General Veterinary Microbiology
VMC-211

COURSE OVERVIEW

VMC 211: GENERAL VETERIANRY MICROBIOLOGY (1+1)

Welcome to General Veterinary Microbiology. This is the first course in Microbiology for the B.V.Sc & A.H students. In theory classes, the students learn the morphology, reproduction, cultivation and genetics of the different types of microorganisms. In practical classes, the students learn to perform the staining techniques used to demonstrate bacteria and fungi. They also learn about sterilization of materials, preparation of media, cultivation methods for bacteria and fungi, biochemical tests, antimicrobial susceptibility testing and evaluation of disinfectants. This course content is developed as per the syllabus prescribed by Veterinary Council of India. Diagrams and pictures are given wherever necessary. At the end of each theory lesson a quiz and power point presentation are included. At the end of this course content, glossary, question bank and a list of reference books are given.

MODULE-1: HISTORY OF MICROBIOLOGYLEARNING OBJECTIVES

Learning objectives

To learn about

- History of development of Microbiology
- Divisions of Microbiology

INTRODUCTION

- Microbiology is the study of organisms of microscopic size called microorganisms or microbes. They are very small in size and hence can not be seen with unaided eyes. We can observe them only through a microscopes. Microbes include bacteria, fungi, algae, protozoa and viruses.
- Microbiology began when people learned to make lenses from glass and combine them to produce magnifications great enough to see microbes.
- Antony Van Leeuwenhoek (1632- 1723) of Holland got the credit for the discovery of the microbial world. He is called the father of Microbiology. He was a merchant by profession. He ground lenses and made microscopes as a hobby. He observed cells with an improved microscope.
- The resolution was enough to allow about 3 or 4 hundred X useful magnification. He put hay and pepper into water and then looked at it through his microscope. Leewenhoek saw bacteria, protozoa, yeasts and described all the microbial forms we now know, excepting viruses.
- He described the microbes he saw under vthe microscope as “animalcules” meaning little animals. He communicated his findings to the Royal Society of London where his observations were translated in English and published. In 1680 he was elected as a member of the society.

THEORY OF SPONTANEOUS GENERATION

- This theory states that living beings are produced spontaneously from non living matter.The formation of life from non living matter is called abiogenesis. It was believed that maggots would emerge from dead flesh with out eggs being laid and snakes and toads could be born in soil without prior contact with snakes or toads. Various workers had conducted experiments to disprove this theory.
- Francesco Redi (1688) an Italian physician disproved this idea of *spontaneous generation* by showing that rotting meat carefully kept from flies will not spontaneously produce maggots. He filled six jars with decaying meat and covered three of them with lids . After several days meat in the open jars had maggots while the meat in the sealed jars had no maggots.
- Spallanzani (1769), a monk, boiled and sealed broths. When he was careful no microbes developed. Needham, a Welchman of the Royal Society criticized this work. He said that other factors, excluded by Spallanzani, were needed for Spontaneous Generation, notably air. Needham conducted the same experiments but sloppily done, in which microbes grew from contamination. Spallanzani died before he could clearly disprove Needham.
- Louis Pasteur (1861) took up the challenge and utilized broths allowing air but disallowing microbes. He used broths closed with cotton and showed that the germs accumulated on cotton. He did the Swan neck flask experiment.

THEORY OF BIOGENESIS

- Rudolph Virchow (1858) claimed that living cells can only arise from preexisting living cells.This was proved by Louis Pasteur and other scientists also proved this concept called biogenesis.

GERM THEORY OF DISEASE

- Germ theory states that infectious diseases are caused by microbes. During the golden age of microbiology(1857-1914) the germ theory of disease was also proposed in opposition to the then belief that disease was a punishment for crimes or caused by demon.
- Joseph Lister proved that pathogenic microbes could be transmitted from patient to patient in surgical wards if physicians hands were not wahed, instruments were not sterilized and surgical wounds were not disinfected.

HISTORY OF THE DEVELOPMENTS

1677 Antony Van Leeuwenhoek	Observed "little animals".
1688 Francesco Redi	Disproved the idea of spontaneous generation
1796 Edward Jenner	Small pox vaccination In his time milkmaids and villagers often said that “if you want to marry a woman who will never be scarred by the pox, marry a milkmaid." Hence he thought that previous infection with cowpox could offer protection against smallpox. He collected scrapings of cowpox lesions from the fingers of Sarah Nelmes, a young milkmaid and injected it into James Phipps, an 8-year-old boy. James got mild fever and typical cow pox lesions. A few weeks after recovery, Jenner injected James with the live smallpox virus and found that the boy was protected from the disease. This method was very efficient that by 1840 the British government had banned alternative preventive measures against smallpox.. Jenner invented the word "Vaccination" for his treatment (from Latin *vacca*, a cow). Pasteur adopted this word for immunization against any disease.
1850 Ignaz Semmelweis	Advocated washing hands to stop the spread of disease
1861 Louis Pasteur	Disproved spontaneous generation
1862 Louis Pasteur	He proved that life itself did not " spontaneously come into being " through a series of experiments using a sterilized flask and showed that life can only be generated from existing life Pasteur also showed that microorganisms caused fermentation - a process used in baking and brewing. Pasteur solved the problem of rancid wines in France's vinyards. He recommended sterile technique and *Pasteurization* (became a central method for controling TB, Diptheria, and other diseases) . Pasteur conducted studies on chicken cholera and discovered one could attenuate cultures and produce artifical vaccines. He also developed vaccines for rabies and anthrax.

1867 Joseph Lister	Antiseptic surgery. By spraying carbolic acid on surgical instruments, wounds and dressings, he reduced surgical mortality due to bacterial infection considerably. Lister used antiseptics on wounds and during surgery. He showed that the healing was faster with the antiseptic treatment.He was also the first to isolate a pure culture by serial dilution: *Bacterium lactis*.
1876 Robert Koch	First proof of Germ Theory of Disease with *B. anthracis* discovery First to cultivate anthrax bacteria outside the body using blood serum at body temperature
1876 John Tyndall	Developed fractional sterilization (Tyndallization)
1881 Robert Koch	Growth of Bacteria on solid media
1882 Robert Koch	Kochs postulates The agent must be present in every case of the disease. The agent must be isolated and cultured *in vitro*. The disease must be reproduced when a pure culture of the agent is inoculated into a susceptible host. The agent must be recoverable from the experimentally-infected host.
1882 Paul Ehrlich	Developed acid-fast Staining technique
1883 Elie Metchinikoff	Discovered phagocytosis
1884 Christian Gram	Developed Gram Staining technique
1885 Louis Pasteur	First Rabies vaccination
1887 R.J. Petri	Invented Petri Dish
1892 Dmitri Iosifovich Ivanovski	Discovery of viruses
1899Martinus Beijerinck	Recognized viral dependence on cells for reproduction
1900 Jules Bordet and Octave Gengou	Discovered complement fixation reaction
1906 August Von Wassermann	Introduced Complement fixation test for syphilis
Frederick W.Twort (1915 and Felix d'Herelle (1917)	Independently discovered Bacteriophages

1928 Alexander Fleming	Discovery of Penicillin
1931 Ernst Ruska	First electron microscope
1977 W. Gilbert & F. Sanger	DNA sequencing
1983 Kary Mullis	Polymerase Chain Reaction

DISCOVERY OF CAUSATIVE ORGANISMS

Year	Disease	Organism	Scientist
1876	Anthrax	*Bacillus anthracis*	Robert Koch
1880	Typhoid	*Salmonella typhi*	Eberth
1882	Glanders	*Burkholderia mallei*	Loeffler and Schutz
1885	Tetanus	*Clostridium tetani*	Nicolaier
1894	Plague	*Yersinia pestis*	Kitasato and Yersin
1895	Fowl typhoid	*Salmonella gallinarum*	Moore
1897	Brucellosis	*Brucella abortus*	Bang

FIELDS AND DIVISIONS OF MICROBIOLOGY

Fields according to organisms studied

Bacteriology	Bacteria (singular: bacterium)
Phycology (phyco, seaweed)	Algae (singular: alga)
Mycology (myco, a fungus)	Fungi (singular: fungus)
Protozoology (proto, first; zoo, animal)	Protozoa (singular: protozoan)
Virology	Viruses

Divisions of Microbiology

- Medical Microbiology
- Veterinary Microbiology
- Plant Microbiology
- Dairy Microbiology
- Industrial Microbiology
- Soil Microbiology
- Microbiology of water and sewages
- Exobiology

MODULE-2: CLASSIFICATION OF ORGANISMS, MORPHOLOGY OF BACTERIA

Learning objectives

To learn about

- Developments in the classification of organisms
- Bacterial structure
- Bacteial shapes and arrangement.

CLASSIFICATION OF ORGANISMS

- Over the years classification systems had changed based on the information available. Greek philosopher Aristotle grouped organisms as plant or animal. Microscopic organisms were not known then. Fungi were included in plants. This system distinguished between plants and animals on the basis of movement, feeding mechanism, and growth patterns.
- In 1735 Carolus Linnaeus adopted the use of two Latin names to identify each organism, a system called binomial nomenclature. He grouped the organisms in to two kingdoms. At that time though single-celled organisms were observed they were not classified.
- Plantae containing Plants and Fungi
- Animalia containing Animals
- In 1866 Ernst Haeckel proposed a third kingdom, Protista, which included all single-celled organisms. Some taxonomists also grouped simple multicellular organisms, such as seaweeds, in Kingdom Protista. Bacteria formed a separate group within Protista called Monera.
- Protista - All single-celled organisms, such as amoebas and diatoms, and sometimes simple multicellular organisms such as seaweeds.
- Plantae - Plants
- Animalia - Animals
- In 1938 Herbert Copeland proposed a fourth kingdom, Monera, to include only bacteria.

	PROKARYOTES	EUKARYOTES		
Kingdom	Monera(Prokaryote)	Protista	Plantae	Animalia
Organisms	Bacteria	Amoebas, diatoms, and other single-celled eukaryotes, and sometimes simple multicellular organisms, such as seaweeds.	Plants Fungi	Animals

- In 1957 Robert H. Whittaker proposed a fifth kingdom – Fungi .

Kingdom	Monera(Prokaryote)	Protista	Fungi	Plantae	Animalia
Organisms	Bacteria	Amoebas, diatoms, and other single-celled eukaryotes, and sometimes simple multicellular organisms, such as seaweeds.	Multicellular, filamentous organisms that absorb food	Multicellular organisms that make food through photosynthesis	Multicellular organisms that ingest food

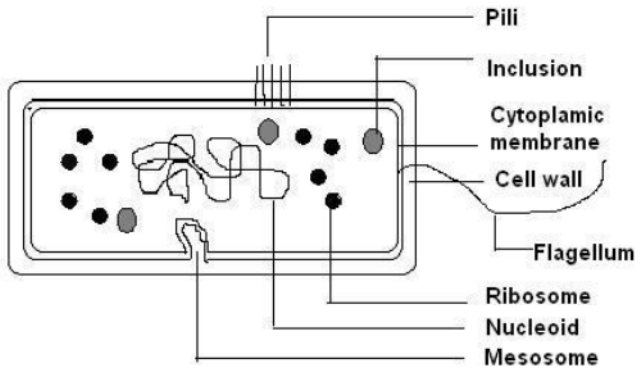
- In 1990 Carl Woese formed a new category, called a Domain, to reflect evidence from nucleic acid studies that precisely revealed evolutionary, or family, relationships. He found that a group of organisms previously classified under Bacteria belong to a separate taxon. They are the Archea . They have distinct molecular structures and physiological characteristics. They live in extremely hot, saline, or acidic anaerobic environments. He proposed three domains, Archaea, Bacteria, and Eucarya, based largely on the type of ribonucleic acid (RNA) in cells.

	PROKARYOTES		EUKARYOTES			
Domain:	Archaea	Bacteria	Eucarya			
Kingdom:	Crenarchaeota	Euryachaeota	Protista	Fungi	Plantae	Animalia
Organisms:	Ancient bacteria that produce methane	Ancient bacteria that grow in high temperatures				

BACTERIA

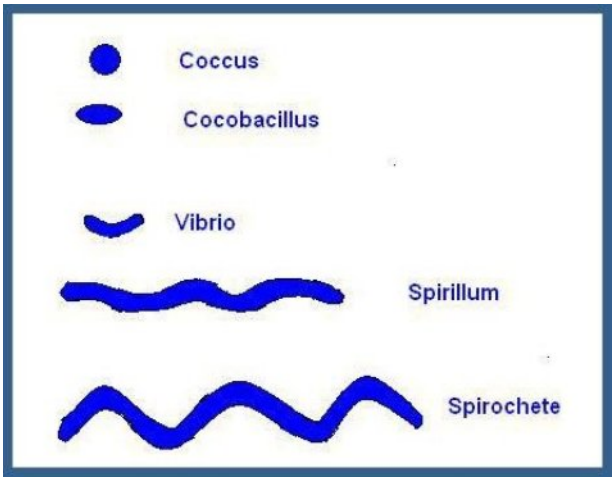
- Bacteria are very small. They have a diameter of 0.5 – 1 um. Due to this small size the surface area /volume ratio is very high compared to that of large organisms of similar shape.
- The large surface area compared to a small volume facilitates easy nourishment of all areas of the cell. So there is no circulatory mechanism to distribute the nutrients. There is little or no cytoplasmic movement with in the cell.

Diagram of bacterial cell structure



SHAPE

Spherical	cocci (coccus) *Greek word kokkos, means a berry*
Straight rods	bacilli (bacillus) *Latin word bacillus, means a stick*
Helically curved rods	spirilla (spirillum)



- Variations in these basic forms are also seen. Eg: cocobacillary, ovoid and filamentous.
- There is variation in the size of bacteria. Bacilli range from 2 - 5 µm in length and 0.5 - 1µm in width. Length of spirochetes is up to 20 µm. But their width is 0.1 – 0.2 µm. Cocci are approximately 1 µm in diameter. Based on size bacteria can be grouped as below:
  - Large bacteria – Spirochetes, *Bacillus, Clostridium*
  - Medium – *Escherichia coli, Proteus*
  - Small – *Brucella, Pasteurella, Haemophilus*
  - Very small – *Rickettsia, Chlamydia, Mycoplasma*

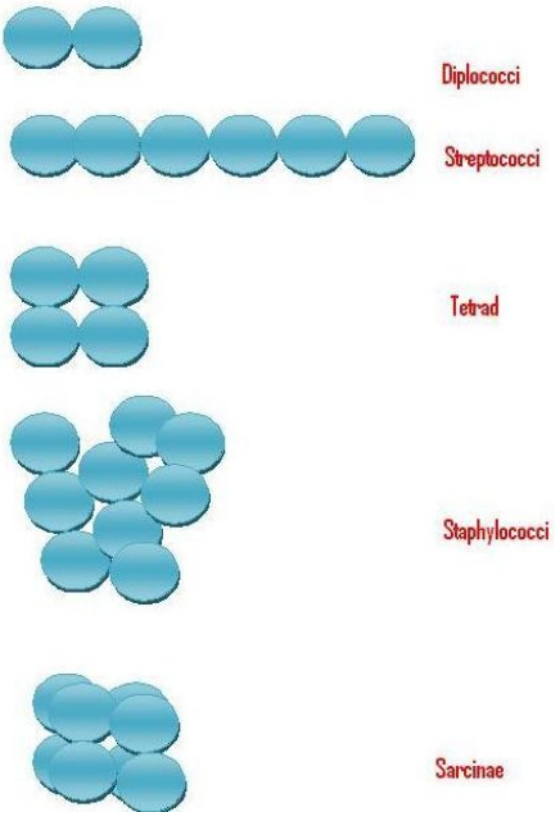
ARRANGEMENT

Bacteria are usually arranged in a manner characteristic of their species.

Cocci

It occurs in several characteristic arrangements depending on the plane of cellular division and whether daughter cells stay together following division.

- Diplococci: cells divide in one plane and remain attached predominantly in pairs.
- Streptococci: Cells divide in one plane and remain attached to form chains.

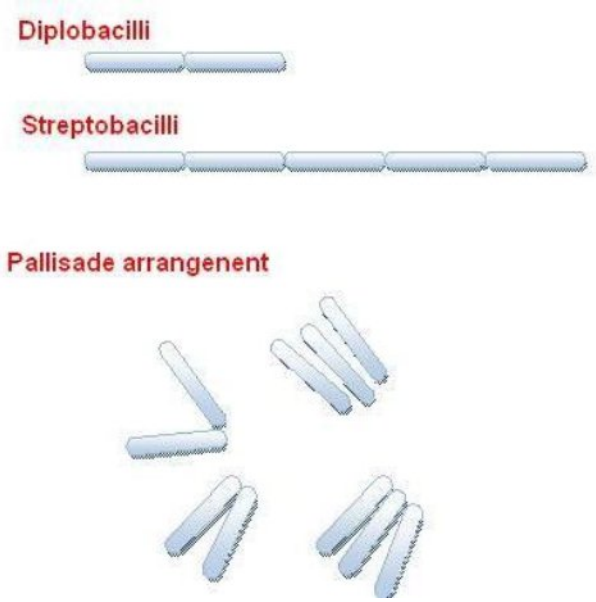


- Tetracocci: Cells divide in two planes and characteristically form groups of four cells.
- Staphylococci: Cells divide in three planes, in an irregular pattern, producing “bunches” of cocci
- Sarcinae: Cells divide in three planes, in a regular pattern, producing cuboidal arrange ment of cells.

Bacilli

They occur mostly singly or in pairs (diplobacilli). Some form chains (streptobacilli) (eg: *Bacillus subtilis*).

- Some trichomes – similar to chains but have a much higher area of contact between adjacent cells. (eg: *Saprospira species*).
- Palisade arrangement – cells are lined up side by side like match sticks and at angles to one another(corynebacterium)
- Streptomycis species form long,branched,multinucleate filaments called hyphae. The hyphae collectively form a mycelium.



- Curved rods are usually curved with a twist or turn. Bacteria with less than one complete twist or turn are called vibrioid (eg: vibrio). Those with one or more complete turns have a helical shape.
- Spirilla : rigid helical bacteria.
- Spirochetes: highly flexible. They can twist and contort their shape.

MODULE-3: STRUCTURE OF BACTERIAL CELL - STRUCTURES EXTERNAL TO CELL WALL

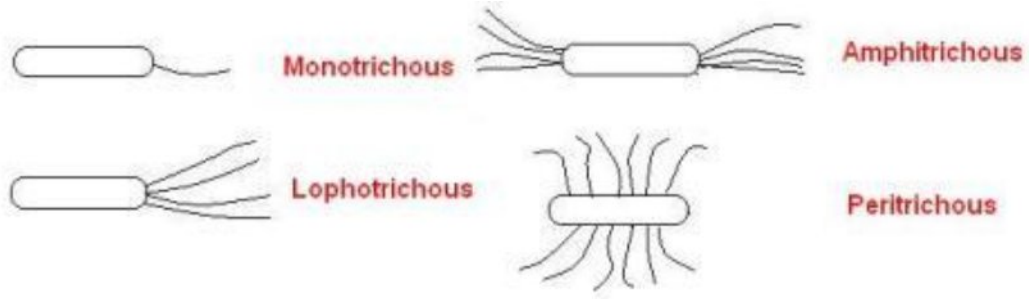
Learning objectives

To learn about the structures present outside the bacterial cell wall and their function.

FLAGELLA

Flagella are hair like helical appendages that protrude through the cell wall. They are responsible for bacterial motility. The diameter of the flagellum is 0.01-.0.02 um. It is thinner than the flagella or cilia of eukaryotes. Location of the flagella varies depending on the species.

- *Monotrichous:* Single polar flagellum. Eg: *Pseudomonas aeruginosa*
- *Lophotrichous:* A cluster of polar flagella. Eg: *Pseudomonas fluorescens*
- *Amphitrichous:* Single or clusters of flagella at both cell poles. Eg: *Aquaspirillum serpens*
- *Peritrichous:* Surrounded by lateral flagella. Eg: *Salmonella typhi*
- *Atrichous:* No flagella



STRUCTURE OF FLAGELLUM AND MOTILITY

- It is made of three parts.
  - Basal body associated with the cytoplasmic membrane and cell wall.
  - A short hook
  - Helical filament which is several times as long as the cell.

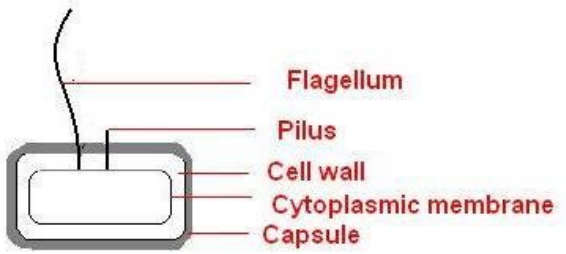
- Chemical composition of the basal body is not known. Gram negative bacteria have four basal rings. Gram positive bacteria have only two rings. Hook and filament are composed of protein subunits arranged in helical fashion. The protein of the filament is known as flagellin. Bacteria move by rotating the helical flagella. The process is analogous to rotation of a cork screw. The nature of the rotary motor that spins the flagellum is not known. The rings in the basal body are probably involved. The enery for thia motor is from proton motive force.
- Bacteria with polar flagella swim in a back and forth manner. They reverse the direction by reversing the direction of flagellar rotation. Bacteria with lateral flagella swim in a more complicated manner. The flagella form a bundle that extend behind the cell. When the motors reverse conformational changes occur along the flagella. The bundle flies apart and the cell tumbles wildly. Finally the flagellar motors resume their normal direction, the bundles again forms and the cell begins to swim in a different direction.
- This sequence of events occurs repeatedly so the motility becomes a series of swimming (runs) punctuated by periods of tumbling (twiddles) with a change in direction after each tumble.
- Spirochetes exhibit swimming motility though they lack external flagella. They have flagella like structures within the cell just beneath the outer cell envelope. They are called as periplasmic flagella. Other names are axial filament or endoflagella. Spiroplasmas also exhibit motility but they lack any organelle for motility even periplasmic flagella. Mechanism of this motility is not known.
- Gliding motility is seen in some bacteria.Eg: Cytophaga species. They are motile only when they are in contact with solid surface. They exhibit a sinuous, flexing motion. It is a slow process. The movement is only a few um/second. The mechanism of this motility is not known.

PILI (FIMBRIAE)

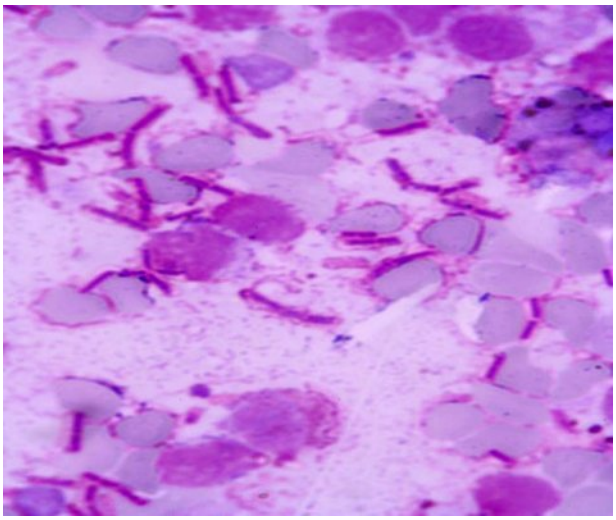
- They are hollow, non helical, filamentous appendages. They are shorter, thinner and more numerous than flagella. They are not involved in motility.They composed of a protein monomer called pilin.
- Pili are found in both motile and non motile bacteria. They play a major role in attachment of bacteria to epithelial cells lining the respiratory, intestinal and genitourinary tracts.
- This helps bacteria from being washed away by the flow of mucous or body fluids and permits infection to be established. Sex pili are involved in conjugation.

CAPSULE

- Some bacteria are covered by a viscous substance. It is called glycocalyx. This forms an envelope around the cell wall. It may be in the form of a capsule or a slime layer. Slime layer is loosely associated with the bacterial cell. It can be easily washed off. Capule is tightly attached to the cell and has definite boundaries. Capsule can be classified in to two types. If it is seen by special staining methods it is called a capsule. If it is too thin to be seen by light microscopy then it is called as microcapsule.
- Most capsules are made of polysaccharides. Capsules made of single type of sugar are called homopolysaccharides. They are synthesized outside the cell from disaccharides by extracellular enzymes.
- Capsules made of many types of sugars are called heteropolysaccharides. These are synthesized from sugar precursors that are activated within the cell, attached to a lipid carrier, transported outside and polymerized.
- A few capsules are made of polypeptides. Eg: Capsule of *Bacillus anthracis.* It is made of a polymer of glutamic acid.



Blood smear - *Bacillus anthracis* - Pink stained capsule



Functions

- Provide protection against temporary drying by binding water molecules.
- Block attachment of bacteriophages.
- Capsule is antiphagocytic. It inhibits engulfment by leuckocytes. So it contributes the virulence.
- Promote attachment of bacteria to surfaces.
- When the capsule is having compounds with an electric charge the stability of the bacteria suspension is increased. This is by prevention of cells from aggregating and settling out because cells bearing similar charges repel each other.

SHEATH, PROSTHECAE AND STALK

Sheath

- It is seen in some bacteria in fresh water or marine environments. The chains or trichomes are enclosed by a hollow tube.
- Sometimes the sheaths may be impregnated with ferric or manganese hydroxides. This strengthens them.

Prosthecae (Prostheca)

- They are semi rigid extensions of the cell wall and cytoplasmic membrane.
- The diameter of these extensions is less than that of the cell. It is seen in many aerobic bacteria in fresh water and marine environments.

Stalk

- Nonliving ribbon like or tubular appendages that are excreted by the cell. They aid in attachment.

MODULE-4: CELL WALL

Learning objectives

To learn about cell wall structure of Gram positive and Gram negative bacteria

INTRODUCTION

- Cell wall is present external to the cytoplasmic membrane. It is a rigid structure which gives shape to the cell. It prevents the cell from expanding due to uptake of water, since most bacteria live in hypotonic environments.
- Cell wall constitutes as much as 10-40% of the dry weight of the cell. It is usually essential for bacterial growth and division.
- Bacterial cells whose cell walls have been completely removed are incapable of normal growth and division.

STRUCTURE AND CHEMICAL COMPOSITION

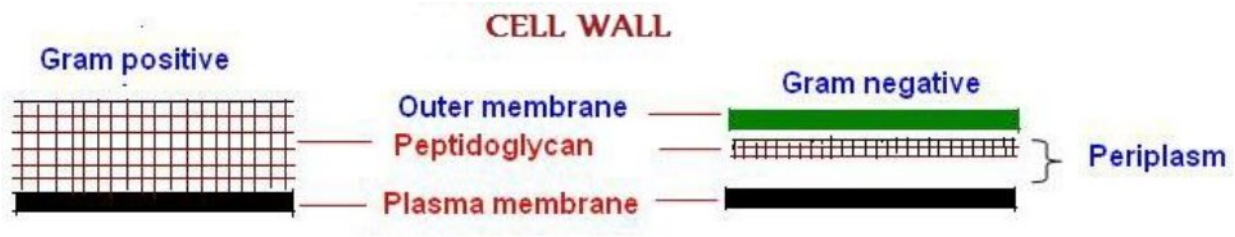
- The shape determining part of the cell wall is mainly peptidoglycan. It is also called as murein. It is an insoluble, porous, cross linked polymer of high strength and rigidity. It is found only in prokaryotes. It occurs in the form of a “bag shaped macromolecule” surrounding the cytoplasmic membrane.
- Peptidoglycan is a polymer of N-acetylglucosamine, N-acetylmuramic acid, L- alanine, D- alanine, D- glutamate and a diaminoacid. It occurs in a dynamic state. Portions of the peptidoglycan is continually degraded by wall associated hydrolytic enzymes so that new polymer can be added. Peptidoglycan structure

CELL WALL STRUCTURE IN GRAM POSITIVE BACTERIA

- The cell wall of Gram positive bacteria have higher amount of peptidoglycan than gram negative bacteria. The peptidoglycan may be 50% or more of the dry weight of some Gram positive species.
- In Gram negative bacteria it is only about 10%. Other molecules are also present in the cell wall. Cell wall of *Streptococcus pyogenes* has polysaccharides covalently linked to the peptidoglycan.
- *Staphylococcus aureus* and *Streptococcus faecalis* contain teichoic acids. Teichoic acids are acidic polymers of ribitol phosphate or glycerol phosphate. They are covalently linked to peptidoglycan.
- They bind magnesium ions which help to protect the bacteria from thermal injury by stabilization of the cytoplasmic membrane. Very little lipid is present in the cell walls of most Gram positive bacteria.
- Mycobacterium and Corynebacterium have high lipid content in the cell wall. Mycolic acids give the acid fast character. A mycolic acid derivative called cord factor (trehalose dimycolate) is toxic. It plays a role in the disease caused by these organisms.

CELL WALL STRUCTURE IN GRAM NEGATIVE AREA

- Gram negative bacteria have more complex cell wall compared to Gram positive bacteria. The main difference is the outer membrane. This membrane surrounds a thin layer of peptidoglycan. Gram negative bacteria are rich in lipids because of this membrane (11 – 22% of the dry weight of the cell wall).
- Outer membrane is an impermeable barrier which prevents escape of important enzymes such as those involved in cell wall growth from the space between the cytoplasmic membrane and outer membrane. This space is called as periplasmic space.
- Outer membrane is attached to the peptidoglycan by Braun's lipoprotein. Outer membrane is a bilayer made of phospholipids, proteins and lipopolysaccharide (LPS). LPS is toxic. It is also known as endotoxin. It is present in the outer layer of the membrane. LPS is made of Lipid A, core polysaccharide and polysaccharide O antigens.



PROTOPLAST AND SPHEROPLAST

Protoplast

- Bacterial cell without cell wall can be prepared from Gram positive bacteria by treatment with lysozyme to dissolve the cell wall or by growing the cells in the presence of an antibiotic such as penicillin which inhibits cell wall synthesis. Such cells are termed protoplasts.
- The osmotic pressure of the medium should be high to prevent the protoplasts from bursting. The protoplasts under such circumstances are soft, fragile and spherical.

Spheroplast

- In Gram negative bacteria also the peptidoglycan of the cell wall can be broken down by lysozyme or its synthesis can be inhibited by antibiotics.
- But the flexible outer membrane remains. This generates cells with two membranes, the cytoplasmic membrane and outer membrane. They are called as spheroplasts

L-forms

- They are bacterial variants with defective cell walls and irregular growth and multiplication. They are produced by the degradation of peptidoglycan by bacteriolytic enzymes or affecting its synthesis by inhibitors, or by mutations in essential genes for cell wall synthesis.

MODULE-5: STRUCTURES INTERNAL TO CELL WALL

Learning objectives

To learn about

- Structures present inside the bacterial cell wall namely, cytoplasmic membrane, mesosome, cytoplasm and nuclear material.
- Bacterial spores and structure of endospore.

CYTOPLASMIC MEMBRANE

- Cytoplasmic membrane is 7.5 nm thick. It is composed of phospholipids and protein.
- Phospholipids constitute 20-30% and proteins constitute 60-70%. Phospholipids form a bilayer.
- There are two types of proteins – *Integral* and *Peripheral proteins.*
    - Integral proteins are tenaciously held and can be removed only by destruction of the membrane by treatment with detergents.
    - Peripheral proteins are loosely attached and can be removed by mild treatments like osmotic shock.
- The lipid part of the membrane has fluidity. Hence the components can move around laterally. This fluidity appears to be essential for various membrane functions.
- Cytoplasmic membrane acts as a hydrophobic barrier to most water soluble molecules. Specific proteins in the membrane facilitate the passage of small molecules across the membrane.
- Enzymes involved in respiratory metabolism and synthesis of capsular and cell wall components are present in the cytoplasmic membrane. It is impermeable to protons and act as the site of generation of proton motive force.
- Cytoplasmic membrane is a very important functional structure. Damage by physical and chemical agents can result in the death of the cell.

MESOSOMES

- Membrane invaginations in the form of systems of convoluted tubules and vesicles are called mesosomes. They are present in many bacteria especially gram positive bacteria.
- There are two types - *Central mesosomes* and *Peripheral mesosomes*.
    - Central mesosomes penetrate deeply in to the cytoplasm. They are located near the middle of of the cell and appear to be attached to the nuclear material.
    - Peripheral mesosomes are shallow penetrations in to the cytoplasm. They are not restricted to central location and not associated with nuclear material.
    - Mesosomes are believed to play a role in cell division.
    - However electron microscopic studies reveal that mesosomes may be an artifact. Mesosome structure in electron microscopy

CYTOPLASM

- It is the cell material surrounded by the cytoplasmic membrane. It may be divided into
    - Cytoplamic area – granular in appearance.
    - Chromatic area – rich in DNA.
    - The fluid portion with dissolved substances.

- In the cytoplasm some ribosomes are free while some especially those involved in synthesis of proteins to be transported out of the cell are associated with the inner surface of the cytoplasmic membrane. The sedimentation coefficient of the bacterial ribosomes is 70 S. It is made of 50 S and 30 S subunits. (In eukaryotes - 80 S made of 60 S and 40 S subunits.)
- In some bacteria concentrated deposits of certain substances are found in the cytoplasm.
    - Volutin granules or metachromatic granules - They are composed of polyphosphate. They stain intense reddish purple with methylene blue. In electron microscopy they appear as round dark areas. They serve as reserve source of phosphate.
    - Poly beta hydroxybutyrate(PHB) granules - They are found in aerobic bacteria. They are made up of chloroform soluble lipid like material which can serve as a reserve of carbon and energy source. PHB granules can be stained with lipid soluble dyes such as Nile blue. In electron microscopy they appear as clear round areas.
    - Polysaccharide granules – They are made up of glycogen.The granule can be stained with iodine and appear brown in colour. In electron microscopy they appear as dark granules.
- Gas vacuoles are found in some bacteria living in aquatic habitats. They provide buoyancy. They appear as bright, retractile bodies in light microscope. In electron microscope they appear as hollow, rigid cylinders with more or less conical ends and have a striated protein boundary.
- Intra cellular globules of elemental sulphur are found in some bacteria growing in hydrogen sulphide rich environments.

NUCLEAR MATERIAL

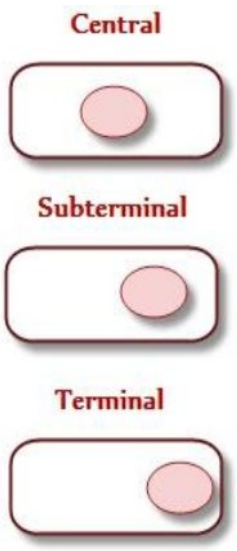
- Procaryotic cells do not have membrane enclosed nucleus. An area near the centre of the cell contains the DNA of the cell. It is considered as a nuclear structure.
- This structure is called as nucleoid, chromatin body, nuclear equivalent or bacterial chromosome.
- Nucleoid can be seen in light microscope by Feulgen staining which is specific for DNA. In electron microscope it is seen as a light area with delicate fibrillar structure.
- The nuclear material is very long sometimes more than 1000 times the size of the cell. So to accomodate this within the cell it is highly coiled.

SPORES

- *Endospore:* Spore produced within the cell
- *Exospore:* External to the cell

Endospores

- Endospores are unique to bacteria. They are thick walled highly retractile bodies produced by *Bacillus*, *Clostridium*, *Sporosarcina* and few other genera.
- Shape and location of the spore within the cell vary depending on the species – central, sub terminal and terminal.
- Examples
    - terminal -*Clostridium tetani*
    - central - *Bacillus cereus*
    - subterminal - *Bacillus subtilis*




Gram stained smear showing unstained spores

- Sometimes the endospore can be so large the cell can be distended around the endospore, this is typical of *Clostridium tetani* .
    - Endospores can not be stained by regular staining due to the impermeability of the endospore wall to dyes and stains. The rest of a bacterial cell may stain but the endospore is left colourless. Endospores can be stained by the Schaffer and Fulton stain, which stains endospores green and bacterial bodies red.
    - Endospores are resistant to dessication, heat, radiation, disinfecting agents and staining. The degree of heat resistance of endospores varies with bacterial species.
    - Most spores can resist 90 C for atleast 10 minutes. Dehydrated state and dipicolinic acid (DPA) may be responsible for heat resistance.
    - DPA is unique to endospores. DPA constitutes 10-15% of the spore's dry weight. DPA occurs in combination with large amount of calcium.
    - Calcium dipicolonate may play a role in heat resistance. DPA synthesis and uptake of calcium occur during advanced stages of sporulation.
    - Spores are usually produced by cells growing in rich media which are approaching the end of active growth.

Sporulation

- When environmental conditions are become unfavourable sporulation is started. It takes about eight hours. The DNA is replicated and a membrane wall called *spore septum* begins to form between it and the rest of the cell.
- The plasma membrane of the cell surrounds this wall and pinches off to leave a double membrane around the DNA. The developing structure is now known as a forespore.
- Calcium dipicolinate is incorporated into the forespore during this time. Next the peptidoglycan cortex forms between the two layers and the bacterium add a spore coat to the outside of the forespore.
- Sporulation is now complete, and the mature endospore will be released when the surrounding vegetative cell is degraded.

Germination

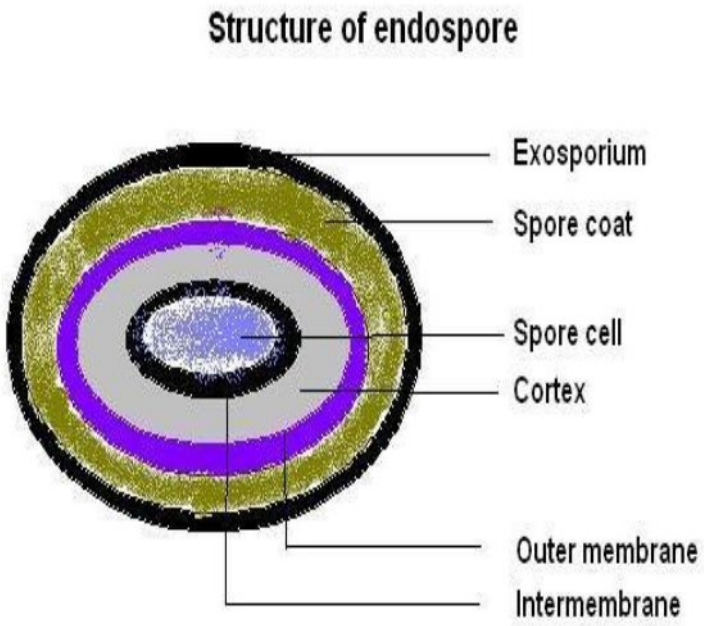
- Formation of vegetative cells from spores is called germination. During germination endospores loose their resistance to heat and staining.
- Outgrowth occurs, characterized by synthesis of new cell material and development of the organism into a new cell.

Exospores

- Methylosinus genus forms exospores by budding at one end of the cell. They are heat and dessication resistant but they do not contain DPA.

STRUCTURE OF ENDOSPORE

- Endospores are made up of a central spore cell, which is surrounded by various layers. The outermost layer is the *exosporium* - a thin protein covering.
- Below this is the spore coat which is composed of highly cross-linked keratin and layers of spore-specific proteins. The cortex consists of loosely cross-linked peptidoglycan and contains dipicolinic acid (DPA), which is particular to all bacterial endospores.
- Core - The core contains the cell wall and, cytoplasmic membrane, nucleoid, and cytoplasm. The core only has 10-30% of the water content of vegetative cells; therefore the core cytoplasm is in a gel state. The low water content contributes to the endospores success in dry environments. However, the low water concentration and gel cytoplasm contributes to the inactivity of cytoplasmic enzymes. Small acid soluble spore proteins (SASPs), are formed during sporulation and bind to DNA in the core. SASPs protect the DNA from UV light, desiccation, and dry heat. SASPs also serve as a carbon energy source during germination.



MODULE-6: BACTERIAL NUTRITION

Learning objectives

To learn about

- Nutritional requirements of bacteria
- Types 0f media
- Physical conditions required for bacterial growth

BACTERIAL NUTRITION

Nutritional requirements

- All organisms require a source of energy. Those dependent on chemical compounds for energy are called *chemotrophs*. Those which use radiant energy are called phototrophs. Both types are present in bacteria.
- All organisms require a source of electrons. Based on the source of electrons bacteria can be grouped as
    - Lithotrophs which use reduced inorganic compounds as electron donors. Chemolithotrophs and photolithotrophs.
    - Organotrophs use organic compounds as electron donors. Chemoorganotrophs and photoorganotrophs.
- All organisms require carbon in some form for synthesis of cell components. Based on the source of carbon bacteria can be grouped as
    - Autotrophs use CO2 as major or even sole carbon source.
    - Heterotrophs require organic compounds as their carbon source.

	Energy source	Carbon source	H/ electrons
Photoautotrophs (photo lithotrophic autotrophs)	Light	CO_2	Inorganic
Photoheterotrophs (photo organotrophic heterotrophs)	Light	Organic compound	Organic
Chemoautotrophs (chemolithotropic autotropha)	Chemical energy Source (inorganic)	CO_2	Inorganic
Chemohetrotrophs (chemo organotropic heterotrophs)	Chemical energy source (organic)	Organic compounds	Organic

NUTRIENTS REQUIRED

- Nitrogen is required by all organisms. Bacteria are versatile in this respect. Some bacteria can use atmospheric nitrogen. Others use inorganic nitrogen compounds such as nitrates, nitrites and ammonium salts. Some can use nitrogen from organic compounds like amino acids.
- *Water* is required by all organisms. All nutrients must be in aqueous solution before they can enter the cells. Water is a highly polar compound and efficient in dissolving and dispersing cellur components and to provide suitable conditions for metabolic reactions. High specific heat of water provides resistance to sudden transient temperature changes in the environment. It is also required for many hydrolytic reactions in the cell.

- *Oxygen* can be obtained from water, atoms in various nutrients or molecular oxygen.
- *Sulphur* is needed for synthesis of amino acids cysteine,cystine and methionine. Some bacteria require organic sulfur compounds. Some can use inorganic sulfur compounds. Some can even use elemental sulfur.
- *Phosphorus* is a component of nucleotides, nucleic acids, phospholipids, teichoic acids and other compounds. It is usually supplied as phosphate.
- All organisms require metal ions like potassium, calcium, magnesium and iron. Zinc, copper, manganese, nickel and cobalt are needed at very low concentrations. They are often called as trace elements. Iron, magnesium, zinc, manganese and cobalt are cofactors for different enzyme systems.
- Vitamins and vitamin like compounds function as coenzymes or as building blocks for coenzymes. Some bacteria can synthesize vitamins from other compounds in the medium. Others need the vitamins and will not grow without the required vitamins.

MEDIA

- *Chemically defined medium*
  - A medium composed of known chemical compounds. It is also called as synthetic medium.
  - It is needed for cultivation of autotrophs.
  - It is also useful for defining nutritional requirements of heterotrophs.
- For routine cultivation of heterotrophs complex materials like peptones, meat extract and yeast extract are used in the media. Such media support the growth of many heterotyphic bacteria.
- *Beef extract*
  - It is an aqueous extract of lean beef tissues concentrated to a paste.
  - It contains water soluble substances from animal tissues. This includes carbohydrates, organic nitrogen compounds, water soluble vitamins and salts.
- *Peptone*
  - It is prepared by digestion of proteinaecious substances like meat,casein and gelatin.
  - Digestion is done by acids or enzymes. Peptone is the principle source of organic nitrogen.
  - Vitamins and carbohydrates may also be present depending on the protein digested.
- *Agar*
  - It is the complex carbohydrate obtained from certain marine algae. It is used as a solidifying agent for media. Aqueous solution of agar gels when the temperature is reduced below 45 C. It is not source of nutrient for bacteria.Agar is used as a non nutritive solidifying agent to make solid media eg: nutrient broth(liquid) to nutrient agar (solid).

- *Yeast extract*
  - It is an aqueous extract of yeast cells. It increases nutrient quality.
  - It contains B vitamins and other growth promoting substances.
  - It is a very rich source of B vitamins.
  - It also contains organic nitrogen and carbon compounds.
- *Other supplements* – blood, serum, extracts of plant or animal tissues. They may be needed for cultivation of some fastidious heterotrophs.
- Some bacteria like *Haemophiles* require X (Haematin) & V (NAD) factors additionally.

TYPES OF MEDIA

Classification according to consistency of media

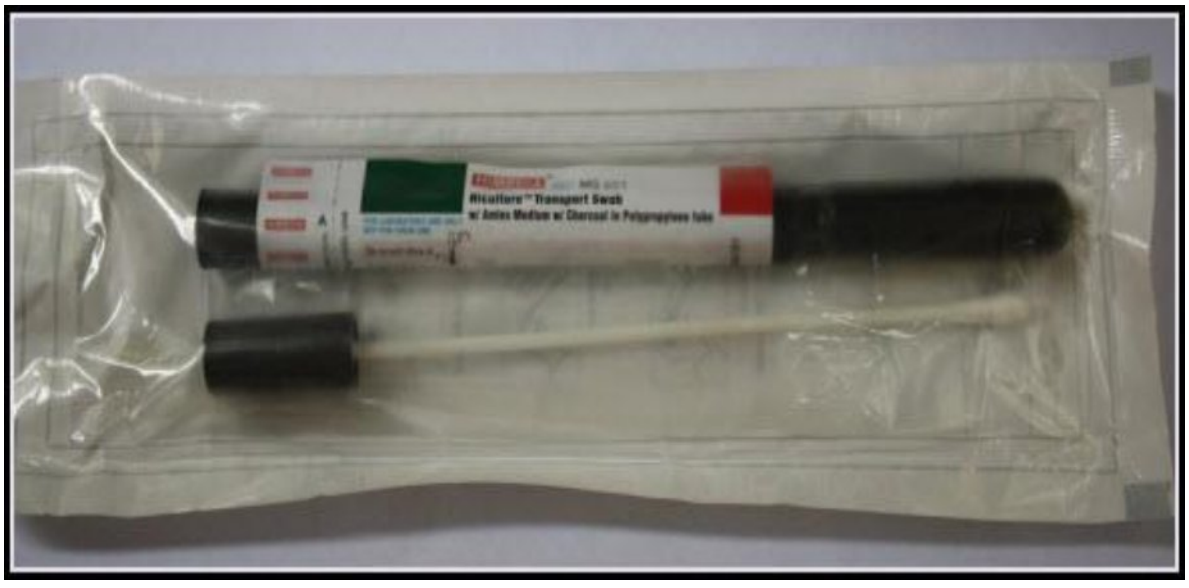
- Liquid media
  - A liquid medium is called a broth. Eg: Nutrient broth
- Semisolid media
  - The media contain agar at a concentration of 0.5 percent or less. These media have custard like consistency. It is useful for cultivation of microaerophilic bacteria and determination of motility.
- Solid media
  - They are useful for isolation of bacteria and determination of the characteristics of colonies. Agar is used at a concentration of 1.5 – 2 percent level. It forms a firm transparent gel. It is not degraded by most bacteria. Eg: Nutrient agar.
  - Classification according to the requirements
    - General purpose media/ basal media
    - Nutrient broth and nutrient agar

Composition of nutrient broth and nutrient agar

Component	Nutrient broth	Nutrient agar
Beef extract	5 g	5 g
Peptone	10 g	10g
Sodium chloride	5 g	5 g
Agar	-	15 g
Water	1000 ml	1000 ml

- Enriched media
  - These media contain extra nutrients in the form of blood, serum, egg yolk etc, to basal medium. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Eg: Blood agar, chocolate agar, serum agar

- Selective media
  - The medium is designed to suppress the growth of some microorganisms while allowing the growth of others (i.e., they select for certain microbes). Solid medium is employed with selective medium so that individual colonies may be isolated. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen to be isolatead. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these. Eg.: Mannitol salts agar (selects against non-skin flora), MacConkey agar (selects against gram-positives)
- Enrichment media
  - They differ from selective media in not out and out suppressing the growth of non-enriched microorganisms. Enrichment culture is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium. Unlike selective media, enrichment media are liquid media. Eg.: Selenite F broth, tetrathionate broth
- Differential media
  - Certain reagents or supplements when incorporated in to the media allow differentiation of different kinds of bacteria. Eg: blood agar. Some bacteria may hemolyse the red blood cells and others do not. So the hemolytic and non hemolytic bacteria can be differentiated.
- Special media
  - Media used for the cultivation of specific organisms. Eg: Dorset egg medium for Mycobacterium tuberculosis, PPLO agar for Mycoplasma, EMJH medium for Leptospira
- Transport media
  - Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors.



PHYSICAL CONDITIONS REQUIRED

Temperature

- Optimum growth temperature is the temperature that allows for most rapid growth during a short period of time (12-24 hours).
- *Psychrophiles:* organisms which grow at 0-20°C.
- *Mesophiles:* they grow best at the temperature range of 25-40°C. All pathogenic bacteria are mesophiles. Most of them grow best at 37° C.
- *Thermophiles:* they grow best at temperature above 45°C. Growth range of many thermophiles extend in to mesophilic region. They are called facultative thermophiles. Other thermophiles which can not grow in the mesophilic region are called true thermophiles or obligate thermophiles or stenothermophiles.

Gaseous requirements

- Based on oxygen requirement bacteria can be divided in to
  - Aerobic bacteria
  - Anaerobic bacteria
  - Facultative anaerobic bacteria
  - Microaerophilic bacteria
  - Capinophilic bacteria
- *Aerobic bacteria* require oxygen for growth and can grow when incubated in an air environment (eg: 21 percent oxygen).
- *Anaerobic bacteria* do not use oxygen to obtain energy. Oxygen is toxic to them and can not grow in an air atmosphere. Some tolerate low levels of oxygen. They are called *aerotolerant anaerobes.* Others can not tolerate even low levels and may die up on brief exposure to air. They are called *strict or obligate anaerobes.*
- *Facultative anaerobic* bacteria do not require oxygen but may use it for energy production if it is available. They are not inhibited by oxygen. They grow well in air atmosphere as they do in the absence of oxygen.
- *Microaerophilic bacteria* require low levels of oxygen for growth but can tolerate the level of oxygen present in air atmosphere.
- *Capinophilic bacteria* require low level (5-10%) carbon dioxide for growth.

Oxygen toxicity

- Aerobic and facultative anaerobic bacteria have mechanisms that protect them against oxygen toxicity. Microaerphiles and anaerobes are deficient.
- Toxic derivatives of oxygen
  $O_2 + e^- \rightarrow O_2^-$

$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$

$O_2^- + H_2O_2 \rightarrow O_2 + OH^- + OH.$

chelated iron

pH

- Most pathogenic bacteria grow best at a neutral or slightly alkaline pH(7.2-7.6)
- Based on optimum pH for growth bacteria can be classified as.
    - Acidophiles pH 0 – 5.5 eg. *Lactobacillus, Helicobacter pylori.*
    - Neutrophiles pH 55 – 8.0 most pathogenic bacteria.
    - Alkalophiles pH > 8.5 –*Vibrio cholerae and alcligens faecalis.*
- Obligate acidophiles, some *Thiobacillus* species, actually require a low pH for growth since their membranes dissolve and the cells lyse at neutrality. Several genera of Archaea, including *Sulfolobus* and *Thermoplasma* are obligate acidophiles.
- Yeast and molds grow rapidly in acidic pH of 4-6.

MODULE-7: BACTERIAL GROWTH AND CULTIVATION

Learning objectives

To learn about

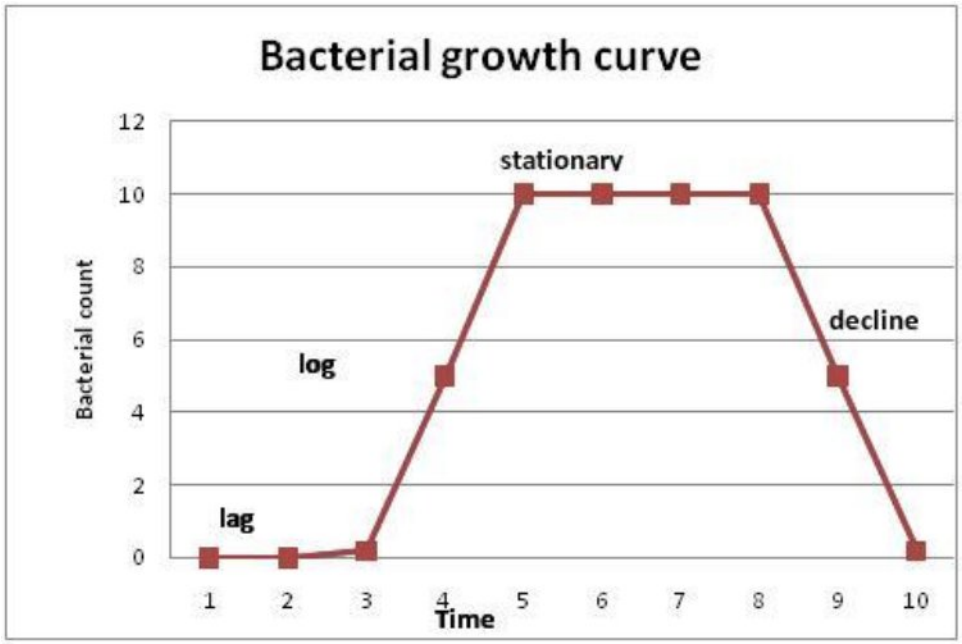
- Bacterial cell division and growth
- Growth curve
- Methods for measurement of bacterial growth

REPRODUCTION AND GROWTH

- The mode of bacterial cell division is by binary fission. The bacterial cell divides after developing a transverse septum (cross wall). It is an asexual reproduction process.
- Some bacteria reproduce by budding. In this process a small protuberance (bud) develops at one end of the cell.This bud then enlarges and finally develops in to a new cell which separates from the parent cell. Eg: *Rhodopseudomonas acidophilia*.
- Bacteria that produce extensive filamentous growth eg: *Actinomyces and Nocardia* reproduce by fragmentation of the filaments in to small bacillary or coccoid cells. They give rise to new growth.
- Before cell division nucleic acid, proteins and other macromolecules are synthesized. There is increase in cell size and mass.New cell wall components are synthesized. Then binary fission starts. The septum formation is triggered by completion of DNA replication.
- First the inward growth of cytoplasmic membrane at the middle of the cell occurs. The mesosome is usually attached to the cytoplasmic membrane at this location particularly in gram positive cells.
- It may play a role in the synthesis of new membrane material. Then the inward growth of cell occurs to form a septum leading to the separation of two daughter cells.
- In gram negative cells a pleb or fold of the outer membrane occurs at the site where the septum will be formed. The outer membrane does not invaginate until the final stages of septum formation.

BACTERIAL GROWTH CURVE

- Growth of bacteria following inoculation in to fresh medium exhibits a growth curve having four pahses. The phases are
  - lag phase
  - log phase
  - stationary phase
  - decline phase



Lag Phase

- Immediately after inoculation of the cells into fresh medium, the population remains temporarily unchanged. Although there is no apparent cell division occurring, the cells may be growing in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity.
- The length of the lag phase depends the size of the inoculum; time necessary to recover from physical damage or shock in the transfer; time required for synthesis of essential coenzymes or division factors; and time required for synthesis of new enzymes that are necessary to metabolize the substrates present in the medium.

Exponential or log Phase

- In this phase the cells are dividing regularly by binary fission and cell numbers increase in geometric progression. The cells divide at a constant rate depending upon the composition of the growth medium and the conditions of incubation. The rate of exponential growth of a bacterial culture is expressed as generation time, also the doubling time of the bacterial population. This is the prefered phase of bacterial growth for performing antimicrobial sensitivity.

Stationary Phase

- Exponential growth cannot be continued for ever. Growth is limited by exhaustion of available nutrients and accumulation of toxic metabolites or end products. Hence in stationary phase the cell numbers remain unchanged. This may be due to death of some cells and production of equal number by division or the calls have simply stopped growing and dividing. The stationary phase, like the lag phase, is not necessarily a period of quiescence. Secondary metabolites (metabolites produced after the active stage of growth), such as antibiotics are produced during the stationary phase of the growth. Spore-forming bacteria induce the activity of genes involved in sporulation process.

Death or Decline Phase

- Stationary phase is followed by the death or decline phase. During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the log phase.

Generation time

G (generation time) = t (time) / n(number of generations)

  - t = time interval in hours or minutes
  - n = number of generations (number of times the cell population doubles during the time interval)
- Generation times for bacteria vary from about 12 minutes to 24 hours or more. The generation time for *E. coli* in the laboratory is 15-20 minutes. For most known bacteria that can be cultured, generation times range from about 15 minutes to 1 hour.

Bacteria	Generation Time (Mins)	Time required for one cell to grow to a colony
Clostridium	10 Mins	8
E. coli	20 mins	16
M. tuberculosis	20 hrs	2(weeks)

CONTINOUS CULTURE

- The culture volume and the cell concentration are kept constant by allowing fresh sterile medium to enter the culture vessel at the same rate that the spent medium containing the cells is removed from the growing culture.
- By this method the rate of at which new cells are produced in the culture vessel is exactly balanced by the rate at which cells are being lost through the over flow from the culture vessel.
- Widely used continuous cultivation system is the chemostat.

MEASUREMENT OF BACTERIAL GROWTH

Bacterial growth can be measured by assessing one of the following

- Cell count
- Cell mass
- Cell activity

CELL COUNT

- It can be measured directly by direct counting using a microscope or by using electronic particle counter.
- Indirect measurement can be made by colony count.

Direct microscopic count

- Bacteria can be counted using the Petroff – Hausser chamber. It is a special slide ruled into squares that are 1/400 mm^2 in area; a glass coverslip rests 1/50 mm above the slide. So the volume over the square is 1/20,000 mm^3.
- A suspension of unstained bacteria can be counted using a phase contrast microscope. Direct microscopic count can be made rapidly with minimum equipment. Morphology of the bacteria can also be observed.

Electronic enumeration

- Bacterial suspension is placed inside an electronic particle counter in which bacteria are passed through a tiny orifice 10 - 20 um in diameter. This orifice connects the two compartments of the counter which contain an electrically conductive solution.
- When a bacterium passes through the orifice, electrical resistance between the two compartments increases. This leads to generation of an electrical signal which is counted. The disadvantage of direct counting is that there is no way to find out whether the cells are live.

Plate count

- A known volume of bacterial suspension is added to the petridish. Then the agar medium is added and thoroughly mixed by rotating the plate.
- When the medium solidifies the organisms are trapped in the medium.
- Each one grows to produce a colony. Count of the colony reveals the viable bacterial number in the inoculum.

Membrane filter count

- It is a variation of the plate count method. Membrane or molecular filters are used in this method.
- The pore size of the membrane is small to trap microorganisms. This method is very useful in counting the number of bacteria in a large volume of sample that has a very small number of viable cells eg: bacteria in large volume of air or water can be counted by simply filtering them. Then the membrane with the trapped bacteria is placed in a special plate having a pad saturated with appropriate medium.

CELL MASS

Turbidimetry

- A spectrophotometer or colorimeter can be used for turbidimetric measurements of cell mass.
- It is a simple, rapid method for following growth.
- The culture must be dense enough to register some turbidity in the instrument. Both live and dead cells contribute to turbidity.

Determination of nitrogen content

- There is approximately 14 percent nitrogen on dry weight basis.
- Cells are harvested and washed free of medium and then nitrogen estimated. This method is applicable only to concentrated populations. It is restricted primarily to research.

Determination of dry weight

- It is the most direct approach for quantitative measurement.
- It can be used only with very dense suspensions and cells have to washed free of all extraneous matter.
- Dry weight may not always be indicative of living material in cells.

CELL ACTIVITY

Measurement of specific chemical change

- In this method acid or other end products made by the bacteria is measured.
- It is an indirect approach and only applicable in special conditions.

CULTURAL CHARACTERISTICS

Colony characteristics

- *Size:* Small pin point colonies, a fraction of mm in size to large colonies (5-10 mm in diameter).
- *Margin or edge:* Evenly like the edge of a droplet, rounded projections, notches, thread like or root like projections.
- *Surface texture:*
  - Smooth (shining, glistening)
  - Rough (dull,granular or mottled)
  - Mucoid (slimy or gummy)
  - Wrinkled surface exhibited in some species.
  - Smooth to rough variation.
- *Elevation:* Thin or thick and the surface may be flat or exhibit varying degrees of convexity. Colony morphology illustration
- *Consistency:* It can be determined by touching a transfer needle to the colony.
  - Butyrous – butter like consistency
  - Viscous, stringy or rubbery
  - Some species form dry, brittle or powdery colonies that break up when touched with the needle.
- *Emulsifiability* - ability to form uniform suspension in water. Eg: Staphylococcus aureus colonies are easily emulsifiable where as Mycobacterium tuberculosis colonies are not easily emulsifiable.
- *Optical features:* Opaque, translucent or opalescent

Chromogenesis or pigmentation

- Some species produce water insoluble pigments intracellularly so the colonies become pigmented.
  - Eg : *Staphylococcous aureus* – gold
  - *Micrococcus luteus* – yellow
- Some produce water soluble pigments which diffuse in to the agar and stain it. Eg: *Pseudomonas aeruginosa* – blue water soluble pigment called pyocyanin.
- Some water soluble pigments are fluorescent ie the agar medium around the colonies glow white or blue green when exposed to UV light.
  - Eg: *Pseudomonas aeruginosa* – pyoverdin is fluorescent.

Characteristics in broth culture

- Amount of growth – scanty, moderate or abundant.
- Distribution and type of growth
  - Uniform distribution through out the medium (evenly turbid)
  - Confined to the surface of the broth as a scum or film (pellicle)
  - Accumulate as sediment which may be viscous or granular

Mucoid colonies

Dew drop like coonies (*P.multocid*

MODULE-8: DISTRIBUTION OF BACTERIA, SOURCE AND METHODS OF TRANSMISSION

Learning objectives

To know about

- Sources and routes of infection
- Transmission of micro organisms
- Toxins

SOURCES OF INFECTION

Sources of infection are animal and inanimate in nature.

Animal sources

- Normal flora
- Animals in incubation period -Animals in incubation period may excrete the pathogen.
- Animals with overt disease.
- Convalescent carrier animals – In these animals shedding of the pathogen occurs for varying periods after clinical recovery. The period may vary from weeks to months.
- Contact carrier or subclinical infections – They acquire pathogenic organisms from other animals with infectious disease without contracting the disease themselves. Such animals are called as contact or subclinical carriers. The carrier state may be temporary for a few days or lasting for months.

Inanimate sources (fomites)

- Contaminated utensils, feed and water troughs and vehicles

TRANSMISSION

- Disease can be transmitted by direct or indirect contact.
- Direct contact
  - Disease spread occurs by contact with the infectious agent on the infected host ie contact with discharges from the animal. The other means is coitus.
  - Vertical transmissiom from mother to offspring.
- Indirect contact
  - Organisms excreted by the infectedc animal are carried in various vehicles like water, milk, food, litter, air or dust. Such contaminated objects are called as fomites.
  - Contaminated instruments may also spread the infection.

PORTALS OF ENTRY

- Inhalation
- Ingestion
- Inoculation or infection through the skin or mucous membrane
- Through coitus or contaminated instruments, catheters, semen in Artificial insemination.
- Transplacenatal infection via the umbilicus
- Hospital acquired infections are called nosocomial infections.
- Physician induced infections are known as iatrogenic infections.

MICROORGANISM AND HOST

- Saprophytes and parasites
- *Saprophytism* – Living on dead or decaying organic matter.
- *Parasitism* – Living on or within another living organism. In parasites there are different host parasite relationships.
    - *Commenselism* – The organism lives on the host without causing any disease.
    - *Symbiosis* – Mutally beneficial relationship
    - Opportunistic pathogen – The organism is generally harmless but can cause disease when it gains access to other sites or tissues.
    - *Obligate pathogen* – The organism always causes disease .
- *Infectivity* – It is the capacity of the organism to become established in the tissues of the host. It is the ability to penetrate the tissues, to survive in the host defenses and to multiply and disseminate in the animal.
- *Pathogenicity* – It is the capacity of the microbial species to produce disease.
- *Virulence* – It is the measure of degree of pathogenicty. It refers to the capacity of of a microbial strain to produce disease
- Depending on the susceptibilty or resistance of the host the microorganisms will produce disease.

BACTEREMIA

Bacteremia

- Bacteremia is the presence of bacteria in blood.
- The pathogenic organisms may gain entry in to a blood capillary or venule by active or passive means from the initial site of entry.
- Once in blood stream the organism can spread to various parts of the body and cause localized infections.Eg: Leptospira can reach the kidneys by means of bacteremia.
- Organisms can directly gain access to the blood by first infecting the lymphoid system.

Septicemia

- In septicemia the bacteria in the blood stream actively multiply and produce toxic products.
- One of most severe septicemia is anthrax in which the number of bacteria in blood may often exceed the erythrocytes in blood.
- Septicemic infections often start as localized infections that later become generalized. Eg:streptococcal pharyngitis, bubonic plague.

Toxemia

- Toxemia is the presence of toxins in blood.

TOXINS

- Toxins are of two types, exotoxin and endotoxin. Exotoxins are toxic proteins secreted by living microorganisms.

Exotoxins

- Exotoxins can be divided based on site of action and the cell type affected.
- Neurotoxins – tetanus toxins, enterotoxin – *Vibrio cholera*. Diphtheria toxin kills several types of cells and hence termed cytotoxin.
- Some cytotoxins kill leukocytes and hence called leukocidins. Those which lyse the erythrocytes are called hemolysins.
- Formaldehyde treatment of exotoxins causes inactivation of the toxigenicity without affecting the antigenicity.
- The formaldehyde treated toxin is called toxoid. It induces antitoxins which react with toxins and neutralize them.Eg: tetanus and diphtheria toxoids.

Endotoxins

- The lipopolysaccharides of the Gram negative bacterial cell released in to the medium cause the toxic effect. This is due to the lysis of cells which occurs during late growth stages of culture.
- All endotoxins exhibit similar pharmacological effects. They cause pyrexia, blood changes and shock. Pyrogenicity is the ability to change body temperature - fever response.

CHARACTERISTICS OF ENDOTOXINS AND EXOTOXINS

Feature	Exotoxins	Endotoxins
Chemical nature	Protein	Lipopolysaccharide
Heat stability	Heat labile	Withstands autoclaving, heat stable
Immunogenicity	Readily converted to toxoid and neutralized by antitoxin	Can not form toxoid, neutralization with antitoxin not possible or only with difficulty
Mechanism of action	Each toxin has a highly characteristic action	All act similarly
Lethal dose	Small	Large

MODULE-9: BACTERIAL METABOLISM – I

Learning objectives

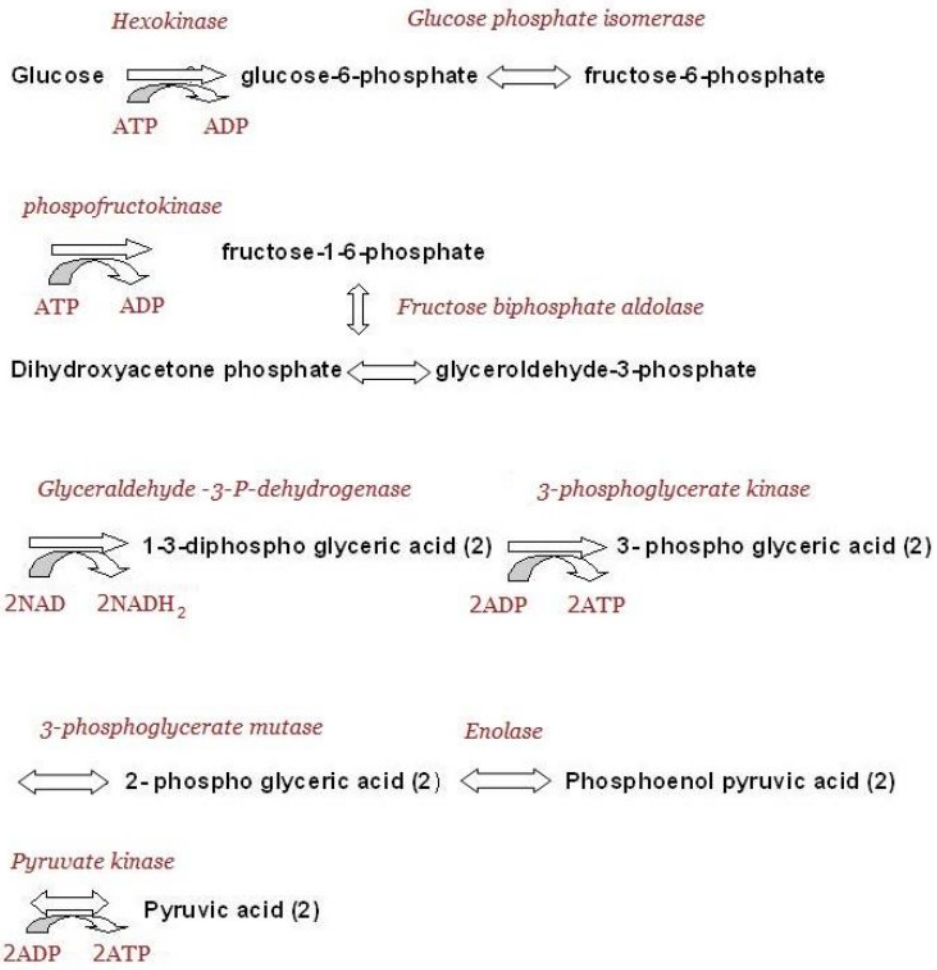
To learn about energy production in bacteria by catabolism of sugars.

EMBDEN MEYERHOFF PATHWAY OF GLYCOSIS

Embden Meyerhoff pathway of glycolysis

(Glycolysis – splitting of sugar)

It is the common pathway of glucose metabolism. Found in microorganisms as well as in animals and plants. It does not require oxygen and hence can occur both in aerobic and anaerobic cells.



Net reaction

$C_6H_{12}O_6 + 2\ NAD + 2\ ADP + 2\ Pi \rightarrow\ 2\ CH_3COCOOH + 2\ NADH_2 + 2\ ATP$

(Pi – inorganic phosphate)

PENTOSE PHOSPHATE PATHWAY

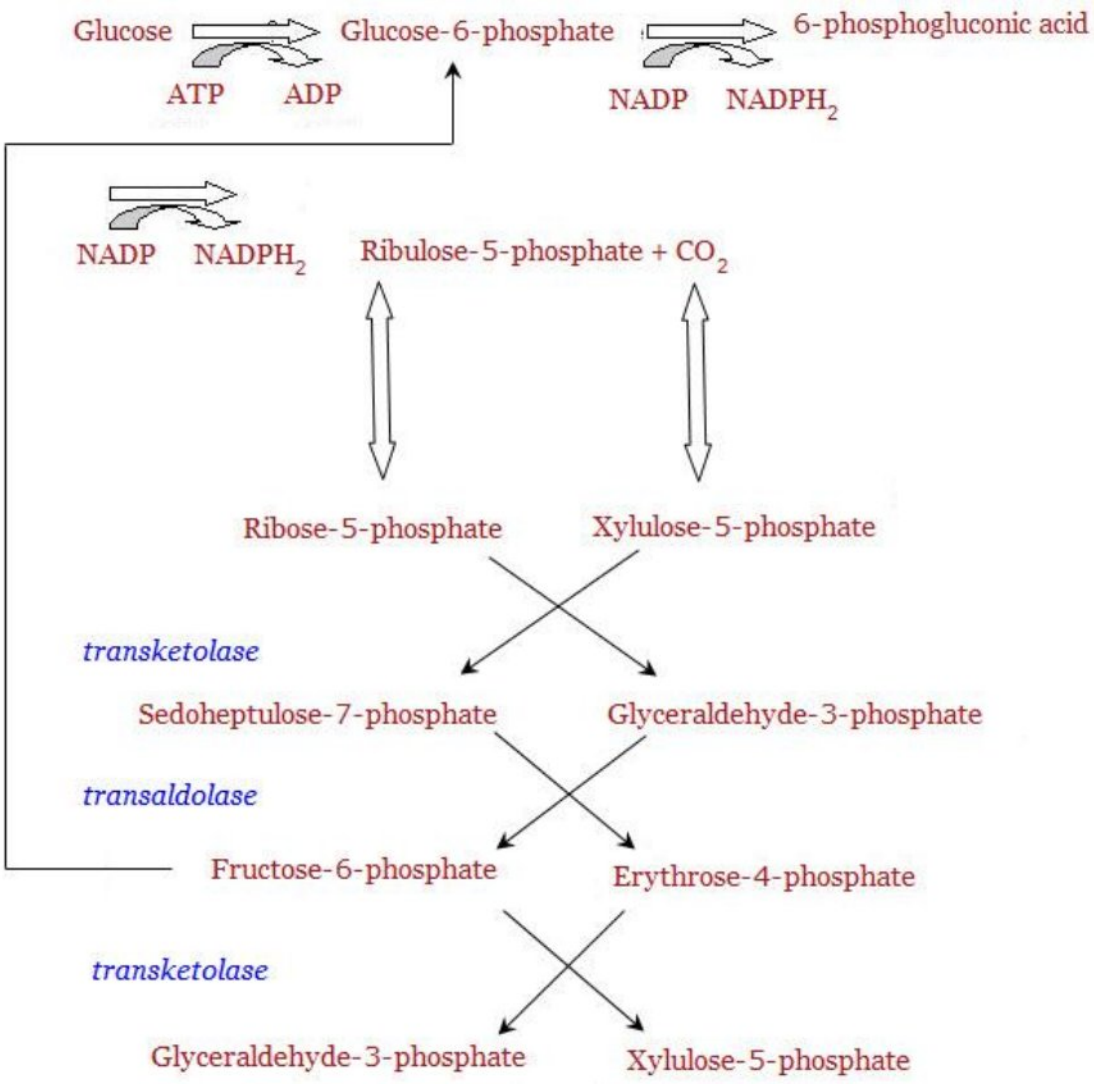
Pentose phosphate pathway

It is present in both prokaryotes and eukaryotes. Since it involves some reactions of the glycolytic pathway it is seen as a shunt of glycolysis. Hence, it is also called as hexose mono phosphate shunt. Other name is phospho gluconate pathway. It provides reducing power in the form of $NADPH_2$ and pentose phosphates for use in nucleotide synthesis. It also acts as a mechanism for obtaining energy from 5-carbon sugars.

Net reaction

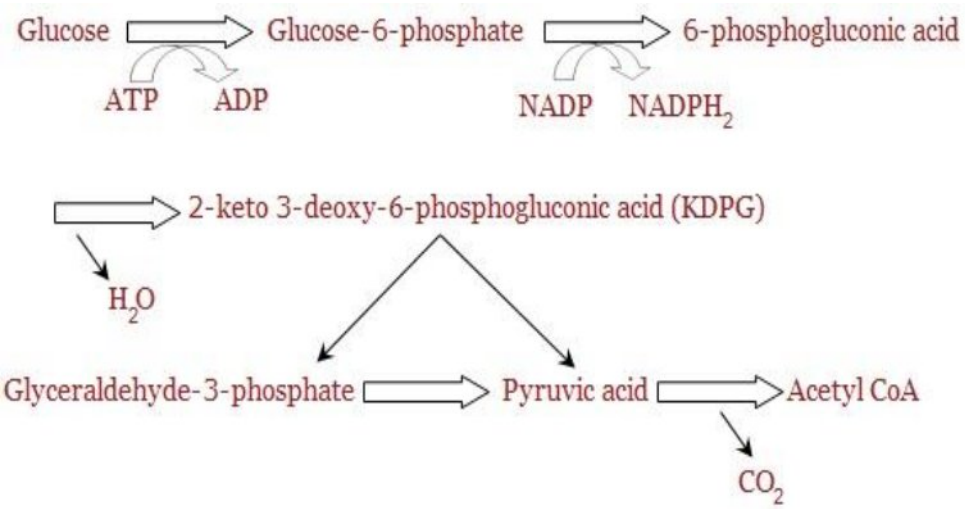
6 glucose-6-hosphate + 12 NADPH+ → 5 Glucose-6-phosphate + 6 CO2 + 12 $NADPH_2$ +Pi

It feeds into glycolytic pathway by means of fructose-6-phosphate and glyceraldehydes-3-phosphate.



ENTNER-DOUDOROFF PATHWAY

- It is found in aerobic and anerobic prokaryotes but not in eukaryotes.
- Glucose is phosphorylated to glucose-6-phosphate and then oxidized to 6-phosphogluconic acid. Glucose-6-phosphate is then dehydrated to yield 2-keto 3-deoxy-6-phosphogluconic acid (KDPG).
- KDPG is cleaved to pyruvic acid and glyceraldehydes-3-phosphate which is metabolized by some Embden Meyerhoff pathway enzymes to produce a second molecule of pyruvic acid.



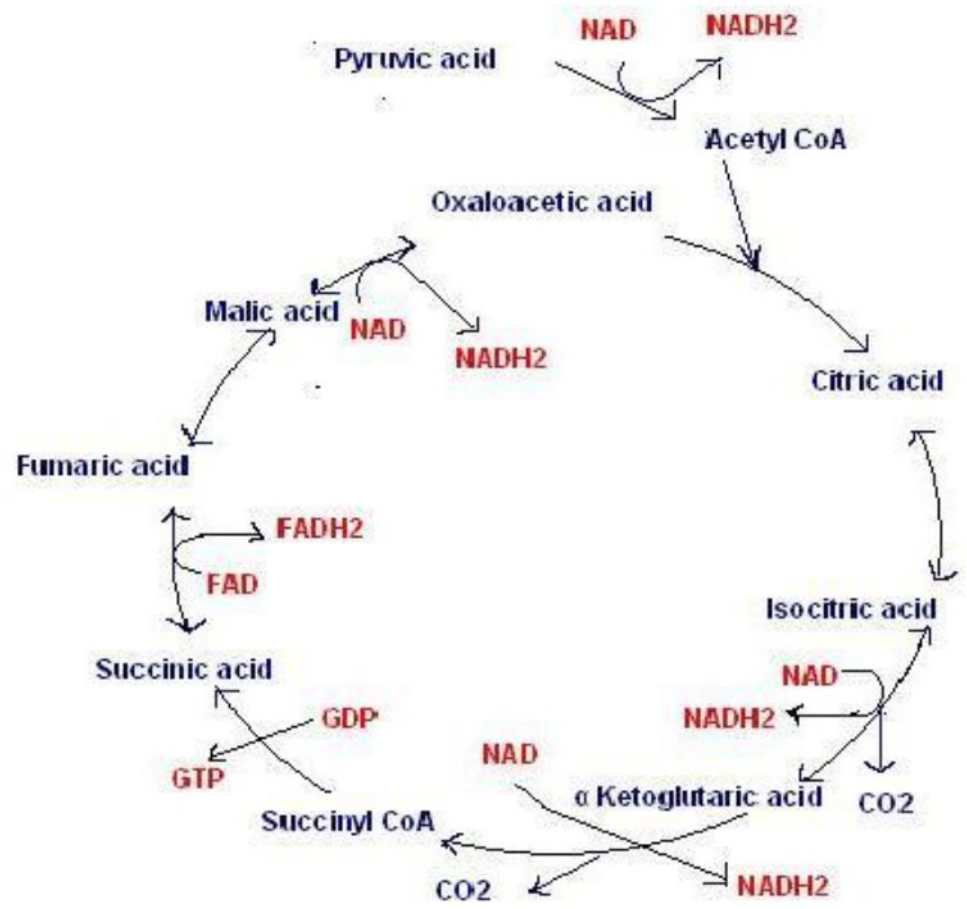
TRICARBOXYLIC ACID (TCA CYCLE)

Tricarboxylic acid cycle (TCA cycle)

It is an amphibolic cycle ie it functions not only in catabolic but also anabolic reactions. Intermediates in the cycle are precursors in the biosynthesis of aminoacids, purines , pyrimidines etc.

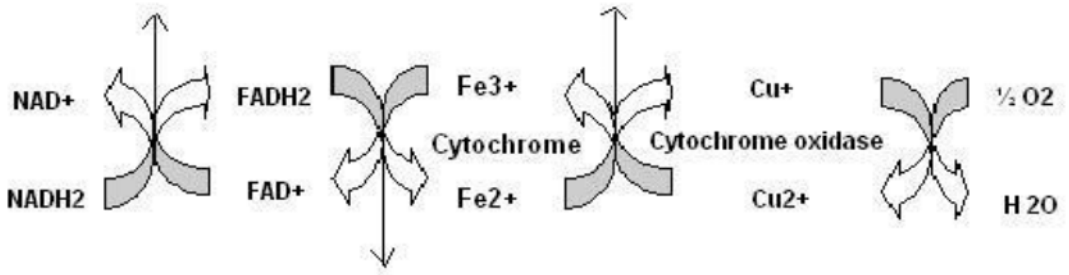
Net reaction

- Acetyl CoA + 3 H_2O_2 + 3 NAD + FAD + ADP + Pi → 2 CO_2 + CoA + 3 $NADH_2$ + $FADH_2$ + ATP
- Since two aetyl CoA are produced from one glucose molecule the overall equation is twice the above.
- Total yield of ATP from the complete oxidation of one glucose molecule is 38.



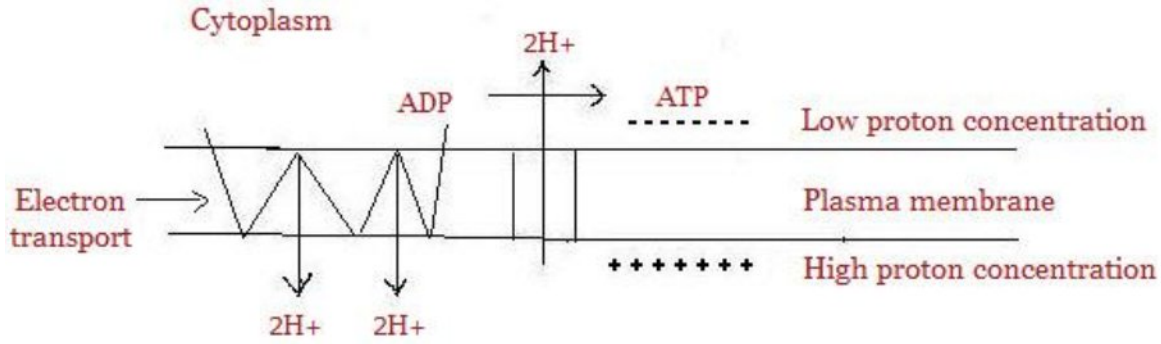
ELECTRON TRANSPORT AND THE TERIMINAL PATHWAY

- Energy is generated by passage of electrons through a series of electron donors and electron acceptors.
- The energy inherent in the substrate is released via
  - Dehydrogenase reactions
  - passage of hydrogens or electrons through the series of oxidation and reduction systems biologically useful energy in the form of ATP is generated. This is called oxidative phosphorylation.



MECHANISM OF ATP SYNTHESIS (CHEMIOSMOTIC HYPOTHESIS)

- This was proposed by British biochemist Peter Mitchell in 1961. the flow of electrons through the system of carrier molecules releases energy which drives positively charged hydrogen ions (H+) or protons across membrane - mitochondria or bacterial cell.
- This causes acidification of surrounding medium and generation of a pH gradient. In addition it produces electric potential gradient across membrane.
- In this way the energy released during transfer of electrons is conserved as “proton motive force”(pmf) - the electric potential gradient produced by pumping hydrogen ions across membrane.
- When the hydrogen ions reenter the organelle or cell they are transported by membrane bound enzyme adenosine triphosphatase. The energy released on reentry drives the synthesis of ATP.



MODULE-10: BACTERIAL METABOLISM – II

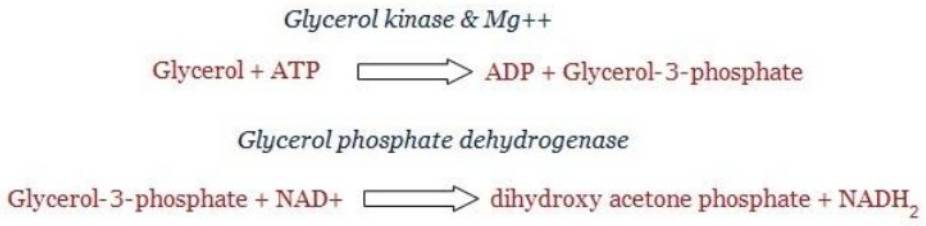
Learning objectives

To learn about

- Catabolism of lipids and proteins
- Mechanisms of nutrient transport

CATABOLISM OF LIPIDS

- Lipids are converted into intermediates of glycolytic or TCA cycle so that minimum number of additional enzymes are needed to effect complete break down. So glycolysis and TCA cycle serve as common centre around which other catabolic pathways are built. First triglycerides are split into glycerol and fatty acids by enzymes called lipases.
- Glycerol is converted to glycerol-3-phosphate which is then converted to dihydroxy acetone phisphate.



- Fatty acids are oxidized by successive removal of 2- carbon fragments in the form of acetyl CoA. This process is called as beta oxidation. Acetyl CoA enters TCA cycle and the hydrogen atoms and their electrons enter electron transport chain leading to oxidative phosphorylation. There is more energy yield per gram of fat than per gram of carbohydrates.
- Relatively few microbial species are effective in breakdown of lipids partly because of limited solubility of lipids.

CATABOLISM OF PROTEIN

- Since proteins are too large to pass into cell bacteria secrete exoenzymes called proteases to hydrolyze proteins to peptides which are then transported into cytoplasm.
- Peptidases break down peptides into individual aminoacids. Aminoacids are then broken down according to specific aminoacid and species and strain of bacteria.

- Carbon skeletons of aminoacids are oxidized to compounds that may enter TCA cycle. Entry into TCA cycle can be via acetyl CoA, alpha ketoglutaric acid, succinic acid, fumaric acid or oxaloacetic acid.

UTILISATION OF ENERGY IN NON - BIOSYNTHETIC PROCESSESS

- Much energy is used in biosynthesis of new cell components including energy storage inclusion granules such as glycogen and beta hydroxyl butyrate.
- Other uses are maintenance of physical and chemical integrity of the cell, transport of solutes across membranes and activity of locomotor organelles. The flagellar motor is driven by pmf.

TRANSPORT OF NUTRIENTS

- Nutrient transport occurs by four mechanisms. They are
  - Passive diffusion
  - Facilitated diffusion
  - Group translocation
  - Active transport

PASSIVE DIFFUSION

- In this method, solute molecules cross the membranes as a result of concentration gradient of molecules across the membrane.
- No substance in the membrane interacts with the solute molecules. Water and some lipid molecules can pass by this method.

FACILITATED DIFFUSION

- The flow occurs from higher to lower concentration but it involves a specific protein carrier molecule called *porter or permease* located in the cytoplasmic membrane.
- Carrier combines with the solute molecule and the solute complex moves across the membrane to release the solute in the inner surface. Carrier then returns to bind a new molecule of solute on the outer surface.
- This mechanism is common in eukaryotic cells but rare in prokaryotic cells.
- Both passive diffusion and facilitated diffusion mechanisms do not require energy.

GROUP TRANSLOCATION

- In this process the solute molecule is altered during transport.
- Eg:Phosphoenol pyruvate - dependent sugar - phosphotransferase system. This is present in many bacterial genera. It mediates translocation of many sugars and sugar derivatives. They enter the enzyme as sugar phosphates and are accumulated in the cell in this form.
- First heat stable carrier protein (HPr) is activated.

$$\text{PEP} + \text{HPr} \xrightarrow{\textit{Enzyme I}} \text{pyruvate} + \text{phosho HPr}$$

- The sugar combines with enzyme II at outer membrane surface and transported to inner membrane surface. Enzyme II is specific for a particular sugar and is an integral part of cytoplasmic membrane. Then it combines with phosphate group carried by the activated HPr. The sugar phosphate is released and enters the cell.

Enzyme II

Sugar + phosphor HPr → sugar phosphate + HPr

(out side cell) (inside cell)

Enzyme I

PEP + Sugar → sugar phosphate + pyruvate

Enzyme II

HPr

ACTIVE TRANSPORT

- Binding of a solute to a receptor on membrane bound carrier protein.
- Translocation of the complex across the membrane
- Coupling the translocation to an energy yielding reaction to lower the affinity of the carrier protein for the solute at the inner surface so that it will release the solute to the cell interior.

MODULE-11: BACTERIAL GENETICS

Learning objectives

To know about

- Bacterial mutations
- Methods of gene transfer
- Plasmids

MUTATIONS

- A change in the nucleotide sequence of a gene is called mutatiion.
- Cell or organism which shows the effect of a mutation is called a mutant. Un mutated cells are called wild type.

Types

- Point mutations
- Frameshift mutations

Point mutation

- Substitution of one nucleotide for another is called point mutation.
- Transition - replacement of a purine by purine or replacement of a pyrimidine by a pyrimidine
- Transversion - replacement of a purine by pyrimidine or vice versa.
- Point mutation can result in
    - Missense mutation – An altered protein is produced.
    - Nonsense mutation – Stop codon formed leading to non production of protein
    - Neutral mutation - There is no change in the protein sequence.

Frame shift mutation

- Addition or loss of one or more nucleotides in a gene is termed insertion or deletion. This results in a shift of the reading frame. Generally this causes production of nonfunctional proteins because an entirely new sequence of aminoacids is synthesized from a frame shift reading of nucleotide sequence of m RNA.

Mutagens

- Mutations commonly occur during DNA replication. Some occur as result of damages caused by UV or X ray. Account for many spontaneous mutaions. Mutation rates can be increased by exposing the culture to radiation.
- Any agent which increases the mutation rate is called a mutagen. Mutation obtained by the use of mutagens is called induced mutation.

- UV light causes formation of dimmers by cross linking between adjacent pyrimidine especially thymine residues in DNA. X rays cause break in the phosphodiester back bone of nucleic acid.
- Base analogues are chemicals similar in structure to normal DNA bases. They get substituted during DNA replication. But they donot have the hydrogen bonding properties as normal bases. This leads to introduction of errors in replication resulting in mutation eg: 5-bromouracil.
- Intercalating agents distort the structures and cause subsequent replication errors. Eg; acridine orange, proflavin and nitrogen mustards.
- Transposons cause deletions or inversions.

Repair mechanisms

- When cells exposed to lethal doses of UV light are immediately exposed to visible light photoreactivation occurs. An enzyme designated PRE induced by visible light splits or unlinks the dimmers formed because of UV light. Endonucleases and exonucleases cut out the damaged segment of DNA. Then polymerases and ligases repair the break. This is called *excision*repair.
- Inducible or SOS repair – It is used by *E.coli*. It is not a single discrete mechanism but diverse responses. All are coordinately regulated.
- Mutation can also be corrected by reverse mutation.

PHENOTYPES OF BACTERIAL MUTANTS

- Antibiotic or drug resistance
- Nutritionally deficient or auxotrophic mutants
- Changes in colonial form or ability to produce pigments
- Antigenc mutants
- Resistance to bacteriophages
- Changes in ability to produce spore, capsule or flagella
- Loss of function but retaining the intracellular enzymatic activities to catalyse the reactions. Eg: loss of permease (cryptic mutants)
- Conditionally expressed mutants
- Altered fermentation ability

BACTERIAL RECOMBINATION

- Recombination results from three types of gene transfer. They are
    - Transformation
    - Transduction
    - Conjugation

TRANSFORMATION

- Griffith in 1928 inoculated mice with a mixture of a few live rough (noncapsulated, nonpathogenic) pneumococci and a large number of heat killed smooth (capsulated, pathogenic) pneumococci. The mice died of pneumonia and live smooth bacteria were isolated from their blood. This showed that some factor responsible for pathogenicity of the smooth bacteria (even though they were dead) was transferred to the living rough bacteria and transformed them in to pathogenic smooth bacteria. The transforming factor could be passed from the transformed cells to their progeny and thus had the characteristics of a gene. Later this transforming principle was identified as DNA by Avery Macleod and Mc Carty in 1944.

- Transformation is the process in which cell-free or naked DNA containing limited amount of genetic information is transferred from one bacterial cell to another. DNA is obtained from donor cell by natural cell lysis or by chemical extraction. In the recipient cell recombination occurs. Bacteria that have inherited markers (specific characteristic) from the donor cell are said to be transformed. DNA is taken in through cell wall and cell membrane. After entry into the cell one strand of the DNA is degraded by deoxyribonucleases while the other strand undergoes base pairing with a homologous portion of the recipient cell chromosome. Since complementary base pairing takes place between one strand of donor DNA and a specific region of the recipient cell chromosome. Only closely related strains of bacteria can be transformed.
- Significance of transformation as a natural mechanism of genetic change is questionable. It is very useful in genetic studies of bacteria in the laboratory. Transformation was subsequently shown to occur in *Haemophilus ,Neisseria, Xanthomonas, Bacillus, Rhizobium* and *Staphylococci.*

TRANSDUCTION

- It is the transfer of a portion of DNA from one bacterium to another by bacteriophage.
- Phages exhibit two types of replication, lytic and lysogenic. In lytic mode after entering into the bacteria they direct the synthesis of phage genomes and proteins which assemble to produce new phages. The newly formed phages are then released by lysing the bacterial cell.
- In lysogenic mode carried out by temperate phages they have their DNA like an episome in bacteria. This viral DNA can be integrated in the bacterial chromosome. Such DNA are known as prophages.
- The bacteria having prophages can be induced by UV light and other agents to make the prophages to replicate rapidly and go through lytic cycle of growth. This leads to lysis of cells and release of virus particles.
- The phages particles may have some bacterial DNA incorporated or in some phages there will be only bacterial DNA. When such phage infects another bacterium the bacterial DNA in then is transferred to the recipient bacterial cell.
- This DNA transfer mechanism was found out by Zinder and Lederberg in 1952 when studying sexual recombination in Salmonella.

Generalized transduction

- All fragments of bacteria DNA have a chance to enter in to the phage in generalized transduction.
- Any part of the bacterial chromosome may be incorporated in to the phage head during assembly and it is usually not associated with any viral DNA.

Specialized transduction

- In this process the phages transduce only those bacterial genes adjacent to the prophage in the bacterial chromosome. This process is also called as restricted transduction.
- When such a phage infects a bacterium it carries with it certain bacterial genes which have become part of phage genome.
- The bacterial genes carried in the phage genome can then recombine with the homologous DNA of the infected cell.

CONJUGATION

- In conjugation, transfer of DNA occurs between a mating pair of cells followed by separation of the cells.
- In this process large fragments of DNA can be transferred. In *E.coli* the donor (male) cells contain a small circular piece of DNA which is extrachromosomal. It is called the sex factor or fertility (F) factor.
- These cells are referred as F^{+}. The recipient (female) cells lack this factor and are referred as F^{-}. Sex pili bind to the F^{-} cell and retract it into F^{+} cell. They also act as tubules through which DNA passes from F^{+} cell to F^{-} cell. Transfer of DNA can also occur at sites of contact between the cells.
- The donor cell replicates its sex factor and one copy of it is transferred to the recipient.
- Now the F^{-} cell is converted it into F^{+} cell and can act as a donor cell.

PLASMIDS

- *Plasmids* are double stranded, closed circular DNA molecules capable of autonomous replication. One exception to circularity is the linear plasmid present in *Borrelia burgdorferi*. Depending on number copies present in the cell plasmids are called low copy number and high copy number plasmids.
- *Episomes* are plasmids that can integrate into bacterial chromosome. F factor is an example of episome.
- Size of plasmids: 1-200 kb
- *Conjugative plasmid:* A plasmid that can mediate its own transfer to a new strain.
- *Non conjugative plasmid:* A plasmid which cannot mediate its own transfer to a new strain.
- *Cryptic plasmids:* They have no identifiable function other than self replication.
- *Resistance plasmids:* They have ability to impart resistance to drugs. R factor provides resistance to many drugs. A single R factor may carry traits for resistance to as many as seven or more chemotherapeutic agents. R factors in the normal flora of human and animals may be transferred to pathogenic organisms leading to sudden appearance of multiply resistant strains.R plamids play a major role in the development of resistance to antibiotics.

MODULE-12: CLASSIFICATION AND NOMENCLATURE OF BACTERIA

Learning objectives

To learn about

- Classification of bacteria and the methods used for classification
- Nomenclature

INTRODUCTION

- A strain consists of all the descendents of a pure culture. It is usually a succession of cultures derived from an initial colony. Species is a collection of strains having similar characteristics. Species is the basic taxonomic group. Type strain is the permanent reference specimen for the species. All other strains are considered sufficiently similar to the type strain to get included in the species. Bacterial genus is a collection of similar species. One of the species is designated as the type species. It forms the permanent example of the genus. The other species should be sufficiently similar to the type species to be included in the genus.
- The higher taxonomic groups are also formed in a similar manner. They are
  - Family – a group of similar genera
  - Order – a group of similar families
  - Class - a group of similar orders
  - Division – a group of similar classes
  - Kingdom – a group of similar divisions

METHODS USED IN CLASSIFICATION OF BACTERIA

- Three methods are used for classifying bacteria.

The intuitive method

- The microbiologist thoroughly familiar with the properties of the organism he is studying for many years decides that it represents a species or genera.
- In this the characteristics of organism that seem important to one person may not be considered important by another person.

Numerical taxonomy

- Many characteristics of an organism are studied (usually 100 – 200) and each characteristic is given equal weight. Then the percent similarity of each strain to every other strain is calculated. The formula is

$$\%S = NS/NS + ND$$

- NS is the number of characteristics that are the same (positive or negative) for both the strains. ND is the number of characteristics that are different. Sometimes a rigorous method also is used to calculate the percent similarity where the NS is taken as the number of positive characteristics that are same for both the strains. What the organisms can do is important than what they cannot do.
- The strains having a high %S are placed into groups. The groups having a high %S to each other are in turn placed into larger groups and so on. This method of classification has great practical utility and it is also relatively unbiased. It gives high degree of stability and predictability.

Genetic relatedness

- This is the most objective of all the classification methods. The DNA of the different organisms with respect to the sequence of their component nucleotides is compared.
- The methodologies used are
    - DNA homology experiments
        - The double stranded DNA from two organisms are heated to separate the strands and then mixed. This leads to the formation of heteroduplexes ie one strand from one organism will base pair with a strand from another organism.
        - If the organisms are not closely related then heteroduplexes will not be formed. This method is useful at species leve classification.
    - Ribosomal RNA homology
        - The nucleotide sequence of ribosomal RNA is highly conserved.
        - The degree of similarity can therefore be used to measure the relationship between organisms a level higher than the species.

NOMENCLATURE

- Bacteria are named as per the rules prescribed by the international code of nomenclature for bacteria.
- The name is written in Latin or latinized binomial. The first letter in the binomial is the genus name in which the first letter is capitalized.
- The second word indicates the species and it is not capitalized. Both the genus and species names are italicized in print and underlined in writing.
- Eg. *Escherichia coli*

CLASSIFICATION OF BACTERIA

- A manual on the identification of bacterial species was compiled and published in 1923 by David Bergey and colleagues in the University of Pennsylvania. It was named as Bergey's manual of determinative bacteriology. The manual was periodically revised and 8 editions were published.
- In 1984 , it was renamed as Bergey's manual of systematic bacteriology with the approach to the classification more systematic than determinative. The first edition was published in 4 volumes. The prokaryotes were placed in the kingdom prokaryotae. The kingdom was divided into four divisions.
    - *Gracilicutes* – bacteria with gram negative cell wall
    - *Firmicutes* – bacteria with gram positive cell wall
    - *Tenericutes* – bacteria lacking cell wall
    - *Mendosicutes* – bacteria lacking peptidoglycan in the cell wall
- The committee empowered for the classification of bacteria is ICTB or TCNB. International committee for taxonomy/ nomenclature of bacteria.

MODULE-13: MORPHOLOGY OF FUNGI

Learning objectives

To learn about the fungal cell structure and morphology of yeast and mould.

MORPHOLOGY OF FUNGI
INTRODUCTION

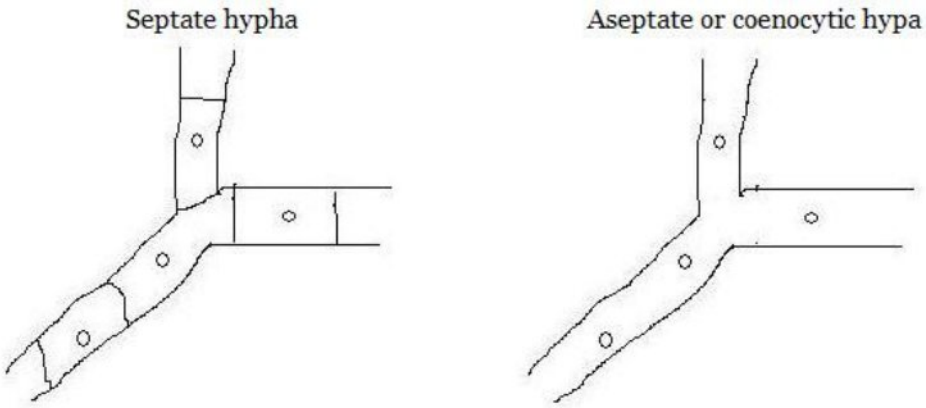
- Fungi are eukaryotic, chemoorganotrophic organisms. They do not have chlorophyll.
- There are two types, yeasts and moulds. Yeasts are unicellular. Moulds are multicellular. The thallus or body of the mould consists of filaments 5-10 um in diameter. The filaments are branched.
- The fungal cells have a true cell wall. Some fungi are dimorphic. They exist in two forms. In the host they have yeast like unicellular form. When growing saprophytically in soil or on laboratory medium they have mould form.

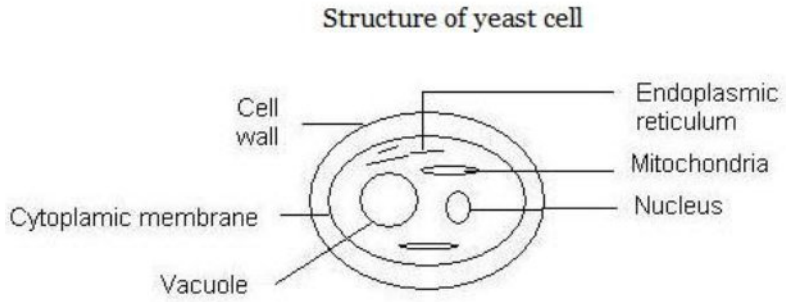
MORPHOLOGY - FUNGI

- Morphologically the fungi causing infections in animals are classified in to four groups.
- Yeast is the simplest form of fungi. They are ovoid or ellipsoid and unicelluar. On cultivation in mycotic media produces colonies similar to bacteria. Temperature of incubation is 37C. Eg: Crytococcus neoformans
- Yeast like fungi - these are fungi resembling yeast with presence of pseudohyphae. Up on cultivation in mycotic media they produce colonies similar to bacteria with fungal fringes. Optimun temperture is 37C. Eg: Candida albicans
- Moulds/filamentous fungi - this group of fungi are called true fungi because it produces true filamentous hyphae with mycelia. Optimun temperature for the growth is 22-25C. Eg: Aspergillus fumigatus
- Dimorphic fungi - one and the same fungi can exist in two different morphologies based on the conditions of the growth. At 37C they grow as yeast/yeast like organisms and at 22C--25C they grow as moulds. Eg: Sporothrix schenckii, Histoplasma capsulatum
- Yeast cells are larger than bacterial cells. They vary in size ranging from 1-5 um in breadth and 5-10 um or more in length.
- Yeast cells do not have flagella or organelle for locomotion.
- In moulds the thallus consists of the mycelium and the spores. The mycelium is a complex of many filaments called hyphae. The hypha is 5-10 um in diameter. It is made up of an outer tube like wall enclosing a cavity called the lumen. The lumen is filled with protoplasm.
- A double layered membrane the plasmalamella surrounds the protoplasm and present between the wall and the protoplasm. The hypha is divided into cells by cross walls. They are formed by the centripetal invagination from the cell wall.
- The cross walls constrict the plasma membrane and grow in ward to form an incomplete septum which has a central pore through that protoplasmic streaming occurs. Even nuclei can migrate from one cell to another in the hypha.

HYPHA

- Three forms of hyphae
  - Nonseptate or coenocytic- they do not have septa.
  - Septate with cells having single nuclei.
  - Septate with multinucleate cells. Each cell has more than one nucleus in each compartment.
- Mycelia are of two types – vegetative and reproductive. Some hyphae in the vegetative mycelium penetrate in to the medium to obtain nutrients.
- Soluble nutrients are absorbed through the wall. Insoluble nutrients are first digested by the secreted enzymes. Reproductive mycelia usually extend from the medium into air. They form the spores.





CELL WALL

- The cell wall is the important structure in fungus. It determines the shape and the process of fungal morphogenesis. The wall consists of microfibrils made of hemicellulose and chitin. The matrix material of the wall where the microfibrils are embedded consists of proteins, lipids and other substances.
- Many varieties of fungi share the same polysaccharides and hence have the same surface antigens. But many unique antigenic determinants resulting from the different branching patterns of the polysaccharide are also found within a certain group. These are useful for classification.

CAPSULE

- Some fungi have an external coating of slime or a more compact capsule.
- They are composed of amorphous polysaccharides that may cause the cells to adhere and clump together.
- The capsule may be antigenic and antiphagocytic in some fungi.

CYTOPLASMIC MEMBRANE

- It is similar in structure and composition to the cell membranes of higher eukaryotes.
- The fungal membrane contains mainly ergosterol and zymosterol. Mammalian cell membrane has cholesterol.

MODULE-14: REPRODUCTION IN FUNGI AND CLASSIFICATION

Learning objectives

To learn about

- Asexual and sexual reproduction in fungi
- Fungal spores
- Classification of fungi

REPRODUCTION ASEXUAL

- Fungi reproduce by many ways.

Asexual reproduction

- In asexual reproduction or somatic or vegetative reproduction there are no sex cells or organs and it does not involve union of nuclei. It can occur by
  - fission of somatic cells or
  - budding of somatic cells
  - fragmentation or disjointing of hyphal cells
  - spore formation
- Asexual spores are produced in large numbers.
- The different types of asexual spores are
  - *Sporangiospores* – single cell spores formed within structures called sporangia at the end of special hypha called sporangiophores. Motile sporangiospores are called zoospores. They have flagella.
  - *Conidiospores or conidia* – small single celled conidia are called microconidia. Large multinucleated conidia are called macroconidia. Conidia are formed at the tip or side of a hypha.
  - *Arthrospores or oidia* – single celled spores formed by disjointing of hyphal cells.
  - *Chlamydospores* – thick walled single celled spores formed from cells of vegetative hypha. They are resistant to adverse conditions.
  - *Blastospores* – they are formed by budding.

SEXUAL REPRODUCTION

- Sexual reproduction occurs by fusion of the compatible nuclei of two parent cells. The process begins with the joining of two cells and fusion of protoplasts (plasmogamy). The two haploid nuclei fuse together to form a diploid nucleus. Then meiosis occurs to reduce the number of chromosomes to the haploid number.
- The sex organs are called gametangia. They may form differentiated sex cells, gametes or may contain instead one or more gamete nuclei. If the male and female gametangia are morphologically different, the male gametangium is called antheridium and the female gametangium is called the oogonium.
- The different methods of sexual reproduction are
  - gamete copulation – fusion of naked gametes one or both of which are motile.

  - gamete – gametangial copulation – gametangia come in to contact but do not fuse. The male nucleus migrates through a pore or fertilization tube in to the female gametangium.
  - gametangial copulation – two gametangia or their protoplasts fuse and give rise to a zygote that develops in to a resting spore.
  - somatic copulation – fusion of somatic or vegetative cells.
  - spermatization – union of a special male structure called spermatium with a female receptive structure. The spermatium empties its contents in to the latter during plasmogamy.
- Sexual spores occur less frequently and in small numbers than asexual spores. The different sexual spores are
  - Ascospores – single celled spores produced in a sac called ascus. Usually eight ascospores in each ascus.
  - Basidiospores – single celled spores are borne on a club-shaped structure called a basidium.
  - Zygospores – large thick walled spores formed when the tips of two sexually compatible hyphae
  - Oospores – formed in a special female structure called oogonium. Fertilization of eggs or oospheres by male gametes formed in an antheridium gives rise to oospores. One or more oospores are present in each oogonium.

CLASSIFICATION OF FUNGI

- Classification of fungi is mainly based on the characteristics of the sexual spores and fruiting bodies present during the sexual stage of reproduction.
- The complete or perfect life cycles of many fungi are not known yet. Hence the imperfectly described fungi must be classified on bases other than the characteristics of the sexual stage.
- The morphology of the asexual spores and the thalli become important their sexual traits are found out. So the imperfect higher fungi are provisionally placed in a special class called form-class *Deuteromycetes*.
- Taxonomy of fungi follows the Committee on International Rules on Botanical Nomenclature.
  - Division: -*mycota*
  - Subdivision: -*mycotina*
  - Class: -*mycetes*
  - Subclass: -*mycetidae*
  - Order: -*ales*
  - Family: -*aceae*
  - Genera and species have no standard endings.
- *Division I Gymnomycota* (ingest particulate nutrients and lack cell walls in vegetative stage)
- *Division II Mastigomycota* (flagellated lower fungi)
- *Division III Amastigomycota* (terrestrial fungi, non-motilte)
- Subdivision Zygomycotina
  - Nonseptate, asexual and sexual reproduction present
- Class
- Order Mucorales
  - Asexual reproduction by sporangia , bread mould (*Rhizopus, Absidia,Mucor*)
- Order Entomophthorales
  - Asexual reproduction by conidia
- Subdivision Ascomycotina
  - Septate mycelium, sexual reproduction by sac-like structure called ascus.
- Subdivision Basidiomycotina
  - Septate mycelium, sexual reproduction by basidium

- Subdivision Deuteromycotina (Fungi imperfecti)
  - Septate mycelium, sexual reproduction not found.
- Class Blastomyces
  - Imperfect yeasts Eg: *Candida, Malassezia, Trichosporon*
  - Some have true hyphae and others pseudohyphae.
- Class Hyphomycetes
  - Produce septate hyphae, reproduce asexually by conidia, most pathogenic fungi Eg : *Aspergillus, Blastomyces, Geotrichum, Histoplasma, Microsporum, Trichophyton, Penicilium* and many darkly pigmented (dermatiaceous) fungi.

Classification based on disease

- Pathogenic fungi can be grouped into those causing Superficial mycoses, cutaneous mycoses, subcutaneous mycoses and systemic mycoses.
- Superficial mycoses are limited to the outermost layers of the skin and hair.
- Cutaneous mycoses extend deeper into the epidermis, as well as invasive hair and nail diseases.
- Subcutaneous mycoses involve the dermis, subcutaneous tissues, muscle and fascia.
- Systemic mycoses originate in the lung and may spread to many organ systems.

MODULE-15: GROWTH AND NUTRITION OF FUNGI

Learning objectives

To learn about nutritional requirements and cultivation of fungi.

GROWTH AND NUTRITION

- Fungi can withstand certain extreme conditions than other organisms. Moulds can grow in medium containing high concentration of sugar that inhibits most bacteria. Fungi also can tolerate more acidic conditions that other microbes. They are usually aerobic organisms. Some yeasts are facultative. They can grow both under aerobic and anaerobic conditions. The optimum temperature for saprophytic fungi is 22-30 C. For pathogenic fungi it is 30-37C. Some fungi can even grow at 0 C and can cause deterioration of meat and vegetables in cold storage.
- Fungi are heterotrophic. They can not use inorganic compounds like carbon dioxide as their sole carbon source. Carbon must come from an organic source such as glucose. Some fungi can use inorganic compounds of nitrogen but all fungi can use organic nitrogen.
- Most fungi can grow aerobically in the usual bacteriological media at temperatures ranging from 20 -30 C. Since many fungi grow more slowly compared to bacteria in media which support growth of bacteria and fungi, fungi may be over grown by bacterial contaminants in a mixed culture. Hence for isolation of fungi the medium which favours their growth but not suited for bacterial growth is preferred. Acidic media having high sugar concentration are tolerated by moulds but not bacteria.

MEDIA AND CULTIVATION

- Media used for the cultivation of fungi are called as mycotic media. They can be classified into three groups
- Natural media: Presterilized natural substance can be used for the cultivation of fungi. Eg: Rice, straw, sugar cane baggasises, hair, paddy, grains and dung.
- Semisynthetic media: This group of media contain natural and synthetic substances in its composistion. Eg: Potato dextrose agar, Corn meal agar
- Synthetic media: this group of media contain synthetic organic substances. Eg: Sabouraud's dextrose agar. It is one of the best known and oldest medium for fungal cultivation was formulated by Sabouraud. This medium contains maltose and peptone as main constituents.
- The common modification suggested by Emmon's contains less concentration of glucose. It is widely used for isolation of moulds and yeasts. It is useful for growing pathogenic fungi from infected body fluids and exudates.
- The partial selective action of fungal media is due to the high sugar concentration and low pH (pH 5.6 - 6.4).
- Inoculation of a small fragment of mycelium on the medium is enough to start new mould colony. A transfer needle is used for inoculation.
- The needle used for moulds is stiffer and has a flattened tip for cutting the mycelium. For inoculation of yeasts ordinary inoculating loop used for bacterial cultures is sufficient.
- For the cultivation of mould point inoculation is employed where as for the cultivation of yeast streak plate method of isolation is followed.

MODULE-16: GENERAL PROPERTIES OF VIRUS

Learning objectives

To learn about

- Composition and symmetry of viruses
- Bacteriophage,Viriods and Prions

INTRODUCTION

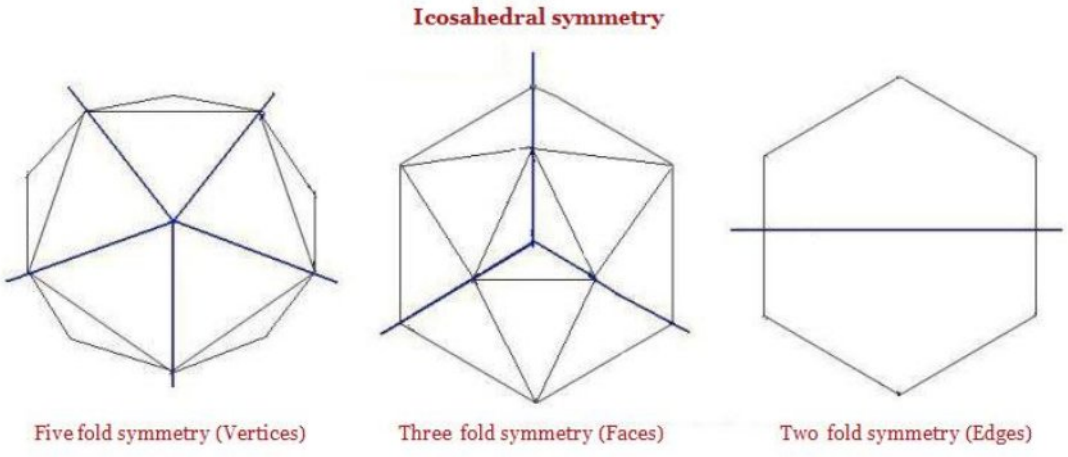
- Viruses are obligate intracellular parasites which are ultramicroscopic in size. They can reproduce only in living cells. In its simplest form the virus particle is made of nucleic acid enclosed in a protein coat. The nucleic acid is called genome and the protein coat is called capsid. In some viruses there is an additional covering over the capsid called envelope.
- The envelope is made of lipid bilayer derived from the host cell. Some viral derived proteins are studded in the envelope. The outermost proteins of the virus allow the virus to recognize the correct host cell and gain entry into the cytoplasm. The viral genome nucleic acid is either DNA or RNA.

SYMMETRY

- Viruses show basically four types of symmetry, icosahedral, helical.complex and binal symmetry.

Icosahedral

- An icosahedran is a polyhedran made of 20 triangular faces with 5 each at the top and bottom and 10 at the middle. There are 12 vertices and 30 edges. Each triangle is symmetrical and can be inserted in any orientation which ever way it is inserted. The vertices have five fold symmetry. This means that rotation of the icosahedran by one fifth of a revolution presents a position which is indistinguishable from the starting orientation. Each of the 20 faces has a three fold axis of symmetry and each of the 30 edges has a two fold axis of symmetry.



Helical

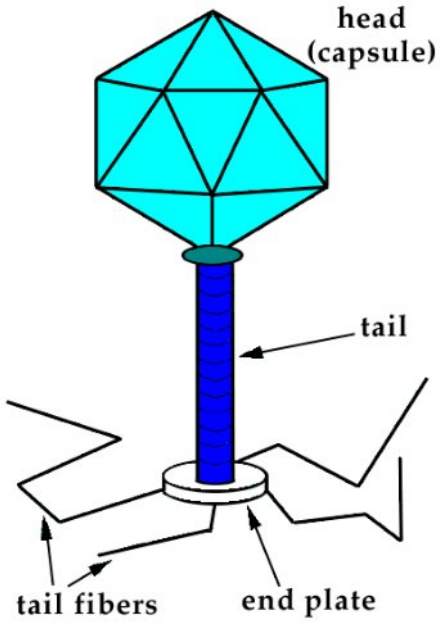
- In this type of symmetry the capsomeres are set in a helical manner around the genome nucleic acid.

Complex

- Certain viruses have complex symmetry. Eg: pox viruses have the most complex virion structure containing different proteins and lipoproteins.

Binal symmetry

- Certain viruses have binal symmetry. Eg: bacteriophages - viruses which infect bacteria. They have an icosahedral head with a helical body.



BACTERIOPHAGES

- Bacteriophages are viruses that infect bacteria. They were discovered independently by Frederick W.Twort in England in 1915 and by Felix d' Herelle at Pasteur institute in France in 1917.
- Twort discovered that bacterial colonies some times underwent lysis and that this effect could be transmitted from colony to colony. Even high dilutions of the material from a lysed colony after filtration through bacterial filter could transmit the agent.
- Twort suggested that the agent might be a virus. D'Herelle rediscovered this phenomenon in 1917 and coined the word bacteriophage meaning "bacteria eater".

LYTIC AND LYSOGENIC INFECTION

- In lytic infection by a phage the virus replication occurs and the progeny are released by lysing the bacterial cell.
- In lysogeny the viral DNA is incorporated in to the host DNA and becomes a prophage in the bacterial chromosome.
- The bacterium multiplies normally and the viral DNA is transmitted to daughter cells in successive generations. Some times the viral DNA is removed from the host chromosome and the lytic cycle occurs. This process is called *spontaneous induction*.
- Infection of the bacterium with temperate phage can be detected by the resistance to infection by the same or related phages and induction to produce phage particles. Induction can be done by exposure to UV light or some chemicals.

VIRIODS

- Viriods are nucleic acid entities of low molecular weight ($1.1 – 1.3 \times 10^5$) having unique structure. They cause many important diseases of plants. Eg: potato spindle tuber and cucumber pale fruit diseases.
- Viriods are the smallest known agents of infectious disease. They do not have a protein coat and exist only as short infectious molecules of RNA.
- Some are single stranded covalently closed circular RNA molecules and some are single stranded linear RNA molecules. It is not known how they cause disease. They may cause host symptoms by interference with gene regulation in infected host cells.

PRIONS

- Prions are composed of protein only. Nucleic acids have not been detected. They are small, proteinaceous particles. Examples of prion diseases are
    - Human - Kuru, Gerstmann-Straussler
    - Sheep - Scrapie
    - Cattle - Bovine Spongiform Encepalopathy (BSE)

MODULE-17: CLASSIFICATION, VIRAL HAEMAGGLUTINATION, INTERFERENCE

Learning objectives

To learn about

- Virus classification
- Virus cell interactions

CLASSIFICATION

- Initially virus classifications were made based on the host normally infected and also based on trophism. Eg: neurotrophic virus (having affinity for nerve tissue). Later when the methods for studying the physical, chemical and biological properties of viruses were developed more information on viruses became available to develop a classification system on the basis of these properties. The main properties used are nature of
- Nucleic acid (DNA or RNA, single or double stranded, single or segmented nucleic acid, positive or negative sense and molecular weight)
- Structure of virion (helical, icosahedral or complex symmetry, enveloped or non enveloped, complexity; number of capsomeres for icsahedral, diameter of nucleocapsids for helical virions)
- Site of replication (nucleus or cytoplasm)
- The other properties used are host range, trophism, mode of transmission, surface structures (eg: antigenic properties).
- In 1971, David Baltimore proposed a virus classification based on how the viral genome is replicated and expressed. Viruses were grouped into six classes based on their method of transcription(m RNA synthesis). This grouping is based primarily on whether the genome is single or double stranded and whether the genome is converted to an intermediate form or not before the m RNA is produced.
- An International Committee on Nomenclature of Viruses (ICNV) was formed at the ninth International Congress for Microbiology in 1966. This committee is now called International Committee on Taxonomy of Viruses (ICTV).

DNA VIRUSES

Viruses with ds DNA genome

- Family: *Adenoviridae*
  - Non enveloped, icosahedral with fibre protein at each vertex, ds stranded linear DNA, replication in nucleus
- Family: *Asfarviridae*
  - Enveloped,200 nm icosahedral particle with 70 nm isometric core, linear ds DNA, cytoplasmic replication.
  - African swine fever virus
- Family: *Herpesviridae*
  - Enveloped 200 nm particle with spikes,enclosing a tegument and an icosahedral nucleocapsid of 100 nm. Linear ds DNA, replication in nucleus. Large family, latency common, some are oncogenic.
  - Three subfamilies – alphaherpesvirinae, betaherpesvirinae and gammaherpesvirinae.

- Family: *Papilomaviridae*
  - Non enveloped, 55 nm icosahedral, covalently closed circular ds DNA genome, replication in nucleus, some are oncogenic.
- Family: *Polyomaviridae*
  - Nonenveloped, 40-45 nm icosahedral, covalently closed circular ds DNA , replication in nuceus oncogenic.
  - Simian virus 40 (SV 40)
- Family: *Poxviridae*
  - Brick shaped or ovoid virions of 220-450 nm x 140-260 nm wide, some enveloped. Complex structure enclosing two lateral bodies and a biconcave core. Linear ds DNA genome, cytoplasmic replication.
  - Two subfamilies – Chordopoxvirinae (viruses of vertebrates) and Entomopoxvirinae (viruses of insects).

Viruses with ss DNA genomes

- Family: *Circoviridae*
  - Nonenveloped small icosahedral, circular ss DNA genome
  - Porcine circovirus 1 and chicken anaemia virus
- Family: *Parvoviridae*
  - Nonenveloped icosahedral 18-26 nm, linear ss DNA genome replication in nuceus.
  - Two sub families – Parvovirinae (viruses of vertebrates) and Densovirinae (viruses of mainly insects)

Viruses with DNA genome that replicate through RNA intermediate

- Family: *Hepadnaviride*
  - 42-50 nm enveloped particle with projections, isometric nucleocapsid with DNA polymerase and protein kinase activities, partially ds DNA that is not covalently closed. Complete negative sense strand of 3000 nt and a variable length positive sense strand of 1700 – 2800 nt, contains reverse transcriptase.
  - Hepatitis B virus of humans, duck hepatitis B virus

RNA VIRUSES

Viruses with ds RNA genome

- Family: *Birnaviridae*
  - Nonenveloped icosahdral 60 nm particles, two segments of linear ds RNA genome, replication in cytoplasm.
  - Infectious bursal disease virus

- Family: *Reoviridae*
  - Nonenveloped 60-80 nm icosahedral capsid, 11,12 or 13 segments of linear ds RNA , cytoplasmic replication.
  - Bluetongue virus

Viruses with positive sense ssRNA

- Family: *Arteriviridae*
  - Enveloped (45-60 nm)particle containing icosahedral nucleocapsid, linear positive sense ss RNA genome, cytoplasmic replication
  - Equine arteritis virus
- Family: *Caliciviridae*
  - Nonenveloped icosahedral 27-40 nm particle with calyx like (cup shaped) depressions. Linear ss RNA, cytoplasmic replication.
  - Vesicular exanthema of swine virus
- Family: *Coronaviridae*
  - Enveloped 120-160 nm with club shaped sparse protein spikes, helical nucleocapsid with linear ss RNA genome, cytoplasmic replication.
  - Avian infectious bronchitis virus
- Family: *Flaviviridae*
  - Enveloped 40-60 nm with isometric nuclecapsid of 25-30 nm linear ss RNA, cytoplasmic replication
  - Yellow fever virus, classical swine fever virus, hepatitis C virus
- Family: *Picornaviridae*
  - Nonenveloped icosahedral 30 nm, linear ss RNA, cytoplasmic replication
  - Poliovirus, foot-and-mouth disease virus
- Family:*Togaviridae*
  - Enveloped 70 nm particles with icosahedral nucleocapsid, linear ss RNA, cytoplasmic replication
  - Semliki forest virus, Rubella virus

Viruses with negative sense ssRNA

- Family: *Arenaviridae*
  - Enveloped isometric particles (usually 120 nm) with club shaped spikes. Genome present two helical nucleocapsids, larger one with 75,000 nt negative sense ss RNA, smaller one with 3500 nt ambisense RNA
  - Lymphocytic choriomeningitis virus
- Family: *Bunyaviridae*
  - Enveloped isometric 100 nm with 10 nm spikes, three nucleocapsids present each with one molecule of negative sense ss RNA, cytoplasmic replication
  - Nairobi sheep disease virus, rift valley fever virus
- Family: *Filoviridae*
  - Enveloped bacilliform or filamentous and sometimes branched particles 800 – 900 (sometimes 14000) x 80 nm with helical nucleocapsid of 50 nm diameter, negative sense ss RNA,
  - Ebola virus, Marburg virus
- Family: *Orthomyxoviridae*
  - Enveloped pleomorphic 120 nm particles with a dense layer of protein spikes, 6-8 helical nucleocapsids , 9 nm diameter with transcriptase activity, each contains a linear, negative sense ss RNA, RNA synthesis nuclear.
  - Influenza viruses
- Family: *Paramyxoviridae*
  - Enveloped pleomorphic particles usually 150- 200 nm diameter with dense layer of protein spikes. One helical nucleocapsid 12-17 nm diameter with one molecule of linear negative sense ss RNA, cytoplasmic replication

  - Newcastle disease virus of poultry, rinderpest virus, canine distemper virus, peste des petits ruminants virus
- Family: *Rhabdoviridae*
  - Enveloped bullet shaped particles 100-430 nm x 45-100 nm with 5-10 nm spikes, helicle necleocapsid with linear, negative sense ss RNA, cytoplasmic replication.
  - Rabies virus

Viruses with RNA genomes that replicate through DNA intermediate

- Family: *Retroviridae*
  - Enveloped 80-100 nm particles with spikes, nucleocapsid is isometric or truncated cone, contains two identical copies of positive sense ss RNA, virions contain reverse transcriptase and integrase enzymes.
  - Subfamilies – Orthoretrovirinae and Spumavirina
  - Avian leukosis virus, bovine leukemia virus, humal immunodeficiency viruses

VIRAL HAEMAGGLUTINATION

- Some viruses bind to red blood cells of certain animal species and cause agglutination. Binding occurs by interaction of viral protein located on the capsid or envelope with receptors on RBC.
- Agglutination is result of bridging effect of one virion binding to two RBCs simultaneously and these inturn bound to other RBCs by additional virions. This causes a lattice-like aggregate of RBCs.
- Haemagglutination is influenced by pH and temperature. Haemagglutination is useful in concentration and purification of some viruses and to detect virus in suspected materials.

INTERFERENCE

- It is the prevention of virus multiplication. It is due to defective interfering particles or interferons. Interferons are proteins secreted by cells in response to virus infection.
- The trigger for interferon production is double stranded RNA produced during RNA virus replication. Inferferons protect adjacent cells from infection and activate T cell mediated immunity.
- There are number of types of interferons. Alpha and beta are produced by most cells when infeted with viruses. The secreted interferon diffuses to nearby cells and bind to interferon receptors and trigger antiviral activities.

MODE OF INTERFERON ACTION

- The antiviral activities of interferon are
  - Activation of genes that encode double stranded RNA dependent protein kinase R and RNase L.
  - Stimulation of MHC I production and proteosome proteins. These enhance the presentation of viral peptides on infected cell surface to T cells.
  - Activation of NK cells.
  - Induction of apoptosis.
- Another type of interferon called gamma interferon is produced mainly by T cells and NK cells when trigerred by certain molecules released during immune responses(eg:IL-2).
- It has number of effects including stimulation of antigen presentation and activation of phagocytes and NK cells.

MODULE-18: CULTIVATION OF VIRUSES

Learning objectives

To learn about virus cultivation in different substrates.

INTRODUCTION

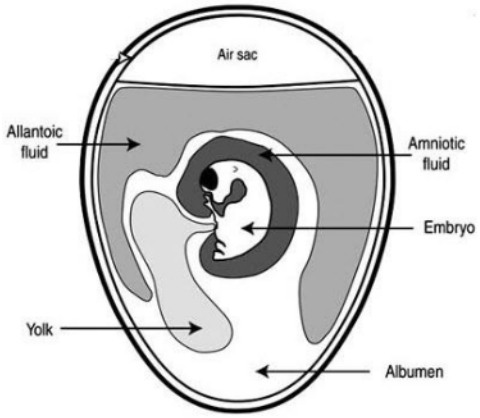
- Viruses can grow only in living cells. The substrates used for virus cultivation are
  - Animals – host or experimental
  - Embryonated eggs
  - Tissue cultures

ANIMALS

- Some viruses can not be cultivated in cell cultures or embryonated eggs. They must be propagated in living animals.
- The host animal or experimental animals such as mice, guinea pigs or rabbits are used. By animal inoculation we can observe the symptoms , lesions and histopathology sections of infected tissues can be examined.
- The disadvantages are the need for containment facility,ethical issues and variation in susceptility.

EMBRYONATED EGGS

- One of the most economical and convenient methods for growing many animal viruses is the chicken embryo inoculation. This method was devised in 1931.
- Different routes of inoculation are used namely yolk sac, allantoic, amniotic, chorioallantoic and intravenous route. Chick embryos have different types of cells in which various viruses can replicate. This method has been used in the production of vaccines against various animal and human diseases.
- Limitation is that not all animal viruses can be grown by this method. In the case of poultry viruses presence maternal antibody in embryonated chicken eggs may interfere with virus multiplication.



TISSUE CULTURES

- Cell cultures are the preferred method for virus cultivation at present. Convenience, economy of maintenance compared to animals, cytopathic effect and choice of cells for their susceptibility to particular viruses are the reasons for the preferred use of the cell of cultures.
- There are three types
  - Primary culture
  - Diploid cell strains
  - Continuous cell lines

PRIMARY CULTURE

- When normal tissue is processed and grown *in vitro* the first monolayer formed is called a *primary culture*. Monolayer is a confluent layer of cell growing on the surface of a tissue culture flask. Cells from subcultures are called *secondary cultures*.
- Primary cultures can subcultutred only a limited number of times before cell death. For some types of cells only a few divisions are possible while in some 50-100 divisions can occur.Cultures from embryonic tissues are capable of greater number of divisions than those from adult tissues.

DIPLOID CELL STRAINS

- Derived from primary cell culture established from a particular type of tissue such as lung or kidney of embryonic origin. They are single cell type and can undergo 50-100 divisions before dying.
- They posses normal diploid karyotype. Diploid cell strains are used for viral studies and human vaccine production.

CONTINUOUS CELL LINES

- These cells are capable of infinite number of divisions. Continuous cell lines arise from mutation in a cell strain or by establishment from malignant tissue.
- The karyotype of these cells is aneuploid (variable multiple of haploid chromosome number)
- These cell lines are morphologically different from the cells of origin and usually nutritionally less fastidious. Some cell lines are nonadherent and can grow in suspension. Continuous cell lines are useful in many viral studies. Eg: vero,BHK21 and HeLa

CYTOPATHIC EFFECTS(CPE)

- Virus growth in cell culture can be detected by CPE. The CPE produced by some viruses are cell death, formation of giant cells, inclusion bodies etc.
- Inclusion bodies are aggregates of unassembled virus subunits or intact virus. They may be intracytoplamic or intranuclear and may be acidophilic or basophilic. Eg: Negri bodies in rabies - intracytoplasmic acidophilic inclusion bodies.
- *Plaque:* Infected area surrounded by normal cells in monolayer cell cultures.
- *Pock:* Infected area surrounded by normal area in chorioallantoic membrane.

MODULE-19: REPLICATION OF VIRUSES

Learning objectives

- To learn about the steps in virus replication

INTRODUCTION

Virus replication process can be divided into seven steps.

- Attachment of the virion to the cell
- Entry in to the cell
- Uncoating of the genome
- Transcription of early mRNAs
- Translation of early viral proteins
- Genome replication
- Transcription of late mRNAs
- Translation of late viral proteins
- Assembly of genome surronded by late proteins
- Maturation and release of virions from cell

ATTACHMENT

- Attachment occurs via one or more of virus surface proteins to specific molecules of host cell. These cellular molecules are known as receptors.
    - The recognition of the receptor by the virion is highly specific. Some viruses have to to bind to a second type of cell surface molecule called coreceptor in order to infect a cell.
    - In some viruses binding to the receptor causes conformational changes in the virus protein that enables it to bind to the coreceptor.
    - Receptors and coreceptors are cell surface molecules usually glycoproteins. Eg: intrcellular adhesion molecule–I (ICAM-I),CD 155, CD 4, sialic acid containg glycoproteins, signaling lymphocyte activation molecule (SLAM)
- Virus attachment sites of naked viruses are on the capsid surface. For some viruses attachment occurs through specialized structure such as fibres and knobs of adeno virus and the spikes of rotavirus.
- For enveloped viruses the attachment sites are on the surface glycoproteins present on the envelope.
- Initially a virion is weakly bound to a cell at only one or few receptors. At this stage the attachment is reversible. Subsequently binding to many receptors occurs and the attachment becomes irreversible.

ENTRY

- Naked viruses can deliver their genomes directly in to the cell through a pore in plasma membrane. Many naked viruses enter through endocytosis.
- The plasma membrane flows around the virus. More receptors bind and eventually the virion is completely enclosed in the membrane which pinches off as an endosome.
- In the case of enveloped viruses entry is by fusion of the viral envelope with plasma membrane or endocytosis followed by fusion of virus envelope with the endosome membrane.

GENOME UNCOATING

- Genome uncoating is the complete or partial removal of the capsid to release the virus genome . Depending on the virus the process can take place
    - At the cell surface with the capsid remaining at the exterior surface of the cell.
    - Within cytoplasm
    - At nuclear pore
    - Within nucleus
    - From the stage of penetration till the appearance of mature progeny virions the virions cannot be demonstrated inside the host cell. This period during which the virus seems to disappear is known as eclipse phase.

TRANSCRIPTION

- David Baltimore grouped viruses into classes based on genome type and mode of transcription.
    - Class I ds DNA viruses - mRNA
    - Class II ss DNA viruses – dsDNA – m RNA
    - Class III ds RNA viruses - m RNA
    - Class IV ss positive RNA viruses – negative RNA – mRNA
    - Class V ss negative RNA viruses - mRNA
    - Class VI positive RNA – negative DNA - ds DNA – m RNA ds DNA – m RNA - positive RNA – negative DNA – ds DNA
- Viruses with positive RNA genomes(class IV&VI) have the same sequence as the virus m RNA. However only in class IV the genomes can function as m RNA. They are called as *plus strand or positive sense RNA viruses.*
- The class V are referred as *minus strand or negative sense RNA viruses.* Class VI viruses must first reverse transcribe their ss genomes to ds DNA before m RNA can be transcribed. Because they carry out transcription in reverse (RNA to DNA) they are known as retro viruses.
- Later some DNA viruses were also found to have the ability to carry out reverse transcription. These viruses are known as pararetroviruses and class VII was formed to accommodate them.
- Some single stranded viruses have a mixture of positive and negative polarity within the strand. Genomes of this type are known as ambisense. Eg: ss DNA – geminiviruses (plant virus), ss RNA – arenaviruses (animal virus)
- Viruses carrying out trnacription in the nucleus use cell RNA polymerase II. These viruses include retro and many DNA viruses. DNA viruses replicating in cytoplasm use virus encoded transcriptase.
- In RNA viruses each virus in class III,IV and V encode its own enzyme. All viruses that carry out transcription except the positive sense RNA viruses have the transcriptase in the virion.
- The enzyme is immediately available to transcribe the virus genome when the cell is infected. Before starting transcription the positive RNA viruses must translate copies of the from the genome RNA.

CAPPING AND POLYADENYLATION

Capping

- Most eukaryotic and viral m RNA have a cap at their 5' end. The cell enzymes that carry out the capping activities are guanylyl transferases (add guanosine 5' triphosphate) and methyl transferases (add methyl groups).
- Most viruses carrying out transcription in the nucleus use the cell enzymes. Some of the viruses replicating in cytoplasm eg: pox,reo and corona viruses encode their own capping and methylating enzymes.
- Negative sense RNA viruses with segmented genomes " snatch" caps from cell m RNAs eg: influenza. In this case the viral RNA polymerase binds to the cellular capped m RNA and cleaves the RNA 10-20 nucleotides from the 5' end.
- The capped oligonucleotide acts as primers to initiate transcription of viral m RNA. Not all m RNAs are capped. For example picorna viruses donot cap their m RNAs.

Polyadenylation

- Series of adenosine residues are to 3' end of most m RNAs of eukaryotes and viruses. This probably increase stability of m RNA and plays a role in the initiation of translation.
- Some viruses (eg: reoviruses) do not polyadenylate their m RNA.

GENOME REPLICATION

- Most DNA virus genomes are replicated in the nucleus but some ds DNA virus genomes are replicated in cytoplasm. Genomes of most RNA viruses are replicated in the cytoplasm but those of the minus strand RNA viruses with segmented genomes are replicated in the nucleus. In retro and pararetro viruses RNA to DNA replication occurs in the cytoplasm and DNA to RNA replication occurs in the nucleus.
- In the case of many RNA viruses the first nucleotide of the new strand base pairs with a nucleotide in the viral RNA. This initial nucleotide effectively acts as a primer for RNA replication.
- Some ss DNA viruses (parvoviruses) use self priming. There are regions at their 3' end with complementarity sequences that can base pair. The hydroxyl group of the nucleotide at the 3' end forms a linkage with the first nucleotide.
- Some DNA viruses (polyoma viruses) use cellular enzyme primase to synthesise their RNA primers. Others viruses such as herpes viruses encode their own primers.
- Retroviruses use the cellular t RNA to prime DNA synthesis and then use the 3' –OH in a partly degraded positive RNA template to prime positive DNA synthesis. RNA primer synthesized by cell primase is used for RNA synthesis from proviral DNA.
- Some viruses use hydroxyl group of a serine or tyrosine in a protein as a primer. Eg: DNA – adeno, RNA – picorna. Hepadna viruses use protein to initiate negative DNA replication and use RNA primer to initiate positive DNA.

ASSEMBLY AND RELEASE

Assembly

- After synthesis of genomes and viral proteins they are assembled into virus particles in the nucleus and/or cytoplasm of the infected cell.
- DNA viruses (except poxviruses – assembled in cytoplasm) are assembled in the nucleus. RNA viruses are generally assembled in the cytoplasm. Maturation step may follow the initial assembly process.

Release

- It is the final step in virus replication. Naked virions are released by lysis of the cell. Enveloped animal viruses are released by budding through the host cell membranes.
- In a few viruses the host cells are not destroyed. The virions leave the cells through cytoplasmic channels over an extended period of time.

MODULE-20: VIRAL GENETICS

Learning objectives

To learn about mutation, recombination and other interactions of viruses.

MUTATION

- Spontaneous mutations
  - These mutations occur naturally during virus replication. DNA viruses are stable genetically than RNA viruses. This may be due to the lack of error correction mechanisms in RNA replication.
- Induced mutations
  - Chemicals.
  - Nitrous acid – direct action on bases.
  - Base analogues – indirect action, mispairing thus generating mutations.
  - Physical.
  - UV and X rays.

TYPES OF MUTATIONS

- Point mutations or insertion deletion mutations.Phenotypic changes seen are
  - Conditional lethal mutants
    - The mutant multiplies under certain conditions but not others where as the wild type grows at both conditions. Eg : temperature sensitive mutant – grows at low temperature 31C but not at 39C. The reason is the altered protein can not maintain functional conformation at the high temperature.
  - *Host range mutant* - grows only in a subset of cell types.
  - *Plaque size* - plaques may be larger or smaller than the wild type.
  - *Drug resistant mutants.*
  - *Enzyme deficient mutants.*
  - *Hot mutants* – grow better at elevated temperature than wild type.
  - *Attenuated mutants* – cause much milder symptoms or no symptoms compared to parental virus.

RECOMBINATION

Genetic recombination

- Exchange of genetic information by breaking and joing nucleic acid bonds is seen in DNA and retroviruses. Recombination systems for DNA are available in the cell.
- This type of recombination is very rare in RNA viruses. Picornaviruses have a type of very low efficiency recombination. This is not like the standard DNA recombination. It is probably a *copy choice* type. In that the polymerase switches templates when copying the RNA.

- Recombination is also common in the coronaviruses. In this also the mechanism is different from that of DNA recombination.
- In the negative stranded RNA viruses recombination leading to viable progeny not reported.

Genetic reassortment

- It is a non-classical type of recombination. When dual infection occurs in a cell by two variants of a virus having segmented genome (eg.Orthomyxovirus) , progeny virions can be produced with some segments from one parent and some from the other.

COMPLEMENTATION

- It is interaction at a functional level. There is no exchange of genetic material.
- If we infect two mutants with a *ts (temperature-sensitive)* lesion in different genes in a cell each mutant provides the missing function of the other and hence both can replicate. But the progeny virions will still contain *ts* mutations and will be temperature-sensitive.

MULTIPLICITY REACTIVATION

- When UV inactivated double stranded DNA viruses are infected at a very high multiplicity of infection production of live virus progeny is noticed. This happens because the inactivated viruses cooperate as genes inactivated in one virion may still be active in one of the others.

PHENOTYPIC MIXING

- When two viruses infect a cell the progeny viruses may contain coat components derived from both parents and will have coat properties of both the parents. This is called *phenotypic mixing*.
- It involves no alteration in the genetic material. The progeny of such virion is determined by which parental genome is packaged and not by the nature of the coat proteins.
- Phenotypic mixing can occur between related or between unrelated viruses. Some times the coat is entirely that of another virus. Eg: retrovirus nucleocapsid in a rhabdo virus envelope. This type is also called pseudotype (pseudovirion) formation.
- The pseudotype described has adsorption –penetration – surface antigenicity characteristics of rhabdo virus but after infection behaves as a retro virus and produce progeny retro viruses.

DEFECTIVE VIRUSES

- Defective viruses lack the full complement of genes. They need another virus to provide the missing functions- *helper virus*.

Defective interfereing (DI)particles

- The defective virus particles compete with the helper virus for the functions the helper provides. This may lead to decrease in the replication of the helper virus. This phenomenon is known as interference and such defective particles are called defective interfrering (DI) particles.
- Not all defective particles cause interference. DI particles can modulate natural infection.