UNIT 1

GENERAL MICROBIOLOGY AND INSTRUMENTATION

Introduction: -Microbiology is a branch of science that deals with microbes, the microscopic forms of life. Microbiology gives its focus on the occurrence and distribution of microorganisms in nature; their form, structure, physiology, reproduction, metabolism and classification; their helpful or harmful relationships with other living things; their significance and usefulness in science, medicine and industry; their beneficial and harmful effects on human being and domestic animals and cultivated plants.

Microbes: -Microbes are invisible creatures, too small to be seen with the naked eye. They can be seen only by magnifying their image with a microscope. They are the micro-organisms. They include viruses, bacteria, algae, fungi and protozoa. The science of viruses is called Virology; that of bacteria is called Bacteriology; that of algae is called Phycology; that of fungi is called Mycology and that of protozoans is called Protozoology.

Distribution: -Microbes are widely distributed in the world. They are omnipresent. They are present everywhere in air, water, soil, in living plants and animals, in dead matter, etc. They are also distributed in Arctic regions, in hot tropical areas, forest, frosty atmosphere or even in wholly arid region. They are present on our body, in our body, on our clothing's, in the air we breathe, in the food we eat, in the water we drink, in our mouth and in our intestine. Actually, one third, of the dry weight of human faeces is bacteria.

Levels of Organization: -The microbes are either unicellular or multicellular or non-cellular forms. Protozoa and bacteria are unicellular forms and are made up of single cells. The algae and fungi are multicellular forms where the organism is assembled out of many cells. But viruses lack a cellular structure and hence they are non-cellular particles and actually viruses occupy a border line between living and non-living things.

Concepts of Microbiology

1. Theory of Spontaneous Generation; -The formation of life from non-living substances is called spontaneous generation or abiogenesis. Aristotle (384-322 BC) suggested that maggots and grubs develop spontaneously in decaying matter. Epicurus (342-271 BC) wrote that worms and many other animals originated from soil and manure by the action of sun and rain.

The theory of spontaneous generation was disproved by Louis Pasteur. They experimentally proved that micro-organisms cannot arise spontaneously from decaying meat and flesh. Worms, maggots and micro-organisms develop from decaying flesh only from the eggs and spores already deposited by the insects, air, etc. When the decaying flesh is sealed from external agents, life cannot appear spontaneously from the flesh. Thus, Louis Pasteur and others disproved the theory of spontaneous

generation and they argued that life originated from pre-existing life only. **This concept is called biogenesis.**

2.Germ Theory of Pasteurization:-Pasteurization is the process of heating milk or wine or other liquids to moderate temperature for a definite time, which kills pathogenic bacteria and considerably delays other bacterial growth.This process was originally introduced by Louis Pasteur in 1866 to sterilize wine. He postulated that souring of wine was due to extraneous organisms that produced acid; thus undesirable secondary fermentations were the cause of the so called wine disease. This was prevented by pasteurization.

3.Germ Theory of Disease:-Germ theory states that infectious diseases are caused by microbes. Formerly microbes were not considered as the causative agent for diseases. But it was Robert Koch in 1876 showed that certain microbes cause diseases. He proved that Bacillus anthracis causes anthrax. Robert Koch extensively studied anthrax. He discovered the typical bacilli in the blood of cattle died of anthrax. He isolated these bacteria, cultured them in his laboratory and examined them microscopically to be sure he had only one kind present.

Robert Koch injected these cultured bacteria into other animals to see if these animals became infected and developed clinical symptoms of anthrax. From these experimentally infected animals, he isolated microbes like those he had originally found in sheep that died of anthrax. This was the first experiment to prove that a bacterium was the causative agent of an animal disease. This series of experiment led to the establishment of a concept called Koch's postulates. It provides guidelines to identify the causative agent of an infectious disease. They are as follows:

1. A specific microbe can always be found in association with a given disease.
2. The microbe can be isolated and grown in pure culture in the laboratory.
3. The pure culture of microbe will produce the disease when inoculated into a susceptible animal.
4. It is possible to recover the microbe in pure culture from the experimentally infected animal.

Scope of Microbiology :-Microorganisms have gained tremendous significance. This has been partly due to the increasing recognition of the economic importance of microorganisms and partly to the development of new techniques of investigation. Today, microorganisms are the basic tools of genetic engineering and biotechnology.

1. Biogeochemical Cycles:-The flow of chemicals between living and non-living things is called biogeochemical cycles. Microbes decompose dead plants and animals into simple chemical nutrients that can be used by plants and photosynthetic organisms. The plants use these simple chemical

nutrients and incorporate them into complex organic compounds which are the ultimate source of food for all animals.When these plants and animals die, the microorganisins decompose the complex organic compounds of dead bodies into simple chemical nutrients. These nutrients are again available for the use of plants. This recycling process allows the earth, with its limited supply of nutrients, to sustain a continuum of life.

2. Cellulose Digestion: -Ruminants cannot digest cellulose present in plants because they do not contain the enzyme cellulase. The stomach of ruminants contains microbes which help in the digestion of cellulose. If the ruminants are deprived of these microbes, the ruminants will starve and die.

3. **Food Production:** -Some of our foods are actually the by-products of microbial growth. Cheese is produced by the growth of microorganisms such as Leuconostoc citrovorum and Streptococcus lactis. The type and flavour of the cheese is largely determined by the microorganism used for processing. The blue mould Penicillium provides both the flavour and colour to Roquefort cheese. Yoghurt results from the growth of bacteria such as Lactobacillus bulgaricus and Streptococcus thermophilus in milk. The leavening of bread is accomplished by Saccharomyces cerevisiae (baker's yeast) which produces CO, as a waste product. This gas is trapped as tiny bubbles that cause the rise. The baker's yeast is also responsible for the production of alcoholic beverages.

4. **Energy Production**:-Microbial methane generators are used to convert manure to combustible fuel for powering vehicles and heaters. Biological fuel generators convert our wastes into usable energy to supply power to residential communities and industry.

5. **Industrial Products**:-Microorganisms are extensively used in industries to produce products useful to mankind. These products include fermented foods, alcohol, alcoholic beverages such as wine, beer, whiskeys, etc., antibiotics, pharmaceuticals, steroids, vaccines, vitamins, organic acids, amino acids, enzymes, proteins, organic solvents, synthetic fuels, etc.

6. **Microbes in Medicine:** -Microbes and their products are of enormous significance in medicine. Some important drugs synthesized by microorganisms are antibiotics such as penicillin, streptomycin, chloramphenicol, tetracyclines, neomycin, actinomycin, etc.,

7. **Microbes in Pesticides:** -Certain microbes like bacteria, fungi, viruses, protozoa and even nematodes infect insects and kill them. They may be called microbial pesticides and are used as biopesticides.

8**. Microbes in Improvement of Soil:**-Most of the bacteria and fungi live saprophytically on dead organic matters of soil. They decompose complex organic matter into simpler substances. Certain microbes increase the fertility of soil by converting atmospheric nitrogen into ammonia, nitrites and nitrates. This is brought about by microbes like Nitrosomonas, Nitrobacter, Rhizobium, etc.

9. **Microbes to Better Sanitation**;-Microbes like bacteria, algae, fungi and protozoa are used in improvement of sanitary methods. They act on the solids and semisolids of sewage and decompose them.

10. **Biomining:**-Microbes are now used in extracting valuable metals like uranium from rocks. Pseudomonas and baker's yeast are used to absorb or adsorb heavy metals.

Contributions of Robert Koch:-He investigated the anthrax disease cycle in 1876, and studied the bacteria that cause tuberculosis in 1882 and cholera in 1883.

- He discovered bacteria such as the anthrax bacilli, tubercle bacilli and cholera bacilli.
- Koch observed the phenomenon of acquired immunity.
- He introduced solid media for culture of bacteria. Koch pioneered the use of agar as a base for culture media. He developed the pour plate method and was the first to use solid culture media for culture of bacteria.
- Koch also developed media suitable for growing bacteria isolated from the body. Because of their similarity to body fluids, meat extracts and protein digests were used as nutrient sources. The result was the development of nutrient broth and nutrient agar media that are still in wide use today.
- He also introduced methods for isolation of bacteria in pure culture.
- He described hanging drop method for testing motility.
- He introduced staining techniques by using aniline dye.
- He invented the hot air oven and steam sterilizer, and also introduced methods to find out the efficacy of antiseptics.
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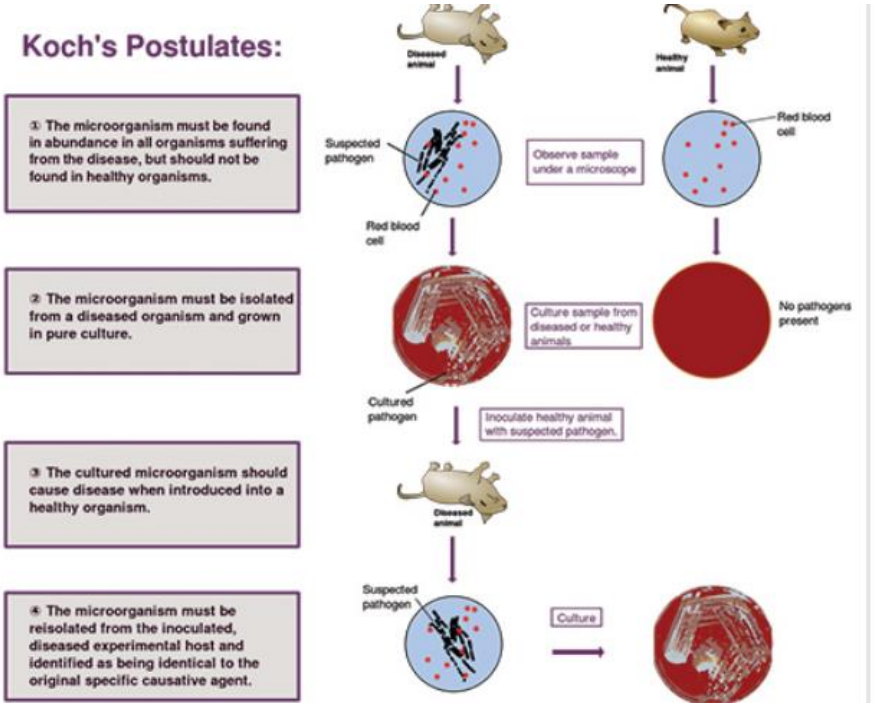
Experimental evidence:- In the experiment, Koch injected healthy mice with a material from diseased animals, and the mice became ill. After transferring anthrax by inoculation through a series of 20 mice, he incubated a piece of spleen containing the anthrax bacillus in beef serum. The bacilli grew, reproduced, and produced spores. When the isolated bacilli or spores were injected into mice, anthrax developed. During Koch's studies on bacterial diseases, it became necessary to isolate suspected bacterial pathogens. His criteria for proving the causal relationship between a microorganism and a specific disease are known as Koch's postulates.



Antonie van Leeuwenhoek (24 October 1632 – 26 August 1723) The full name of Antonie van Leeuwenhoek is Antonie Philips van Leeuwenhoek.Antonie van Leeuwenhoek was a moderately educated owner of a textile business and a scientist.He is also known as **"the Father of Microbiology".**He was one of the first microscopists and microbiologists.

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- **Single-lens microscope:** Antonie van Leeuwenhoek discovered the world's first Single-lens microscope after Hooke's illustration and very popular book Micrographia in 16. which was approximately 5 cm long. He created 25 single-lens microscopes, which of them only nine were survived. Those microscopes, which were survived they have the magnification power up to 275 times. He also made 500 optical lenses.

- **Single-Celled Life:** In 1674, Antonie van Leeuwenhoek discovered first single-celled life forms, nowadays which are grouped with the protists – these are mainly single-celled plants and animals. In the beginning, the Royal Society refused Leeuwenhoek's Single-Celled Life. But, In 1677 his microscopic creatures were fully accepted.

- **The shape and size of Red Blood Cells:** Leeuwenhoek was the first person who determines the shape and size of Red Blood Cells accurately In 1674.

- **Bacteria:** In 1676, Leeuwenhoek first discovered bacteria in water and called them 'animalcules' (from Latin 'animalculum' meaning tiny animal).

- **Spermatozoa:** Leeuwenhoek discovered the Spermatozoa in 1676.
- **Lymphatic Capillaries:** Leeuwenhoek discovered the lymphatic capillaries in 1683, which carries “a white fluid, like milk.”.
- He observed the life-cycles of maggots and fleas, and proved creatures are not spontaneously generated. By dissecting aphids Leeuwenhoek discovered parthenogenesis. Leeuwenhoek observed the flow of blood in tiny capillaries and confirmed the blood circulation theory of William Harvey.

Edward Jenner: -Edward Jenner was an English country doctor who introduced the vaccine for smallpox. Previously a keen practitioner of smallpox inoculation, Jenner took the principle a stage further by inducing immunity against this killer disease via exposure to a harmless related disease, cowpox. His technique provided safer and more reliable protection than traditional inoculation.

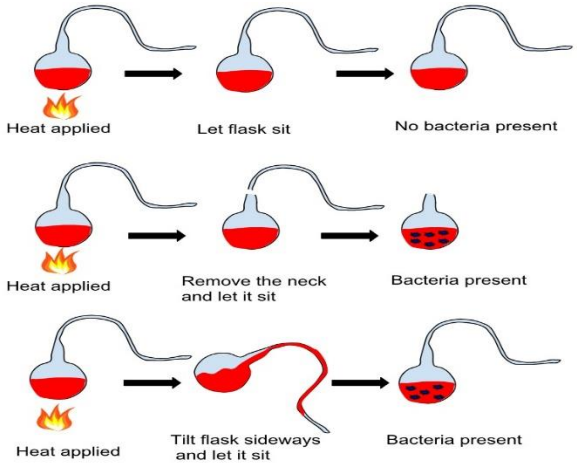
For many years, he had heard the tales that dairymaids were protected from smallpox naturally after having suffered from cowpox.obeserving this, Jenner concluded that cowpox not only protected against smallpox but also could be transmitted from one person to another as a deliberate mechanism of protection. He then presented the hypothesis that infection with cowpox protects against subsequent infection with smallpox. He discovered vaccination.

Louis Pasteur was a French chemist and microbiologist whose work changed medicine. He proved that germs cause disease; he developed vaccines for anthrax and rabies; and he created the process of pasteurization. The studies on fermentation led Pasteur to take interest to work in microbiology. His contributions to microbiology are as follows: **He disproved the theory of spontaneous generation of disease and postulated the germ theory of disease**: He stated that disease cannot be caused by bad air or vapor but it is produced by the microorganisms present in air.

The doctrine of spontaneous generation was disapproved by his experiments that showed that without contamination, microorganisms could not develop.

- He proposed the principles of fermentation for preservation of food,grains,wines to give rise to different types of wines.
- He introduced the sterilization techniques and developed steam sterilizer, hot air oven and autoclave.
- He described the method of pasteurization of milk and wine.
- He reduced mortality from puerperal fever. He had also contributed for the vaccine development against several diseases, such as anthrax, fowl cholera and rabies.
- Liquid media concept: He used nutrient broth to grow microorganisms.
- He was the founder of the Pasteur Institute, Paris.
- Discovered the causative agent pebrine disease of silk worm.

Besides in microbiology, Pasteur made significant discoveries in chemistry, most notably on the molecular basis for the asymmetry of certain crystals and racemization.



Louis Pasteur's spontaneous generation experiment illustrates the fact that the spoilage of liquid was caused by particles in the air rather than the air itself. These experiments were important pieces of evidence supporting the idea of germ theory of disease.

Alexander Fleming:-Alexander Fleming (1881-1955) was a medical microbiologist. He made one of the greatest accidental discoveries ever. It led to a breakthrough in medical science. Got Nobel prize for medicine. Fleming **observed the bacterial-killing effects of penicillin** in his laboratory

Discovering penicillin:-In 1928, Fleming discovered penicillin, the first form of antibiotics. He grew cultures of bacteria on petri dishes in the hospital where he worked. A fungal spore happened to contaminate one of the bacteria cultures and grew into a fungal colony. The bacteria round the fungal colony failed to grow as well as the bacteria on the other areas of the petri dish. From this, Fleming deduced that a substance produced by the fungus must be slowing down the bacteria's growth. He called this substance penicillin after the fungus on the petri dish, Penicillium notatum.

He studied the wound infection. He demonstrated the use of antiseptics on wounds, he washed with mild saline solution. Fleming demonstrated that antiseptic agents were only useful in treating superficial wounds, but were harmful when applied to deep wounds. A saline solution – salt water – should be used to clean deep wounds, because this did not interfere with the body's own defenses and in fact attracted white cells.

He discovered lysozyme-an enzyme present in the body fluids such as saliva,tears has a antiseptic activity. The effect of lysozyme was to destroy certain types of microbe, rendering them harmless to people. The presence of lysozyme in our bodies prevents some potentially pathogenic microbes from causing us harm. It gives us natural immunity to a number of diseases. However, lysozyme's usefulness as a medicine is rather limited, because it has little or no effect on many other microbes

that infect humans. lysozyme is used as a food and wine preservative. It is naturally present in large concentrations in egg-whites, offering protection to chicks against infection.

Dmitri Ivano sky, in studying a disease that affects tobacco plants, paved the way for the discovery of the infectious particle known as a virus Ivanovsky is one of two biologists usually credited with discovering viruses. In 1890, he was trying to find the cause of tobacco mosaic disease, an infection that causes tobacco leaves to discolour. From affected leaves, he prepared a solution containing the infectious agent, and he passed it through a new kind of filter, known as a Chamberland filter, made of porcelain, which has pores so small it can filter out bacteria. Ivanov sky found that after passage through the filter, the solution was still fully capable of infecting more tobacco plants, meaning the agent was much smaller than a bacterium. Six years later, in 1898, a Dutch biologist named Martinis Bierock independently performing the same experiments, announced he had found a new kind of infecting organism, and he named it a virus. Not until the advent of electron microscopy in the 1950s would it be discovered that the tobacco mosaic virus is a tiny hollow rod, formed by a single spiralling strand of RNA, surrounded by a protein coat.

GENERAL CHARACTERS: -Although bacteria (singular bacterium) show variations in detailed structures and behaviour, there are some characters common to all. These common characters are :

1. Bacteria are unicellular in nature but grouping of cell can be observed.
2. They do not have a distinct nucleus due to lack of nuclear membrane and hence prokaryotic.
3. They possess a cell wall outer to the cell membrane and also there is a slime capsule layer external to cell wall in some cases.
4. They contain peptidoglycans with N-acetyl muramic acid and N-acetyl glucosamine in the cell wall which is absent in archaea and eukaryotes. It also contains diamino pimelic acid (DAP)
5. The DNA is a ds DNA and circular in nature which acts as its chromosome as well as genome.
6. No histone protein
7. They do not possess any cell organelles like chloroplast, mitochondria, golgi bodies etc. However, the inward foldings of the inner cell membrane forms mesosomes that function as respiratory organelle.
8. All bacteria possess 70S ribosome which are free or in polysomes found in its protoplasm.
9. Most bacteria have an absorptive mode of nutrition. The autotrophic and chemosynthetic group possess the capacity to prepare their food.
10. There is no nucleosome formation in bacteria.
11 Bacteria mainly reproduce by binary fission. There is no sexual reproduction, however some bacteria conjugate to transfer their genetic material to other bacteria.
12. Plasmids or extra genomic DNA are found in some bacteria.
13. Some bacteria are motile and possess flagella on their cell surface which is made up of 8 parallel chains of flagellin protein.

STRUCTURE OF BACTERIA: -

A. Capsule and Slime Layers: -The capsule or slime layer is known as glycocalyx whose literal meaning is 'sugar coat'. Capsule is a rigid covering with high molecular weigth polysaccharides like glucose, amino sugars, muramic acid, rhamnose, poly D-glutamic acid etc. Capsule shows immunological specificity. When this outer layer is a loose, irregular diffused layer and can be deformed or removed, it is called as slime layers.

Functions of Capsule: It protects bacteria and forms a defensive mechanism (against phagocytosis. It protects from desiccation due to its high water content (98%) and acts as osmotic barrier.It also acts as reserve food in adverse environmental conditions. It prevents soil erosion as it binds to the soil particles. It protects against attacks from bacterial viruses. The presence of capsule makes the bacteria virulent and most of the pathogenic bacteria are capsulated type.

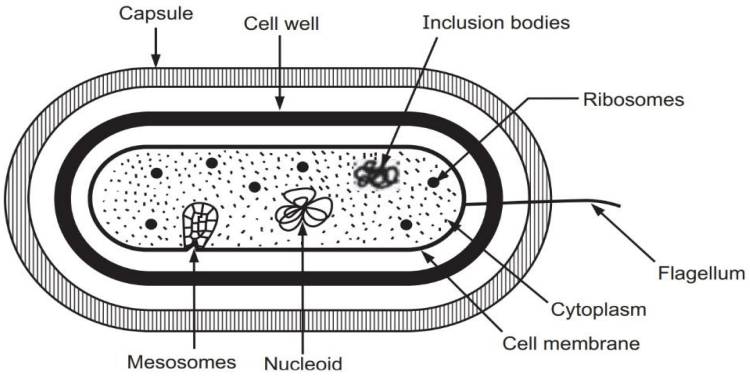


Fig: Ultra Structure of Bacterial Cell

Surface Appendages There are some external structures of the cell. These are called surface appendages and may be flagella, fimbriae or spinae

Flagella: -The most prominently observed external structures protruding from the surface. They are responsible for bacterial fertility. A flagellum has whip like filament, a motor complex and a hook. The filament is 50nm diameter

Fimbriae, Pilli and spinae:-These are short, fine and hair like surface structures in bacteria

These are non-locomotive structures. These structures are very thin and only seen under electron microscope. Each fimbriae is made up of a protein called pilin. Fimbriae help in attaching to substrates, help in forming biofilms, help in transfer of genetic materials, and in few case help in gliding motion.Sex pili are a type of fimbriae which are longer than the normal fimbrae.One or two sexpilli are found and their presence is genetically determined. Their presence is associated with the presence of 'F' factor (a plasmid) in the bacterial Pili help in formation of conjugation bridge.

Cell wall is an unique feature of bacterial cell which is present inner to capsule or slime layer and very close to the cell membrane.The presence of cell wall made bacteria to be included under plant kingdom for a considerable period of time. However the cell wall of bacteria is different from the plants. The bacterial cell wall contains peptidoglycans - which are polymers consisting of N-acetyl

glucosamine (NAG) and N-acetyl muramic acid (NAM) and short chains of amino acids.NAG and NAM are arranged alternating to each other by ß 1-4 bonds to form the carbohydrate back bone or the glycan. Rows of NAG and NAM are linked by polypeptides in a cross bridge manner.The structure of the poly peptide cross-bridges may vary but they have always a tetrapeptide side chain consisting, of 4 amino acids (a alanine, D-alamine, D glutamine acid and Diamino pimelic acid) attached to NAM. The structural rigidity of cell depends on the cross linking of basic structural repeating units. While peptidoglycan is present in all bacterial cell walls, there are two basic variations seen so as to divide all bacteria into two major-groups - Gram +ve and Gram -ve due to differential staining behaviour to grams stain. In addition to peptidoglycans, peptides; some other compounds found are teichoic acid, teicharonic acid, transmembrane protein called porin, some lipoproteins etc., are found in less amount in the cell wall. Cell wall is an important structure of a bacteria. It give shape,rigidity and support to the cell.

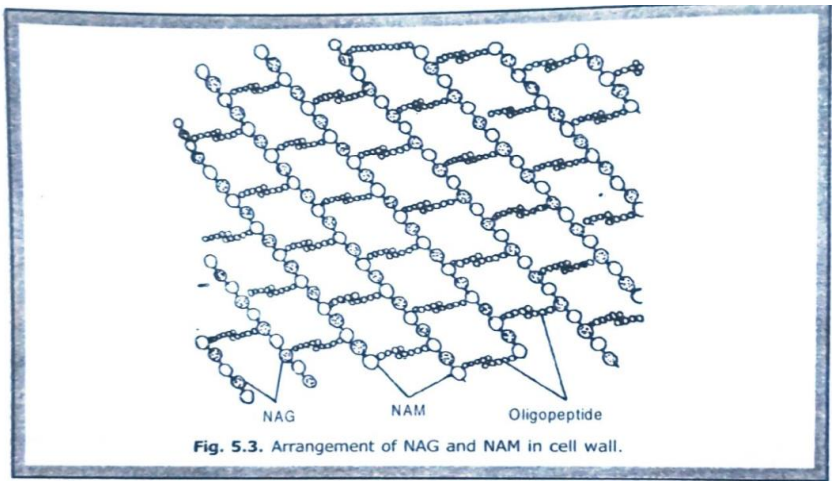


Fig. 5.3. Arrangement of NAG and NAM in cell wall.

Plasma membrane:-The plasma membrane is triple layered structure with a bimolecular lipid middle layer sandwiched by two layers of proteins on either side.The plasma membrane contains 60% proteins, 30% lipids, 10% carbohydrates.

Cytoplasm;- Since there is no distinct nucleus due to absence of nuclear membrane, there is no cytoplasm rather the entire thing is the protoplasm which has often been refered as 'matrix' However for convenience 'cytoplasm' term can be used for the mass of substances along with its inclusions inner to cell membrane. **It contains a number of substances in aqueous medium which include - proteins, lipoproteins, nucleoproteins, amino acids, peptides, enzymes, varieties of ions, purines pyrimidines, vitamins, glucose, ribose, and so many other inclusions** like volutine granules. Ribosomes are the only cellular organelles found in a bacterial cell. A bacterial ribosome is a typical 70S (prokaryotic) type with 2 sub-units of 50s and 30s. A ribosome is 250-350 A in length and 250 A in diameter. The number of ribosome units vary from 5000 to 10000 per cell which may occur both as free or in polyribosome

Photosynthetic apparatus: -Well organised photosynthetic organelle, 'chloroplast' is absent in bacteria. However integration of photosynthetic pigments into lamellar systems are observed in

photosynthetic bacteria such as cyanobacteria, purple bacteria and green bacteria. Lamellae are flat sheets that arise as invaginations from plasma membrane and chromosomes are non unit membrane enclosed structure seen in green sulphur bacteria. These two systems contain photosynthetic pigments chlorophyll c, d, e, bacterio-chlorophyll a.

Bacterial genome (or Bacterial chromosome):-**It refers to the circular ds DNA molecule that forms the visible mass called 'nucleoid' not bounded by any membrane.The length of the bacterial DNA is found to be more than 1000 times the length of the cell itself and therefore reliably oriented and highly organized within the cell.** A single bacterial chromosome is usual The electron microscopic photographs reveal that this is separated from cytoplasm but not bounded and some DNA strands extrude from the nucleoid into the cytoplasm much like the loose pieces of a tight yarn.The nucleoid contains 60% DNA, 30% RNA and 10% protein by weight. vi) The bacterial chromosome is without histone and does not have introns.

Plasmids: - Plasmids are extra or additional DNA molecules in the bacterial cell other than the bacterial nucleoid. Nucleosomes are found. It can be defined as a self replicating circular DNA molecule carrying few useful but non essential genes in the cytoplasm in addition to the main genome.There may be one molecule of plasmid and the size varies form 1 kbp to 400 kbps.These plasmids can be easily removed from the cell and manipulated for which they form the main tool in recombinant DNA technology. A copy of the plasmid can be transferred from one bacterial cell to other during conjugation process.

Classification based on morphology

1. Coccus (Pl. cocci): The spherical unicellular bacteria are called cocci which may be arranged in chains or in groups. They are comparatively resistant than other forms. They may be found in pairs called as diplococcus, or in chain called as strepto coccus or in clusters called staphylococcus. When cocci divide in two planes successively at right angles they form a group of four cells which are called as tetracoccus or Gaffkya. Sometimes a cubical mass of cocci is formed due to three divisions at right to each other and this cubical cocci is called as sarcinae.
2. Bacillus (Pl. Bacilli): They are unicellular rod like cells which may be long and narrow. Their size ranges from 1.5 u width to 10 u in length. Like cocci, they may occur single (called microbacillus) or in pairs known as diplobacillus, or in chains Lactobacillus of rods called as streptobacillus. The common rod shaped bacteria are - (in milk and curd), Clostridium (in soil), Bacillus subtilis etc.
3. Vibrio (Pl. Vibrios) are rod shaped bacteria which are little bit curved at one end to give a comma shape. They are small within 10 μ diameter. E.g. Vibrio comma.
4. Spirillum (Pl. spirilla) they are rod shaped forms which become coiled like a spring with more than one turn of helix. They are motile with cork screw motion. Their size varies 10-50 μ in length and 0.3 to 0.5 μ in breadth. E.g. Spirillum undulum, S. volutans.

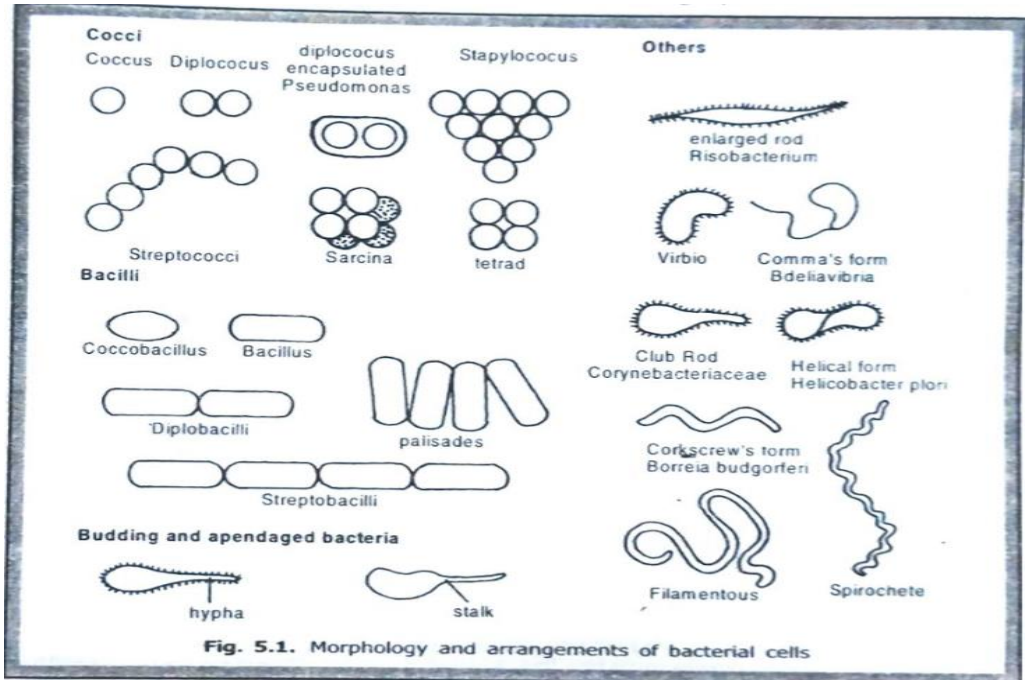


Fig. 5.1. Morphology and arrangements of bacterial cells

On the basis of cell wall composition, bacteria are classified into two major group

1) **Gram Positive**
2) **gram negative**

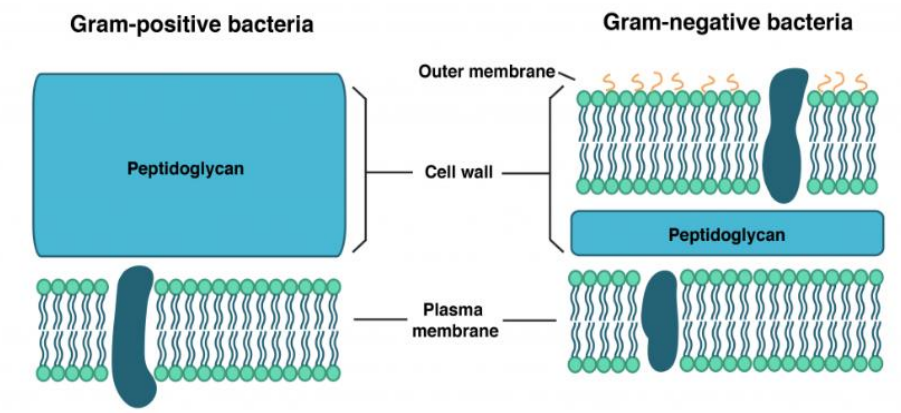
- The gram-positive bacteria retain the crystal violet colour and stain purple whereas the gram-negative bacteria lose crystal violet and stain red. Thus, the two types of bacteria are distinguished by gram staining.
- Gram-negative bacteria are more resistant to antibodies because their cell wall is impenetrable.
- Gram-positive and gram-negative bacteria are classified based on their ability to hold the gram stain. The gram-negative bacteria are stained by a counterstain such as safranin, and they are de-stained because of the alcohol wash. Hence under a microscope, they are noticeably pink in colour. Gram-positive bacteria, on the other hand, retains the gram stain and show a visible violet colour upon the application of mordant (iodine) and ethanol (alcohol).
- Gram-positive bacteria constitute a cell wall, which is mainly composed of multiple layers of peptidoglycan that forms a rigid and thick structure. Its cell wall additionally has teichoic acids and phosphate. The teichoic acids present in the gram-positive bacteria are of two types – the lipoteichoic acid and the teichoic wall acid.
- In gram-negative bacteria, the cell wall is made up of an outer membrane and several layers of peptidoglycan. The outer membrane is composed of lipoproteins, phospholipids, and LPS. The peptidoglycan stays intact to lipoproteins of the outer membrane that is located in the

fluid-like periplasm between the plasma membrane and the outer membrane. The periplasm is contained with proteins and degrading enzymes which assist in transporting molecules.

- The cell walls of the gram-negative bacteria, unlike the gram-positive, lacks the teichoic acid. Due to the presence of porins, the outer membrane is permeable to nutrition, water, food, iron, etc.

Difference between Gram-Positive and Gram-Negative Bacteria

- The cell wall of gram-positive bacteria is composed of thick layers peptidoglycan.
- The cell wall of gram-negative bacteria is composed of thin layers of peptidoglycan.
- In the gram staining procedure, gram-positive cells retain the purple coloured stain.
- In the gram staining procedure, gram-negative cells do not retain the purple coloured stain.
- Gram-positive bacteria produce exotoxins.
- Gram-negative bacteria produce endotoxins.



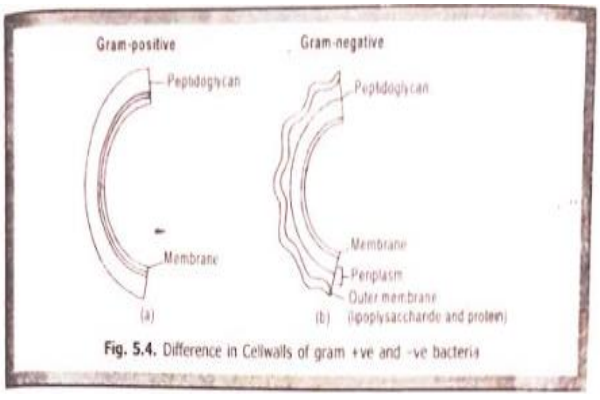


Fig. 5.4. Difference in Cellwalls of gram +ve and -ve bacteria

Classification based on nutrition: -

Nutritional Types of Bacteria: -Bacteria need enough suitable nutrients for its growth and reproduction. Enough moisture and temperature, suitable pH and enough nutrients such as a carbon

source, a nitrogen source electron donors and trace elements are necessary for the growing bacteria. Bacteria are extraordinarily diverse in their specific nutrient requirements. The various nutritional types of bacteria are discussed below:

On the basis of nutrition, bacteria are divided basically into two groups. They are autotrophs and heterotrophs. The bacteria which utilize carbon dioxide as the source of carbon are called autotrophs. The bacteria which use organic compounds as the source of carbon are known as heterotrophs.

The autotrophic bacteria which use sunlight as the energy source are called **photoautotroph or photosynthetic bacteria.** Green sulphur bacteria containing pigment bacteriouridin, they use sunlight as the sources of energy to prepare food from CO_2 and H_2S.

$$6CO_2 + 12H_2S \rightarrow C_6H_{12}O_6 + 6H_2O + 12S.$$

The autotrophic bacteria which derive energy by oxidizing certain inorganic compounds an known as chemoautotrophs or chemolithotrophs or chemosynthetic bacteria. Nitrifying bacteria, sulphur bacteria, iron bacteria, etc. are examples of chemosynthetic bacteria. The nitrifying bacteria oxidize ammonium salts into nitrites to draw from CO_2 .

$NH_3 + 3O_2 \rightarrow HNO_2 + 2H_2O$ + Energy (70K cal).

The sulphur bacteria oxidize hydrogen sulphide to get energy for chemosynthesis.

$H_2S + 2O_2 \rightarrow H_2SO_4$ + Energy.

The iron bacteria oxidize ferrous iron compounds into ferric hydroxides to get energy for chemosynthesis.

$Fe^{2+} + H^+ + {}^1/_2O_2 \rightarrow Fe^{3+} + H_2O$ + Energy.

The carbon bacteria in the damp coal oxidizes carbon monoxide into carbon dioxide to get energy for doing chemosynthesis.

$CO + ½\ O_2 \rightarrow CO_2$ + Energy (74 K cal).

The heterotrophic bacteria make use of organic compounds such as simple sugars and amino acids as the source of carbon. The bacteria which lives in the cells of other organisms by feeding on their cellular components are called parasitic bacteria. Those living on dead organic matter to draw energy and carbon source are called saprophytic bacteria or organotrophs. The bacteria which live in association with other organisms are known as symbiotic bacteria. Eg. Rhizobium in the root nodules of legumes.Some heterotrophic bacteria use sunlight as the energy source and organic compounds as the source of carbon. Such bacteria are known as photoheterotrophs or photo-organotrophs. Eg. Non-sulphur purple bacteria.Some heterotrophic bacteria use organic compounds as the source of carbon as well as energy. Such bacteria are known as chemoheterotrophs or chemo-organotrophs.

Classification based on Respiration

1**. Aerobic Respiration:-**The organic food substances such as sugars are oxidized into CO_2 and H_2O by using molecular oxygen. Here the food substance acts as an electron donor and the oxygen acts as an electron acceptor. Anaerobic respiration in bacteria is very similar to that in higher plants. The overall reaction of aerobic respiration is given below

$C_6H_{12}0_6$ ----→$6CO_2 + 6H_2O$ + D686 Kcal

The glucose is broken into 2 molecules of pyruvic acid by glycolysis. The pyruvic acid molecules are oxidized into CO, and H,O by Krebs's cycle. During these two series of reactions. NADH, molecules are generated. The electron transport systems present in the plasma membrane oxidize the NADH, into NADH and release energy. This energy is stored in the form of ATP molecules. Aerobic bacteria grow on the surface of the culture media.

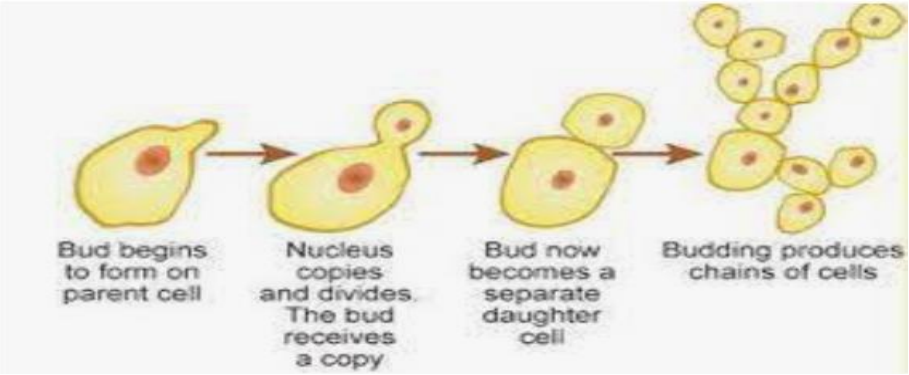
2. **Anaerobic Respiration:-**The generation of energy from organic food substances using electron acceptors other than free oxygen is called anaerobic respiration. Here NO, SO^2, CO_2, S^o and Fe^3 are used as the final electron acceptor in the electron transport chain.The glucose is broken into two molecules of pyruvic acid by glycolysis. The pyruvic and undergoes fermentation and forms ethyl alcohol and CO_2 or lactic acid. It consumes NADH The NADH, produced in the glycolysis passes through the electron transport chain that relea energy. At the final step the electron goes to any one of the above said electron acceptors. For example, in Desulfuromonas, elemental sulfur S^o accepts the electron and becomes HS Pseudomonas and Bacillus use Fe^{3+} as electron acceptor and hence Fe^{3+} becomes Fe^{2+} A large amount of energy is wasted in the form of heat during anaerobic respiration. Further it is an incomplete respiration that makes little ATPs. Anaerobic microbes form colonies at the bottom of the culture media.

3. Facultative Anaerobes:-Some bacteria do aerobic respiration when free oxygen is present and proceed anaerobic respiration when there is no oxygen. Such bacteria are called facultative anaerobes. Eg Methanogens and Desulfovibrio. They are growing well in the middle of the culture media. Their electron transport system is best suited for aerobic and anaerobic respirations.

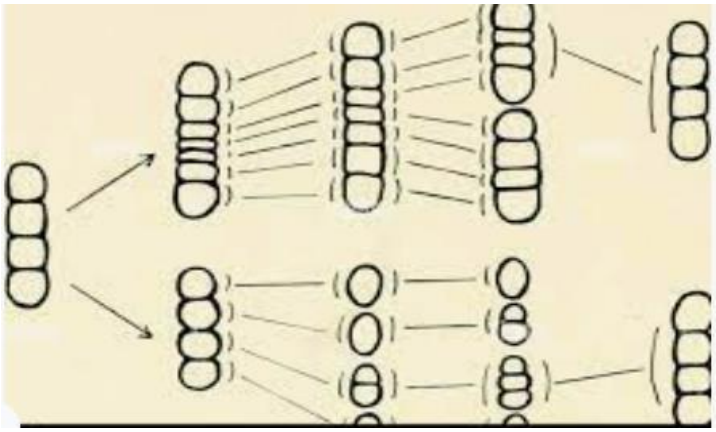
REPRODUCTION IN BACTERIA: -Reproduction in bacteria or multiplication of cell numbers in bacteria occurs mainly by vegetative reproduction and asexual method. There is no sexual reproduction in bacteria due to lack of sex organs or gamete formation.

(A) Vegetative Methods of Reproduction: **-It occurs by three methods Budding, Fragmentation and Binary Fission.**

(a) Budding : Budding occurs by formation of a protuberance called bud at one end of the cell. Genome replication occurs and a copy of the genome enters into the bud. The bud then enlarges and finally gets separated from the parent cell to give rise to a daughter independent bacterial cell.



(b) Fragmentation : During unfavourable conditions bacterial protoplasm undergoes compartmentalization forming minute bodies called gonidia. Each gonidium grows to a new bacterium under favorable conditions. Prior to this, the genome has under gone several replications so as to be included one in each gonidium. The parent body divides into several fragments and each fragment develops into a new organism



(c) Binary Fission: It is the most common method of reproduction in all bacteria

1. The bacterium before binary fission is when the DNA is tightly coiled.
2. The DNA of the bacterium has uncoiled and replicated.
3. The DNA is pulled to the separate poles of the bacterium as it increases in size to prepare for splitting.
4. The growth of a new cell wall begins to separate the bacterium.
5. The new cell wall fully develops, resulting in the complete split of the bacterium.
6. The new daughter cells have tightly coiled DNA rods, ribosomes, and plasmids, these are brand new organisms. (ii) Before binary fission the cell constituents get doubled. (iii) The entire event occurs in a proper sequence - first DNA replication, then DNA partitioning and lastly cross wall formation.(iv) First the bacterial chromosome gets attached to the cell membrane and replicates into two.(v) As the cell enlarges, the daughter DNAs still attached to the wall get separated by a simple pinching off process.(vi) A cross wall is then formed. First the transverse plasma membrane is laid

down followed by the centripetal growth of the cell wall which splits the plasma membrane into two halves.(vii) The binary fission occurs when the environmental condition is favorable and it takes nearly 20-30 minutes to form two cells. This is the generation time. (viii) Due to binary fission the single bacterial cell grows in a geometric progression and a big colony is seen in the culture plate in a short time

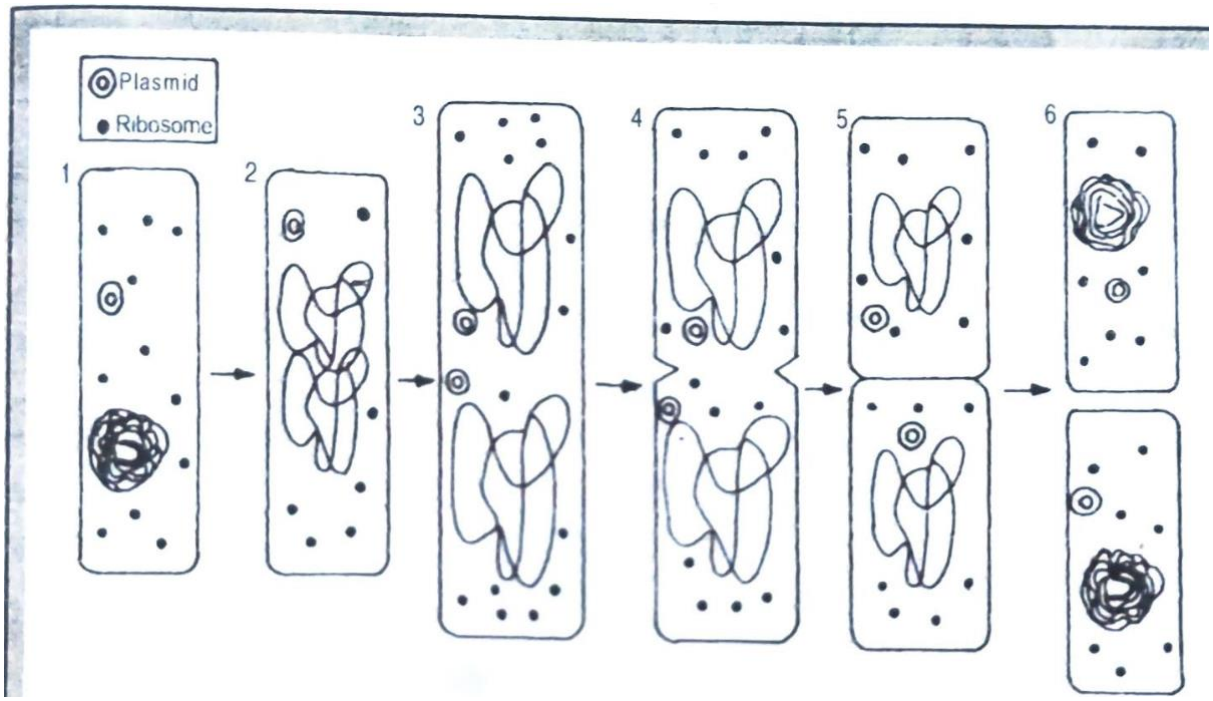


Diagram of Binary fission

d) **Endospore formation**:-Some bacteria are able to produce spores called endospores. Generally spore producing bacteria are rod shaped and species of Clostridium and Bacillus form endospores under unfavourable circumstances. Only one endospore is formed in one cell.

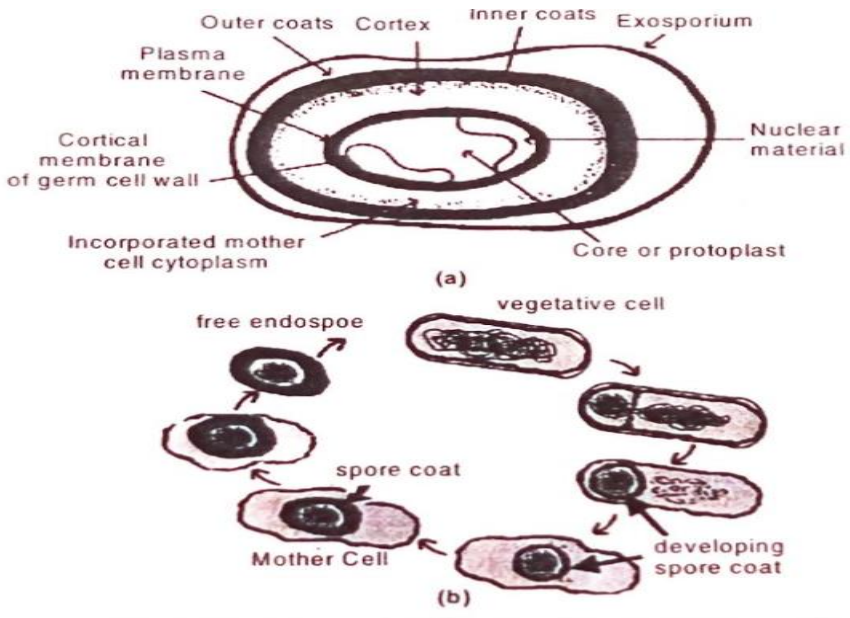
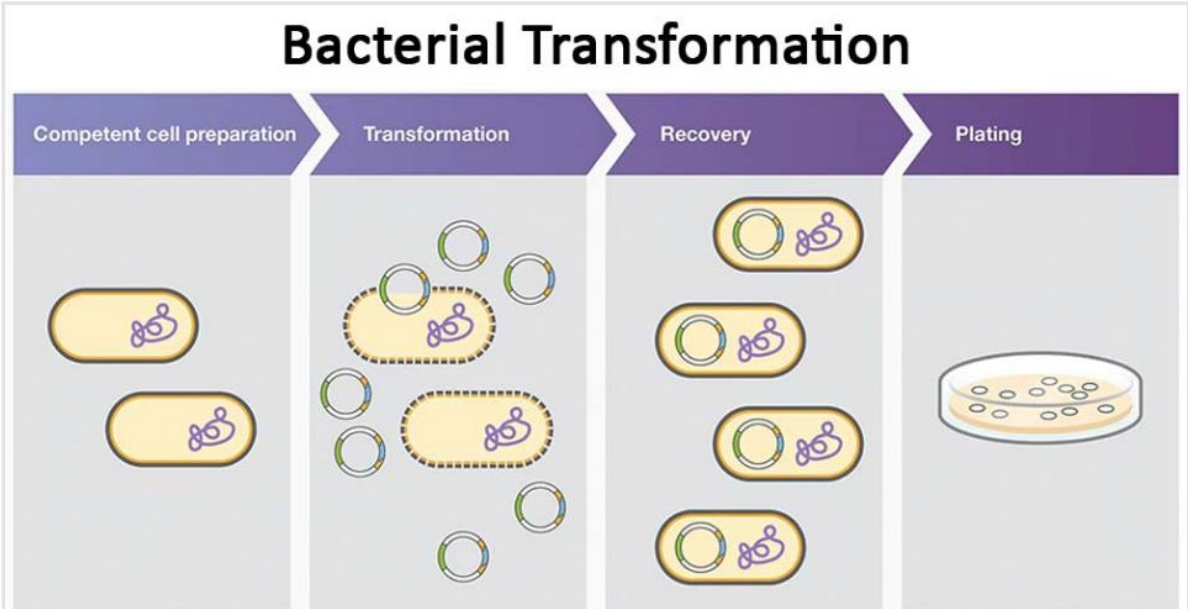


Fig. 5.8. Endospore formation and structure

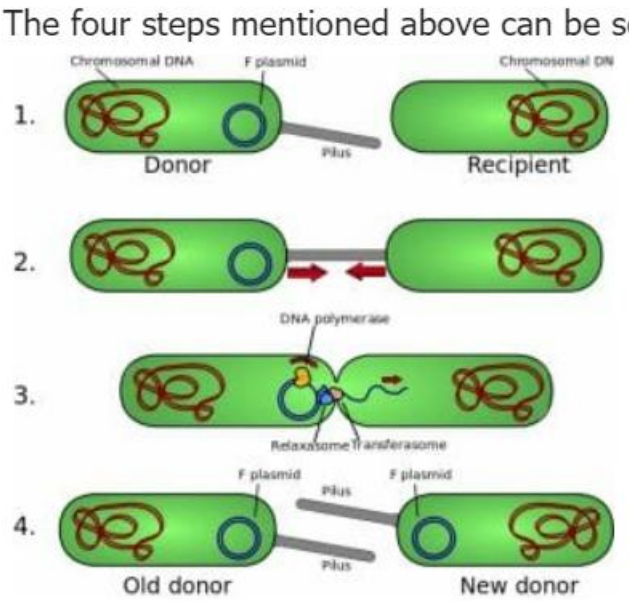
i) During the process, a part of the protoplast becomes concentrated around the (ii) A hard resistant wall is secreted around it. (iii) The rest of the bacterial cell degenerates.
(iv) Each endospore is either spherical, cylindrical or ellipsoidal in shape. (v) The endospore has a complex structure.(vi) The outer layer is thin, delicate and called exosporium. It is followed by the spor coat. Inner to spore coat the cortex contains concentric rings of wall. Below th cortex, the core cell wall is present surrounding the cell membrane and the protoplasm (vii) Endospore contains especially dipicolinic acid (DPA) which in association with calcium ions make the endospore heat resistant.(viii) Under favourable conditions, the endospore germinates by imbibing water, activating the cytoplasm and synthesizing metabolites and then due to swelling bursting or wall occurs giving rise to the new vegetative cell

Sexual method of reproduction(genetic recombination in bacteria):-
Recombination is the arrangement and reshuffling of genes resulting in the new genotypes. Bacterial recombination is brought about by the transfer of genetic materials from one bacterium to another. The bacterium which denotes the genetic material is called donar and the bacterium is called recipients. It occurs by 3 methods
1)Transformation:- Bacterial transformation is the transfer of free DNA released from a donor bacterium into the extracellular environment that results in assimilation and usually an expression of the newly acquired trait in a recipient bacterium. Bacterial transformation is based on the natural ability of bacteria to release DNA which is then taken up by another competent bacterium.
The success of transformation depends on the competence of the host cell. Competence is the ability of a cell to incorporate naked DNA in the process of transformation. Organisms that are naturally transformable spontaneously release their DNA in the late stationary phase
This process doesn't require a living donor cell and only requires free DNA in the environment. The recipient that successfully propagates the new DNA is called the transformant. During extreme environmental conditions, some bacterial genera spontaneously release DNA from the cells into the environment free to be taken up by the competent cells. The competent cells also respond to the changes in the environment and control the level of gene acquisition through a natural transformation process. Transformation is adopted as the most common method of gene transfer as it is the best way for the transfer of artificially altered DNA into recipient cells. The process of transformation can transfer DNA regions of one to tens of kilobase.Several bacteria, including Escherichia coli, can be artificially treated in the laboratory to increase their transformability by chemicals, such as calcium, or

by applying a strong electric field



2)Conjugation:-Conjugation is the transfer of a plasmid or other self-transmissible DNA element and sometimes chromosomal DNA from a donor cell to a recipient cell via direct contact usually mediated by a conjugation pilus or sex pilus. Recipients of the DNA transferred by conjugation are called transconjugants. The process of conjugation can transfer DNA regions of hundreds to thousands of kilobases and has the broadest host range for DNA transfer among the methods for bacterial exchange.Conjugation occurs in and between many species of bacteria, including Gram-negative as well as Gram-positive bacteria. The process of bacterial conjugation is based on the principle that the plasmid or any other genetic material is transferred from the donor cell to the recipient cell through close physical contact. Of all the conjugative plasmids, the F (fertility) plasmid of *E. coli* was the first discovered and is one of the best-studied. The F plasmid is present in one or two copies per cell and is very large (about 100 kilobases). *E. coli* harbouring the F plasmid are referred to as donor (F^+ or male) cells and E. coli lacking the F plasmid are referred to as recipient (F^- or female) cells. Only donor cells are capable of transferring the F plasmid to recipient cells.For transfer of the F plasmid from donor to recipient, intimate contact between cells, resulting in mating-pair formation, is required. The transfer of genetic material is then brought by membrane fusion of the two cells by the action of different enzymes.Following the membrane fusion, the replication of donor DNA occurs and is transferred into the recipient cell.



3)Transduction: - The transfer of genetic material from one bacterium to another through bacteriophage is called transduction. Here the bacteriophage infects a bacterium and multiplies within it. During this process, the new phages pick up certain genes from the bacterial genome. After lysis of the bacterium the new phages infect another bacterium. During this process, the host gene picked up the phage is injected into the bacterium.Therefore, the bacterium shows a some characters of the old host of the phage virus. Transduction is a method of gene transfer in bacteria from donor to recipient using bacteriophage. In transduction at first bacteriophage infects donor bacteria and then carries some part of donor genome with it. When this bacteriophage infects new bacterial cell, it transfer that DNA in to recipient cell. The phages involved in transduction are all temperate, that is, those which either lyse the cell at once or remain in the host cell without killing it. They can multiply inside the bacterial cell and finally cause lysis of the host cell. A bacterium which harbours temperate phages is said to be lysogenic.