throughout life, thereby affecting brain structure and function.

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ANTIMICROBIAL ACTIVITY OF PLANT *Tinospora cordifolia*

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Tinospora cordifolia, which is well-known by the familiar names gurjo, heart-leaved moonseed, guduchi, and giloy, Tinospora cordifolia is a medicinal plant whose importance in the field of natural medicine and Ayurveda is of the peak order. *Tinospora cordifolia*, a shrubs plant of great medicinal stuff which is extensively and generally used in the ayurvedic and local forms of medicine is calculated in the phytochemical and diverse components that exhibit the belongings that have been famous and sustained in the old age civilizations and medicinal practices. *Tinospora cordifolia* is a plant that is natural to India. In Ayurvedic medicine used as a root, stems, and leaves. *Tinospora cordifolia* is commonly used for Diabetes, Hypersensitive Rhinitis, High Cholesterol, Upset Stomach, Fever, Gout and other Cancers, Rheumatoid Arthritis (RA), Hepatitis, Syphilis, Lymphoma, Gonorrhea, Peptic Ulcer Disease (PUD), and to enhance the Immune System.

Botanical classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Ranunculales
Family	:	Menispermaceae
Genus	:	*Tinospora*
Species	:	*cordifolia*

Medicinal Products of *T. cordifolia* and their uses

Name of Market Product	Biological Roles
Tinospora cordifolia Pellets	A number of diseases
Guduchi	The immune system and the body's resistance to infections
Abhaibhubejhr	Anti-stress
Safe herb	Cure by Anemia and sexual disabilities
Brave Heart Capsule	It lowers the lipid levels especially cholesterol and LDL cholesterol in body, diuretic
Cirrholiv capsules	Hepatoprotective
Cirrholiv-ds syrup	Hepatoprotective
Mussaffen	Blood purifier and anti-allergic
Madhu Mehari	Cure by urinary problems, maintain blood sugar, fatigue
Tonplex	Increase immunity
Rebuild	Anti- stress and anti-oxidant

Antimicrobial activities

Methanolic intangible of *T. cordifolia* has been associated with microbial infection. Antibacterial action of *T. cordifolia* obtain has been biotested alongside *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, *Salmonella typhi, Proteus vulgaris, Salmonella paratyphi, Shigella flexneri, Salmonella typhimurium, Enterobacter aeruginosa, Pseudomonas aeruginosa* and *Enterobacter aerogenes*. Additional, *T. cordifolia* obtained has been related against bacterial growth and increased phagocytic and intracellular bacterial roles of neutrophils.

Some of the chemical antimicrobial agents are irritant and toxic. Although, plants are easily obtained, reliable, and non-hazardous in most cases, but do not have as successful as other chemical agents. Hence, there is essential need and much concern in finding ways to create new types of secure and cost-effective biocidal materials. While Number of researchers are reported *T. cordifolia* have shown antimicrobial action against infectious microbial strains. *T. cordifolia* is a good antimicrobial efficiency and thus has a capability to be used as antimicrobial agent anti wide range of microbes over common antibiotics.

Anti-HIV Activities

Extract from roots of this plant has been indicated a drop in the usual resistance anti-HIV. This anti-HIV influence was shown by decrease in eosinophil count,

encouragement of B lymphocytes, macrophages, level of hemoglobin and polymorphonuclear leucocytes.

Immunomodulatory Activities

T. cordifolia is also known as for its immunomodulatory response. This property has been well documented by scientists. A large change of compounds which are accountable for immunomodulatory and cytotoxic impacts are N-methyle-2-pyrrolidone,11- hydroxymuskatone, Nformylannonain, cordifolioside A, tinocordioside, magnoflorine and syringin. These biological complexes have been related to improve the phagocytic movement of macrophages, augmentation in nitric acid manufacture by incentive of splenocyte, and manufacture of Reactive Oxygen Species (ROS) in human neutrophil cells.

Anti-Cancer Activities

T. cordifolia indicates anti-cancer activity, this action is commonly shown in animal types. Extract from roots of *T. cordifolia* has been identify radio protective part due to excessive increase in body weight, tubular diameter tissue weight. Dichloromethane gets of TC indicates cytotoxic impacts due to lipid peroxidation and release of LDH and decline in GST. Root extract has generally changed radiation, caused increase in lipid peroxidation, and followed in the decline of GSH in testes. Commonly synthetic chemotherapeutic causes laid toxic influences on the living organisms. The impact of Giloy has been stated better than doxorubicin care.

Anti-Diabetic Activities

The stem of this plant is usually used to diagnosed diabetes by controlling level of blood glucose. It has been described to play as anti-diabetic drug all through illustrative oxidative stress, stimulating insulin excretion by preventing gluconeogenesis and glycogenolysis. Properties of anti-diabetics shown by this plant species are attached due to the occurrence of alkaloids (Magnoflorine, Palmetine and Jatrorrhizine), tannins, flavonoids, cardiac glycosides, saponins, steroids etc. The raw obtain of stem in ethyl acetate, chloroform, dichloromethane, and hexane reduces the enzymes as a salivary, amylase and glucosidase subsequentrise in post-prandial glucose level and shows likely events against Diabetes mellitus disease. Extract from roots of this plant has also been stated to have anti-diabetic estates which reduce the level of hydroperoxides, glycosylated hemoglobin and vitamin E.

The *Tinospora cordifolia* plant had been valued to increased levels for the therapeutic, healing, medicinal, curative, and relieving nature. However, the plant has a strong protection anti microorganisms, pests and organisms, the plant in himself is not invulnerable and is bound to be influenced by several diseases and one of the causal organisms that clearly involved. The variations in the sick and normal plant is not only limited to the basic form but the abnormal procedure the phloem and xylem methods are obviously seen and the sieve Parts and the vascular bundle carry varying measures to the normal cells.

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OMICS

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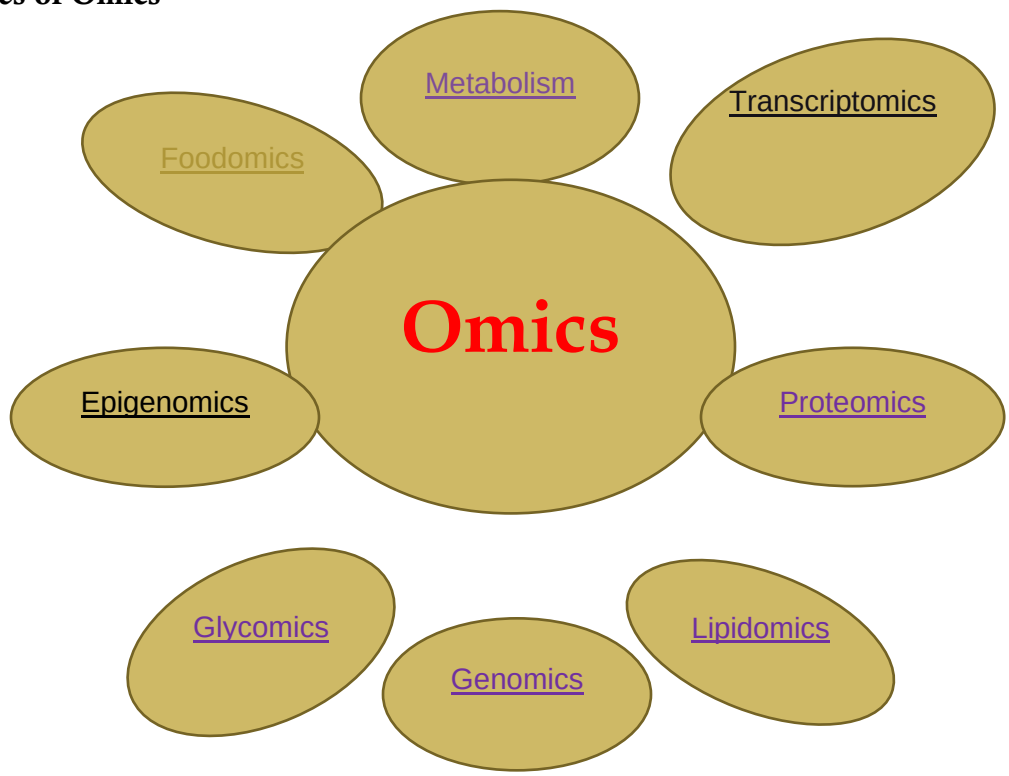
Omics the words refers to a field of study in biological sciences that ends with -*omics*, such as genomics, transcriptomics, proteomics, or metabolomics. The field of science known informally as omics are a Combination of area in biology whose names end in the suffix -*omics*, such as genomics, proteomics, metabolomics, and glycomics. Omics aims at the collective classification and quantification of pools of biological molecules that explain into the structure, function, and dynamics of an organism or organisms.

Suffix -ome is used to address the things of study of such field, such as the genome, proteome or metabolome in that categorize. In molecular biology the suffix –*ome* as use totality of some sort; the "neo-suffix" is a example of formed by idea from various Greek terms in -ωμα, a series that does not form an certain suffix in Greek. Third definition originated from OED as a back-formation from *mitome*, Early attestations consist of *biome* (1916) and *genome* (first discoverd term as German *Genom* in 1920).

Bioinformaticians and molecular biologists figure with the first scientists to apply the "-ome" suffix generally. Before time advocates included bioinformaticians in Cambridge, UK, where there were various near the commencement bioinformatics labs such as the MRC centre, Sanger centre, and EBI (European Bioinformatics Institute). For example, the MRC centre approved out the first genome and proteome projects.

Types of Omics



Genomics

a) ***Cognitive genomics:*** Study of the changes in cognitive processes linked with genetic profiles.
b) ***Comparative genomics:*** Study of the association of genome structure and function across unusual biological species or strains.
c) ***Functional genomics:*** Describes gene and protein functions and interactions (frequently uses transcriptomics).
d) ***Metagenomics:*** Study of metagenomes, i.e., genetic material recovered usually from environmental samples.
e) ***Neurogenomics:*** Study of genetic influence on the growth and function of the nervous system.
f) ***Pangenomics:*** Study of the complete collection of genes or genomes establish within a given species.
g) ***Personal genomics:*** Field of genomics concerned with the sequencing and study of the genome of a character.
h) ***Epigenomics:*** The epigenome is the sustaining arrangement of genome, as well as protein and RNA binders, other DNA structures, and chemical modifications on DNA. For example, Epigenomics & Nucleomics
i) ***Lipidomics:*** To the study of pathways and networks of lipids in large- scale.

j) ***Proteomics:*** To the study of proteins is called proteomics.
k) ***Immunoproteomics:*** Study of proteins (proteomics) concerned in the immune response.
l) ***Nutriproteomics:*** The molecular targets identification of nutritive and non-nutritive mechanism of the diet.
m) ***Proteogenomics:*** is a field of biological research that utilizes an arrangement of proteomics, genomics, and transcriptomics to help in the invention and discovery of peptides.
n) ***Structural genomics:*** Study of 3-dimensional structure of every protein encoded by a given genome using a combination of experimental and modeling approaches.
o) ***Glycomics:*** Glycomics is the complete study of the glycome for example. sugars and carbohydrates.
p) ***Transcriptomics:*** Transcriptome is the complete set of all RNA molecules, with mRNA, rRNA, tRNA, and other non-coding RNA, produced in one or a population of cells.
q) ***Metabolism:*** To the study of chemical processes concerning metabolites.
r) ***Metabonomics:*** Pathophysiological stimuli or genetic modification

Pharmacology and Toxicology

- ***Pharmacogenomics:*** Investigate the effect of the sum of variations within the human genome on drugs;
- ***Toxicogenomics:*** To the study of the collection, interpretation, and storage of in sequence about gene and protein activity within particular cell or tissue of an organism in response to toxic substance.
- ***Psychogenomics:*** To the study of drug addiction, the final goal is to extend more effective treatment for these disorder as well as objective diagnostic tools, defensive measures, and finally cures.
- ***Stem cell genomics:*** The study of stem cell biology.
- ***Connectomics:*** The study of the neural connections in the brain.
- ***Microbiomics:*** The study of the genomes of the community of microorganisms that live in a specific environmental niche.
- ***Cellomics:*** The study using bioimaging methods, bioinformatics and quantitative cell analysis.
- ***Ethomics:*** Study of Animal behaviour.
- ***Multiomics:*** Combination of different omics in a single study or analysis pipeline.

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ALGAE – FUTURE FOOD

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The concept of functional food is conquering the world and inspires both science and industry to search for innovative ingredients capable of certain physiological effects to incorporate into food products. In a search for more sustainable food systems, microalgae are considered one of the most auspicious sustainable sources of food ingredients. Photoautotrophic microalgae have a higher development effectiveness contrasted with conventional crops, don't require arable land, and significantly add to catching climatic CO_2. Microalgae is an ingredient full of potential, being rich in numerous health-beneficial compounds such as omega-3 long-chain polyunsaturated fatty acids. In addition, microalgae are also a perfect vegan alternative source of ω3-LC-PUFA and other nutrients, such as proteins, vitamins and minerals.

The most abundant utilization of seaweeds as food is observed in Asia, particularly Japan, Korea and China, where industrial seaweed cultivation is a part of the country's economy. *Porphyra* (a red alga) is a cold-water species and it contains of 30 % – 35 % proteins, 40 % – 45 % carbohydrates and a lot of vitamins makes it a very popular food ingredient. Dulse (*Palmaria palmata*) is red algae, Dried *Palmaria palmata*is served as a salty cocktail snack in the bars and it is eaten raw or cooked. *Laminaria* (a brown alga), also known as kelp, contain about 2 % fat with 10 % of protein and a significant amount of essential mineral. The market demand for this brown alga is huge as it constitutes the main ingredients of food products known as "kombu" or "konbu", which are extensively used in Japanese cuisines as one of the basic ingredients necessary to make dashi, a soup stock. The Japanese food product "sarumen" is based on the *Alaria* genus seaweeds, and *Alaria esculenta* particularly provides both protein of excellent quality and iodine Another genus of microalgae is *Monostroma* (a green alga), and along

with the *Ulva* genus is a leafy plant of one-cell thick form. Commonly it is processed into sheets and dried and then boiled with the addition of sugar, soy sauce and other ingredients to prepare "nori-jam".

For algae to be considered as a potential new food sources, one crucial factor is their composition and nutritional content. The nutritional composition varies tremendously among algal species, and even within the same species nutritional content can vary significantly based on the growth environment, both the media composition as well as temperature and light regime. Microalgae biomass is a super concentrated source of many active ingredients. Some algae have a very high percentage of their dry biomass as protein (eg: *Arthrospira platensis* have 70 % of their biomass as protein content). The composition of protein is much richer in essential amino acids compared to common plant proteins (eg: *E. gracilis* contain all of the essential amino acids, making a complete protein source). Algae can accumulate lipids to very high levels (eg: *Auxenochlorella protothecoides* accumulate 70 % of dry biomass as lipids). *Phaeodactylum tricornutum* can accumulate up to 30 % to 40 % of total the fatty acids produced as EPA, and other species like *Schizochytrium* sp. can accumulate about 50 % of the total lipids of the cell as DHA. The green alga *Dunaliella tertiolecta,* which has great source of vitamin A, vitamin B1, vitamin B9 and vitamin E. There are other nutrients that have a positive impact in human wellbeing that can be provided by algae, like antioxidants (e.g., lycopene, b-carotene, and astaxanthin) or polysaccharides (β-Glucans). This makes microalgae a future food that is more feasible and with a lower carbon footprint than other food raw material resource. However, for that become a reality, algae need to undergo a series of improvements to enhance growth yields, nutritional quality, organoleptic traits, and perhaps most importantly, social acceptance of algae as food.

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ANTIMICROBIAL ACTIVITY OF SOME ESSENTIAL OILS AGAINST *Propionibacterium acnes*

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Acne is a common skin disease that can affect people of all ages, with a prevalence is up to 85 % of teenagers and 11 % of adults. It can lead to both physical and psychological effects, resulting in depression, poor self-esteem, and suicidal tendencies. *Propionibacterium acnes* is a Gram positive, anaerobic microorganism, *Propionibacterium acnes* assumes a huge role in the pathogenesis of acne. *Propionibacterium acnes* can activate certain inflammatory mediators and metabolize sebaceous triglycerides into fatty acids, which induce the attraction of white blood cells to the plugged follicle, leading to skin inflammation. When the wall of the hair follicle is broken down, sebum, dead cells, and bacteria are secreted leading to a spectrum of acne severity. For many years antibiotics and hormones were usually applied to treat acne. However, these agents are often accompanied by severe side effects and drug resistance. Current medicines for skin break out (acne) incorporate skin treatments (comedolytic specialists, anti-microbials, and anti - inflammatory drugs) and fundamental treatments (anti-microbials, zinc, and hormones). Be that as it may, the advancement of anti-toxin opposition, the expense of the traditional treatments, and their results have prompted look for elective cures, including plants, plant extricates, and phytomolecules. Specifically, some basic oils showed an intriguing antibacterial action against *Propionibacterium acnes*. The

examination was to evaluate the *in vitro* inhibitory impact of some basic oils got from plants against a strain of *Propionibacterium acnes*.

Propionibacterium acnes is a Gram positive anaerobic bacterium; although it is traditionally implicated in the pathogenesis of acne, its exact role in the pathophysiology of this disease is still debated. In general, it is present in acne lesions, but it is also a major commensal of the normal skin flora, often with similar concentrations in patients with acne and healthy subjects; moreover, some acne lesions do not contain viable bacteria. On the other hand, antibiotics that reduce *Propionibacterium acnes* total count on acneic skin result in clinical improvement, and the presence of antibiotic-resistant strains is associated with reduced efficacy of these treatments. These controversial data may depend on inappropriate sampling methodologies used in qualitative and quantitative studies involving the role of *Propionibacterium acnes* in acne. Moreover, Fitz-Gibbon *et al*. compared the skin microbiome (at the strain level and genome level) of *Propionibacterium acnes* between a group of acne patients and a group of healthy individuals: although there was no statistically significant difference in the relative abundances of these bacteria in the cohorts studied, a strong association between specific bacterial ribotypes and acne was demonstrated. At present, direct involvement of *Propionibacterium acnes* appears certain; presumably, it is not involved in the initiation of acne lesions but would mediate later inflammatory events, leading to worsening of the lesions. In particular, *Propionibacterium acnes* stimulates the production of host Antimicrobial Peptides (AMP), small molecules with antimicrobial activity and immune-modulatory properties; moreover, a lipase secreted by the bacterium is responsible for the hydrolysis of sebum and the subsequent release of free fatty acids, which have an irritating and pro-inflammatory effect.

Essential oils are one of the most famous regular items utilized for clinical purposes. Joined with their well-known use in dermatology, their accessibility, and the improvement of antimicrobial obstruction, business Essential oils are regularly a possibility for therapy. Antimicrobial action of different unpredictable oils against *Propionibacterium acnes*. Tea tree oil and Thyme oil is broadly utilized in skin break out consideration items. It is powerful against different strains of *Propionibacterium acnes* and has anti‑inflammatory movement.

To overcome the problem of antibiotic resistance, essentials oils and medicinal plant extracts have been extensively studied as an alternative. Herbs are safe, efficacious and multifunctional. The ingredients in topical acne treatments, particularly herbs and naturally derived compounds have received considerable interest as they have fewer adverse effects than synthetic agents

From our study, all oils (Cinnamon oil, Tea tree oil, Rosemary oil, Thyme oil, Rose oil, Chamomile oil, Lavender oil, Jasmine oil, Grapefruit, Lemon oil, Ginger oil and Mint oil) showed antibacterial activity against *Propionibacterium acnes*. The maximum inhibition zone was observed with Thyme oil, followed by cinnamon oil and Tea tree oil, respectively. The data acquired from the disc diffusion test method indicated that thyme essential oil exhibited the strongest inhibitory activities. The cinnamon essential oil also possessed considerable antibacterial activity. Jasmine oil showed the least inhibitory activity.

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TANDEM FLUORESCENT PROTEINS AS ENHANCED FRET-BASED SUBSTRATES FOR THE ACTIVITY OF BOTULINUM NEUROTOXIN

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Clostridium botulinum and some of its related species (*Clostridium baratii*, *Clostridium butyricum*, *Clostridium argentinense*) are known to produce Botulinum Neurotoxins (BoNTs) which are the most formidable toxins causing the well-known neuroparalytic disease, botulism and have been recognized with great potential as a bioterrorism agent. Botulinum toxins are assigned serotypes A-G and some serotypes namely A, B, E, and F have the potential to cause disease in humans, and C, D, G are mostly toxic to animals. Even though it is the most harmful toxin, selective serotypes (BoNT/A and BoNT/B) are being used in cosmetic applications known as Botox treatment. Botulinum neurotoxins are synthesized as holotoxins of 150 kDa and get activated through proteolysis occurring post-translation event and consist of a dimeric structure consisting of heavy (approx.100 kDa) and light chains (approx. 50 kDa) linked via a disulfide bond. BoNTs are known to interfere with cholinergic transmission at the neuromuscular junction. The toxin gets absorbed by the gastrointestinal tract and travels to general circulation and then gets concentrated and rapidly endocytosed by presynaptic

nerve cells at cholinergic nerve endings at the neuromuscular junction. Upon endocytosis, the disulfide bond is reduced and its light chain is released as an active zinc-dependent endopeptidase into the cytosol. The toxin acts like a molecular scissor which causes proteolysis of SNARE (Soluble N-ethylmaleimide sensitive factor attachment protein receptor) proteins so when there is an action potential, the vesicles cannot release their contents which fails neurotransmission due to disfunction of presynaptic membrane exocytosis leading to flaccid paralysis and eventually death. Different BoNTs are cleaving different SNARE proteins at individual sites-BoNTs A, C, E are known to cleave SNAP25 (synaptosomal associated protein of 25kDa) whereas B, D, F, G cleaves VAMP-2/synaptobrevin2 (vesicle-associated membrane protein 2). BoNT/C also cleaves syntaxin and is the only known serotype with two substrates. Each serotype has unique cleavage sites on the respective substrate molecules demonstrating that the different BoNTs can be detected by analyzing the generated cleaved products.

Detecting these dangerous BoNTs is essential for prompt diagnosis and effective treatments. The detection is challenging due to their high potency and large variation at the molecular level with several serotypes and subtypes. The toxicity of BoNTs is conciliated by enzymatic cleavage of distinct synaptic proteins involved in the release of neurotransmitters at specific cleavage sites of serotypes. Sensitive and rapid detection of serotypes is required for the serotype identification and timely treatments by administering appropriate antitoxins to the infected individuals as well as for epidemiological information. Monitoring and distinguishing of active toxins can be done via *in vitro* assays by detecting the substrate cleavage products. Currently known and employed gold standard method to detect the BoNT toxins is mouse bioassay or mouse lethality test which is based on toxin's enzymatic activity and is an effective method. The drawback of this assay is that it is slow and requires around 6 days to get the results and the delay may prevent immediate response and treatments. Additionally, animal handling and maintenance of animal facilities are labor-intensive and costly. The time and expense of the analysis increase more because of the use of selectively immunized animals to each serotype. There are many other techniques too but there is a need for a new detection technique for the rapid screening process and early serotyping as there are fewer tools that have been developed for serotype other than BoNT/A and BoNT/E. A recently reported technique known as FRET (Förster Resonance Energy Transfer) is gaining attention and is an effective approach towards botulinum toxins detection. The *in vitro* and *in vivo* disconnect found in recent high-throughput screening (HTS) of small molecule libraries for BoNT/A inhibitors brought to light the urgent need for a quick and authentic in vitro assay for identifying physiologically relevant inhibitors.

The catalytic light chains of BoNTs (BoNT-LC) are zinc-dependent proteases that recognize extended regions of their substrates for cleavage. Recognition between BoNT/A and SNAP25 involves two extended exosites for optimal substrate binding and recognition. The minimal size of SNAP25 known to retain full activity as a BoNT/A substrate is the C-terminal 66-mer peptide (residues 141–206) with both exosites. Short peptides containing the cleavage site of the substrate can be converted into active site-based FRET substrates for inhibitor screening, but this approach would miss inhibitors specifically targeting the exosites, which are the unique features of BoNT/A necessary for distinguishing BoNT/A from other metalloproteases in cells first reported the feasibility of using the CFP-YFP pair with full-length SNAP25 as a FRET-based substrate for BoNT/A in a cell-based assay or with the 66-mer peptide as a FRET substrate in an in vitro assay. However, the length of the 66-mer peptide does not permit for high FRET efficiency under optimal enzymatic cleavage conditions. Simple CFP-SNAP25(141–206)-YFP substrate (CsY) provides only a little change in FRET signal after BoNT/A-LC treatment, and also the FRET signal is highly dependent on the concentration of substrate and reaction conditions. An approach to ameliorate the FRET in a substrate such as CsY is to shorten the linker peptide between the two fluorophores since the efficiency of FRET (E_{FRET}) is dependent on the distance (r) between the donor and the acceptor as described in the Förster equation: E_{FRET} ¼ 1/[1 þ $(r/R_o)^6$], where R_o is the Förster distance at which 50% of FRET would be detected. When two fluorophores are attached to the other ends of the 66-mer peptide as in CsY, they are separated by over twice the Förster distance of 48 Å for the CFP-YFP pair, presuming the peptide is folded into an alpha-helix such as that found in a SNARE complex. Although shorter SNAP25-derived peptides are reported to be substrates of BoNT/A and synthetic peptides with proper acceptor/donor pairs of fluorescent dyes, like the commercially available SNAPtide, have been used to monitor the activity of BoNT/A, researchers have found that CFP-YFP tethered through a 17-mer peptide was immune to cleavage by BoNT/A-LC. Other strategies for optimization of FRET efficiency include the employment of fluorescent protein variants or circularly permuted mutants of fluorescent proteins.

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STUDY OF NATURAL HAIR OIL AGAINST DANDRUFF CAUSING ORGANISM *Malassezia furfur*

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Dandruff is a problem that possesses serious concern in both developed and developing countries. Dandruff manifests as profuse white to silvery powdery scales in the scalp region often with moderate to severe itching. *Malassezia furfur* is a yeast-like lipophilic basiodiomyceteous fungus considered to be the causative agent for dandruff. The present work was undertaken to study the effect of antidandruff activity in natural hair oil that was prepared by using the natural hair oils. It is believed that, *Malassezia furfur* converts the sebum lipid into fatty acids and triglycerides. These fatty acids may presumably accelerate hyper proliferation of keratinocytes natural hair oil is reported and known to have activity against *Malassezia furfur* in *in-vitro* studies. The common topical preparation used to treat dandruff includes- ketoconazole, fluconazole, zinc-pyrthrone, selenium sulphide, coal tar etc. Both synthetic and natural drugs are dispensed in different formulation like shampoos, cream, lotions, emulsions, hair oils and other cosmetic formulation. Along with synthetic treatment, natural elements are more preferred for the management of dandruff.

Natural hair oil is an herbal product, formulated specifically for all ages of men, women and children. Natural antidandruff hair oil is a very good blend of herbs that have been used from centuries to provide dandruff free scalp giving sufficient strength and vitality to hair. These formulations are herbal based, with viable substitutes for synthetic drugs. Over the last few decades, natural products have shown a tremendous increase in herb based cosmetics. There are many herbal hair oils available nowadays, containing herbal ingredients like essential oils and extracts of plants. Many plants have beneficial effects on hair. Medicinal plants are rich in antioxidants, known to treat different diseases. The antioxidant potential is tested at various levels. The need for herb based natural hair oil is vastly increasing due to the lack of side effects and natural goodnes. The underlying research deals with the preparation and assessment of Poly-herbal anti-dandruff formulation containing the goodness of Amla, Bhringraj, Reetha, Hibiscus, Neem, Tulsi, Ginger, Eucalyptus, *Aloe vera* and Tea tree oil.

Natural Anti-dandruff Hair oil

There are some plant extracts, volatile oils and isolated compound found to be effective for treatment and management of dandruff. There are number of plants that proved scientifically to be effective against *Malassezia furfur*. Amla, Bhringraj, Reetha, Hibiscus, Neem, Tulsi, Ginger, eucalyptus*, Aloe vera* and Tea tree oil. However, in Indian traditional medicines literature there are many plants which used traditionally for dandruff. The Natural hair oil enriched with natural oils prevents dandruff by eliminating microbial infections of the scalp. In the formulation, the plant species have been selected those used in Ayurvedic hair oils for the treatment of ailments of the head and scalp conditions. Systematic standardization (quality control) from raw materials to the final product the plants are proven to contain constituents that can remove dandruff.

Components of the formulation

On the basis of literature survey, there can possible of formulating a poly herbal hair oil having antidandruff activity. The herbs used in the study for making herbal oil were dried, crushed and passed through 80 mesh stainless steel sieves and olive oil was used as base. The hair oil was prepared utilizing three different methodologies. First is the direct boiling method in which the crude drugs were powdered, weighed and directly boiled in olive oil with continuous stirring and heating until the drug had completely extracted in the oil base.

Isolates from the dandruff was inoculated by swabbing on the surface of gelled media plates. Wells of 6 mm in diameter was performed in the SDA media, and each well filled with 50 µl of poly herbal hair oil. The plates were kept in laminar air flow for 30 min for proper diffusion of the extract and thereafter incubated at 37º C for 3 - 5 days.

The radius for the zone of inhibition was measured in millimeters and recorded against the corresponding concentration.

The antifungal activity coupled with Natural oil very effective in the management of dandruff. Observations were made by using the Amla, Bhringraj, Reetha, Hibiscus, Neem, Tulsi, Ginger, Eucalyptus, *Aloe vera* and Tea tree oil. In this study, *Malassezia furfur* were found to be very sensitive to the Polyherbal hair oil. Natural hair oil showed good activity against dandruff causing organism *Malassezia furfur*. From the results, we conclude that natural oils have antifungal activity and could be safely used for treating dandruff. Further studies can be made on the active molecules of plant extracts responsible for antidandruff activity. Toxicity evaluation of these extracts can also be carried out.

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ANTIMICROBIAL ACTIVITY OF AJWAIN OIL, LEMON PEEL OIL AND VETIVER OIL

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Medicinal plants constitute a large part of natural flora and are considered an important resource in various fields such as the flavor, pharmaceutical, fragrance, and perfumery industries. At present, more than 80 % of the global population depends on traditional plant-based medications for treating various human health problems. According to an estimate, the worth of herbal products on the global market is approximately 62 billion USD, and it is predicted to grow up to 5 trillion USD by the year 2050. More than 9000 native plants have been identified and recorded for their curative properties, and about 1500 species are known for their aroma and flavor. Essential-oil–based products or natural aroma chemicals are in higher demand in the cosmetic, food, perfume, and pharmaceutical industries, and more than 250 types of essential oils, at a value of 1.2 billion USD, are traded annually on the international market.

More recently, the prevalence of antimicrobial drug resistance has prompted researchers to discover novel antimicrobial lead molecules to treat various human pathogens. Some of the presently available synthetic drugs fail to inhibit Evidence-Based Complementary and Alternative Medicine many pathogenic microbes. In addition, the use of synthetic chemicals for the control of pathogenic microorganisms is limited because of their carcinogenic effects, acute toxicity, and environmental hazard potential. In this regard, the exploitation of essential oils to control epidemic multi drug resistant

Pathogenic microorganisms can be useful to combat various infectious diseases. Therefore, the present review details the antibacterial, antifungal, and antiviral potentials of Ajwain oil, lemon peel oil, and vetiver oil.

The antimicrobial effects of Ajwain oil, lemon peel oil, and vetiver oil are the basis of copious applications, in various sectors such as pharmaceutical, nutraceutical, cosmetic, perfume, and sanitary industries. In the following section, we have reviewed the antibacterial, antifungal, and antiviral effects of Ajwain oil, lemon peel oil, and vetiver oil

Antimicrobial activity of Ajwain oil, Lemon peels oil and Vetiver oil

Ajwain have large amounts of Thymol or Carvacrol in its total essential oil, mentioned phenolic compounds is reported to be either bactericidal or bacteriostatic agents depending on the concentration. Agar well diffusion method showed the antimicrobial activity of the pure ajwain seed extract with zone of inhibition against *Salmonella, Staphylococcus aureus, Escherichia coli* and *Shigella*, respectively. This showed that the ajwain seed extract has antimicrobial properties against various pathogenic and food spoilage microbes. *Trachyspermum ammi* (Ajwain) seed extract is a potent inhibitor of growth and biofilm formation in *Candida albicans*. Ajwain oil has been documented to be effective against filamentous fungi like *Aspergillus niger, Fusarium moniliforme* and *Curvularia lunata*, with Thymol as most potent component, along with other compounds like p-cymene, γ-terpinene and β-pinene.

Lemon is an important medicinal plant of the family Rutaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts of Lemon against clinically significant bacterial strains has been reported. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities. The antibacterial activity of the peel of the dried fruit of *Citrus limon* was done by using disk diffusion method. The lemon peel extracts is done by different solvents such as ethanol, methanol and acetone which are subjected to antibacterial assay. Methanolic extract shows higher antimicrobial activity against tested microorganisms (*Escherichia coli, Staphylococcus aureus*, *Candida albicans* and *Trichophyton rubrum*).

Vetiver is a key ingredient for the perfume industry nowadays. However, with the constant and rapid changes of personal tastes, this appeal could vanish and this sector could decline quite quickly. From review study twenty bacterial strains and two *Candida* species using the in vitro micro broth dilution method, vetiver oil demonstrated notably some outstanding activities against Gram-positive strains and against one *Candida*

glabrata strain. Based on these findings, vetiver essential oil appears to be an appropriate aspirant for the development of an antimicrobial agent for medicinal purposes and for the development of a cosmetic ingredient used for its scent and displaying antimicrobial activity as an added value.

The solvent extracts of *Vetiver* can be used as a potential antimicrobial drug against infectious agents and can be used in treatment of various infectious diseases. They are safe and sustainable methods that may be applied to control the growth of microorganisms directly to the infection site are generally inexpensive and non-toxic for humans and effective in small concentrations.

Ajwain oil, lemon peel oil, and vetiver oil contain a wide variety of phytochemicals, such as alkaloids, phenolic compounds, tannins, saponins, flavonoids, terpenoids, β and γ-sitosterol, glycosides and volatile oils, and many other active components. Thus, essential oils and their constituents can hopefully be considered in the future for more clinical assessment and possible applications in search for effectiveness against the virus like coronavirus.

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NANOTECHNOLOGY AND FUTURE EFFECTS OF NANOPOLLUTION/NANOTOXICOLOGY

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Introduction

Nanotechnology is designed to provide a novel and improved approach to cancer diagnosis and treatment. Nanotechnology is a relatively recent development in scientific research, but the development of its central concepts happened over a longer period of time. As nanotechnology is an emerging field, there is great debate regarding to what extent industrial and commercial use of nanomaterials will affect organisms and ecosystems. The discovery of carbon nanotubes and graphene, and our increasing understanding of the properties of materials on the nanoscale, is leading to an explosion in the commercialization of next generation technologies. Now over 400 products in the U.S alone are labelled as nanobased. Although the nano boom will bring a wealth of positive changes, it is simultaneously giving rise to a persistent from of pollution which is too small to detect or contain easily.

Nanopollution

Nanotechnology is a generic name for all waste generated by nanodevices or during the nanomaterials manufacturing process. Nanowaste is mainly the group of particles that are released in to the environment, or the particles Nanopollution that are thrown away when still on their products.

Health hazards

Nanotechnology is the field which studies potential health risks of nanomaterials. The extremely small size of nanomaterials means that they are much more readily taken up by the human body than larger sized particles. How these nanoparticles behave inside the organism is one of the significant issues that need to be resolved. The behaviour of nanoparticles is a function of their size, shape and surface reactivity with the surrounding tissue from what happens if non-degradable or slowly degradable nanoparticles accumulate in organs, another Apart concern is their potential interaction with biological processes inside the body; because of their large surface, nanoparticles on exposure to tissue and fluids will immediately adsorb on to their surface some of the macromolecules they encounter. The large number of variables influencing toxicity means that it is difficult to generalise about health risks associated with exposure to nano materials each new nanomaterial must be assessed individually and all material properties must be taken in to account. Health and environmental issues combine in the workplace of companies engaged in producing or using nanomaterials and in the laboratories engaged in nanoscience and nanotechnology research. It is safe to say that current workplace exposure standards for dusts cannot be applied directly to nanoparticle dusts.

The extremely small size of nanomaterials also means that they are much more readily taken up by the human body than larger sized particles. How these nanoparticles behave inside the body is one of the tissues that needs to be resolved. The behaviour of nanoparticles is a function of their size, shape, and surface reactivity with the surrounding tissue. They could cause overload on phagocytes, cells that ingest and destroy foreign matter, thereby triggering stress reactions that lead to inflammation and weaken the body's defence against other pathogens. Apart from what happens if non – degradable nanoparticles accumulate in organs, another concern is their potential interaction with biological processes inside the body: because of their large surface, nanoparticles on exposure to tissue and fluids will immediately adsorb on to their surface some of the macromolecules they encounter. This may for instance, affect the regulatory mechanisms of enzymes and other proteins.

Nanotechnology is a relatively recent development in scientific research, but the development in scientific research, but the development of its central concepts happened over a longer period of time. As nanotechnolgy is an emerging field, there is great debate regarding to what extent industrial and commercial use of nanomaterials will affect organisms and ecosystems. Nanotechnology environmental impact can be split in to two

aspects: the potential for nanotechnological innovations to help improve the environment, and the possibly novel type of pollution that nanotechnological materials might cause if released in to the environment. I will focus more on nanopollution and nanotoxicology.

The health effects of nanopollution and nanotoxicology are yet to be fully understood, making nanopollution and nanotoxicology yet another man-made environment impact with uncertain effects in the long term. Some of us living in urban areas are already been exposed to high levels of nanopollution due to the exhaust from cars and manganese oxide from construction sites.

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HALOPHILIC MICROORGANISMS AND THEIR INDUSTRIAL IMPORTANCE

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Halophiles are extremophiles that inhabit and proliferate in hyper saline environments. The group includes both prokaryotic and eukaryotic microorganisms. There are aerobic as well as anaerobic halophiles, heterotrophic, phototrophic, and chemoautotrophic types. Phylogenetic analysis revealed that besides domain Achaea, the halophilic and halo tolerant bacteria are dominantly found in domain Bacteria which are spread within the phyla Cyanobacteria, Proteobacteria, Firmicutes, Actinobacteria, Spirochaetes and Bacteroidetes. The largest family of halophilic Achaea is represented by Halobacteriaceae comprising of 36 genera with 129 species. Halophilic microorganism has been found to inhabit hyper saline water where the salt concentration is 2-5 times greater than normal sea water which is approximately 0.5 M salinity. These regions differ with salt concentration, soil composition, and nutrient types and thereby has a huge impact on the types of microorganism. The cultivable diversity of halophilic Archaea which includes 47 genera and 165 species of the family Halobacteriaceae.

Halophiles have the capability to balance and thus regulate the osmotic pressure of the environment and thereby resisting the denaturing effects of high salinity. Halophiles are extremophiles that require more than 0.2 M sodium chloride (NaCl) to survive and grow. The three halophilic classifications are based ranging from slightly to extremely halophilic. Most of the bacterial strains can live up to 15 % salt concentration and some can even adapt higher than that and thus they have the ability to adjust to

rapid change in external salt concentration. Proteins in these kind of organism shows stability only in presence of salt and overall surface negative charges prevents salting out of proteins. However, halophiles should not be confused with halotolerant microorganisms which can live and proliferate in the presence as well as absence of salt.

Cyclic tetrahydropyrimidine (1, 4, 5, 6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid) popularly known as ectoine, is major solute in moderate halophilic and halotolerant bacteria. This compound can be considered a marker for halophilic bacteria. It is synthesized by a wide range of bacteria, both halotolerant and halophilic varieties. This solute was first detected in the halophilic phototrophic *Halorhodospora halochloris*. Later many halophilic and halotolerant bacteria were detected to synthesize this solute. Most recently, it has also been observed in the moderately halophilic methylotrophic bacteria *Methylarcula marina*, *Methylarcula terricola* and *Methylophaga* sp. Ectoine comparatively to other solutes have wide range of beneficiary function and application, mainly in industrial point of view. The first commercial product which was derived from ectoine was as a skin care ingredient. Ectoine plays an important role, especially in sun protection and anti-aging products where ectoine is widely used and reports even suggested that recently, the use of ectoine in health care products and derivatives of ectoine has been use as nasal spray and against eye irritation causing redness and thus has become of increasing importance. Most interesting application of ectoine in medicine point of view is this product may serve as a stable and effective precursor for development of amyloid (aggregates of proteins folding which allows many copies of that protein to stick together forming fibrils) inhibiting novel compounds for the treatment of disorders such as Alzheimer's (amyloid-β peptide), Parkinson´s, Diabetes type 2 (islet amyloid polypeptide, IAPP) and disease caused by many common prions (PrP).Positive result has also been inferred in a clinical trial on skin effect (skin aging) of ectoine.

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'ESKAPE' PATHOGENS: A BIGGEST PUBLIC HEALTH CHALLANGE

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The 'ESKAPE' mnemonic stands for *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumanni, Pseudomonas aeruginosa* and *Enterobacter* species. The mnemonic represents deadly bacterial pathogens with rapidly growing multidrug resistance properties which have led to various 'difficult to treat infections' like central line associated blood stream infections, cathater associated urinary tract infection, ventilator associated pneumonia and surgical site infections mostly prevalent in hospital setting. This group of Gram positive and Gram negative bacteria can evade commonly used antibiotics due to their increasing Multidrug Resistance (MDR). As a result, throughout the world they are major causes of life-threatening nosocomial infection particularly in immune compromised and critically ill patients. Because of natural and unnatural pressure, and other factors, antibiotic resistance in bacteria usually emerges through genetic mutation or acquired through horizontal gene transfer - a genetic process by which antibiotic resistance can spread *via* transfer of Antibiotic Resistant Genes (ARGs). The main cause of rising antibiotic resistance which have led to the emergence of the 'ESKAPE' pathogens is the excessive use of antibiotics not only in healthcare but also in animals and agriculture sectors. Although antibiotic resistance is the result of adaptation of pathogens to nature genetically but misuse and overuse of antimicrobials is enhancing this process. Main factors related to this are in appropriate antibiotic choices, poor adherence to treatment guidelines and inadequate antibiotic

dosing without professional oversight. From the global perspective, injudicious and persistent use has provoked the emergence of Multi-drug Resistance (MDR) and Extremely Drug Resistant (EDR) bacterial species leading to 16% of hospital acquired infection cases. This all has created unprecedented challenges for the human civilization. Specifically, the opportunistic nosocomial 'ESKAPE' pathogens, the majority of whose isolates are MDR, correspond with the highest risk of mortality. Two pathogens within the 'ESKAPE' group-carbapenem resistant *Acinetobacter* and carbapenem resistant Enterobacteriaceae are currently in top five antibiotic resistant bacteria on the CDC's 2019 urgent threat list, whereas Vancomycin resistant *Enterococcus faecium* (VRE) and Methicillin Resistant *Staphylococcus aureus* (MRSA) are in the list of high priority group.

Issues of antibiotic resistance are rising rapidly, affecting adversely the goals of sustainable development. In the last three decades pace of resistance has increased manifold and unfortunately the pace of adding new antimicrobials to the armory of existing drugs has slowed down. Emergence of new antimicrobial mechanisms have engulfed the globe thus posed a threat in treating even a common infectious disease that were once sensitive to small doses of typical antibiotics. This public health issue has such a gigantic face that WHO has dedicated awareness weak to address this concern. Combating this threat is a public health priority and needs a collaborative approach. Researchers and scientists around the world should put their hands together to find new solutions so as to fix this grave public health issue. It is need of hour to keep an eye on the development of 'ESKAPE' pathogens and other ABR pathogens. There is also an utmost need to perform deep researches on alternative combination therapies like the use of antimicrobials in combination and the use of adjuvants, nanoparticles, peptides, biologics and phytochemicals so that new ways and agents could be traced out to cope up with emerging superbugs.

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A JOURNEY TO MAKE ACHEESE!!

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It was around 8000 BC when the rise of agriculture led to domesticated sheep's and goat's which ancient farmers harvested for milk. But left in warm conditions for several hours that fresh milk began to sour it's lactic acids caused proteins to coalesce, binding in to soft clumps upon discovering this peculiar transformation the farmers drained the remaining liquid later named ''whey'' and found the yellowish lumps could be eaten fresh as a soft spreadable meal, these clumps or cruds, became the building blocks of cheese. Which would eventually be aged, pressed, ripened and whizzed into a diverse cornucopia of dairy enchantments. The discovery of cheese gave Neolithic people an enormous survival advantage, milk is rich with essential proteins, fats and minerals, also contains high quantities of lactose a sugar which is difficult to process for many ancient and modern stomachs, cheese, however, could provide all of milks advantage with much less lactose and since millennia before refrigeration, cheese become a way to preserve milk. These essential nutrients could be eaten throughout scarce famines and long winters. By the end of the bronze's age, cheese was a stranded commodity in maritime trade throughout the eastern Mediterranean in the densely populated city-states of Mesopotamia, cheese became a staple of culinary and religious life.

''Rennet'' this animal by-product, produced in the stomachs of certain mammals that can accelerate and control coagulation. Eventually this sophisticated chess making tool spread all around the globe giving way to a wide variety of new harder cheese and though some traditionalist food cultures rejected the dairy intricacy, many more embraced cheese and quickly added their own local flavours to it. Nomadic Mongolians

used yak's milk to create it hard, sundried slices of byaslag. Egyptians loved goats milk cottage cheese straining the whey with reed mats, in south Asia milk was coagulated with a variety of food acids, such as lemon juice, vinegar and many more then hung to dry into loafs of paneer this soft clement cheese could be added to sauces or simply fried as a quick vegetarian cuisine. The Greeks produced bricks of salty brined feta cheese along with the harder variety comparable to today's pecorino Romano this abrasive cheese was produced in Sicily and used in various cuisine. Under the Roman rule, dry cheese or caseusaridus became an essential food nearly for 500,000 soldiers shielding the vast borders of the Roman Empire. In the hundreds of Benedictine cloisters disintegrated across the Europe medieval monks experimented endlessly with different types of milk cheese making and aging procedures. That leads to many of the today's popular chesses – Parmesan, Roquefort, Munster and several Swiss types were all superior and perfected by these cheese making clergymen. In the Alps Swiss chess making was particularly successful producing a myriad of cow's milk chesses. By the end of the 14th century alpine cheese from the gruyere region of Switzerland had become so profitable that the neighbouring state occupied the gruyere highlands to take control of growing cheese trade. Cheese endured popular through the renaissance took production out of the monastery into machinery. Today the world produces coarsely 22 billion kilograms of cheese in a year, transported and consumed around the earth but,10,000 years after its invention, the local farms are still following the paths of their Neolithic ancestors hand crafting procedures is one of humanity's oldest and favourite foods.

''Making of cheese'' involves 6 important steps: (i) Acidification, (ii) Coagulation, (iii) Curds and whey, (iv) Salting, (v) Shaping and (vi) Ripening. In first step a starter culture is added to milk that will change lactose into lactic acid which changes the acidity levels of milk, cheese makers usually add an enzyme called rennet naturally found inside of cows, goats, and some other mammals to help this process along eventually those little curdles turns into bigger curds ,the solid part is called curds and liquid part is whey this curds cut into pieces the smaller the curds ,the harder cheese like 'cheddar' by cooking the curd changes its texture, prevent crumbly and makes it tender, the whey is drained by leaving the curds to become cheese and salt is added for extra preservation and for flavour then it is put into baskets or moulds to form it into a specific shapes, cheese ripening is a slow and expensive process it gets completed in several steps where numerous biochemical reactions like glycolysis, lipolysis, proteolysis are initially involved and biophysical changes occurs all done by enzymes like microbial lipase, lactases, microbial serine proteinases from organisms *Aspergillus niger*, *Streptococcus lactis* and *Bacillus subtilis*.

Bacteria are the key for making cheese, to make cheese, milk is inoculated with bacteria which gobble the lactose (sugar) and produce lactic acid, along with many other chemicals as the milk gets more and more acidic its proteins start to aggregate and curdle that's why spoiled milk is clumpy, different strains of bacteria creates a different kinds of cheese.

'White-brined cheese' has different varieties of cheese-beyazpeynir, domiati, feta, Iranian white cheese, these are salty and acid taste, white in colour, closed and semi hard or semi soft in texture with 55 - 60 % moisture and 4.5 to 4.8 pH, for these kind of cheese the starter culture contains a mixture of *Lactococcus lactis, cremoris* or *thermophilic lactic* culture including *Streptococcus thermophilus.*

'Hard cheeses' these are of two types hard and semi hard types and extra hard type, includes cheddar, Colby, Cantal, Asiago, Parmesan, Romano which has uniform and close texture, yellow in colour, majority is dry salted, matures more than 2 years very hard and grainy texture, strong and slightly rancid flavour with 35 to 40 and 25 to 30 % moisture and 5.3 to 5.5 pH it has microorganisms like *Lactobacillus helveicus, Streptococcus thermophilus,* etc.

A cheese with eye is 'Swiss' chesses includes beau-ford, gruyere comte, emmental these are characterised large eyes produced by propionic acid bacteria like *Propionibacterium freudenreichii* subspecies *hermanii* with 35 % moisture and 5.6 pH, it has strong flavours and another type of eye cheese is 'dutch' cheese are gouda and edam it has small eyes, semi hard, colour full, sweet and buttery flavour, citrate positive *Lactococci* and *Leuconostoc* are used as starter culture with 42 % moisture and 5.8 pH.

'Blue cheeses' has variety of Roquefort, Stilton, Gorgonzola, Cabrales, Danablu are characterized *Penicillium roqueforti* in fissures throughout the cheeses, soft in texture and flavour with methyl keteones is prepared with a mixture of *Lactococcus lactis* and *Penicillium roqueforti* with 40 % moisture and 6.3 pH.

'Surface mould cheeses' has varieties of Camembert, Brie with 50 % moisture and 7.0 pH it grows white mould *Penicillium camemberti* on surface and complex microflora, soft in texture, mixtures of *Lactococcus lactis* and *Penicillium camemberti, Geotrichum candidum* used as starter culture.

'Smear cheeses' are Tilsit, Comte, Trappist, Havarti, Taleggio, Limburger, Munster they are Soft in texture, red or orange in colour, mixed complex flora on their surface with mixture of *Lactococcus lactis, Brevibacterium linens, Debaryomyces hansenii,* coryneform bacteria, has 6.5 pH and 42 % moisture

'Pasta-filata cheeses' are Mozzarella, Provolene, Kaskaval, Kashar,these are Heated and kneaded curd, closed texture, piquant taste, low salt which has starter culture of *Thermophilic lactic* cultures including *Streptococcus thermophilus, Lactobacillus helveticus* and Non-starter lactic acid bacteria as secondary microorganisms with 45 to 50 % moisture and 5.3 pH

'Functional cheeses' these are Probiotic cheese Based on the selected cheese as carrier Mixtures of *Lactococcus lactis* and subspecies *cremoris* and *Lactobacillus acidophilus.* The growth of microorganisms in cheese includes water activity, concentration of salt oxidation–reduction potential, pH, NO_3^-, temperature. Perhaps the production of bacteriocins by some microorganisms are the factors which controls the growth.

'Spoilage of cheese by *microbes'*the most common spoilage occurs by early or late gas development but this problem can be solved with better hygiene in milk production and better quality control in cheese plants. Early gas formation occurs within 1or 2 days after manufacture, it is characterized by the appearance of gas which creates small in the cheese inside and it is caused by coliform bacteria and yeast (H_2 and CO_2) these gases are produced from lactose, it is a more problematic in soft and semi-soft cheeses than in hard cheeses, early gas formation can be controlled by adding nitrate to milk. Late gas formation also called late blowing it occurs only when the ripening gets late and it is due to the formation of butyrate CO_2 and H_2 by *Cltyrobutyricum* and *Clostridium butyricum*. Butyrate creates large holes in cheese and it also responsible for off flavour development, these type of production of gas is particularly prevalent in swiss-type of cheese as clostridia can grow during the hot-room ripening period, Thermophilic cultures stimulate the growth of *Clostridia* by the production of peptides and amino acids, it can be controlled by the addition of NO_3^- and lysozyme which occurs naturally in milk, saliva, tears which hydrolyses the cell walls of sensitive bacteria, like *Clostridium tyrobutyricum*, causing them to lyse. Commonly used in Italian cheeses and it is added to the milk with the starter at a level of 25 mg l^{-1}. The bacteriocin, nisin, produced by some strains of *Lactococcus lactis* is effective in controlling the growth of *clostridia* and used in processed cheese, *Enterococcus malodoratus* bacteria produce bad flavours and found in Gouda cheese, a little damage created by moisture and unwrapping which is an ideal environment for the growth of molds and yeasts, can be wash off the surface of cheese with a dilute brine solution.

Cheese is a great source of calcium, fat, proteins with high amounts of vitamin A, vitamin B-12, zinc phosphorus, riboflavin along with omega -3 fatty acids, cheese helps in protection of teeth from cavities, the casein present in the cheese protects tooth enamel by forming a thin film on the surface of tooth which prevents tooth decay. It

contains 'Conjugated linoleic acid' that helps in prevention of cancer, the presence of calcium in cheese helps in weight gain, increase bone density, prevents osteoporosis and gives strength to muscles, as cheese rich in vitamin A and B it improves eye vision and helps in the growth of cells making skin to glow by reducing blemishes which gives a healthy skin, cheeses like blue, Swiss, cheddar are packed with selenium an antioxidant which plays an important role in boosting the immune system and also maintains body function. There are many nutrition facts vary from one type to another for example, Mozzarella contains 85 calories and 6.3 grams of fat per ounce, 1 gram of carbohydrates, 6 grams of protein, 143 mg calcium and 138 mg sodium, Brie has 95 calories and 7.9 grams of fat per ounce,1 grams of carbohydrate, 5 grams of protein, 150 grams of calcium and 170 grams of sodium. Lower calories of cheese like part skim Mozzarella, Swiss cheese and feta cheese.

Few people are sensitive to cheese as it contains lactose, a sugar that cannot be digested by lactose intolerant people because their body lacks the lactase enzyme which break down the lactose which may lead to health issues, certain cheese like parmesan are very low in lactose, people with allergic to casein can't tolerate low lactose cheeses, it is a calorie dense food by eating it gets 100 calories per ounce and usually it is loaded with sodium which makes it easy to overeat and can be an issue people with high blood pressure, cheese do not contains Fiber excessive intake and of pasteurized dairy may cause constipation, it is expensive to purchase.

Cheese market has been one of the most dynamic food segments in the last 20 year with steady growth in consumption international trade and production, the world cheese production was doubling since 1980s, this trend continues in the upcoming years. The European union are in top to producer of cheese, Switzerland produces 191,000 metric tons of cheese, Greece produces 222,000 metric tons of cheese it is popular for feta cheese, Australia produces 370,000 metric tons, Portuguese brought cheese to brazil which produces 780,000 metric tons, India has less production of cheese, Belgium produces 121,000 metric tons. Gruyere cheese wins the title of world's best cheese from Switzerland.

A food which consisting of coagulation, compressed, usually ripened curd of milk separated from whey with various textures and flavours, which has nutrients, health benefits where the tiny microorganisms play an important role. The all-time favourite food for children and adults CHEESE!!

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MICROBIAL DEGRADATION USING INDUSTRIALLY OBTAINED SOLID WASTE

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Microbial degradation is the process of degradation of organic contaminants from contaminated solid waste, the process employs or allow natural bacterial strain which degrade naturally. There are special bacteria which use undesirable organic compound as a source of carbon and energy for their growth. The present study was conducted to find out the utilization of effective bacteria for waste degradation as organic manure or compost. The sample was collected from different potential habitat in Porto Novo region of Cuddalore for isolating the effective solid waste degrading bacteria. 10 isolates were screened in different selective or nutrient agar medium. (MD1-MD10) were isolated and characterization of these strain were also studied by observation of colony morphology, biochemical assay followed by Bergey's manual of systematic bacteriology classification. Specific bacteria namely *Pseudomonas aeruginosa* MDPs3 strain changes of colour, odour, weight loss, temperature and pH of decomposing waste were noted for selecting the most effective strain.

The solid waste about 500 grams weighed in a consortium and 10ml of bacterial strain MDPs3 were used in under controlled condition. It took around 45 days to degrade completely to prefer as compost or manure. The decomposed mixture was used as compost or organic manure to observe their effects on biomass production. From present study, it can be concluded that useful bacteria might be isolated from the surrounding environment for friendly bioconversion of solid organic waste.

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GIARDIASIS - AN IGNORED QUESTIONABLE DISEASE

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Giardiasis, also known as 'Beaver fever', is a parasitic intestinal illness which is caused by a parasite known as *Giardia duodenalis.* It is usually found in soil, food or water that had been contaminated with feces from the infected humans or animals. It has been revealed that cysts of causative organism can survive in cold water for more than three months. The transmission of this disease occurs mostly by drinking water possessing cysts of parasite. Fecal oral route is primary route of transmission. Children are more affected than Adults. Most infections caused by this parasite are usually asymptomatic and that is the cause why people mostly ignore the disease and finally the transmission is carried out in Hidden manner. Asymptomatic person can also pass very large numbers of cysts in stool, thus transmit disease silently. The disease can affect people of all ages but mostly infants and young children have an increased susceptibility to the Giardiasis. It is more common in developing countries than in developed ones because developing countries are having poor Sanitization. In India, prevalence rates of *Giardia* infection in patients with Diarrhea ranges from 0.4 % to 70 %. The Asymptomatic cyst passage has been found to be as high as 50 % in Rural southern India. Giardia parasite has been found in about 80 % of raw water supplies from lakes, streams, and ponds. The average duration of symptoms in all age groups ranges from 3-10 weeks. Giardia completes its life cycle in only one host (Human), with no intermediate host. Its life cycle is mainly composed of two stages, the Trophozoite which

exists freely in small intestines and the Cystic stage in which cysts are passed out the through feces.

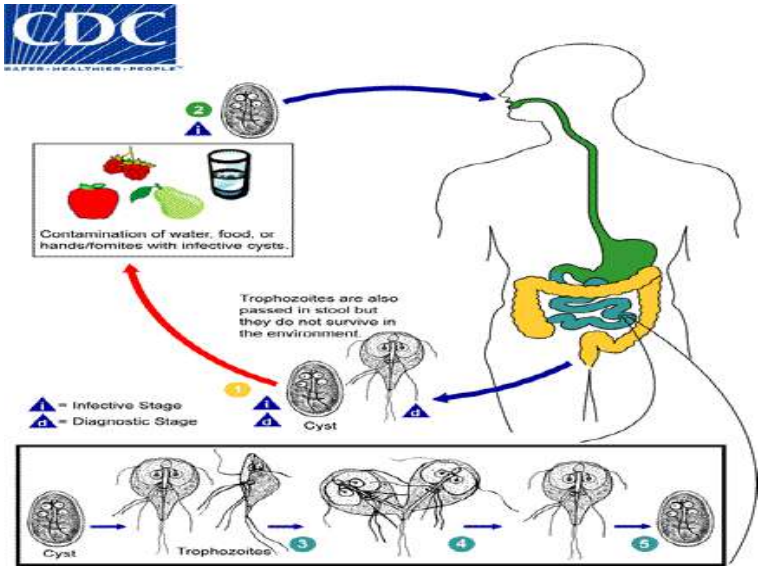

Figure – 1: Life cycle of Giardiasis (Adopted from www.cdc.gov)

Cyst form is considered infectious and once it has entered the host, transforms into trophozoite state. The later gets attached to intestinal wall and start replicating which results in delayed expression of brush border enzymes, enhanced intestinal permeability, alteration of microvilli, and apoptosis of intestinal epithelial cells. Both cysts and trophozoites are limited to intestinal lumen and don't invade beyond that. Alteration of villi renders them unable to absorb water and nutrients thus leading to Diarrhea, one of the prominent symptoms. In the case of asymptomatic Giardiasis, there can be malabsorption. Diarrhea, Abdominal cramps, vomiting, weight loss, low grade fever (infrequent), weakness, foul/greasy stools, flatulence are main symptoms of Giardiasis. In chronic infections, frequent symptoms are Malaise (weight loss) seen in 66% of symptomatic patients followed by nausea (post infection lactose deficiency) seen in 20-40% of cases. There is no contribution of physical examination to which Giardiasis can be diagnosed, although weight loss may be the evidence. For diagnostic purpose, a stool examination, ideally 3 specimens from different days are done because of potential variations in fecal excretion of cysts. Almost 80 – 85 % of Giardiasis can be diagnosed by Microscopic examination of stool for ova and parasite (O and P) examination. There are other various methods also used for the diagnostic purposes e. g. detection of antigens on the surface of the parasite in stool specimen is sensitive technique. Treatment of Giardiasis involves the use of nitroimidazole medication with metronidazole as first line treatment. Maintaining appropriate fluids and electrolytes is

critical in this disease, particularly in patients with diarrheal stools. The disease can be most frequently controlled by prophylaxis strategy which includes proper disposal of wastes, feces, and other suspected materials, frequent handwashing, drinking clean and boiled water. Effect of probiotics on giardiasis induced diarrhea and effect of giardiasis on normal microbiota of intestine are the subjects of current research.

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ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANT AGAINST WOUND INFECTED PATHOGENS

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Many medicinal plants have antimicrobial properties uses in traditional Indian system of medicine mostly in Ayurveda and Unani. Leaves are also using for therapeutic purpose. It is being used as a source of medicinal agents for antibacterial, antihelminthic, astringent, emetic, febrifuge, sedative and stimulant. Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug; Antibiotics provide the main basis for the therapy of bacterial infections. *Acalypha indica* L. It has been reported to be useful in treating pneumoniae, asthma, rheumatism and several other ailments. The dried leaves of *Acalypha indica* is added to oil or lime and used to treat a variety of skin disorders. The leaves of *Acalypha indica* have been reported to possess contraceptive activity. Several chemical and biological investigations have been carried out on this plant.

The leaves extract of the plant reduces mutagenecity in *Escherichia coli*. The leaves are laxative while a moisturizer for the diagnosis of facial paralysis. The leaves of the plant can be used in the diagnosis of jaundice, piles and also externally skin eruptions, ring worms. Leaf be use like painkiller the same as an antipyretic, it be a tonic used for malaria also fever. It is too apply inside anthrax, blood poisoning, along with anti-dysentric, anti-dibetic and leprosy intended for the elimination of abdominal obstacle

and they are also helpful in the treatment of asthma. The leaves of the plant contain too be report toward seize the superiority of contraceptive action . The extract of the origin is to be able to condensed the blood sugar stage *via* 30 %. Leaves possess anti-periodic with laxative properties and the leaf extract can be applied to insect bites. Root is of use during agitation, heart disease plus retain excretions. The 50 % ethanol extracts of pods reveals the anti-fertility activity in female albino rats. Cough can be easily cured by making burnt pods with little salt along with honey 3 - 4 times. Roots are worn during the cure of antipyretic, diabetes, helpful in coffer complaint, gorge plight, eyes defect along with the diagnosis of cardiac disorder, stiff situation, ulcer, wound, boils furthermore diverse crust disease. The extract of the root bark among alcohol be capable of exist use meant for backwart fever. The seed is slightly sweet and also possess laxative, carminative, cooling improves the appetite Seed powder is used in amoebiasis. The extract of the flower inhibits the ovarian utility with excite the utility of uterine in albino rats. The pulp of the fruit around the seed is used in curing diabetes. Outwardly it is of use used for the emigration in flatulent tummy ache, while salad dressing intended for gouty otherwise stiff joint. The fruit flesh is used for constipation, tummy ache, chlorosis along with urinary disorder. The bark seizes stimulant also anti dysentric property helpful in administering of leprosy, jaundice and heart disease.

The *Acalypha Indica* L. shows utmost action versus *Pseudomonas aeruginosa Bacillus cereus*, *Vibrio cholerae*, *Salmonella typhi*, *Staphylococcus aureus, Shigella flexneri Pseudomonas aeruginosa*. A different study reveals so as to acetone along with water extracts of stem, bark, leaves, stem, of *Acalypha Indica* L. are effective against *Staphylococcus aureus, Escherichia coli.* The water moreover acetone extracts exhibit superior antibacterial activity alongside gram positive with this is while strong when numerous viable drugs. Thus, they are able to exist used in cure of transmittable disease cause through veteran strain as well as latent anti-microbial agent can exist prepared.

The agar well diffusion method, 5 mm size wells were cutted in cultural strain swabbed plates. Then, 5 µl of plant extract (acetone, water plant extract) was added in the well by using micro pipette. Methanol added well considered as a control. 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 (5 mm).

Plant extracts showed antibacterial activity against the bacterial strain. The water and acetone extract of *Acalypha indica* showed antibacterial activity against tested organisms. In this present investigation the comparison between to the acetone 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

Pseudomonas aeruginosa (20 mm) and *Staphylococcus aureus* (17 mm), and acetone extract of *Acalypha indica* showed the maximum zone of inhibition for *Staphylococcus aureus* (16 mm) and minimum for *Pseudomonas aeruginosa* (9 mm) *Acalypha indica* was found to be active against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

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BACTERIOCINS MAY REPLACE ANTIBIOTICS IN FUTURE

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Antibiotics were an excellent achievement in controlling infectious disease which helped a lot to control morbidity and mortality in humans. However, Antimicrobial resistance has emerged rapidly over the years and has become a critical health issue, leading to increasing morbidity and mortality once again as the cure only has become ineffective. Methicillin Resistant *Staphylococcus aureus* and Vancomycin resistant enterococci are some of the examples which suggests the need for development of new therapeutic approaches to treat diseases. Bacteriocins can provide a pathbreaking achievement in tackling diseases as they are potential antimicrobial agents against different bacteria, fungi and even bacterial biofilms. These are natural ribosomally synthesized peptides, produced by bacteria in a competitive polymicrobial environment to eliminate other bacterial species. Almost all bacteria can produce bacteriocin which allows a wide range of antimicrobial molecules. Nisin, a bacteriocin produced by some strains of *Lactococcus lactis* is only bacteriocin which is approved by FDA to be added in food additives as it inactivates Clostridium botulinum and is nontoxic to humans as they are degraded by proteolytic enzymes of mammalian gastrointestinal tract. Also, unlike broad range antibiotics, specific pathogens can be targeted without any impact on commensal microflora. Bacteriocins have also been reported as a therapeutic agent in case of *Mycobacterium* sp. and *Streptococcus pyogenes* which suggests its potential use against pathogenic infections in place of antibiotics, many of which are ineffective now due to antibiotic resistance. Bacteriocins are classified according to their size, molecular

composition, structure and modification process. In Gram positive bacteria, Class 1 bacteriocin are lantibiotics i.e., contain lanthionine and due to which confer thermostable property to bacteriocin and are of low molecular weight are associated with inhibition of many food borne pathogens. Class 2 which do not contain lanthionine, causes destabilization and permeabilization of bacterial membrane and pore formation too provide potential use against pathogens. Class 3 bacteriocins has endopeptidase, resulting in death of target cell and Class 4, which are combined with carbohydrates or lipids too disrupt cell membrane. Plasmids or chromosomes carry genes for bacteriocins where a cluster of genes are involved in its synthesis and transportation via bacteriocin operon. Bacteriocins of Gram positive bacteria usually binds to lipid 2 during peptidoglycan synthesis and forms pore in plasma membrane. Nisin uses lipid 2 as a docking material and increases membrane permeability leading to cell death. Another Class Bacteriocin, Lactococcin A, targets receptor Mannose-PTS and helps in permanent opening and continuous efflux of intracellular molecules leading to cell death. All these can provide a vast range of application of bacteriocin against human pathogens which may replace antibiotics. In Gram negative bacteria, colicins are important bacteriocin which forms pores in bacterial cell wall and can also degrade nucleic acid structures. Another class of bacteriocin produced by gram negative bacteria are Microcins, which act by inhibiting various enzymatic functions like ATP synthase complex such as Microcin C, which can act against aspartyl-tRNA synthatase, ultimately blocking protein synthesis and killing the target cell. However, for microcins to work, it must enter the target cell and using specific receptors they inhibit many important enzymes such as DNA gyrase, etc. Thus, each of them can be used a potential therapeutic agent where many bacteriocins can be used against various pathogens as these can kill selectively using different mechanisms. Thanks to the studies, several bacteriocins have been already reported against several human pathogens. Bacteriocin VJ13, isolated from *Pediococcus pentosaceus* has demonstrated antibacterial activity against *Listeria monocytogens*, *Staphylococcus aureus*, *Clostridium* sp. and *Klebsiella pneumoniae*. Moreover, the antilisteric activity is not affected by lipase and is stable at pH 2 to 8. Another bacteriocin PJ4, produced by *Lactobacillus helveticus* is active against *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. All these 3 bacteria pose a harmful effect to humans and bacteriocin PJ4 is effective against these. Another major breakthrough is Paracaseicin A, from Lactobacillus paracass which is active against Multi drug resistant bacteria paving way for the replacement of antibiotics as they are not cure any more in these cases. Gallidermin by Staphylococcus gallinarum is also effective against Multi drug resistant bacteria and as they act against atl and ica genes, necessary for biofilm formation can be used against infections caused by biofilms too. Several microcins which are produced by gram negative bacteria are too have been reported for act against several pathogenic organisms such Microcin E492 act against

Klebsiella pneumoniae and Microcin J25 against pathogenic strains of *Escherichia coli*. Thus, bacteriocins can play a major role in tackling harmful pathogens especially in cases where antibiotics are no more am option as a therapeutic agent. As, microbial resistance is a cause of worry which limits therapeutic options, bacteriocins can provide a major breakthrough in tackling the diseases which even act against multi drug resistant strains of bacteria and with more research, several undiscovered and unutilized bacteriocins can provide a potential therapeutic use and can become replacement of antibiotics in future.

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ACTINORHIZAL PLANTS

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Introduction

- Actinorhizal plants are a group of angiosperms characterized by their ability to form a symbiosis with the nitrogen-fixing actinobacteria *Frankia*. The Actinorhizal symbioses are mutualistic relationships between the actinomycete genus *Frankia* and a number of dicotyledonous plants. This mutualistic association leads to the formation of nitrogen-fixing root nodules.
- Actinorhizal plants are dicotyledons distributed among three angiosperm orders, 8 families, 24 genera, and some 230 species. Major Actinorhizal plant families and genera are: Betulaceae, on 47 *Alnus* species; Casuarinaceae, on 16 spp. of *Casuarina*, and 54 spp. of *Allocasuarina*; *Myricaceae*, 28 spp. of *Myrica*, and *Rhamnaceae*, 31 spp. of *Ceanothus* (Russo, 2005). These genera are widespread in ecosystems on all continents except Australia, and in Oceania

Actinorrhizae/ Frankia

Domain: Bacteria
Phylum: Actinobacteria
Class: Actinobacteria
Order: Actinomycetes
Family: Frankiaceae
Genus: *Frankia*

- Atinorrhizae also called *Frankia* which is the dominant Actinorhizal genus. In 1886, this genus was originally named to honor the German biologist Albert Bernhard Frank.
- *Frankia* is a genus of nitrogen-fixing, bacteria that live in symbiosis with Actinorhizal plants, similar to the Rhizobium bacteria found in the root nodules of legumes in the family Fabaceae. *Frankia* also help to start of root nodules.
- *Frankia* are Gram-positive, aerobic, and fix nitrogen in both under symbiotic and free-living aerobic conditions.
- Morphology of *Frankia* is different in the nodule according to the host plant. The size of hyphae and the presence or the absence of vesicles depends on the bacterial association.
- They can adapt themselves to grow under the most diverse environmental conditions and geographical zone. The growth of *Frankia* is very slow and it is filamentous and Microaerophilic.
- Some *Frankia* strains are very tolerant to salinity and can be used as biofertilizers in land affected by salt. Mycelial growth of Frankia binds to the soil structure and it became more important in the CPN cycle (Carbon, phosphorus, and nitrogen cycle).

Nodule formation in Actinorhizal Plants:

- Actinomycetes bacteria have nod genes which is produce nod factor and initiate nodule formation.
- Attachment of *Frankia* cells to the mucilage layer in root region/ Attachment of Frankia spores to the root hairs.
- Curling of root hairs (which provides a pathway for the Rhizobium to travel into the root epidermal cells).
- Actual entry of *Frankia* has not been seen, but hyphae are seen as simple or multiple thread after branching inside the deformed root hair.
- Such threads expand from host-derived cell wall material & continue with the root hair cell wall.
- Threads could be seen penetrating cortex or other root section.
- Prenodule formation in 10-14 days.
- Later around the primary nodule, primordium appear.
- Later, if meristem undergo progression & branching & form a typical adult nodule called "Rhizothamnion".

Actinorhizal nodule

- Actinorhizal nodule is a lobe-like structure which have 5 to 6 cm in diameters and resembling a tennis ball.
- Actinorhizal nodules have nitrogen-fixing actinomycete *Frankia* on the roots of several actinorhizal plant species. Actinorhizal nodules have multiple lobes, which represents a modified root without root-cap. The root nodule is an organ where the plant anatomy and metabolism has adapted to facilitate the hosting of Frankia. The plant provides the nodules with photosynthesis mostly in the form of sucrose.

Importance of Actinorhizal Plants with actinobacteria *Frankia*

- Actinorhizal plants are also pioneering species and enhance plant establishment on sites, to improve soil fertility and stability.
- Degraded soils have one of the rare nutrients like nitrogen. The symbiotic relationship with nitrogen-fixing bacteria Frankia increases soil fertility and increases the performance of trees during their plantation in degraded lands.
- The positive aspects of actinorhizal plants on the remediation of degraded ecosystems.
- Certain actinorhizal plants like *Hippophae rhamnoides* (sea buckthorn), *Hippophae* L., and *Rubus ellipticus* (yellow Himalayan raspberry) are used as food ingredients and medicinal purposes.
- Actinorhizal trees are used as windbreaks to protect adjacent crops and fix with soil in Senegal, Tunisia, Egypt, China, and India.
- Actinorhizal trees are also used in the production of smokeless fuelwood with a high energy, and hardwood in the construction of houses and in the production of paper pulpwood in India.
- Association with *Frankia* increased plant growth and biomass in this symbiotic relationship, bacteria confer to plants a high resistance to abiotic and biotic stresses.
- Actinorhizal plants are also important for symbiotic association between Casuarina-*Frankia*, farmers have begun to use these biofertilizers through inoculation with crushed nodules.
- These plants are used for Fruits edible and made into refreshing drinks, bark used to intoxicate fishes and yield a yellow dye.
- The wood is useful as fuel and dry branches for hedges and also used in local beverages, flowers used as a source of bee forage.
- Edible Fruits of actinorhizal plants are used in cough and bronchitis and seed powder used as an expectorant.

- These plants use for degraded lands reclamation, Because of their nitrogen-fixing captivity.

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SINGLE CELL PROTEIN

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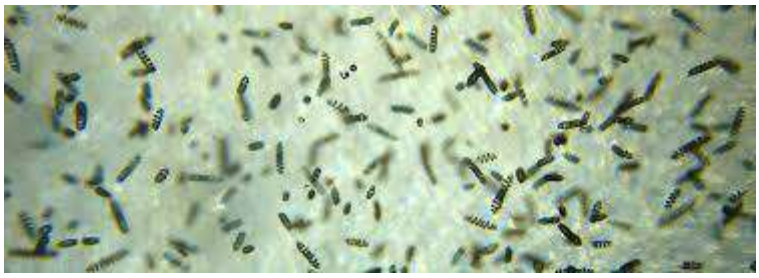
Single Cell Proteins (SCP) are the proteins derived from cells of microorganisms such as yeast, fungi, algae, and bacteria which are grown on various carbon sources for synthesis. The first development of SCP production was achieved during war times when conventional foods were in short supply. It is called as single cell protein because of that organism is single is singe celled. It refers to edible unicellular organisms. The dried cells of microorganisms or the whole organism is harvested and consumed. Single cell protein is a protein source for human supplements and animal feeds. Single cell proteins have the capacity of feeding the over increasing world population. A huge quantity of SCP can be produced in a single day. Production of SCP has important advantages over other protein sources such as its considerable shorter time doubling and small land requirements.

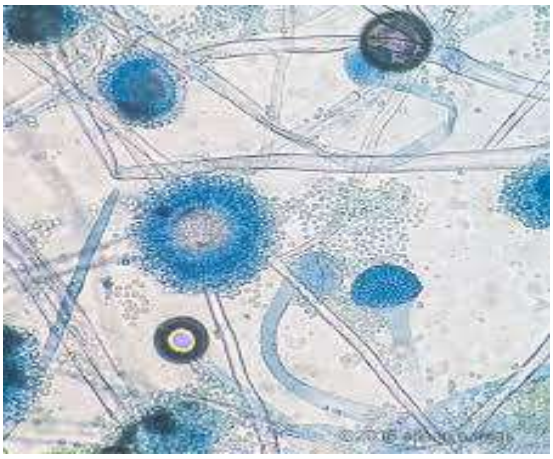
Algae protein

Algae is a key member in SCP family, and has been utilized by people for centuries due to its high nutritional content of proteins as well as fatty acid. For example, the famous Blue-green algae *Arthrospira platensis*, more commonly known as "*Spirulina*". Algae uses photosynthesis, which means their main substrates are carbon dioxide and sunlight. The commonly used algae protein is *Chlorella pyrenoidosa*, *Candida utilis*, etc.



Fungi protein

Edible fungi are arrived at the commercial food market only a few decades ago. Fungi are not picky eaters. They can grow on various different substrates like sugars, cellulose and even lipids. The most common way of cultivating fungi is in the fermenter. Then proteins derived from fungi are called "mycoproteins' Mycoproteins are generally more accepted as human food. Filamentous fungi have been used in traditional Asian foods like "tempeh" and "oncom", and they are actually responsible for the food health benefits. The most common examples of fungi protein are *Aspergillus fumigatus*, *Aspergillus niger*, *Rhizopus cyclopean* etc.



Mushroom protein

Mushroom is a protein rich in food and has been considered as a source of single cell protein. They are easily digestible and possess a high amount if amino acids. It is directly used as food and plays a major role in the production of SCP. Edible mushrooms are highly nutritious and can be compared with eggs, milk and meat. These possess high quantities of fibers, few sugars and low calories and a high quantity of amino acids such as phenylalanine, threonine, and tyrosine. Examples are *Agaricus bisporus*, *Morchella crassipes*, *Volvariella volvaceae*, etc.

Bacterial protein

Bacterial SCP generally contains 50 – 80 % protein on a dry weight basis and the essential amino acid content, Methionine content is upto 0.3 % which is higher than that of obtained in algal and fungal species in general. In addition to proteins and nucleic acids, bacterial SCP provides some lipid and vitamins from B group. Bacterial species used as SCP are *Cellulomonas*, *Alcaligenes*, etc. They have short generation times and most can double their cell mass in 20 mins to 2 hrs.

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BACTERIOLOGICAL ASSESSMENT OF DRINKING WATER SUPPLIED AT SCHOOLS: A MAJOR HEALTH CONCERN

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Water is the basic and primary need for all vital life processes. With increasing population growth, industrialization and urbanization the water has been contaminated by different human, animal and industrial wastes. As a result, it is a formidable factor in transmission of several waterborne diseases like Dysentery, typhoid fever, cholera, hepatitis etc. According to WHO, contaminated drinking water is estimated to cause 4, 85, 000 diarrheal deaths each year.

The purity of drinking water is evaluated by testing for the presence of coliforms as an evidence for fecal contamination. The coliform bacteria include *Escherichia coli, Streptococcus faecalis, Clostridium welchii* etc. The only natural source of coliform is the intestines of humans and other mammals. Although these coliforms are non-pathogenic in healthy individuals, when ingested, their presence indicates fecal contamination. Thus, provides possible route for other pathogenic strains to cause infection.

Bacteriological examination of water for coliforms is performed by membrane filtration technique. Water sample is passed through a thin sterile membrane filter of pore size 0.45µm kept in a special filter apparatus contained in a suction flask. The filter

disk that contains the trapped microorganisms is aseptically transferred in a sterile petri dish having an absorbent pad saturated with a selective, differential media like Endo agar and is incubated at 37ºC for 24 hours. Pink to red colonies on agar represent coliforms (Table 1) (Figure 1).

Table 1: Microbiological assessment of water

Sample code	Totalcoliform count	Coliforms	Remarks
1. C3	6	Detected	Non-potable
2. U2	38	Detected	Non-potable
3. E1	46	Detected	Non-potable
4. B1	0	Not detected	Potable

WHO guidelines for drinking water illustrate Fecal counts of 0, 1–10, 10–100, 100–1000, >1000 per 100mL correlate with no risk, low risk, intermediate risk, high risk, and very high risk respectively. However, for every 100mL of drinking water tested, no total coliforms, fecal coliforms or *E. coli* should be detected. Most coliform bacteria do not cause disease. However, some rare strains of *E. coli*, particularly the strain 0157:H7 can cause serious illness.

Many commercial counterfeit filters and water purifiers are available in the market which claims to kill 100% disease causing germs. However, filtrate water sample U2 and E1 obtained from water purifier reported significant coliforms count that alarms the distribution and use of counterfeit water purifier with deficient germicidal activity. It was found that many types of water treatment devices available had never undergone laboratory testing and certification for removal of biological and chemical contaminants. Lack of water surveillance for possible physical, chemical and biological contaminants explains the scenario of unsafe water supply. Inadequate and unsafe distribution of water poses a threat to human beings. The parasitic infestations mostly seen in children has been associated with consumption of contaminated water. Besides, the worse clinical effects brought by infection with cholera, dysentery, polio, hepatitis cannot be ignored. With growing urban sectors, the unmanaged sewage drainage system leads to possible contamination of drinking water supply by fecal contaminants. Water from improved and safe sources always safeguard the health and economy. Particularly children who are at high risk to waterborne diseases, provision of safe water at home and school can address their health, sanitation and therefore brings improvement in school attendance and positive academic achievements.In spite of chlorination of water in reservoir, the faulty water supply channels across the sewage, drainage supply have direct role in fecal contamination of water. In addition, counterfeit water purifiers with

poor germicidal activity does not guarantee of safe water distribution. So, regular monitoring and microbiological analysis of water with installation of improved and reliable water purifier is recommended for distribution of safe drinking water at schools, homes and community.

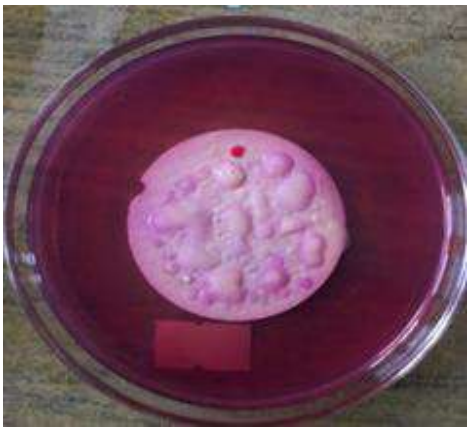

Figure 1: Coliforms colony

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Helicobacter pylori INFECTION: GASTRITIS TO GASTRIC CANCER

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Helicobacter pylori, previously known as *Campylobacter pylori* is a Gram negative, microaerophilic bacterial pathogen that selectively colonizes the gastric epithelium of 50 % of the world's population. The bacterium is urease, catalase, and oxidase positive, is spiral shaped, and possesses 3 to 5 polar flagella that are used for motility. *H. pylori* has evolved with the ability to colonize the highly acidic environment found within the stomach by metabolizing urea to ammonia via urease enzyme, which generates a neutral environment protecting the bacteria. Oral-oral and fecal-oral route transmission have been known behind the transmission of this bacteria from one host to another. Infection with *Helicobacter pylori* causes chronic inflammation and significantly increases the risk of developing duodenal and gastric ulcer disease which can progress to gastric cancer. *Helicobacter pylori* infection induce gastrointestinal diseases, ranging from chronic active gastritis without clinical symptoms to peptic ulceration, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma.

Infection with *Helicobacter pylori* is the strongest known risk factor for gastric cancer, which is the second leading cause of cancer-related deaths worldwide and fourth most common cancer worldwide. Once *Helicobacter pylori* colonizes the gastric environment, it persists for the lifetime of the host, suggesting that the host immune response is ineffective in clearing this bacterium. Robin Warren and Barry Marshall

identified *Helicobacter pylori* in 1982 by culturing an organism from gastric biopsy specimens that had previously been observed for almost a century by pathologists.

Pathogenesis

Infection by this gastric pathogen is mediated by Oxidative DNA damage induced by *Helicobacter pylori* infection has been well documented for gastritis tissues. Bacterial factors critical for *Helicobacter pylori* colonization of the gastric mucosa include urease, flagella, adhesins, and δ-glutamyl transpeptidase. Lipopolysaccharide, urease, and vacuolating cytotoxin are among the factors that allow *Helicobacter pylori* to persist for decades and invoke an intense inflammatory response, leading to damaged host cells. *Helicobacter pylori* can damage the protective lining of the stomach and small intestine. This can allow stomach acid to create an open sore (ulcer). Inflammation of the stomach lining by *Helicobacter pylori* infection can cause inflammation (gastritis). About 10 % of people with *Helicobacter pylori* will develop an ulcer and 1 – 2 % are susceptible to gastric cancer.

Pathology

Pangastritis is the most common gastritis in which entire stomach is affected. Pangastritis can lead to low acid production, chronic inflammation, atrophy, intestinal metaplasia and finally gastric ulcer and gastric cancer. Antral gastritis is another form in which only antral portion of stomach is affected with high acid production. Antral gastritis can progress to chronic gastric inflammation, gastric metaplasia of duodenum and finally duodenal ulcer.

Lifestyle Risk Factors

Salted food, processed meat has been studied with *Helicobacter pylori* infection and gastric cancer. The high salt intake damages mucosa within the stomach and increases the risk for *Helicobacter pylori* infection. Research reported high intake of dietary vitamin C, β-carotene and abstinence from alcohol, smoked and grilled meat have reduced the risk for developing gastric cancer in *Helicobacter pylori*-infected individuals.

Symptoms

Gastritis is clinically characterized by abdominal pain that get worse when stomach is empty. In addition, nausea, loss of appetite, frequent burping, bloating, unintentional weight loss are other symptoms of the infection.

Diagnosis

Helicobacter pylori bacterium is about 3μm long and can be demonstrated in tissue by Giemsa stain, Gram stain, haematoxylin-eosin stain and phase contrast microscopy. It also has ability to form biofilms and can be converted to a possibly Viable But Nonculturable Coccoid Form [VBNC]. It is also diagnosed by Urea breathe Test, histopathological examination of biopsy specimen, Stool Antigen test, Serology for detection of serum antibodies against *Helicobacter pylori.*

Treatment and Prevention

It is highly recommended to maintain healthy weight, physically active, eating healthy diet and limiting alcohol consumption. Accurate diagnosis and appropriate antibiotic therapy can eradicate early phase of infection. Observation from *in vitro* studies have revealed that polyunsaturated fatty acids possess a bactericidal effect against *Helicobacter pylori,* but *in vivo* effects are currently under research.

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FOOD SPOILAGE: A MAJOR ISSUE

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Many of known or unknown diseases come from spoiled food which can causes infection or any gastrointestinal disease due to injection of contaminated food and the causative agent here can be bacteria, viruses, fungi or protozoa as well. WHO stated as "any disease of an infectious or toxic nature caused by or thought to be caused by the consumption of food or water". Are said to be Food borne diseases. Spoilage may occur at any stage along food chain. Food are rich in all nutrients so they are like a proper host for microbes to live on so avoid this varieties of methods are in use from ancient times like using of oil, heating, smoking of meat and upto now in this modern era with proper packaging and adding of different preservatives. Putrefaction, infection, intoxication are terms used in spoilage of food. The habit of food storage at room temperature induces microbial growth to thrive well and resulted in consequent intoxication was predominant in the late 19th century. The growth of pathogenic bacteria can be slowed down by refrigeration to less than 40 °F, which can flourish at warmer temperatures without any notifiable indication of putrefaction to the consumer. Depending on food categories, specific microorganisms thrive, resulting in spoilage and consequent foodborne diseases. Depending on the nature of subjected foods, microorganisms will cause changes in appearance, flavor, odor, and other qualities of foods and this change in food (detoriation in food) will include a) Rancidity, b) Putrefaction and c) Detoxification. Extrinsic factors include, a) Temperature of storage, b) relative humidity of storage environment and c) Gaseous atmosphere. Intrinsic factors include, a) Water activity and b) pH activity. Other factors include, a) Enzymatic and chemical reaction

with light and water, b) Infestation by insect, pest and rodents and c) Contamination during food handling and processing.

Food spoilage causing microbes

Spoilage microbes are often common inhabitants of soil, water, or the intestinal tracts of animals and may be dispersed through the air and water and by the activities of small animals, particularly insects. It should be noted that with the development of new molecular typing methods, the scientific names of some spoilage organisms, particularly the bacteria, have changed in recent years and some older names are no longer in use. Many insects and small mammals also cause deterioration of food but these will not be considered here.

Yeast

Yeasts are a subset of a large group of organisms called fungi that also includes molds and mushrooms. They are generally single-celled organisms that are adapted for life in specialized, usually liquid, environments and, unlike some molds and mushrooms, do not produce toxic secondary metabolites. Yeasts can grow with or without oxygen (facultative) and are well known for their beneficial fermentations that produce bread and alcoholic drinks. They often colonize foods with a high sugar or salt content and contribute to spoilage of maple syrup, pickles, and sauerkraut. Fruits and juices with a low pH are another target, and there are some yeasts that grow on the surfaces of meat and cheese. There are four main groups of spoilage yeasts: *Zygosaccharomyces* and related genera tolerate high sugar and high salt concentrations and are the usual spoilage organisms in foods such as honey, dried fruit, jams and soy sauce. They usually grow slowly, producing off-odors and flavors and carbon dioxide that may cause food containers to swell and burst. *Debaryomyces hansenii* can grow at salt concentrations as high as 24 %, accounting for its frequent isolation from salt brines used for cured meats, cheeses, and olives. This group also includes the most important spoilage organisms in salad dressings. *Saccharomyces* spp. are best known for their role in production of bread and wine but some strains also spoil wines and other alcoholic beverages by producing gassiness, turbidity and off-flavors associated with hydrogen sulfide and acetic acid. Some species grow on fruits, including yogurt containing fruit, and some are resistant to heat processing. *Candida* and related genera are a heterogeneous group of yeasts, some of which also cause human infections. They are involved in spoilage of fruits, some vegetables and dairy products. *Dekkera/Brettanomyces* are principally involved in spoilage of fermented foods, including alcoholic beverages and some dairy products. They can produce volatile phenolic compounds responsible for off-flavors.

Molds

Mold s are filamentous fungi that do not produce large fruiting bodies like mushrooms. Molds are very important for recycling dead plant and animal remains in nature but also attack a wide variety of foods and other materials useful to humans. They are well adapted for growth on and through solid substrates, generally produce airborne spores, and require oxygen for their metabolic processes. Zygomycetes are considered relatively primitive fungi but are widespread in nature, growing rapidly on simple carbon sources in soil and plant debris, and their spores are commonly present in indoor air. notorious for causing rots in a variety of stored fruits and vegetables, including strawberries and sweet potatoes. Some common bread molds also are Zygomycetes. Some Zygomycetes are also utilized for production of fermented soy products, enzymes, and organic chemicals.

Penicillium and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions. They are distinguished by their reproductive structures that produce chains of conidia. Although they can be useful to humans in producing antibiotics and blue cheese, many species are important spoilage organisms, and some produce potent mycotoxins. *Aspergillus* and related molds generally grow faster and are more resistant to high temperatures and low water activity than Penicillium spp. and tend to dominate spoilage in warmer climates. Many aspergilla produce mycotoxins: aflatoxins, ochratoxin, territrems, cyclopiazonic acid.

Bacteria

Spore-forming bacteria are usually associated with spoilage of heat-treated foods because their spores can survive high processing temperatures. Other thermophiles (*Bacillus* and *Geobacillus* spp.) cause a flat sour spoilage of high or low pH canned foods with little or no gas production, and one species causes ropiness in bread held at high ambient temperatures. *Bacillus* spp., putrefaction of canned products, early blowing of cheeses, and butyric acid production in canned vegetables and fruits (*Clostridium* spp.); and "medicinal" flavors in canned low-acid foods (*Alicyclobacillus*). Psychrotolerant spore formers produce gas and sickly odors in chilled meats and brine-cured hams (*Clostridium* spp.) while others produce off-odors and gas in vacuum-packed, chilled foods and milk (*Bacillus* spp.).

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria, including species of *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Oenococcus*, some of which are useful in producing fermented foods such as yogurt and pickles. However, under low oxygen, low temperature, and acidic conditions, these bacteria become the predominant spoilage

organisms on a variety of foods. Undesirable changes caused by LAB include greening of meat and gas formation in cheeses (blowing), pickles (bloater damage), and canned or packaged meat and vegetables. Off-flavors described as mousy, cheesy, malty, acidic, buttery or liver-like may be detected in wine, meats, milk, or juices spoiled by these bacteria. LAB may also produce large amounts of an exopolysaccharide that causes slime on layer on this product. Other example - *Alcaligenes*, *Flavobacterium* and *Acinetobacter*.