Study Material For DTM-111(Fundamentals of Microbiology)

Part of my Book (Accpted for Publication)

Microbiology

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Chapter-1

History of Microbiology

History of microbiology is very old long and rich. In early phases it was centered toward the causes of infectious diseases.

But now a day Now it include practical application of so many science like genetics, Botany zoology and others applied field like biotechnology and molecular genomics. It is not sure that who made the first observations of microorganisms, but the first microscope was available during the mid-1600s,and an English scientist named Robert Hooke made key observations. Some of the important contributions of these Scientist are listed below year wise:

1. Robert Hooke (1635-1703): He was young English scientist,that became the first person to view and describe fungi using a simple compound microscope. He observed tiny cork-like structure resembling 'little boxes which he described cell as basic unit of life. His observation is published in his book"*Micrographia*". For his significant work he was known as 'The father of microscopy'. He also **discovered the Law of Elasticity(or Hooke's Law)**.He also studied the planets, and innted a process for telling the time accurately, as well as being an accomplished architect.

2.Antonie van Leeuwenhoek (1632-1723): He was basically a moderately educated cloth merchant, used lenses to examine cloth and that led to his interest in lens making. He assembled hundreds of microscopes, some of which magnified objects 270 times. As he look the things with his microscopes, he discovered "Microorganisms the organisms so tiny that they were invisible to the naked eye. He called these tiny living organisms as "Animalcules". The organism which was found everywhere, in water, Soil, in bubbles in every particle of nature. He was first to describe bacteria and then Protozoans also.In1674 he sent his discoveries to the Royal Society of London. His primary discoveries led the expanded use of microscopy as a standards cientific tool.

Fig: A.V. Leeuwenhoek , Simple Microscope

Source: Virtual Microbiology class room on science proof online.com

3.Debate over Spontaneous Generation/Non spontaneous origin of life

In ancient times, origin of organisms (such as rats and flies) was thought to begin or being arisen spontaneously. Microorganisms always appeared suddenly in certain materials (meat juices or plant extracts) that had previously been free of them. It was believed at that time that the microbes are the products of spontaneous generation (the formation of living things from inanimate or non living matter).

3.1Non Spontaneous theory was supported by Aristotle (Circa350BC)**.**According to him the "readily observable aphids arise from the dew which falls on plants, fleas from putrid matter, and mice from dirty hay".

3.2. Francesco Redi : (1626-1697)The belief of spontaneous origin of life remained unchallenged for more than 2000 years. Until an Italian Physician, Naturalist and Poet **Francesco Redi** first to formally challenge the accepted belief of spontaneous generation.

Fig: Francesco Redi,

(Images: Redi'sexperiment,Unknown Fracesco Redi portrait.

Source:Wikipedia)

Fig: Coverd Jar Experiment by Redi

Source: Virtual Microbiology Class room on Science Proofonline.com.

3.3.Needham Versus Spallanzani:

John Needhamin (1745) did a experiment to disprove the spontaneous origin of organism that believe remain for almost 70-80 yrs. Every one knew boiling killed microorganisms. Therefore, he boiled chicken broth, put it in a flask, and sealed it. Microorganisms could develop in it only by spontaneous generation. Experiment with gravy seemed to show that life could be generated from non-living materials.

3.4.Lazzaro Spallanzani an Italian priest and professor was not convinced with the above theory of Non Spontaneous origin of Organism. According to him, microorganisms might have entered broth after boiling but before sealing. Therefore, Spallanzani put broth in a flask, sealed it, and then boiled it. No microorganisms appeared in the cooled broth.

Fig:Needham(1713–1781)

(Source:Wilkipedia.org.in)

But the clear-cut indication of origin of life were not persuaded to the critics. Spallanzani didn't disproves Spontaneous generation, he just proved that spontaneous generation required air. Gradually, spontaneous generation was rejected as the origin of visible organisms.

Fig:Spallanzani(1729–1799)

(SourceWilkipedia.org.in)

Fig: Experiment of Needham and Splanzanni

Source: Virtual Microbiology class room on Science proof online.com

3.5.Francesco Reddi (1665): He was a physician in Italy, played a major role to disprove the spontaneous theory. He did experiments with covered and uncovered jars of meat. He showed that maggots (fly larvae) developed only in that meat where flies could reach to lay eggs on. Apparently, Spontaneous generation did not occur, at least in the case of flies. Flies and other living livings things come only from pre existing living things. Still, many people believed that microorganisms were an exception to this rule. They are very simple and they always appear, in large numbers, soon after a plant or animal dies. Could decomposition form microorganisms or the microorganisms causing decomposition? Controversy of spontaneous generation gained momentum during the late18th and 19 th centuries, when further advances in microscopy allowed people to view bacteria and other microorganisms.

4.Pasteur'sExperiments:

The controversy over Spontaneous/ Non spontaneous continued foranother 100 years until Louis Pasteur (1822-1895) who counter the argument that air was necessary for spontaneous generation by doing a Famous experiment as follows:

A. Louis Pasteur used a special flask whose neck was shaped like a neck of a swan, hence the name "Swan Neck Flask."

B. He put a nutrient rich broth in theflask,which he called the"infusion.

C. He put a nutrient rich broth in the flask, which he called the "infusion."

D. He then boiled the infusion killing any microorganisms which were already present.

E. Then he allowed the infusion to sit. Because of the shape of the flask, the infusion was exposed to air. However, dust particles and other things in the air never made it into the infusion. Because they were trapped in the curve of the Swan Neck Flask.

F. Microorganisms never appeared in the infusion by letting the fusion for long time even. However, if he tipped the flask and allowed the things trapped in the neck to get into the infusion then microorganisms began to appear in the infusion and multiply rapidly.

Experiment demonstrated that microorganisms do not appear as a result of Spontaneous Generation. Instead, they are introduced into food through dust particles and other things that happen to land on the food. Thus, Pasteur convinced the scientific world that spontaneous generation of microorganisms does not occur even in the presence of air. Pasteur's simple but elegant experiments grounded microbiology in scientific reality. Thus the most famous contribution to microbiology by

Pasteur is to develop sterilization technique.i.e to preserve food by killing the spoilage microbes by heating process .

Source: Wilkipedia.org.in.

Fig: Louis Pasteur

Image: Louis Pasteur, Pierre Lamy Petit, circa 1866

Fermentation: Another important contribution of Pasteur was to define fermentation by seeing observation in spoilage grapes. He noticed two type of spoilage there a good one and a bad one.good one leads to formation of tasty wine while another lewads to a bitter and yukky wine.

He observed a growing blob on the growth media and asked Are these non-living blobs or living microbes?

Grapes fermenting; ***Saccharomyces* yeast**; **Lactobacillus**,

Source : Public health Image Library #1048

Pasteur's Observations:

1. Blobs were alive because they divide and make more of themselves.

2. Put grape juice + yeast in open and in air-tight containers. Fermentation occurred in both.

   This means that yeast are anaerobic fermenters

3. Took two flasks of sterile grape juice and introduced bacteria into one and yeast into

   another.

   yeast + grapes = yummy wine (ethanol)

   Bacteria + grapes = spoiled wine (lactic acid)

Earlier fermentation was believed to be a pure chemical process in which sugar transformed into alcohol. But in 1857, Pasteur proved that a microorganism caused the souring of milk (lactic acid fermentation). He was also able to prove that living cells, the yeast, were responsible for forming alcohol from sugar. The contaminating microorganisms found in air can turn the fermenter sour. He reported his findings in "MÃ©moire sur la fermentation appelÃ©e lactique" (Memoir on the fermentation of lactic acid) in 1857, and "MÃ©moire sur la fermentation alcoholique" (Memoir on the fermentation of alcohol) in 1860. Pasteur identified the microorganisms responsible for both normal and abnormal fermentation.

Another significant contribution of Pasteur is that to introduced the term **"Pasteurization,"** A process of gentle heating to prevent the spoilage of beer and wine by undesirable microorganisms.

He also made notable contributions in the field of vaccination and immunity. Studying cholera, Pasteur found that attenuated organisms, inoculated into poultry, offered protection against virulent strains. Based on this research, he developed the first rabies and anthrax vaccines.

5. John Tyndall and Tyndallization:

Boiling does not always kill all the microorganisms in broth. John Tyndall was an English Physicist (1820-1893) who confirmed the result of Pasteur experiments. He demonstrated that heating does not killed always all the variants of microorganisms present in the heated materials. It is due to the differential ability of some of the microorganisms to tolerate the heat i.e special ability of some microorganism to form ensdospore. Microorganisms exist in two forms.

1. Heat labile and

2. Heat stable. The former likely to be killed by exposure to heat but former need a special treatment.

3. He introduced a new process to kill that heat stable microorganisms by intermittent heating. He successively heated the broth at the interval of one day and cooling them to allow the growth of resistant microorganisms.

4. By successive heating and cooling technique resistant microorganism could be killed there off. This validates Pasteurs nonspontaneous generation theory of origin.

5. Repeated heating on successive days is known as Tyndallization. The alternate day heating at 121℃ and a pressure 15ponds/sq inch can sterilize the solution.

6. **Germ Theory of Disease**: **(Robert Koch,1843-1910):**

Microbiology changed from an observational science to an experimental science once spontaneous generation of microbes was disproved, microbiology exploded. Now the scientist starts working over to study the cause of infectious diseases. A German physician made several discoveries in the field of Microbiology. The main are

1. The bacteria can be isolated and cause disease while working with anthrax disease of animal.He gave final proof of these concept.

2. He developed Koch's Postulate which means that a specific microorganism is responsible for the cause of specific disease.

3. He made another critically important contribution to microbiology. He developed a technique to obtain a pure culture of a bacterium (one that contains only a single kind of bacteria) and propagate it. In nature many kinds of bacteria are found growing together (mixed cultures). It is difficult to do valid experiments using mixed cultures because they are so complicated, although such studies are now becoming more common.

Robert Koch also proved that microorganisms (germs, as they were and are still sometimes called) cause disease. He isolated and identified specially bacterium therefore; he is regarded as true bacteriologist. He is also known as founder of medical microbiology, bacteriology and virology He showed further that specific microorganisms cause specific diseases. Koch also introduced higher scientific standards of rigor to microbiology, as exemplified by those called Koch's postulates. Since he was a physician and practiced as a doctor he came in knowledge with tha anthrax disease which begins with symptoms similar to cold and cause itching skin blister which turn black and swell later on. The bacteria may move into the blood and cause fever shock and finally death.Koch isolated the bacterium that causes anthrax. He injected laboratory animal with the bacteria laden blood to dead sheep and recorded the same symptom appear in the tested animal.

He isolated few bacilli in a clear sterile virtuous humor of an ox's eye and observed their multiplication by injecting the sample from the pure culture of that organism obtained from the dead Ox. Mice were kept for observation for the development of symptoms. After some time mice were dead and sample were withdrawn and cultured on pure culture media. Similar organism is then observed in the tissue of dead mice as that of dead ox. Koch speculated on the formation of a resistant form of bacterium-an endospore. He studied the anthrax bacterium as small rod shape having ability to form a heat resistant spore named

as Endospore. Similar disease he studied in frog and horses and demonstrated that how disease organisms became concentrated in the lymph sacs and spleen.

Koch's postulates: In 1876 Robert Koch while studying anthrax, a disease of cattle and sheep that also affects humans established "scientific rules" to show a cause and effect relationship between a microbe and a disease known as Koch's postulates as follows:

1. The same organisms must be found in all cases of a given disease.
2. The organism must be isolated and grown in pure culture.
3. The isolated organism must reproduce the same disease when inoculated into a healthy susceptible animal.
4. The original organism must again be isolated from the experimentally infected animal. On the above basis microbiologist concluded that a particular organism is responsible for a particular disease. Koch isolated the bacilli-the causal pathogen of tuberculosis. He was awarded with Nobel Prize in 1905 for his work on tuberculosis. He died in 1910.

7. Immunology : Edward Anthony Jenner (1749-1823) was a British Physician who studied his natural surroundings in Berkeley, Gloucestershire. He first developed the technique of Vaccination in 1795 well before the time of Pasteur or Koch. He studied two disease cow pox and small pox. The disease cow pox was known as vaccine and using this word Pasteur developed the term Vaccination and Vaccine. Jenner is widely credited as the pioneer of smallpox vaccine, and is sometimes referred to as the 'Father of Immunology'. Though Pasteur's achievements in microbial immunity were revolutionary, Jenner is credited with inventing the first vaccine against smallpox in late 1700s. In 1796, Jenner developed a controversial experiment to determine the validity of rumors that were circulating in rural communities. Milkmaids and villagers often recanted, "if you want to marry a woman who will never be scarred by the pox, marry a milkmaid." Jenner speculated that becoming infected with cowpox could offer protection against the more virulent smallpox. To test his hypothesis, he created an inoculation with scrapings of cowpox lesions from the fingers of a young milkmaid and injected it into an young old boy. As expected, boy developed the mild fever and cowpox lesions typical of the disease. After a few weeks of recovery, Jenner injected young boy with the live smallpox virus and found that the boy was indeed protected from the disease. In 1798, Jenner published his findings and presented them to the Royal Society.

8.Growth of Medical Microbiology: The work of above scientists like Pasteur, Koch, Jenner and others led to several important discoveries to human pathology. Hensen (1874) discovered Leprosy bacterium (*Mycobacterium leprae*) Klebs (1883) and Loeffler (1884) isolated diphtheria pathogen(*Corynebacterium diptheriae*) Kitasato isolated the germ of tetanus(*Clostridium tetanii*),and plague(*Pasteurella pestis*).etc Later on Immunization of tetanus and diphtheria was discovered by Von Behring(1890) for which he was rewarded with First Nobel Prize in physiology or Medicines. E. Metchnikoff demonstrated that certain leukocytes eat disease causing bacteria in body and finally introduced the theory of Phagocytosis. Sir Alexander Fleming (1928) invented the wonder drug, the Penicillin during a experiment where he

noticed that colonies of *Staphylococci* were dissolved around a mould of *Penicillium notatum* which was accidently growing on a culture plate. From this plate Fleming extracted the active substance called penicillin. It was the first antibiotic that was discovered for this he was known as Father of Antibiotics. Walkman, A plant pathologist along with his coworker discovered streptomycin. The discoveries of Antibiotics have been one of the revolutionary landmarks in the history of medicine. Word antibiotic signifies anything against life .Microorganisms when present in a natural medium either favor the growth of other microorganism by a process called symbiosis or antagonized their growth by a process of antibiosis by secreting a chemical substance known as antibiotics. So the term Antibiotics can be define as metabolic product obtained from life activity of microorganism and show inhibitory action to life of other organism even in a very small amount. A few antibiotics were useful in treating the human disease but their applications are being found useful in curing and controlling plant and animal diseases. For a number of decades, Scientist continuously working on treatment of disease by exploring the capability of microorganisms to produce antibiotics and since then many disease has been controlled.

9.Staining technique: Ninteen sanctuary was the golden years for the development of histological dyes such as carmine and haemotoxyline for the purpose of staining bacteria. Paul Ehrlich (1854-1915) prepared aniline dye from coal tar distillates. He also developed modern concept of Chemotherapy and Chemotherapeutic agents. The methyl violet dye was first used by Weigert (1875) for staining bacteria. In the year 1853-1933 Hans Christian Gram developed important procedure for differential staining that is known as Gram staining of bacteria. He demonstrated that certain strain of bacteria looses the ability to retain color of crystal violet when treated with alcohol whereas other retains it. So on the basis of retention of color he categorized bacteria in two..Those who retain color of crystal violet names as Gram +ve and those who lost their ability to retain is Gram –ve.

10.Antiseptic Surgery : Joseph Lister (1827-1912), a British surgeon was the pioneer of antiseptic surgery, who promoted the idea of sterile surgery while working at the Glasgow Royal Infirmary. Lister successfully introduced carbolic acid to sterilize surgical instruments and to clean wounds, which led to reduced post-operative infections and made surgery safer for patients.

In the year 1878, Joseph Lister also studied Fermentation of milk and published his study that the specific cause of milk souring is Lactic acid bacteria. His research was conducted using the first method developed for isolating a pure culture of a bacterium, which he named *Bacterium lactis.*

11. Virology:

Virus a entity that surprised many scientist from that date being a entity that neither live nor dead but still had a great impact on the human life.Viruses don't have their own machinery but they depends entirely on the host metabolism to survive.This ability of virus make them to take them as live so they are known to be a connecting link between living and non living organisms.Some of the important contributions of the Scientist in this field are as follows:

Dmitri Ivanowski: (1864-1920): A the Russian microbiologist discovered the tobacco mosaic virus. The Science dealing with Virus is known as Virology. The study of viruses, began in 1892, That was even prior to the development of bacteriology. Ivanovsky was studying a disease of tobacco plants called tobacco mosaic disease. To identify its cause, he filtered juice from diseased plants through filters that retained the smallest bacteria. He found the filtered juice still caused disease. Because bacteria were believed to be the smallest microorganisms, Ivanovsky concluded that these minute disease-causing agents can pass through the filter that retain the bacteria. He called these tiny agents 'filterable viruses'. They could not be seen, even under the most powerful microscopes of that time.

Chamberland : (1884): He established the fact that this filterable agent can cause disease to healthy plant. The observation was further supported by M.W.Beijerinck (1892).

F.W.Twort and Felix d'Harelle (1915-1917) : He discovered the phenomenon of Bacteriophagia-the disease caused by harmful and parasitic activities of viruses on bacteria. He defined those viruses who killed or eat the bacteria is known as Bacteriophage.

W.M Stanley (1904-1971): A biochemist worked on crystallization of virus protein and for this outstanding work he got Nobel Prize in chemistry in the year 19426. He argued that TMV is a type of autocatalytic protein which multiplies only in living cell.

Fraenkel- Conrat and Williams (1955): They were able to separate the protein and acid moieties from the TMV particles.

Bawden and Pirie'(1907-1997) : He described the virus protein as a nucleo- protein and established the relation between nucleoprotein and phosphorus present in TMV. With the advent of new technologies and invention of instruments like electron microscope, X-ray diffraction photographic rotation technique and ultracentrifuge techniques etc.

12. **Modern Microbiology and Biotechnology:** As new laboratory techniques and experimental procedure were developed, Knowledge about the characteristics of microorganisms accumulated rapidly. The discoveries of DNA by as a genetic and hereditary material in the year 1952 when Alfred Hershey and Martha Chase using bacteriophage became so significant that phage became a standard laboratory material. These discoveries led the foundation of double helix model of Watson and Crick in the year 1953 who used X-ray diffraction pattern for the elucidation of their model. For this outstanding symmetrical model of DNA they were awarded with Nobel Prize. Research work one by Nirenberg, Holley and Khorana established the basis for deciphering the **genetic Code.** As DNA from different origin contain only the four nucleotides,A,T,G,C protein from diverse sources are commonly made up of only 20 amino acid (the building block of proteins).the questions then raised is how only four nucleotides were necessary to code for 20 amino acids? That is possible only by combination of four nucleotides in all possible way that makes a total of (4^3) of 64 codons. This code is more than enough to code for 20 amino acids. The codons are degenerate, missense and non sense too. They provide an understanding of how the DNA of various organisms may code for the same amino acids. They also provide numerous

ways to code not only for composition but also for the unique arrangements or sequence of amino acid in the diverse protein of those organisms. The triplet code also provide for more than one code for amino acid. There are few codon which cannot code for amino acid are called missence codons. They are considered as the punctuations for the genetic alphabet or code. The finding that more than codons could code for a given amino acid is known as degeneracy or redundancy in the genetic code. Study on DNA of viruses and bacteria gave insight the knowledge of genetic engineering. Bacteria have an additional DNA as plasmid which may code for many of the important functions as antibiotic resistance or toxin production or host range have become the tool for genetic engineering. These plasmids are used as vehicle to carry external DNA and to transfer them into another DNA. Such DNA is also used to developed gene library. The discovery of restriction endonuclease has revolutionalized the field of biotechnology. This was isolated from bacteria and the technique of biotechnology is based on this enzyme. This enzyme recognizes specific DNA nucleotides sequence and provides a molecular scalpel for cutting DNA at these sites. Some restriction endonuclease provides sticky ends which can anneal with any DNA fragments cut by the same enzyme. The normal function of bacterial endonuclease is the protection against invasion by foreign DNA. This technique provide the basis of cloning of many useful genes that are the causes of many chronic disease in the human being and are being in research now a day.

The End

Branches of Microbiology

Applied microbiology is focused on how various organisms can be used (applied) in given processes or the impact they can have in different industries.

Some of the most important branches of microbiology based on application include:

2.1 Food Microbiology:

Deals with the microorganisms that contaminate/damage food and those that can be used for food processing and modification. It give microbiology gives special attention with public health concern in mind. Microorganisms such as molds, yeasts, and bacteria that either benefit or have negative effects on the quality of food material. Food microbiology is connected to several other fields (immunology and molecular biology etc) and deals with such aspects of food processing and preservation, food ingredients, production and fermentation process.eg.Food spoilage,Fermentation etc

2.2 Medical Microbiology

This is the branch of microbiology that is concerned with the diagnosis, prevention and treatment of diseases caused by different types of infection agents vig Bacteria, Fungi and Virus. This sub-discipline is therefore related to a number of other fields including virology, bacteriology, immunology, and germicrobiology.

2.3 Industrial microbiology

This branch of microbiology is concerned with the use of various microorganisms for different industrial production. Industril Microbiology directed towards the use of these organisms to increase and maximize yields in industries like fuel, pharmaceutical, and chemicals etc. It deals with the Optimum use of Microorganisms to maximize the products and Process to meet the demand of population.eg.Production of Alcohal,Bread,Vinegar etc

2.4.Agricultural Microbiology

Agricultural microbiology is concerned with microbes associated with plants and animal production and also their economic importance for farmers and the industry as a whole. In the process, agricultural microbiology is aimed to solve issues identified in agricultural practices while helping increase yields for farmers. Eg. N_2 fixation,Biofertilizer, Biopesticides etc

2.5. Soil Microbiology- This is the branch of microbiology that deals with thestudy of soil microorganisms and how they impact soil properties.eg. Composting, Soil Sturture and texture,

2.6.**Pharmaceutical Microbiology**- Concerned with the use of microorganisms for inhibiting contamination as well as the development of pharmaceuticals.Such as development of anitibiotics and drugs using Microorganisms.

2.7. **Veterinary Microbiology**- Focus on microbes that cause diseases in animals, Production from animals and birds etc .Such as Mastitis in animal,Milk and Meat production etc.

2.8. **Microbial biotechnology**- Area of microbiology and biotechnology aimed at using microbes for beneficial purposes.It focuses on the utilization of Biological things combine with molecular biology to maximize the products for human population.Such as Plasmid ,Recombinant Enzyme,PCR technology etc.

Chapter-3

Application of Microbiology.

Microrganism have been used since long years before for the purpose of many products viz Curd, Bread,Cheese, Paneer,etc**.** The present chapter dealt with various aspects of Microbiology in Food, Industries and Environment

3.1 Food Microbiology

Food Microbiology includes the study of Microorganisms which have both beneficial and deleterious effects on the quality and safety of foods. It focus on the general biology of the microorganisms that are found in foods including: their growth

characteristics, identification, of pathogenes and their symptom after consuming any foor having loads of Microorganism . The main areas of interest which concern food microbiology are: food poisoning, food spoilage, and food legislation. Pathogens in product, or harmful microorganisms, result in major public health problems worldwide and are the leading causes of illnesses and death. In the United States alone, food borne illness has been estimated to cause 10,000 deaths and 86 million illnesses per year.

3.2. Food borne Illness

Microorganisms can cause a variety of deletoriuos effects in food products including spoilage, which primarily affects product quality, and food poisoning, which is generally caused by pathogens. A food borne illness (or disease) is the term indicates - a disease or illness caused by the consumption of contaminated foods or beverages.

Food borne diseases are primarily of two primary types: food-borne infections and food intoxications. While the former takes place due to ingestion of microbes, followed by growth, tissue invasion, and/or release of toxins, the latter is caused as a result of ingestion of toxins in foods in which microbes have grown. More than thousand different food borne diseases have been described. Most of these diseases are infections, caused by a variety of bacteria, viruses, and parasites. Other diseases are poisonings, caused by harmful toxins or chemicals that have contaminated the food, for example, Aflatoxins released by Poisonous mushrooms, and ither toxins caused by Bacillus and Clostridium species or heavy metal contamination .

3.3.Food Spoilage

Spoilage organisms alter food texture , appearance and organoleptic qualities of the food making it unsuitable for human consumption. Spoilage is often the result of a succession. One organism creates an environment conducive to the growth of another. Common microbial food spoilage are:

I.Putrefaction- Protein + proteolytic micro organism's — amino acids + amines + ammonia + H_2S

II. Fermentation- Carbohydrates + fermenting microorganism's—acids + alcohols + gases

III. Rancidity- Fatty foods + lipolytic microorganism's—fatty acids + glycerol.

3.4 Fermented Foods.

Fermentation is most common method of preservation that the people are using since long day before.These are the foods that have been subjected to the action of micro-organisms or enzymes, in order to bring about a desirable change. Numerous food products owe their production and characteristics to the fermentative activities of microorganisms. Fermented foods originated many thousands of years ago when presumably microorganism contaminated local foods. Furthermore, microbial fermentation can increase nutritional quality and digestibility of food while producing desirable textures and flavours (Organoleptic properties). Fermentation, like spoilage, is dependent on microbial succession. The

physical and chemical nature of the food determines fermentation organisms and inhibits unwanted microbes. Microbes involved are lactobacilli (lactic acid bacteria), acetic acid bacteria, yeasts and occasionally mycelial fungi. In Indian home ' back slop' method is followes in which people retained some material from a previous fermentation and added it to a fresh batch of ingredients. In modern days "Starter cultures (collection of well identified and characterized microorganisms which initiate fermentation) are extensively used in the dairy industry. Some examples of fermented foods are as follows:

Dairy Products- Buttermilk, Sour cream, Yoghurt, Cheese.

Vegetables products_Kimchi,Saukeraut ,Pickels etc

Meat Products: Many European sausages, Cured ham, Salami

Wine, Bread, Sourdough, Tofu, Pickles, Silages etc

3.5. Food Preservation

Food Preservation is the process of treating and handling food to check the growth of microorganism or substantially slowing down the spoilage (loss of quality, edibility or nutritive value) caused or accelerated by microorganisms. However there are some methods which use special bacteria, yeasts or fungi to add specific qualities and to preserve food (e.g., cheese, wine). The aim of preservation is to Maintain or create nutritional value, flavor and texture in food. Generally preservation involves preventing the growth of bacteria, fungi, and other microorganisms, as well as retarding the oxidation of fats which cause rancidity. The process also includes processes to inhibit natural ageing and discoloration that can occur during food preparation such as the enzymatic browning reaction in apples after they are cut out. Food to be sealed after treatment to prevent recontamination with microbes and preventing it from drying that allow food to be stored without any special containment for long periods.

Common methods of preservation includes drying, spray drying, freeze drying, freezing, vacuum packing, canning, preserving in syrup, sugar crystallization, food irradiation, and adding preservatives or inert gases such as carbon dioxide. Other methods that not only help to preserve food, but also add flavor, include pickling, salting, smoking, preserving in syrup or alcohol, sugar crystallization and curing.

3.6. Industrial Microbiology

Humans uses the variability of microbes to make improvements in Agriculture, Industries, Medicines, and environmental protection. To use of microorganisms on a large scale to obtain valuable commercial products by chemical transformations is called industrial Microbiology. The science of Induastrial microbiology dates back and originated with bear and wine making fermentation processes vig alcoholic fermentation and subsequently expanded in the area for the production of pharmaceuticals (e.g. antibiotics), food additives (e.g. amino acids), organic acids (e.g. butyric acid and citric acid), enzymes (e.g. amylases, proteases), and vitamins. All these products are obtained by enhancing the metabolic

reactions of microorganisms that were already capable of carrying out in natural conditions. At present, in addition to traditional industrial microbiology, a new era of Microbial Biotechnology is rapidly expanding in which the genes of the microorganisms responsible for such and other metabolic reactions are being manipulated to give to many new products at commercial level.Fermentation is one of the main processes used in industrial microbiology. Fermentation is any process involving the mass culture of microorganisms, either anaerobic or aerobic. This process requires control of a series of parameters that depend on the desired final product. To improve the Fermentation process, strain selection, culture media improvement, and preservation techniques have contributed to maximize the fermentation process in industry.

3.7 Fermentation in industry

In Production and Process industry, as well as other areas, the uses of fermentation progressed rapidly increasing after Pasteur's era ie. Between 1900 and 1930. Ethyl alcohol and butyl alcohol were the most important industrial fermentations in the world. But by the 1960s, chemical synthesis of alcohols and other solvents were less expensive and interest in fermentations was baned. Interest in microbial fermentations is experiencing a halt and braekthorugh.There are abundanece of Plant starch, cellulose from agricultural waste, and whey from cheese manufacture that may be a sources of fermentable carbohydrates. Additionally these materials, not utilized, represent solid waste that must be buried in dumps or treated with waste water.

3.8. Major products of Industrial microbiology:

The major products of industrial microbiology can be enlisted as follows:

Food and Beverage Biotechnology - fermented foods, alcoholic beverages (beer, wine) and flavors.

Enzyme technology - Production and application of enzymes.

Metabolites from microorganisms - amino acids, antibiotics, vaccines, biopharmaceuticals, bacterial polysaccharides and polyesters, specialty chemicals for organic synthesis (chiral synthons).

Biological fuel generation - Ethanol or methane from biomass, Single cell protein, production of biomass, Microbial recovery of petroleum.

Environmental Biotechnology - Water and wastewater treatment, Composting (and landfilling) of solid waste, Biodegradation/Bioremediation of toxic chemicals and hazardous waste.

Agricultural Biotechnology - Soil fertility, Microbial Insecticides, Plant cloning Technologies.

Diagnostic tools - Testing & Diagnosis for Clinical, Food,Environmental, Agricultural applications, Biosensors

3.9. Environmental Microbiology

Environmental Microbiology is the study of the composition and physiology of microbial communities in the

environment. The environment in this case means the soil, water, air and sediments covering the planet and can also include the animals and plants that inhabit these areas. Environmental microbiology also includes the study of microorganisms that exist in artificial environments such as bioreactors. Microbial life is amazingly diverse and microorganisms literally cover the planet. It is estimated that we know less than 1% of the microbial species on Earth. Microorganisms can survive in some of the most extreme environments on the planet and some can survive high temperatures, often above 100°C, as found in geysers, black smokers, and oil wells. Some are found in very cold habitats and others in highly salt / saline, acidic, or alkaline water. Microbial life is amazingly diverse and microorganisms literally cover the planet. An average gram of soil contains approximately one billion microbes representing probably several thousand species. Microorganisms have special impact on the whole biosphere. They are the backbone of ecosystems of the zones where light cannot approach. In such zones, chemosynthetic bacteria are present which provide energy and carbon to the other organisms there. Some microbes are decomposers which have ability to recycle the nutrients. Microbes have a special role in biogeochemical cycles. Microbes, especially bacteria, are of great importance because their symbiotic relationship (either positive or negative) has special effects on the ecosystem.

3.10. Soil Microbiology

Soil is the top layer of the Earth's lithosphere, formed from weathered rock that has been transformed by living organisms. Soil is composed of mineral and organic solid particles, air, soil solution, and living organisms which occur in this edaphon. The organisms living in soil create a community called the edaphon. These are bacteria, fungi, unicellular algae, vascular plants and animals especially invertebrates that occur in the surface layer of soil. Due to the variety of their metabolic abilities the soil microorganisms ensure the permanence (continuity) of element cycles in nature. The effect of their activities is not only the mineralization of organic compounds but also the changes of mineral compounds, which have a big impact upon the development of the green plants. Edaphon constitutes about 1-10% of the dry mass of the soil organic matter. Both bacteria and fungi are the co-creators of soil's structure as they create humus - the most important component of soil that greatly influences its structure, sorption qualities and the richness in organic compounds. They have a great effect on the way of creation of crumb texture and a spongy structure of soil by producing mucous capsules, and like the filamentous bacteria and the fungi by their form of growth.

3.11. Water Microbiology

Water microorganisms may be underground and/ or surface waters as well as bottom sediments. The underground waters (mineral and thermal springs, ground waters) - due to their oligotrophic character (nutrient - deficient) are usually inhabited by a sparse microflora that is represented by a low number of species with almost a complete lack of higher plants or animals. The surface waters such as streams, rivers, lakes and sea waters are inhabited by a diverse flora and fauna. Microorganisms in those waters are a largely varied group. In the bottom sediment, anaerobic putrefying microflora, cellulolytic bacteria and the anaerobic chemoautotroph's develop.

3.12. Air Microbiology

Air is an unfavorable environment for microorganisms, in which they cannot grow or divide. It is merely a place which they temporarily occupy and use for movement. Therefore, there are no metabolic connections occurring between different microorganisms in air (such as in soil or water). As a result they form only a random collection of microorganisms. Microorganisms get into air as a consequence of wind movement, which sweeps them away from various habitats and surroundings (soil, water, waste, plant surfaces, animals, and other), or are introduced during the processes of sneezing, coughing, or sewage aeration. Air conditions are unfavorable for the microorganisms due to a lack of adequate nutrients, frequent deficit of water, threat of desiccation, and solar radiation. There are 3 main groups of microorganisms that occur in air: viruses, bacteria and fungi. Bacteria may exist as vegetative or resting forms however fungi occur in the form of spores or fragments of mycelium.Virus exist as independent body and totally depends on Host metabolisms for its own growth and multiplications.Air may be helpful in dispersion of all these three particle creating epidemic or pandemicsituation.

TheEnd

Chapter-4

STRUCTURE OF BACTERIA THEIR PARTS AND FUNCTIONS

Structure of bacteria is very typical and there are so many differences between bacterial and eukaryotic structure. First of

all let find out the main difference between these two structure.

3.1. **Concepts of prokaryotes vs Eukaryotes Prokaryotes**(pro-KAR-ee-ot-es) (from Old Greek*pro-*before +*karyon* nut or kernel, referring to the cell nucleus, + suffix-*otos*, pl.-*otes*; also spelled "procaryotes") are organisms without a cell

nucleus (= karyon), or any other membrane-bound organelles. Most are unicellular, but some prokaryotes are multicellular.

Eukaryotes (IPA: [ju?'kæ???t]) are organisms whose cells are organized into complex structures by internal membranes and a cytoskeleton. The most characteristic membrane bound structure is the nucleus. This feature gives them their name, (also spelled "eucaryote,") which comes from the Greek ευ, meaning good/true, and κάρυον, meaning nut, referring to the nucleus. Animals, plants, fungi, and protists are eukaryotes.

Differences between eukaryotic and prokaryotic cells

The most fundamental difference is that eukaryotes do have "true" nuclei containing their DNA, whereas the genetic material in prokaryotes is not membrane-bound.

In eukaryotes, the mitochondria and chloroplasts perform various metabolic processes and are believed to have been derived from endo symbiotic bacteria. In prokaryotes similar processes occur across the cell membrane endosymbionts are extremely rare.

The cell walls of prokaryotes are generally formed of a different molecule (peptido glycan) to those of eukaryotes (many eukaryotes do not have a cell wall at all).

Prokaryotes are usually much smaller than eukaryotic cells.

Prokaryotes also differ from eukaryotes in that they contain only a single loop of stable chromosomal DNA stored in an area named the nucleoid, while in eukaryote DNA is found on tightly bound and organized chromosomes. Although some eukaryotes have satellite DNA structures called plasmids, these are generally regarded as a prokaryote feature and many important genes in prokaryotes are stored on plasmids.

Prokaryotes have a larger surface area to volume ratio giving them a higher metabolic rate, a higher growth rate and consequently a shorter generation time compared to Eukaryotes.

Prokaryotes also differ from eukaryotes in the structure, packing, density, and arrangement of their genes on the chromosome. Prokaryotes have incredibly compact genomes compared to eukaryotes, mostly because prokaryote genes lack introns and large non-coding regions between each gene.

Whereas nearly 95% of the human genome does not code for proteins or RNA or includes a gene promoter, nearly all of the prokaryote genome codes or controls something.

Prokaryote genes are also expressed in groups, known as operons, instead of individually, as in eukaryotes.

Difference in genetic makeup: In a prokaryote cell, all genes in an operon (three in the case of the famous lac operon) are transcribed on the same piece of RNA and then made into separate proteins, whereas if these genes

were native to eukaryotes, they each would have their own promoter and be transcribed on their own strand of mRNA. This lesser degree of control overgene expressioncontributes to the simplicity of the prokaryotes as compared to the eukaryote.

3.2. Structure of Bacteria:

Average diameter of bacteria is 0.5 - 2.0 um in while diameter of RBC is 7.5 um. Surface Area of typical bacteria is ~12 um^2,Volume is ~4 um. Surface Area to Volume is 3:1 Typical Eukaryote Cell SA/ Vol is 0.3:1.Large surface of bacteria enable the food enters through SA and allow quick distribution in all parts of bacteria

Fig: A Typical bacterial cell.

Source: Science direct.com.

3.2.1. Size and shape of Bacteria. Individual bacterial cells are not visible to the unaided eye. In general, bacterial cells do not exceed 1 µm (micrometer or micron) in diameter, though their length may vary widely. Some bacteria for example, " *Epulopiscium fishelsohnii*"measuring 80 µm in breadth and 200 µm in length has been discovered in 1991 and another spherical Archaebacterium, called "*Thiomargarita namibiensis"* has been isolated from sea-bottom in 1999. This organism measures 750 µm in diameter and is visible to the unaided eye. But such giants bacteria are rare exceptions. The minute size of bacteria gives certain advantages to them. Due to their small size, bacteria have a much greater surface/volume ratio than most eukaryotic organisms having larger cells. This has important implications on the activity of cells. Because of a greater surface/volume ratio, the bacteria are able to absorb nutrients and gases from the surrounding media very rapidly and this ability is reflected in their faster growth rate in comparison to larger eukaryotic organisms. For example, the rate of oxygen uptake (µl/mg dry weight/hr) of most aerobic bacteria is approximately 10 times faster than that of another unicellular but larger organism—yeast—and 100 times faster than the cells of animal tissues. This high rate of oxygen uptake reflects the fast rate of metabolic activity of bacteria. Many bacteria under optimal conditions of growth can have a doubling time of 20 to 30 minutes. The small size also helps bacteria to spread quickly by air currents over great distances. In fact, bacteria are

ubiquitous in distribution and they occur in all possible types of environments starting from arctic glaciers to boiling hot springs, and from upper levels of atmospheres to bottom of the seas.

3.2.2. Shape of Bacterial Cells: Bacterial cells are bound externally by a rigid wall which gives bacteria their characteristic shape. The Mycoplasma are exceptions in this regard, because they lack a cell wall and they do not have also any characteristic shape. That the cell wall is responsible for giving shape to bacterial cells is also shown when the wall is removed by enzymes.

Classification of Bacteria according to their Shape: A cylindrical bacterial cell on losing the wall assumes a spherical shape. A bacterium can be spherical or cylindrical.

Coccus: A spherical bacterium is generally known as a coccus .

Bacillus: Cylindrical bacterium, or Rod shaped bacteria named as Bacillus.

Vibrio: If the Cylindrical/Rod cell is curved, it is known as a **vibrio** and if helical it is called a

Spirillum. In all these types, the cell is a rigid structure.

Spirochaetes.: Some bacteria have also flexible cells. Helical bacteria with flexible cells are known as Spirochaetes.

Actinomycetes: A group of prokaryotic organisms characteristically consist of a mycelium, somewhat like that produced by fungi is known as Actinomycetes.They posses some character of bacteria (Gram positive Cell wall and other Prokaryotic structure) and some of that of Fungi.So it is considered to be alink between Bactera and Fungi. They can be terrestrial or aquatic.A mycelium is composed of thin hairy hyphae. An Actinomycete hypha is about 1 µm in diameter, whereas a fungal hypha is, on the average, 8-10 µm broad. A hypha is an elongated, often branched structure, divided into cells by formation of cross-walls. Some hyphae enter into the substratum, (Endomycelium) while others remain in the aerial part (Exomycelium).

Importance of Actinomycete: They are of great economic importance to human because Agriculture and Forest depends on their contribution to soil system.They help in decomposition of organic matter and dead organism after that the smaller molecules are taken uop by plants. It is believed that about 80-90% of abtibiotc are produced from Actinomycetes only like Streptomycin, Rimfapicin, Griseofulvin.Some of them are important in N_2 fixation like Frankiaand other are important pathogen causing Tuberculosis such as *Mycobacterium tuberculae*.

Trichomes: There are also bacteria which characteristically form filaments or hair like structure called as trichomes. A trichome consists of a series of cells arranged one on the other to form a column. The trichome may in some forms be enclosed in a common sheath. Apart from the variations of the forms, bacteria may also possess various irregular

appearances which are, however, characteristic of a group. For example, a group of bacteria produces a narrow extension from the cell which is used either for attachment or for producing daughter cells by budding. Such extensions are called stalks or pros-thecae and the cells are stalked or prosthecate.

Fig: Different forms of bacteria

Source Britannica .com

Arrangement of cell : The arrangement of cells are more complex in cocci than Bacilli. The arrangement of cell depends upon adherence of cells together after cell division.

Coccus : There are several groups of cocci based on the number and arrangement of cells.

Diplococcus : Cell divide in one plane and get attached permanently in pairs.

Chain = Streptococcus : Cells divide in one plane and remain attached permanently in pairs.

Cluster = Staphylococcus : Cell divide in three planes and form a group of four cells.

Sarcinae: cell divide in three planes in regular pattern producing bunches of cocci.

Like wise cell are also arranged in different form in rod shaped bacteria Bacillus

Depending upon form, bacillus are also categorized as:

Monobacillus: The single elongated cells freely present in nature are monobacillus.

Diplobacillus: After division the cell remain adhered and appear in paired form

Streptobacillus: After division the cell remain attached in chains appearing like straws

Coccobacillus: The oval cells looking like cocci are called cocobacilli.

Like wise different forms of Spirilli are :

Vibriod = Bacterial cells having less than one complete twist form vibriod shape.eg. vibrio cholera.

Helical: Cells that have more than one twist form a dinstinct helical shape.eg. Spirillum (with flagella).

Other Forms are:

Pleomorphic.

Trichomes

Palisade.

Hyphae.

Spirillum.

Spirochete.

Square.

Star.

Fig: Plane of division and their types

3.3. Bacterial parts Structures and Functions:

Observation under Microscope reveals several structural components outside and inside the cell wall.

- Flagella
- Pili
- Capsule
- Plasma Membrane
- Cytoplasm
- Cell Wall
- Lipopolysaccharides
- Teichoic Acids
- Inclusions
- EndoSpores

3.3.1.Flagella: Bacteria may be motile or non motile.The motite bacteria swim by means of small flexible,whip like appendages called flagella (singular flagellum). These are hair like structure and are attached to cell wall and apparently originate from grandular body just beneth the cell wall.Bacterial flagellum lacks two central and nine arrangemant of cell. A typical bacterial flagellum measures bout 120A° thick and 5 μ long.The molecular weight of a flagellum is about 40,000

Angustrum. The diameter of molecule is about 40 Angustrum.Chemically they are madeup of protein .The primary role of the flagellum is locomotion, but it also often has function as a sensory organelle, being sensitive to chemicals and temperatures outside the cell.The similar structure in the archaea functions in the same way but is structurally different and has been termed the archaellum. Flagella are organelles defined by function rather than structure. Both prokaryotic and eukaryotic flagella can be used for swimming but they differ greatly in protein composition, structure, and mechanism of propulsion. The word flagellum in Latin means whip. An example of a flagellated bacterium is the ulcer-causing *Helicobacter pylori*, which uses multiple flagella to propel itself through the mucus lining to reach the stomach epithelium. Fimbriae and pili are also thin appendages, but have different functions and are usually smaller.

Three types of flagella have so far been distinguished: bacterial, Archaeal, and Eukaryotic.

Source: https://www.shutterstock.com/search/flagellum

Fig: Flagella and their types

Source: Wikipedia.org

The main differences among these three types are:

Bacterial flagella are helical filaments, each with a rotary motor at its base which can turn clockwise or counterclockwise.They provide two of several kinds of bacterial motility. The bacterial flagellum is made up of the proteinflagellin. Its shape is a 20-nanometer-thick hollow tube. It is helical and has a sharp bend just outside the outer membrane; this "hook" allows the axis of the helix to point directly away from the cell. A shaft runs between the hook and the basal body, passing through protein rings in the cell's membrane that act as bearings.

Detail structure of flagellum: Each bacterial flagellum is structurally differentiated in to three parts: (1) Basal body,(2) a hook and (3) Main filament and shaft.

3.3.1.1.Basal body: Basal body of the flagellum of gram-negative bacterium possesses two sets of rings:(i) A proximal and (ii) A distal set. They are surrounded by a rod. Each consists of two rings and these four rings are named as : Membrane (M) Super membrane ring (S) Peptidoglycan ring (P) and Lipopollysaccharide ring(L).They are arranged from inner side to outer side. P and L ring structurally forma a bearing for the flagellar rod to pass through outer membrane. Gram-positive organisms have only two of these basal body rings, one in the peptidoglycan layer and one in the plasma membrane.

3.3.1.2. The Hook:

Hook connect basal and the main flagellum body shaft. The length of hook of Gram –negative bacterium is shorter than of

gram positive bacterium.

3.3.1.3.The Filament or Shaft: The outermost long region of the flagellum is called filament or shaft. The flagellum is chemically made of protein which occurs in multiple units. The protein is called flagellin. The flagellins are arranged in several chains that inter wine and form a helix around a hollow core. The molecular weight ranges from 30,000-60,000.

Locomotion: Three types of flagellar movement have been identified :(1) Flagellar (2) Spirochetes (3) Gliding.

3.3.1.4. Flagellar Movement: This kind of movement occurs in those bacteria in which the flagella are present all over the bacterial surface (Peritrichous) or at one end (Polar).Prokaryotic flagellum is semi rigid helical rotor that move the cell by rotating from the basal body either clockwise or counter clock wise around its axix. The helical waves are generated from the base to the tips of flagellum. The rotating flagellum forms a bundle that pushes against water and propels the bacterium. The basal body acts as motor and causes rotation. According to Doetsch (1966) the motility of flagellum may be due to rigid helix from the basal body. While berg (1976) suggested that the basal body of the flagellum appears to be the motor which causes rotation. The energy is transferred in the basal part.

3.3.1.4.1. Mechanism of flagellar Movement:

The mechanism of flagellar movement in prokaryotes is different from eukaryotic flagella. The bacterium moves when the helix rotates as the filament is in the shape of rigid helix. The flagella act just like propellers on a boat. The direction of flagellar rotation determines the nature of bacterial movement. The movement in monotrichous bacteria stops and tumble randomly by reversing the flagellar rotation. The polar flagella, rotate counter clockwise during normal forward movement, whereas the cell itself rotates slowly clockwise. Peritrichous bacteria also operate in a similar way. To move forward, the flagella rotate counter clockwise. As they do so, they bend at their hooks to for a rotating bundle that propels them forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles (Fig. 17).

Fig :Bacterial flagellar movement

Source: wilkepedia

Motility enables the bacterium to move toward a favorable environment or away from a particular stimulus called taxis . Chemotaxis (include chemicals) and phototaxis (include light). Bacteria do not always swim aimlessly but are attracted by such nutrients as sugars and amino acids, and are repelled by many harmful substances and bacterial waste products. Movement toward chemical attractants and away from repellents is known aschemotaxis**.** The mechanism ofchemotaxis in *E.coli*has been studied most. Forward swimming is due to counterclockwise rotation of the flagellum, whereas tumbling results from clockwise rotation. The bacteria must be able to avoid toxic substances and collect in nutrient-rich regions and at the proper oxygen levels.*E.coli*has four different chemo receptors that recognize serine, Aspartate and maltose, ribose and galactose and dipeptides respectively. These chemo receptors often are called methyl-accepting chemotaxis proteins (MCPs).Some bacteria can move by mechanisms other than flagellar rotation. Spirochetes are a group of bacteria that have unique structure and motility (*Treponemapallidum*) the causative agent of syphilis and*Borrelia burgdorgeri*, the causative agent of Lyme disease).Spirochetes travel through viscous substances such as mucus or mud by flexing and spinning movement caused by special axial filaments - bundles of fibrils that arise at the ends of the cell beneath the outer sheath and spiral around the cell (fig. 18).The rotation of the filaments produces an opposing movement of the outer sheath that propels the spirochetes by causing them to move like corkscrews.

3.3.1.4.2.Spirochaetal Movement: This type of movement is common in flexible helical bacteria such as Spirochaetes. The flagella of these bacteria perform various types of movement such as flexing and spinning free swimming and creeping on surface. Just within the cell envelop they have flagella like structure which are known as periplasmic flagella or axil fibrils or endoflagella. The axil fibril are present in the space between inner and outer membrane of cell envelop.The mechanism of motility is not known. Berg (1975) postulated that the axil fibrils rotate in periplasmic space and cause the rotation of periplasmic cylinder on the body axix in the opposite direction.

Fig: Spirochaete Source: Wilkipedia.com

3.3.1.4.3. Gliding Movement: A very different type of motility, gliding motility, is employed by many bacteria; cyanobacteria, myxobacteria and cytophagas and some mycoplasmas.

Some bacteria such as species of cyanobacteria (eg; Cytophaga) and Mycoplasma show gliding movement. Except mycoplasma ,in other two gram negative type cell wall is present. In the members of cytophagales and cyanobacteria movement helps to find out the substratum eg wood,bark,shell etc for anchorage and reproduction. They secrete slime with the help of which they get attached to the substratum.The movement is long continued and necessary for life cycle of organisms.

3.3.2. Classification on the basis of arrangement of flagella:

3.3.2.1.Monotrichous:

Presence of flagella at one end of cell.

eg: *vibrio cholarae, Pseudomonas aeruginosa*

–

Fig: Different types of flagella (Wilkipedia.com)

3.3.2.2.Lophotrichous: Presence of bundle of flagella at one end of the cell

– eg. *Pseudomonas fluroscence*

3.3.2.3.Amphitrichous: Presence of single or tuft of flagella at both ends of the cell.

eg: *Aquasprillum*

3.3.2.4.Peritrichous: Presence of flagella all around the bacterial cell.

eg: *E.coli, Salmonella, Klebsiella.*

3.3.2.5.Atrichous: Absence of flagella

eg: Shigella

3.3.Pili: Pili and fimbriae are hair like appendages found on surface of cell wall in gram negative bacteria (eg.Enterobactereriaceae,Pseudomondaceae etc).Eukaryotic cells lack pili.The term fimbriae is used for all hair like structure covering the surface of the cell.Pili are generally governed by plasmids and it is differe from flagella in being shorter and thinner straight and less rigid.But they are larger in number.

Chemistry of Flagella : The fimbriae of E.coli is chemically composed of 100% Protein called as fimbrilin with a molecular weight of about 16,000(Flagellar protein is flagellin which have molecular weight 40,000)Fimbrilin consist of about 163 amino acid which usually occur in L form.They have large number of hydrocarbon chain

3.3.1.Classes of Pili: According to function Pili are of two types:

(a) **Common Pili:** Which act to adhere the cell to surface and b)Sex Pili which join othere bacterial cell for transfer of genome.

Sex Pili: These are filamentous structure and determine the sex factor(Plasmid) such as F,Col I and R factor.The synthesis of sex pili is dependent on these sex factors.There are two types of sex Pili in *E.coli*.They are F Pili and I Pili.

Function of Fimbriae and Pili:

1) They have adhesive properties.These help bacteria to attach themselves to the natural substrate and to other organism.

2) They possess antigenic properties.

3) The sex pili are helpful in chromosome transfer during conjugation probably by acting as conjugation tubes

Fig: Sex Pili during conjugation

Source: https://en.wikipedia.org/wiki/Flagellum

3.4.Capsule or Slime Layer:

Some of the bacterial cell are surrounded by the extracellular polymeric sheath (EPS) which are commonly called capsule or glycocalyx.It forms an envelope around the cell wall and can be observed under light microscope after special staining technique.the presence of capsule may be detected by negative staining also such as indian ink method.Capsule consist of loose aggregate material which may not be a part of bacteria.This sheath is not essential for the survival of the bacteria under unfavourable conditions but it may provide pathogenicity to the some of type of bacteria.The capsule is gelatinous polymer made up of either polysaccharide(*Klebsiella pneumoniae*) or polypeptide(*B.anthracis*) or both.The polysaccharide may be of single type of sugars(homopolysaccharides eg:*Acetobacter xylinum*) or several types of sugars(heteropolysaccharides eg.(*Pseudomonas aeruginosa*).The capsule of pneumococci is made up of hexoses,uronic acid and amino sugars and that of *Streptococci* consist of L-aminoacid.The bacterial capsule is species specific and therefore can be used for immunological differentiations of related cell.If these substance are unorganised and loosely attached to cell wall the capsule is called slime layer.The fresh water and marine bacteria forms trichomes which are enclosed inside the gelationous matrix called sheath.Sheath is also found in cyanobacteria and other algae.

Function of Capsule: Some important function as reported as below:

1)The Capsule may prevent the attachment of bacteriophages.

2)It protect the bacterial cells against desication as it is hygroscopic in nature

3)Due to sticky in nature they can attached on the diverse surface such as plant root,human teeth and tissues (Dental caries and Respiratory tract etc) and rocks and pebbles in the fast running water.

4)They impart bacteria a antiphagocytic feature by inhibiting themseves by engulfument of WBC in this way impart virulence.eg.*Streptococcus pneumoniae* causes pneumonia and uncapsulated strain is phagocytized by WBC.

5)In some of bacteria it acts as storage energy as it breakdown during starve condition to release energy for eg: S.mutans

6)Capsule protect the cell from desication,maintain the viscosity and inhibit the movemnat of nutrients from bacterial cell.

3.5. Cytoplasm: Cytoplasm of prokaryotes refers to the internal matrix of cell inside plasma membrane.Cytoplasm consists of water 80% (Protein,carbohydrates,lipids,inorganic ions and certain low molecular compound.Cytoplasm is thick and semitransparent.The DNA molecules ribosomes and other inclusions are the structure of cytoplasm.In certain cyanobacteria gas vacuoles are found.The Prokaryotic cytoplasm differes from the eukaryotic cytoplasm.The formers lacks cytoskelton and cytplasmic streaming.Compartmentation of organelles are absent in prokaryotes and present in eukaryotes.

3.6. Ribosomes: Ribosomes are small particles,present in large numbers in all theliving cells. They are sites of protein synthesis. The ribosome word is derived -'*ribo'*from ribonucleic acid and '*somes*'from the Greek word '*soma*'which means '*body'*.The

ribosomes link aminoacids together in the order that isspecifiedby the messenger RNA molecules.The ribosomes are made up of two subunits - a small and a large subunit. The small subunit reads the mRNA while the large subunit joins the amino acids to form a chain of polypeptides. Ribosmal subunits are made of one or more rRNA (ribosomal RNA) molecules and various proteins. Ribosome acts as a site of protein synthesis.Allmost all living cell contain ribosomes.A single bacteria may possess approximately 10,000-15,000 which account for about 30% of the total weight of a bacterial cell.It gives a granular structure of cell cytoplasm.Ribosomes are found in free floating condition and are randomly distributed in cytoplasm.Eukaryotic ribosome are attached to the cell membrane called as RER(Rough endoplasmic reticulum whereas in Prokaryotes ribosome are found free in the cytoplasm.Eukaryotic ribosomes are of 80 S type whereas prokaryotic ribosomes are of 70 S type.At low concentration of Mg 70 S is dissociated into its two subunit The larger 50 S and a smaller subunit 30 S.Each 50 s subunit contain 5S and 23S RNA and 34 proteins(L1-L34).The smaller subunits consists of 16S RNA and 21 proteins (S1-S21). The letter S refers to Svedberg unit which indicates the relative rate of sedimentation during ultracentrifugation. Sedimentation rate depend on size,shape and weight of the particles.

Fig: Structure of Ribosome

Source: Science direct .com

Fig: Sedimentation coeffiecient of Ribosome

Source :Sciencedirect.com

- Typically ribosomes are composed of two subunits: a largesubunit and a small subunit.
- The subunits of the ribosome are synthesized by thenucleolus.
- The subunits of ribosomes join together when the ribosomesattaches to the messenger RNA during the processof protein synthesis.
- Ribosomes along with atransferRNA molecule (tRNA), helps totranslatethe protein-coding genes in mRNAto proteins.
- They assemble amino acids to form specific proteins, proteins are essential to carry outcellularactivities.
- The process of production of proteins, the deoxyribonucleic acid produces mRNA by the process of DNA transcription.
- The genetic message from the mRNA is translated into proteins during DNA translation.
- The sequences of protein assembly during protein synthesis arespecifiedin the mRNA.
- The mRNA is synthesized in the nucleus and is transported to the cytoplasm for further process of protein synthesis.

- In the cytoplasm, the two subunits of ribosomes are bound around the polymers of mRNA; proteins are then synthesized with the help oftransferRNA.
- The proteins that are synthesized by the ribosomes present in the cytoplasm are used in the cytoplasm itself. The proteins produced by the bound ribosomes are transported outside thecell.

3.7. Plasmids:

A**plasmid**is a smallDNAmolecule within a cell that is physically separated from achromosomal DNAand can replicate independently. They are most commonly found as small circular, double-stranded DNA molecules inbacteria; however, plasmids are sometimes present inArchaeaandeukaryotic organisms. In nature, plasmids often carry genes that may benefit the survival of the organism, for exampleantibiotic resistance. While the chromosomes are big and contain all the essential genetic information for living under normal conditions, plasmids usually are very small and contain only additional genes that may be useful to the organism under certain situations or particular conditions. Artificial plasmids are widely used asvectorsin molecular cloning, serving to drive the replication ofrecombinant DNAsequences within host organisms. In the laboratory, plasmids may be introduced into a cell viatransformation. Plasmids are consideredreplicons, units of DNA capable of replicating autonomously within a suitable host. Plasmids are transmitted from one bacterium to another (even of another species) mostly throughconjugation.This host-to-host transfer of genetic material is one mechanism ofhorizontal gene transfer, and plasmids are considered part of themobilome. Unlike viruses (which encase their genetic material in a protective protein coat called acapsid), plasmids are "naked" DNA and do not encode genes necessary to encase the genetic material for transfer to a new host. However, some classes of plasmids encode theconjugative "sex"pilusnecessary for their own transfer. The size of the plasmid varies from 1 to over 200 kbp,and the number of identical plasmids in a singlecellcan range anywhere from one to thousands under some circumstances. The relationship between microbes and plasmid DNA is neither parasitic nor mutualistic, because each implies the presence of an independent species living in a detrimental or commensal state with the host organism. Rather, plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provideresistanceto naturally occurringantibioticsin a competitiveenvironmental niche, or the proteins produced may act astoxinsunder similar circumstances, or allow the organism to utilize particular organic compounds that would be advantageous when nutrients are scarce.

History

The term*plasmid*was introduced in 1952 by the Americanmolecular biologistJoshua Lederbergto refer to "any extra chromosomal hereditary material. More elaborately the genetic elements that exist exclusively or predominantly outside of the chromosome and can replicate autonomously.

Fig: plasmid and replication

Source: https://en.wikipedia.org/wiki/Plasmid

3.7.1. Properties and characteristics:

In order for plasmids to replicate independently within a cell, they must possess a stretch of DNA that can act as an origin of replication. The self-replicating unit, in this case the plasmid, is called a replicon. A typical bacterial replicon may consist of a number of elements, such as the gene for plasmid-specific replication initiation protein (Rep), repeating units called introns, DnaA boxes, and an adjacent AT-rich region. Smaller plasmids make use of the host replicative enzymes to make copies of themselves, while larger plasmids may carry genes specific for the replication of those plasmids. A few types of plasmids can also insert into the host chromosome, and these integrative plasmids are sometimes referred to as episomes in prokaryotes. Plasmids almost always carry at least one gene. Many of the genes carried by a plasmid are beneficial for the host cells, for example: enabling the host cell to survive in an environment that would otherwise be lethal or restrictive for growth. Some of these genes encode traits for antibiotic resistance or resistance to heavy metal, while others may produce virulence factors that enable a bacterium to colonize a host and overcome its defences, or have specific metabolic functions that allow the bacterium to utilize a particular nutrient, including the ability to degrade recalcitrant or toxic organic compounds.Plasmids can also provide bacteria with the ability to fix nitrogen. Some plasmids, however, have no observable effect on the phenotype of the host cell or its benefit to the host cells cannot be determined, and these plasmids are called cryptic plasmids.

Plasmids may be present in an individual cell in varying number, ranging from one to several hundreds. The normal number of copies of plasmid that may be found in a single cell is called the copy number, and is determined by how the replication initiation is regulated and the size of the molecule. Larger plasmids tend to have lower copy numbers. Low-copy-number plasmids that exist only as one or a few copies in each bacterium are, upon cell division, in danger of being lost in one of the segregating bacteria. Such single-copy plasmids have systems that attempt to actively distribute a

copy to both daughter cells. These systems, which include the par ABS system and par MRC system, are often referred to as the partition system or partition function of a plasmid.

3.7.2. Classifications and type

Plasmids may be classified in a number of ways. Plasmids can be broadly classified into conjugative plasmids, non-conjugative plasmid and Mobilizable Plasmid.

3.7.2.1. Conjugative Plasmids : It contain a set of transfer or *tra* genes which promote sexual conjugation between different cells.In the complex process of conjugation, plasmid may be transferred from one bacterium to another via sex pili encoded by some of the *tra* genes (see figure).

3.7.2.2.Non-conjugative plasmids : They are incapable of initiating conjugation, hence they can be transferred only with the assistance of conjugative plasmids.

3.7.2.**3. Mobilizable Plasmid**: An intermediate class of plasmids are mobilizable, and carry only a subset of the genes required for transfer. They can parasitize a conjugative plasmid, transferring at high frequency only in its presence.

Plasmids can also be classified into incompatibility groups. A microbe can harbour different types of plasmids, however, different plasmids can only exist in a single bacterial cell if they are compatible. If two plasmids are not compatible, one or the other will be rapidly lost from the cell. Different plasmids may therefore be assigned to different incompatibility groups depending on whether they can coexist together. Incompatible plasmids (belonging to the same incompatibility group) normally share the same replication or partition mechanisms and can thus not be kept together in a single cell.

Fig: Transformation in Bacteria

Source: https://en.wikipedia.org/wiki/Plasmid

3.7.3. Another way to classify plasmids is by function.

There are five main classes:

3.7.3.1. Fertility F-plasmids : which contain *tra* genes. They are capable of conjugation and result in the expression of sex pili.

3.7.3.2. Resistance (R) plasmids: Which contain genes that provide resistance against antibiotics or poisons. Historically known as R-factors, before the nature of plasmids was understood.

3.7.3.3. Col plasmids: Which contain genes that code for bacteriocins, proteins that can kill other bacteria.

3.7.3.4. Degradative plasmids: which enable the digestion of unusual substances, e.g. toluene and salicylic acid.

3.7.3.5. Virulence plasmids: which turn the bacterium into a pathogen.

Plasmids can belong to more than one of these functional groups.

3.7.4. **Plasmid as a Vectors:**

Artificially constructed plasmids may be used as vectors in genetic engineering. These plasmids serve as important tools in genetics and biotechnology labs, where they are commonly used to clone and amplify (make many copies of) or express particular genes. A wide variety of plasmids are commercially available for such uses. The gene to be replicated is normally inserted into a plasmid that typically contains a number of features for their use. These include a gene that confers resistance to particular antibiotics (Ampicillin is most frequently used for bacterial strains), an origin of replication to allow the bacterial cells to replicate the plasmid DNA, and a suitable site for cloning (referred to as a multiple cloning site).

Fig: A Typical artificial plasmid with more than one resistant site.

Source: https://en.wikipedia.org/wiki/Plasmid

A schematic representation of the pBR322 plasmid, one of the first plasmids to be used widely as a cloning vector. Shown on the plasmid diagram are the genes encoded (*amp* and *tet* for ampicillin and tetracycline resistance respectively), its origin of replication (*ori*), and various restriction sites (indicated in blue).

3.7. 5. Cloning: Cloning vector

Plasmids are the most-commonly used bacterial cloning vectors. These cloning vectors contain a site that allows DNA fragments to be inserted, for example a multiple cloning site or polylinker which has several commonly used restriction sites to which DNA fragments may be ligated. After the gene of interest is inserted, the plasmids are introduced into bacteria by a process called transformation. These plasmids contain a selectable marker, usually an antibiotic resistance gene, which confer on the bacteria an ability to survive and proliferate in a selective growth medium containing the particular antibiotics. The cells after transformation are exposed to the selective media, and only cells containing the plasmid may survive. In this way, the antibiotics act as a filter to select only the bacteria containing the plasmid DNA. The vector may also contain other marker genes or reporter genes to facilitate selection of plasmid with cloned insert. Bacteria containing the plasmid can then be grown in large amounts, harvested, and the plasmid of interest may then be isolated using various methods of plasmid preparation.

A plasmid cloning vector is typically used to clone DNA fragments of up to 15 kbp. To clone longer lengths of DNA, lambda phage with lysogeny genes deleted, cosmids, bacterial artificial chromosomes, or yeast artificial chromosomes are used.

3.7.5.1. Protein production

Another major use of plasmids is to make large amounts of proteins. In this case, researchers grow bacteria containing a plasmid harboring the gene of interest. Just as the bacterium produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing the protein the gene codes for, for example, insulin.

3.7.5.2. Gene therapy

Plasmid may also be used for gene transfer into human cells as potential treatment in gene therapy so that it may express the protein that is lacking in the cells. Some strategies of gene therapy require the insertion of therapeutic genes at pre-selected chromosomal target sites within the human genome. Plasmid vectors are one of many approaches that could be used for this purpose. Zinc finger nucleases (ZFNs) offer a way to cause a site-specific double-strand break to the DNA genome and cause homologous recombination. Plasmids encoding ZFN could help deliver a therapeutic gene to a specific site so that cell damage, cancer-causing mutations, or an immune response is avoided.

3.7.5.3. Disease models:

Plasmids were historically used to genetically engineer the embryonic stem cells of rats in order to create rat genetic disease models. The limited efficiency of plasmid-based techniques precluded their use in the creation of more accurate human cell models. However, developments in Adeno-associated virus recombination techniques, and Zinc finger nucleases, have enabled the creation of a new generation of isogenic human disease models.

3.8. Episomes

The term *episome* was introduced by François Jacob and Élie Wollman in 1958 to refer to extra-chromosomal genetic material that may replicate autonomously or become integrated into the chromosome.Since the term was introduced, however, its use has shifted as *plasmid* has become the preferred term for autonomously replicating extrachromosomal DNA.

Today some authors useepisomein the context of prokaryotes to refer to a plasmid that is capable of integrating into the chromosome. The integrative plasmids may be replicated and stably maintained in a cell through multiple generations, but always at some stage they exist as an independent plasmid molecule.In the context of eukaryotes, the termepisomesis used to mean a non-integrated extra chromosomal closed circular DNA molecule that may be replicated in the nucleus.Viruses are the most common examples of this, such as herpesviruses, adenoviruses, and polyomaviruses, Episomes in eukaryotes behave similarly to plasmids in prokaryotes in that the DNA is stably maintained and replicated with the host cell. Cyto plasmic viral episomes (as in poxvirus infections) can also occur. Some episomes, as such herpes viruses, replicate in a rolling circle mechanism, similar to bacterial phage viruses. Others replicate through a bidirectional replication mechanism (Theta typeplasmids). In either case, episomes remain physically separate from host cell chromosomes. Several cancer viruses, including Epstein-Barr virus and Kaposi's sarcoma-associated herpes virus, are maintained as latent, chromosomally distinct episomes in cancer cells, where the viruses express oncogenes that promote cancer cell proliferation. In cancers, these episomes passively replicate together with host chromosomes when the cell divides. When these viral episomes initiate lytic replication to generate multiple virus particles, they in general activate cellular innate immunity defense mechanisms that kill the host cell.

3.9. Yeast plasmids

Yeasts naturally harbour various plasmids. Notable among them are 2µm plasmids — small circular plasmids often used for genetic engineering of yeast — and linear pGKL plasmids from *Kluyveromyces lactis*, that are responsible for killer phenotypes.

Other types of plasmids are often related to yeast cloning vectors that include:

3.9.1 .Yeast integrative plasmid (YIp): yeast vectors that rely on integration into the host chromosome for survival and replication, and are usually used when studying the functionality of a solo gene or when the gene is toxic. Also connected with the gene URA3, that codes an enzyme related to the biosynthesis of pyrimidine nucleotides (T, C)

3.9.2Yeast Replicative Plasmid (YRp): Which transport a sequence of chromosomal DNA that includes an origin of replication. These plasmids are less stable, as they canget lostduring the budding.

3.10. Plasmid DNA Extraction:

Plasmids are often used to purify a specific sequence, since they can easily be purified away from the rest of the

genome. For their use as vectors and for molecular cloning, plasmids often need to be isolated. There are several methods to isolate plasmid DNA from bacteria, the archetypes of which are the miniprep and the maxiprep /bulkprep. The former can be used to quickly find out whether the plasmid is correct in any of several bacterial clones. The yield is a small amount of impure plasmid DNA, which is sufficient for analysis by restriction digest and for some cloning techniques. In the latter, much larger volumes of bacterial suspension are grown from which a maxi-prep can be performed. In essence, this is a scaled-up miniprep followed by additional purification. This results in relatively large amounts (several hundred micrograms) of very pure plasmid DNA. In recent times, many commercial kits have been created to perform plasmid extraction at various scales, purity, and levels of automation.

4. The Cell wall (Outer Membrane): Bacteria are protected by a rigid cell wall composed of peptidoglycans. A wall located outside the cell membrane provides the cell support, and protection against mechanical stress or damage from osmotic rupture and lysis. The major component of the bacterial cell wall is peptidoglycan or murein. This rigid structure of peptidoglycan, specific only to prokaryotes, gives the cell shape and surrounds the cytoplasmic membrane. Peptidoglycan is a huge polymer of disaccharides (glycan) cross-linked by short chains of identical amino acids (peptides) monomers. The backbone of the peptidoglycan molecule is composed of two derivatives of glucose: N-acetylglucosamine (NAG) and N-acetlymuramic acid (NAM) with a pentapeptide coming off NAM and varying slightly among bacteria. The NAG and NAM strands are synthesized in the cytosol of the bacteria. They are connected by inter-peptide bridges. They are transported across the cytoplasmic membrane by a carrier molecule called bactoprenol. From the peptidoglycan inwards all bacterial cells are very similar. Going further out, the bacterial world divides into two major classes: Gram positive (Gram +) and Gram negative (Gram -). The cell wall provides important ligands for adherence and receptor sites for viruses or antibiotics.

4.1.Gram-Negative Outer Membrane.

The Gram-negative cell wall is composed of an outer membrane, a peptidoglygan layer, and a periplasm. Cell wall is composed of a single layer of peptidoglycan surrounded by a membranous structure called the outer membrane. The gram-negative bacteria do not retain crystal violet but are able to retain a counter stain, commonly safranin, which is added after the crystal violet. The safranin is responsible for the red or pink color seen with a gram-negative bacteria. The Gram-negative's cell wall is thinner (10 nanometers thick) and less compact than that of Gram-positive bacteria, but remains strong, tough, and elastic to give them shape and protect them against extreme environmental conditions. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS) in addition to proteins and phospholipids. The LPS molecule is toxic and is classified as an endotoxin that elicits a strong immune response when the bacteria infect animals. In Gram-negative bacteria the outer membrane is usually thought of as part of the outer leaflet of the membrane structure and is relatively permeable. It contains structures that help bacteria adhere to animal cells and cause disease. The peptidoglycan layer is non-covalently anchored to lipoprotein molecules called Braun's lipoproteins

through their hydrophobic head. Sandwiched between the outer membrane and the plasma membrane, a concentrated gel-like matrix (the periplasm) is found in the periplasmic space. It is in fact an integral compartment of the gram-negative cell wall and contains binding proteins for amino acids, sugars, vitamins, iron, and enzymes essential for bacterial nutrition. The periplasm space can act as reservoir for virulence factors and a dynamic flux of macromolecules representing the cell's metabolic status and its response to environmental factors. Together, the plasma membrane and the cell wall (outer membrane, peptidoglycan layer, and periplasm) constitute the gram-negative envelope.

Fig: Cell wall of Bacteria

Source: https://www.shutterstock.com/search/bacteria+cell+wall

Fig .Gram-negative bacteria Cell wall:

Source: https://www.shutterstock.com/search/bacteria+cell+wall

4.2. Cell Wall Gram Positive Bacteria Gram-positive bacteria are stained dark blue or violet by Gram staining. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique, thus forming. It is based on the chemical and physical properties of their cell walls. Primarily, it detects peptidoglycan, which is present in a thick layer in Gram-positive bacteria. Gram-positive results in a purple/blue color while a Gram-negative results in a pink/red color. The Gram stain is almost always the first step in the identification of a bacterial organism, and is the default stain performed by laboratories over a sample when no specific culture is referred. In Gram-positive bacteria, the cell wall is thick (15-80 nanometers), and consists of several layers of peptidoglycan. They lack the outer membrane envelope found in Gram-negative bacteria. Running perpendicular to the peptidoglycan sheets is a group of molecules called teichoic acids, which are unique to the Gram-positive cell wall. Teichoic acids are linear polymers of polyglycerol or polyribitol substituted with phosphates and a few amino acids and sugars. The teichoic acid polymers are occasionally anchored to the plasma membrane (called lipoteichoic acid, LTA), and apparently directed outward at right angles to the layers of peptidoglycan. Teichoic acids give the Gram-positive cell wall an overall negative charge due to the presence of phosphodiester bonds between teichoic acid monomers. The functions of teichoic acid are not fully known but it is believed to serve as a chelating agent and means of adherence for the bacteria. These are essential to the viability of Gram-positive bacteria in the environment and provide chemical and physical protection.

4.3.Myco plasmas and Other Cell-Wall-Deficient Bacteria*:*

Some bacteria lack a cell wall but retain their ability to survive by living inside another host cell. For most bacterial cells, the cell wall is critical to cell survival, yet there are some bacteria that do not have cell walls.Mycoplasma species are widespread examples and some can be intracellular pathogens that grow inside their hosts. This bacterial lifestyle is called parasitic or saprophytic. Cell walls are unnecessary here because the cells only live in the controlled osmotic environment of other cells. It is likely they had the ability to form a cell wall at some point in the past, but as their lifestyle became one of existence inside other cells, they lost the ability to form walls. Consistent with this very limited lifestyle within other cells, these microbes also have very small genomes. They have no need for the genes for all sorts of biosynthetic enzymes, as they can steal the final components of these pathways from the host. Similarly, they have no need for genes encoding many different pathways for various carbon, nitrogen and energy sources, since their intracellular environment is completely predictable. Because of the absence of cell walls,*Mycoplasma*have a spherical shape and are quickly killed if placed in an environment with very high or very low salt concentrations. However, They have unusually tough membranes that are more resistant to rupture than other bacteria since this cellular membrane has to contend with the host cell factors. The presence of sterols in the membrane contributes to their durability by helping to increase the forces that hold the membrane together. Other bacterial species occasionally mutate or respond to extreme nutritional conditions by forming cells lacking walls, termed L-forms. This phenomenon is observed in both gram-positive and gram-negative species. L-forms have varied shapes and are sensitive to osmotic shock.

Fig:Mycoplasma

Source: Wilkepedia

Scientific classification of Mycoplasma

Domain: Bacteria

Phylum: Tenericutes

Class: Mollicutes

Family: Mycoplasmataceae

Genus: Mycoplasma

Nowak gave the term mycoplasma in the year 1929

4.4. Cell Walls of Archaea: Archaeal cell walls differ from bacterial cell walls in their chemical composition and lack of peptidoglycans.

Fig: Archaebacteria

Source: Wilkepedia

As with other living organisms, Archaeal cells have an outer cell membrane that serves as a protective barrier between the cell and its environment. Within the membrane is the cytoplasm, where the living functions of the archeon take place and where the DNA is located. Around the outside of nearly all Archaeal cells is a cell wall, a semi-rigid layer that helps the cell maintain its shape and chemical equilibrium. All three of these regions may be distinguished in the cells of bacteria and most other living organisms. A closer look at each region reveals structural similarities but major differences in chemical composition between bacterial and archaeal cell wall. Archaea builds the same structures as other organisms, but they build them from different chemical components. For instance, the cell walls of all bacteria contain the chemical peptidoglycan. Archaeal cell walls do not contain this compound, though some species contain a similar one. It is assembled from surface-layer proteins called S-layers. Likewise, archaea do not produce walls of cellulose (as do plants) or chitin (as do fungi). The cell wall of archaeans is chemically distinct. Methanogens are the only exception and possess pseudopeptidoglycan chains in their cell wall that lacks amino acids and N-acetylmuramic acid in their chemical composition. The most striking chemical differences between Archaea and other living things lie in their cell membrane. There are four fundamental differences between the archaeal membrane and those of all other cells: (1) chirality of glycerol, (2) ether linkage, (3) isoprenoid chains, and (4) branching of side chains.

5.0. Damage to the Cell Wall:

The cell wall is responsible for bacterial cell survival and protection against environmental factors and antimicrobial stress. The cell wall is the principal stress-bearing and shape-maintaining element in bacteria. Its integrity is thus of critical importance to the viability of a particular cell. In both gram-positive and gram-negative bacteria,cell wall consists of a cross-linked polymer peptidoglycan. The cell wall of gram-negative bacteria is thin (approximately only 10 nanometers in thickness), and is typically comprised of only two to five layers of peptidoglycan, depending on the growth stage. In gram-positive bacteria, the cell wall is much thicker (20 to 40 nanometers thick). While the peptidoglycan provides the

structural framework of the cell wall, teichoic acids, which make up roughly 50% of the cell wall material, are thought to control the overall surface charge of the wall. This affects murein hydrolase activity, resistance to antibacterial peptides, and adherence to surfaces. Although both of these molecules are polymerized on the surface of the cytoplasmic membrane, their precursors are assembled in the cytoplasm. Any event that interferes with the assembling of the peptidoglycan precursor, and the transport of that object across the cell membrane, where it will integrate into the cell wall, would compromise the integrity of the wall. Damage to the cell wall disturbs the state of cell electrolytes, which can activate death pathways (apoptosis or programmed cell death). Regulated cell death and lysis in bacteria plays an important role in certain developmental processes, such as competence and biofilm development. They also play an important role in the elimination of damaged cells, such as those irreversibly injured by environmental or antibiotic stress. An example of an antibiotic that interferes with bacterial cell wall synthesis is Penicillin. Penicillin acts by binding to transpeptidases and inhibiting the cross-linking of peptidoglycan subunits. A bacterial cell with a damaged cell wall cannot undergo binary fission and is thus certain to die.

6. **Endospore:** They are thick walled, highly retractile hard resistant structure serve to tide over unfavorable condition like nutrients deficiency, high heat etc. It is produced by some special bacteria like *Bacillus, Clostridium,Sporosarcina,Thermoactinomycetes* and few other genera under unfavorable condition. It is not a means of reproduction and serves to tide over unfavorable environmental condition. After approaching to favorable condition endospore burst to release one cell (In division two cell will form). All endospore contain large amounts of dipicolinic acid (DPA) which is the hard structure due to deposition of Ca -dipicolinic acid. The calcium DPA complex may possibly play a role in the heat resistance endospores. Release of cell is after approaching favorable environment is known as germination.

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Fig: Endospore in Bacteria

Source: Wilkepedia

Endospore lose their resistance to heat and staining subsequent outgrowth occur, characterized by synthesis of new cell material and development of the organism into a growing cell.

The End

Chapter-5

MICROSCOPY AND SPECIMEN PREPARATION

4.1. The Study of Microbial Structure:

Organisms are classified into taxonomyinto many groups such as Animals,Plants, andFungi orUnicellularmicroorganisms such asProtists,Bacteria, andArchaea. All types of organisms are capable ofreproduction,growth and development.They also have ability for maintenance of their cellular structure mand some degree of response tostimuli. Examples of multicellular organisms that have well developed differentiatespecializedtissuesandorgansare Humans,squids,mushrooms, andvascular plants.

An organism may be either aprokaryoteor aeukaryote. Prokaryotes are represented by two separatedomains–bacteriaand archaea. Eukaryotic organisms are characterized by the presence of a membrane-boundcell nucleusand contain additional membrane-bound compartments calledorganelles(such asmitochondriain animals and plants andplastidsin plants andalgae, all generally considered to be derived fromendosymbioticbacteria). Fungi, animals and plants are examples ofkingdomsof organisms within the eukaryotes. About 2 million to 1 trillion species are estimated to be present on current earth of which only 1.7 million have been documented and more than 99% of all species, amounting to over five billion species, that ever lived are estimated to beextinct.

Structure of organisms may vary from 0.1nm to 10 m.Some organism can be seen by naked eys some with the aid of visual aid like Microscope.A normal eys can see up to 1mm.Smaller than tis size need a optical aid.

4.2. Discovery of Microorganisms : The first microorganism was observed by **Antony van Leeuwenhoek (1632-1723)** by his simple made microscope which was nothing but assembly of glasses. He called them as “Animalcules”.

Fig. First Microscope prepraed by A.V.Leewenhook and structure of Microorganism.

Lenses and the Bending of Light- The property of a Microscope depends on the light property,Lenses and bending of light.The common properties of lights are:

- Light is refracted (bent) when passing from one medium to another
- The bending of light is the property of Refractive indexes of different media.
- A measure of how greatly a substance slows the velocity of light
- Direction and Magnitude of bending is determined by the refractive indexes of the two media forming the interface
- **Lenses-**
- Focus light rays at a specific place called the focal point
- Distance between center of lens and focal point is the focal length.
- Strength of lens related to focal length
- Short focal length ⇒more magnification

4.2.1. **The Light Microscope:** The light microscope is an important tool in the study of microorganisms, particularly for identification purposes.

Principles

The light microscope is an instrument for visualizing fine detail of an object. It does this by creating a magnified image through the use of a series of glass lenses, which first focus a beam of light onto or through an object, and convex objective lenses to enlarge the image formed. In the majority of light microscopes, the image is viewed directly through binocular eyepieces that act as a secondary lens in the form of a magnifying glass to observe the projected image. Such instruments are termed 'compound microscopes,' and the total magnification is the sum of the objective magnification and the eyepiece magnification. The magnification range extends from ×10 to ×1000, with a resolving power of the order of 0.2μm, depending on the type and numerical aperture (area available for passage of light) of the objective lenses. A number of books are available, providing comprehensive details on the theory of the light microscope and guidance to the practical use of the instrument, including

methods of image enhancement and instrument care.

Types of compound Microscope: Based on source of enrgy used light can be

Bright-field Microscope.

Dark-field Microscope.

Phase-contrast Microscope.

Fluorescence Microscopes

These are compound microscopes: The compound light microscope uses visible light to directly illuminate specimens in a two-lens system, resulting in the illuminated specimen appearing dark against a bright background. The two lenses present in a compound microscope are the ocular lens in the eyepiece and the objective lens located in the revolving nosepiece. The image is formed by the action of ≥2 lenses

4.2.2.The Bright-Field Microscope: Produces a dark image against a brighter background. It has several objective lenses. Parfocal microscopes remain in focus when objectives are change. Total magnification is the product of the magnifications of the ocular lens and the objective lens

Fig : A compound Microscope.

Parts of Compound Microscope: A typical Compound Microscope have following parts

1. Illuminator: the light source in the base of the microscope;

2. Abbe Condensor: a two lens system that collects and concentrates light from the illuminator and directs it to the iris diaphragm;

3. Iris diaphragm: regulates the amount of light entering the lens system;

4. Mechanical stage: a platform used to place the slide on which has a hole in the center to let light from the illuminator pass through. Often contains stage clips to hold the slide in place;

5. Body tube: houses the lens system that magnifies the specimens;

6. Upper end of body tube—oculars/eye pieces: what you view through;

7. Lower end of body tube—nose-piece: revolves and contains the objectives.

Essentially, a light microscope magnifies small objects and makes them visible. The science of microscopy is based on the following concepts and principles:

a. Magnification is simply the enlargement of the specimen. In a compound lens system, each lens sequentially enlarges or magnifies the specimen;

b. The objective lens magnifies the specimen, producing a real image that is then magnified by the ocular lens resulting in the final image;

c. The total magnification can be calculated by multiplying the objective lens value by the ocular lens value.

Microscope Resolution- Ability of a lens to separate or distinguish small objects that are close together. In other word It is defined as the inverse of the distance or angular separation between two objects which can be just resolved when viewed through the optical instrument.

Resolving Power of Microscope:

For microscopes, the resolving power is the inverse of the distance between two objects that can be just resolved. This is given by the famous Abbe's criterion given by Ernst Abbe in 1873 as

?

$d=\lambda 2n\sin\theta$

?

Resolving power$=1 \quad d=2n\sin\theta\lambda$

Wherenis the refractive index of the medium separating object and aperture. Note that to achieve high-resolutionn sin θmust be large. This is known as the Numerical aperture.

Thus, for good resolution:

1. sin θ must be large. To achieve this, the objective lens is kept as close to the specimen as possible.

2. A higher refractive index (n) medium must be used. Oil immersion microscopes use oil to increase the refractive index. Typically for use in biology studies, this is limited to 1.6 to match the refractive index of glass slides used. (This limits reflection from slides). Thus the numerical aperture is limited to just 1.4-1.6. Thus, optical microscopes (if you do the math) can only image to about 0.1 microns. This means that usually organelles, viruses and proteins cannot be imaged.

3. Decreasing the wavelength by using X-rays and gamma rays. While these techniques are used to study inorganic crystals, biological samples are usually damaged by x-rays and hence are not used.

The limit set by Abbe's criterion for optical microscopy cannot be avoided. However, using different fluorescence microscopy techniques the Abbe's limit can be circumvented. Stefan Hell used a technique called Stimulated Emission Depletion (STED) and the duo Eric Betzig and W.E. Moerner used superimposed images using green fluorescent proteins to bypass the resolution limit and obtain optical images in never before seen resolution. All three were awarded the 2014 Nobel Prize in Chemistry for their pioneering work. Wavelength of light used is major factor in resolution

shorter wavelength $\Rightarrow$ greater resolution

Working distance: It is the distance between the front surface of lens and surface of cover glass or specimen

Fig: Working distance and use of immersion oil for better resolution

4.2.3.The Dark-Field Microscope: Produces a bright image of the object against a dark background. Used to observe living, unstained preparations.

Preparation and Staining of Specimens: The main function of stain in microscopy is **to** Increases visibility of specimen **and** accentuates specific morphological features of Microorganism **.** It also preserves the specimens for long time to study later on.

Fixation: It is the process by which internal and external structures are preserved and fixed in position.During fixation the organism under observation is killed and firmly attached to microscope slide.There are two methods for fixation of organisms:

A. Heat fixing

Preserves overall morphology but not internal structures

B. Chemical fixing: Protects fine cellular substructure and morphology of larger, more delicate organisms

Dyes and Simple Staining

Dyes: Make internal and external structures of cell more visible by increasing contrast with background**.It have** have two common features**a. A** chromophore groups **B.** chemical groups with conjugated double bonds give dye its color and ability to bind cells.

Types of Staining: **There are many types of Staining depending upon our objectives of observation:**

1.Simple staining: In this process a single staining agent is used**.**Basic dyes are frequently used **ie** dyes with positive charges which have ability to bind with negatively chargesd bacterial surface:e.g., crystal violet

2. Differential Staining: Divides microorganisms into groups based on their staining properties. e.g., Gram stain (Divide bacteria into gram +ve and Gram –ve) e.g., Acid-fast stain (For Mycobacterium tuberculosis)

2.1.1 Gram staining: most widely used differential staining procedure**.** Divides Bacteria into two group as Gram +ve and Gram –ve.In this method a series of Chemicals are used to stain the bacteria and then differentiated based on the property to retian a particular dye colour such as crystal violet in gram +ve and Safranin in Gram negative(A counterstain).

The Steps of gram staining is given below:

Flow chart: Process of Gram staining

Source: Wikipedia.org.in

2.1.2.Acid-fast staining: Particularly useful for staining members of the genus *Mycobacterium*e.g., *Mycobacterium tuberculosis* – causes tuberculosis, e.g., *Mycobacterium leprae* – causes leprosy. Mycobacterium have high lipid content in cell walls that is mainly responsible for their staining characteristics.

3. Staining Specific Structures: Some of the dye have ability to stain special structure such as flagella,Endospore,Capsule etc.Some of them are listed below:

3.1.Negative staining: This staining methofds is Often used to visualize capsules surrounding bacteria**.The** capsules looks colorless against a stained background.

3.2 Spore staining: For Visualizing Bacterial Endospore Double staining technique is used in which bacterial endospore retain one color and vegetative cell retain a different color.

3.3.Flagella staining: Flagella are too thin to be visualized using a bright field microscope with ordinary stains, such as the Gram stain, or a simple stain. A wet mount technique is used for staining bacterial flagella, and it is simple and useful when the number and arrangement of flagella are critical to the identification of species of motile bacteria. The staining procedures require the use of a mordant so that the stain adheres in layers to the flagella, allowing visualization.

Other Microscope:

4.2.4.Phase-contrast microscopy: Itis an optical microscope that converts phase shifts in light passing through a transparent

specimen to brightness changes in the image. Phase shifts themselves are invisible, but become visible when shown as brightness variations.Its an example of Dark field Microscope.The Microscope was invented by Zernick in 1950.

When light waves travel through a medium other than vacuum, interaction with the medium causes the wave amplitude and phase to change in a manner dependent on properties of the medium. Changes in amplitude (brightness) arise from the scattering and absorption of light, which is often wavelength-dependent and may give rise to colors.Phase-contrast microscopy is particularly important in biology. It reveals many cellular structures that are not visible with a simpler bright-field microscope.These structures were made visible to earlier microscopists by staining, but this required additional preparation and thus killing the cells. The phase-contrast microscope made it possible for biologists to study living cells and how they proliferate through cell division. It is one of the few methods available to quantify cellular structure and components that does not use fluorescence. After its invention in the early 1930s, phase-contrast microscopy proved to be such an advancement in microscopy that its inventor Frits Zernike was awarded the Nobel Prize in Physics in 1953. The basic principle to making phase changes visible in phase-contrast microscopy is to separate the illuminating (background) light from the specimen-scattered light (which makes up the foreground details) and to manipulate these differently.

Fig: A Phase Contrast Microscope with working Principal.

Source: Wikipedia.org.in

Fig: Phase Constart Microscope

Olympus Made.

Steps to observe image under Phase Contarst Microscope: **Diffrence in working of** A Dark field Microscope and Phase Contrast Microscope:

1.Dark Field Microscoe consists of a detector,lenses to magnify,A Phase ring and a light source.

2.The phase ring stops part of the light so that it canot reach the detector anymore

3.When a sample is inserted ,it scatters part of the light even if it is transparent.

4.The scatterd light refocused on the detector,

5. The image of the sample appereds on the screen.

6. Dark field allows increasing the contrast of image of unstained and uncoloured objects.

Phase Contrast Microscope function:

1. When the sample is inserted it scatters part of the light which is then also refocused to the detector

2. To distinguish direct light from that of scattered by the sample,a phase plate is inserted.

9. The scattered part crosses a thicker part of the plate.This shift its phase compared to the direct light.

10. Scattered light then interfares with direct light which create a phase constarst.

11. The resulting intensity differences allow to visualize transparaent samples

4.2.5.Fluroscence Microscope: The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (i.e., of a different color than the absorbed light). The illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission

filter. Typical components of a fluorescence microscope are a light source (xenon arc lamp or mercury-vapor lamp are common; more advanced forms are high-power LEDs and lasers), the excitation filter, the dichroic mirror and the emission filter (see figure below). The filters and the dichroic beam splitter are chosen to match the spectral excitation and emission characteristics of the fluorophore used to label the specimen. In this manner, the distribution of a single fluorophore (color) is imaged at a time. Multi-color images of several types of fluorophores must be composed by combining several single-color images.

Fig: Working of Flurescence Microscope

Source:Wikipedia.org.in

Fig: An upright fluorescence microscope (Olympus BX61)

with the fluorescence filter cube the objective lenses,

Coupled with a digital camera.

1. Light is allowed to pass through excitation filter to fall upon Object under investigation (Blue Light).
2. Object reflected the light of higher Wavelenght(Green light)
3. Detected as green object in two dimensional and three dimensional figure.
4. Even small quantity of cell component can be visualized by this microscope by moving above the ocular lenses over each part of the cell component.

5.Electron Microscopy: A beams of electrons are used to produce images .Since wavelength of electron beam is much shorter than light, resulting in much higher resolution. It is that type of microscope that uses a beam of accelerated electrons as a source of illumination. As the wavelength of an electron can be up to 100,000 times shorter than that of visible light photons, electron microscopes have a higher resolving power than light microscopes and can reveal the structure of smaller objects. A scanning transmission electron microscope has achieved better than 50 pm resolution in annular dark-field imaging mode and magnifications of up to about 10,000,000× whereas most light microscopes are limited by diffraction to about 200 nm resolution and useful magnifications below 2000×. Electron microscopes use shaped magnetic fields to form electron optical lens systems that are analogous to the glass lenses of an optical light microscope. It is used to investigate the ultrastructure of a wide range of biological and inorganic specimens,cells, large molecules, metals, and crystals. The First Electron Microscope was developed in 1931 by the physicist Ernst Ruska and the electrical engineer Max Knoll.

Fig: A Typical Electron Microscope.Image of a fly under EM

Electron Micrscope are of two types:

5.1. Transmission Electron Microscope and Scanning Electron Microscope

A. Transmission electron microscope (TEM), is a known electron Microscope that uses a high voltage electron beam to illuminate the specimen and create an image. The electron beam is produced by an electron gun, commonly fitted with a

tungstenfilamentcathodeas the electron source. The electron beam is accelerated by ananodetypically at +100 keV(40 to 400 keV) with respect to the cathode, focused byelectrostaticandelectromagneticlenses, and transmitted through the specimen that is in part transparent to electrons and in partscattersthem out of the beam. When it emerges from the specimen, the electron beam carries information about the structure of the specimen that is magnified by theobjective lenssystem of the microscope. The spatial variation in this information (the "image") may be viewed by projecting the magnified electron image onto a fluorescent viewing screen coated with aphosphororscintillatormaterial such as zinc sulfide.

Alternatively, the image can be photographically recorded by exposing aphotographic filmorplatedirectly to the electron beam, or a high-resolution phosphor may be coupled by means of a lens optical system or afibre opticlight-guide to the sensor of adigital camera. The image detected by the digital camera may be displayed on a monitor or computer.

Fig: Working of Transmission Electron Microscope

Source: Brittannica .com

Specimen Preparation: Aanalogous to procedures used for light microscopy**.** For transmission electron microscopy, specimens must be cut very thin**.** Specimens are chemically fixed and stained with electron dense material

5.2. The Scanning Electron Microscope:

SEM produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen. When the electron beam interacts with the specimen, it loses energy by a variety of mechanisms. The lost energy is converted into alternative forms such as heat, emission oflow-energy secondary electronsand high-energy backscattered electrons, light emission (cathodoluminescence) orX-rayemission, all of which provide signals carrying information about the properties of the specimen surface, such as its topography and composition. The image displayed by an SEM maps the varying intensity of any of these signals into the image in a position corresponding to the position of the beam on the specimen when the signal was generated.

Advantage :

1. Althoug the image resolution of an SEM is lower than that of a TEM. But as the SEM images the surface of a sample rather than its interior, the electrons do not have to travel through the sample. This reduces the need for extensive sample preparation to thin the specimen to electron transparency.

2. The SEM is able to image bulk samples that can fit on its stage.

3. The SEM also has a great depth of field, and so can produce images that are good representations of the three-dimensional surface shape of the sample.

4. Another advantage of SEMs comes withenvironmental scanning electron microscopes(ESEM) that can produce images of good quality and resolution with hydrated samples or in low, rather than high, vacuum or under chamber gases. This facilitates imaging unfixed biological samples that are unstable in the high vacuum of conventional electron microscopes.

The End

Chapter-6

CLASSIFICATION, NOMENCLATURE AND IDENTIFICATION

5.1 **Introduction**

Taxonomy (Greek taxis, arrangement or order, and nomos, law, or naming, to distribute or govern) is defined as the science of biological classification. In a broader sense it consists of three separate but interrelated parts: classification, nomenclature, and identification.

5.1.2 Classification

Classification is "the arrangement of organisms into groups or taxa."

5.1.3 Nomenclature

Nomenclature is "the branch of taxonomy concerned with the assignment of names to taxonomic groups in agreement with published rules.

Identification

Identification is "the practical side of taxonomy, the process of determining that a particular (organism) belongs to a recognized taxon."

5.2 History of Taxonomy

Carolus Linnaeus (1707–1778), Linnaeus, 18th century taxonomist, classified organisms by their structure. He is credited with developing the modern system of naming known as binomial nomenclature and is called the 'Father of Taxonomy'.

Two-word name (Genus and species)

– Genus species

– Latin or Greek

– Italicized in print

– Capitalize genus, but NOT species

– Underline when writing

Carolus Linnaeus distinguished two kingdoms of living things: Animalia for animals and Vegetabilia for plants (Linnaeus also included minerals, placing them in a third Kingdom, Mineralia). He divided each kingdom into classes, later grouped into phyla for animals and divisions for plants.

Edouard Chatton (1883-1947), a French biologist, contributed to our knowledge of single-celled protoctists, especially ciliates and dinoflagellates, free-living and/or symbiotic, in relation to the marine invertebrate animals in which they reside. More than the description of many new families, genera and species, and of their life cycles, he anticipated several major concepts of cell biology, including the fundamental difference between prokaryote and eukaryote protists, long time before

the advent of electron microscopy. It gradually became apparent how important the prokaryote/eukaryote distinction is, and Stanier and van Niel popularized Chatton's proposal in the 1960s to divide them.

Ernst Heinrich Philipp August Haeckel (1834–1919), was an eminent German biologist, naturalist, philosopher, physician, professor and artist who discovered, described and named thousands of new species, mapped a genealogical tree relating all life forms, coined many terms in biology, including phylum, phylogeny, ecology and the kingdom Protista. In 1866, Ernst Haeckel divided animals, plants, and microorganisms into 3 kingdoms namely Animalia, Plantae and Protista.

Robert Harding Whittaker (1920–1980), recognized an additional kingdom for the Fungi. The resulting five-kingdom system, proposed in 1969, has become a popular standard and with some refinement is still used in many works and forms the basis for newer multi-kingdom systems. It is based mainly on differences in nutrition; his Plantae were mostly multicellular autotrophs, his Animalia multicellular heterotrophs, and his Fungi multicellular saprotrophs. The remaining two kingdoms, Protista and Monera, included unicellular and simple cellular colonies.

In biological taxonomy, kingdom and/or regnum is a taxonomic rank in either (historically) the highest rank, or (in the new three-domain system) the rank below domain. Each kingdom is divided into smaller groups called phyla (or in some contexts these are called 'divisions'). Currently, many textbooks from the United States use a system of six kingdoms (Animalia, Plantae, Fungi, Protista, Archaea, Bacteria) while British and Australian textbooks may describe five kingdoms (Animalia, Plantae, Fungi, Protista, and Prokaryota or Monera). The classifications of taxonomy are life, domain, kingdom, phylum, class, order, family, genus, and species.

5.3 Classification Systems

Hierarchical classification: In classification taxonomist follow a hierarchy of designations; means in ascending sequence. The full description of a given organism's place among all the world's organisms does not end with its binomial designation. There exists a hierarchy of designations only the last of which describe genera and species denomination. A category in any rank unites groups in the level below it based on shared properties. The major designations, listed in terms of increasing specificity, include

Domain (empire/super-kingdom)

Kingdom

Phylum

Class

Order

Family

Genus

Species

Example

Genus:Escherichia

Species:coli

Family: Enterobacteriaceae

Class: Scotobacteria

Division: Gracilicutes

Kingdom: Procaryotae

5.3.1 Five kingdoms of life:

Living organisms as suggested by are subdivided into 5 major kingdoms, including the Monera, the Protista (Protoctista), the Fungi, the Plantae, and the Animalia. Each kingdom is further subdivided into separate phyla or divisions. Generally 'animals' are subdivided into phyla, while 'plants' are subdivided into divisions. These subdivisions are analogous to subdirectories or folders on your hard drive. The five kingdom system of classification for living organisms, including the prokaryotic Monera and the eukaryotic Protista, Fungi, Plantae and Animalia is complicated by the discovery of archaebacteria. The prokaryotic Monera include three major divisions: The regular bacteria or eubacteria; the cyanobacteria (also called blue-green algae); and the archaebacteria. Lipids of archaebacterial cell membranes differ considerably from those of both prokaryotic and eukaryotic cells, as do the composition of their cell walls and the sequence of their ribosomal RNA subunits. In addition, recent studies have shown that archaebacterial RNA polymerases resemble the eukaryotic enzymes, not the eubacterial RNA polymerase.

5.3.2 **Six kingdoms**

Around 1980, there was an emphasis on phylogeny and redefining the kingdoms to be monophyletic groups, groups made

up of relatively closely related organisms. The Animalia, Plantae, and Fungi were generally reduced to core groups of closely related forms, and the others placed into the Protista. Based on RNA studies, Carl Woese divided the prokaryotes (Kingdom Monera) into 2 kingdoms -Eubacteria and Archaebacteria. Carl Woese attempted to establish a 3 Primary Kingdom system in which Plants, Animals, Protista, and Fungi were lumped into one primary kingdom of all eukaryotes. The Eubacteria and Archaebacteria made up the other two kingdoms. The initial use of 'six kingdom system' represents a blending of the classic five kingdom system and Woese's three domain system. Such six kingdom system has become standard in many works. A variety of new eukaryotic kingdoms were also proposed, but most were quickly invalidated, ranked down to phyla or classes, or abandoned. The only one which is still in common use is the kingdom Chromista proposed by Cavalier-Smith, including organisms such as kelp, diatoms, and water moulds. Thus the eukaryotes are divided into three primarily heterotrophic groups, the Animalia, Fungi, and Protozoa, and two primarily photosynthetic groups, the Plantae (including red and green algae) and Chromista. However, it has not become widely used because of uncertainty over the monophyly of the latter two kingdoms.

5.3.3 Three domain system

In 1970, Carl Woes, by analyzing RNA, developed the 3 domain classification system

Archaebacteria

Bacteria

Eucarya

Woese stresses genetic similarity over outward appearances and behaviour, relying on comparisons of ribosomal RNA genes at the molecular level to sort out classification categories. A plant does not look like an animal, but at the cellular level, both groups are eukaryotes, having similar subcellular organization, including cell nuclei, which the Eubacteria and Archaebacteria do not have. More importantly, plants, animals, fungi, and protists are more similar to each other in their genetic makeup at the molecular level, based on RNA studies, than they are to either the Eubacteria or Archaebacteria. Woese also found that all of the eukaryotes, lumped together as one group, are more closely related, genetically, to the Archaebacteria than they are to the Eubacteria. This means that the Eubacteria and Archaebacteria are separate groups even when compared to the eukaryotes. Therefore, Woese established the three domain system, clarifying that all the Eukaryotes are more closely genetically related compared to their genetic relationship to either the bacteria or the archaebacteria, without having to replace the 'six kingdom system' with a three kingdom system. The three domain system is a 'six kingdom system' that unites the eukaryotic kingdoms into the Eukarya Domain based on their relative genetic similarity when compared to the Bacteria Domain and the Archaea Domain. Woese also recognized that the Protista kingdom is not a monophyletic group and might be further divided at the level of kingdom. Others have divided the Protista kingdom into the Protozoa and the Chromista, for instance.

Some important term:

Serovar

A serovar is a strain differentiated by serological means. Individual strains of Salmonella spp. are often distinguished and distinguishable by serological means.

Biovar (biotype)

Biovars are strains that are differentiated by biochemical or other non-serological means.

Morphovar (morphotype)

A morphovar is a strain which is differentiated on the basis of morphological distinctions.

Isolate

An isolate is a pure culture derived from a heterogeneous, wild population of microorganisms. The term isolate is also applicable to eucaryotic microorganisms as well as to viruses.

METHODS OF CLASSIFICATION

Introduction

Two common approaches used in biological world are referred to as phenotypic and phylogenetic classification.

Phenotypic (phenetic) classification:

Phenotypic classification is concerned with grouping individual species into phenotypic categories (taxons) based on how organisms 'look'. In the recent past, taxonomists were not equipped to classify beyond the level of phenotypic groupings except via inference from phenotypic similarities. Phenotypic similarity and evolutionary relationship do not always map one to one upon each other. This approach is guided by a set of morphological and biochemical tests, constituting the cornerstone of 'Determinative Microbiology'.

Limitations: From the standpoint of many areas of microbiology, a determinative classification is sufficient. For example, in clinical microbiology the identification of organisms permits the physician to assess pathogenicity and to select a treatment. In this context, the purely determinative nature of a classification is not crucial. If the organism has previously been described and hence is already in the classification, then it can be identified and treated. However, from a biological point of view, the lack of a natural system does not permit the projection of properties of previously described organisms onto new ones that

might be closely related, but not identical, to those known before. In addition, it does not help us understand an organism that we have been unable to cultivate in the laboratory. Finally, it does not permit studies of the origin and evolution of cellular functions (e.g. drug resistance, aerobiosis or photosynthesis), because there is no evolutionary (historical) framework.

Phylogenetic (phyletic) classification

Phylogenetic classification is concerned with grouping individual species into evolutionary categories. Since the early 1980s, phylogenetic classfication has been made much more facile by the invention of molecular taxonomy. The evolutionary classification of organisms is based on the nucleotide sequence divergence at individual loci (genes).

Phylogeny from Phenotype

These two approaches (phenotypic and phylogenetic classification) often fully match. This is because there is usually a correlation between evolutionary relatedness and phenotypic relatedness. However, such things as convergent evolution can create confusion between the two classification philosophies since convergent evolution, by definition, produces phenotypic similarity in the absence of close evolutionary relatedness. The trick to solving these discrepancies is to concentrate on true homologies and ignore convergence. Conflicts between phenotypic and phylogenetic classification are at the root of the various monophyly-paraphyly debates.

Molecular phylogeny

A homology is a similarity between two organisms that exists because the two organisms are closely evolutionarily related (that is, the feature in question existed in the common ancestor to the two organisms). The similarity of the DNA (or RNA) of organisms may be determined by a number of means including determinations of base composition, nucleotide sequence, or DNA hybridization rates. Typically these means include very powerful ways by which organisms may be classified, either in terms of distinctions between organisms (i.e. the organisms may be classified as representing two or more species) or similarities (i.e. it may be concluded from evidence of genotypic similarity that the organisms are closely related, i.e. evolutionarily related); the latter similarities we would classify as a genetic homology. The downside of genetic homology is that the acquisition of data often requires a laboratory and at least a little effort. The upside is that genetic homology describes evolutionary relationships with only minimal interference from phenotype.

Numerical Taxonomy

It is a classification system in biological systematics which deals with the grouping by numerical methods of taxonomic units based on their character states. It aims to create a taxonomy using numeric algorithms like cluster analysis. The concept was first developed by Robert R. Sokal and Peter H. A. Sneath in 1963. Phenetics is a closely related discipline and draws heavily from the methods of numerical taxonomy.

Although intended as an objective classification method, in practice the choice and weighing of morphological characteristics is often guided by available methods and research interests. Furthermore, the general consensus has become that the

taxonomic classification should reflect evolutionary (phylogenetic) processes. Some connections between phylogenetic trees and the spectral decomposition of the variance-covariance matrix of quantitative traits subject to Brownian motion over time have been established, providing a theoretical link between phylogenetic methods and numerical taxonomy. The specific phenetic algorithms proposed in numerical taxonomy, however, often fail to properly reconstruct the phylogenetic history of organisms.

5..4 All Species Inventory

In 2001 an international project was launched to identify and record every species on earth in the next 25 years. It is a very challenging undertaking considering that to date 1.5 million organisms have been named. It is estimated that anywhere from 7 – 100 million living species exist.

The End

CHAPTER-7

BACTERIAL GROWTH

Bacterial growth is a complex process that involves numerous anabolic and catabolic reactions, which result in cell division. The increase in numbers or bacterial mass can be measured as a function of time under pure culture conditions, where the nutrients and environmental conditions are controlled. Growth of bacterial cultures is defined as an increase in the number of bacteria in a population rather than in the size of individual cells. The growth of a bacterial population occurs in a geometric or exponential manner: with each division cycle (generation), one cell gives rise to 2 cells, then 4 cells, then 8 cells, then 16, then 32, and so forth. Typically bacteria divides by Binary fission by a series of steps as below:

Fig: Binary Fission in Bacteria.

Source: Wilkepedia.org.in

Source: wilkepedia

1. Prokaryote cells grow by increasing in cell number (as opposed to increasing in size).

2. Replication is by binary fission, the splitting of one cell into two.

3. Therefore, bacterial populations increase by a factor of two (double) every generation time.

6.1. Generation time : The time required to for a population to double (doubling time) in number.

Ex. *Escherichia coli* (*E. coli*) double every 20 minutes

Ex. *Mycobacterium tuberculosis* double every 12 to 24 hours

6.2. Principles of Bacterial Growth

Growth can be calculated

$$N_t = N_0 \times 2^n$$

Where (N_t) number of cells in population

(N_0) original number of cells in the population

(n) number of divisions

Example, $N_0 = 10$ cells in original population

$n = 12$

4 hours assuming 20 minute generation time

$N_t = 10 \times 2^{12}$

$N_t = 10 \times 4{,}096$

$N_t = 40{,}960$

6.2. Growth in Batch Culture : The growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be studied with four different phases: **lag phase** (A), **log phase** or **exponential phase** (B), **stationary phase** (C), and **death phase** (D).

1. During **lag phase**, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs. During the lag phase cells change very little because the cells do not immediately reproduce in a new medium. This period of little to no cell division is called the lag phase and can last for 1 hour to several days. During this phase cells are not dormant.

2. The **log phase** (sometimes called the logarithmic phase or the *exponential phase*) is a period characterized by cell doubling.The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time.The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Under controlled conditions, cyanobacteria can double their population four times a day and then they can triple their population.Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

3. The **stationary phase** is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. The result is a "smooth," horizontal linear part of the curve during the stationary phase. Mutations can occur during stationary_phase. Bridges et al. (2001) presented evidence that DNA_damage is responsible for many of the mutations arising in the genomes of stationary phase or starving bacteria. Endogenously generated reactive oxygen species appear to be a major source of such damages.

4. **Death phase** (Decline phase) bacteria die. This could be caused by lack of nutrients, environmental temperature above

or below the tolerance band for the species, or other injurious conditions.

This basic batch culture growth model draws out and emphasizes aspects of bacterial growth which may differ from the growth of macrofauna. It emphasizes clonality, asexual binary division, the short development time relative to replication itself, the seemingly low death rate, the need to move from a dormant state to a reproductive state or to condition the media, and finally, the tendency of lab adapted strains to exhaust their nutrients. In reality, even in batch culture, the four phases are not well defined. The cells do not reproduce in synchrony without explicit and continual prompting (as in experiments with stalked bacteria and their exponential phase growth is often not ever a constant rate, but instead a slowly decaying rate, a constant stochastic response to pressures both to reproduce and to go dormant in the face of declining nutrient concentrations and increasing waste concentrations.

Batch culture is the most common laboratory growth method in which bacterial growth is studied, but it is only one of many. It is ideally spatially unstructured and temporally structured. The bacterial culture is incubated in a closed vessel with a single batch of medium. In some experimental regimes, some of the bacterial culture is periodically removed and added to fresh sterile medium. In the extreme case, this leads to the continual renewal of the nutrients. This is achemostat, also known as continuous culture. It is ideally spatially unstructured and temporally unstructured, in a steady state defined by the rates of nutrient supply and bacterial growth. In comparison to batch culture, bacteria are maintained in exponential growth phase, and the growth rate of the bacteria is known. Related devices includeturbidostatsandauxostats. When*Escherichia coli*is growing very slowly with a doubling time of 16 hours in a chemostat most cells have a single chromosome.

Bacterial growth can be suppressed withbacteriostats, without necessarily killing the bacteria. In asynecological, true-to-nature situation in which more than one bacterial species is present, the growth of microbes is more dynamic and continual.

Liquid is not the only laboratory environment for bacterial growth. Spatially structured environments such as biofilms oragar surfaces present additional complex growth models.

Growth of bacteria in abatch culture can be summarized as below:

1. Bacteria growing in **batch culture** produce a **growth curve** with up to four distinct phases.
2. Batch cultures are grown in tubes or flasks and are **closed systems** where no fresh nutrients are added or waste products removed.
3. **Lag phase** occurs when bacteria are adjusting to them medium. For example, with a nutritionally poor medium, several anabolic pathways need to be turned on, resulting in a lag before active growth begins.
4. In **log** or **exponential phase**, the cells are growing as fast as they can, limited only by growth conditions and genetic potential. During this phase, almost all cells are alive, they are most nearly identical, and they are most affected by outside influences like disinfectants.
5. Due to nutrient depletion and/or accumulation of toxic end products, replication stops and cells enter a **stationary phase** where there is no net change in cell number.

6. **Death phase** occurs when cells can no longer maintain viability and numbers decrease as a proportion

Fig :Growth in Batch Culture

6.2.1Mean Generation Time and Growth Rate: The mean generation time (doubling time) is the amount of time required for the concentration of cells to double during the log stage. It is expressed in units of minutes.

Growth rate (min^{-1}) =

Mean generation time can be determined directly from a semilog plot of bacteria lconcentration vs time after inoculation

6.3.Environmental factors affecting growth: Various factors affects growth below:

Temperature

Oxygen requirement

pH

Water availability

6.3.1.Temperature:

Enzymes, the machinery of the cell, are influenced by external factors and can be shown to have a range where they function that includes an optimal value that produces the highest activity.The range of enzyme activity determines the range for growth of specific bacteria, analogously leading to a value for optimal growth rate.In the case of temperature, bacteria are divided into categories based on the temperature range where they can grow and the temperature that provides optimal growth:

A.Psychrophile/cryophiles: Psychrophilesor**cryophiles**(adj. psychrophilic or cryophilic) areextremophilicorganismsthat are capable ofgrowthandreproductionin low temperatures, ranging from - 20°Cto +10°C. They are found in places that are permanently cold, such as the polar regions and the deep sea. Psychrophile is Greek words means 'cold-loving'.Many such

organisms are bacteria or archaea, but some eukaryotes such as lichens, snow algae, fungi, and wingless midges, are also classified as psychrophiles.

B. Psychrotroph: Psychrotrophs are cold-tolerant bacteria that have the ability to grow at low temperatures but have optimal and maximal growth temperatures above 15°C and 20°C, respectively. **This type of organisms can survive below temperature less than**

20°C to 30°C. Important in food spoilage.

C. Mesophile. A **mesophile** is an organism that grows best in moderate temperature, neither too hot nor too cold, with an optimum growth range from 20 to 45°C (68 to 113°F). They are More common and are disease causing

D. Thermophiles: A **thermophile** is an organism—a type of extremophile—that thrives at relatively high temperatures, between 41 and 122°C (106 and 252°F). Many thermophiles are archaea. Thermophilic eubacteria are suggested to have been among the earliest bacteria.

Thermophiles are found in various geothermally heated regions of the Earth, such as hot springs like and deep sea hydrothermal vents, as well as decaying plant matter, such as peat bogs and compost. Thermophiles can survive at high temperatures, whereas other bacteria would be damaged and sometimes killed if exposed to the same temperatures. The enzymes in thermophiles function at high temperatures. Some of these enzymes are used in molecular biology, for example the taq polymerase used in PCR. "Thermophile" is derived from the Greek: θερμότητα (*thermotita*), meaning heat, and Greek: φίλια (*philia*), love.

G. **Hyperthermophiles:** A **hyperthermophile** is an organism that thrives in extremely hot environments—from 60 °C (140 °F) upwards. An optimal temperature for the existence of hyperthermophiles is often above 80 °C (176 °F). Hyperthermophiles are often within the domain Archaea, although some bacteria are able to tolerate temperatures of around 100 °C (212 °F), as well. Some bacteria can live at temperatures higher than 100 °C at large depths in sea where water does not boil because of high pressure. Many hyperthermophiles are also able to withstand other environmental extremes such as high acidity or high radiation levels. Hyperthermophiles are a subset of extremophiles. It is believed that the cell structure of these type of bacteria contain high level of saturated fatty acid that retain its shape at high temperature.

Fig: Types of bacteria and according to temperature requirement

Source: Science direct.com

6.3.2.Oxygen requirements: Oxygen is used by aerobic bacteria during the process of cellular respiration as a final electron acceptor. For **aerobic** organisms, oxygen is an absolute requirement for their energy-yielding properties. Certain microorganisms grow in oxygen-free environments and are described as **anaerobic.** Organisms such as these produce odoriferous gases in their metabolism, including hydrogen sulfide gas and methane. Certain pathogenic species, such as *Clostridium* species, are anaerobic. Certain species of microorganisms are said to be **facultative.** These species grow in either the presence or absence of oxygen. Some bacteria species are **microaerophilic**, meaning that they grow in low concentrations of oxygen. In some cases, these organisms must have an environment rich in carbon dioxide. Organisms such as these are said to be **capnophilic.** Many ecosystems are still free of molecular oxygen. Some are found in extreme locations, such as deep in the ocean or in earth's crust; others are part of our everyday landscape, such as marshes, bogs, and sewers. Within the bodies of humans and other animals, regions with little or no oxygen provide an anaerobic environment for microorganisms (Figure1). We can easily observe different requirements for molecular oxygen by growing bacteria in **thioglycolate tube cultures**. A test-tube culture starts with autoclaved **thioglycolate medium** containing a low percentage of agar to allow motile bacteria to move throughout the medium. Thioglycolate has strong reducing properties and autoclaving flushes out most of the oxygen. The tubes are inoculated with the bacterial cultures to be tested and incubated at an appropriate temperature. Over time, oxygen slowly diffuses throughout the

thioglycolate tube culture from the top. Bacterial density increases in the area where oxygen concentration is best suited for the growth of that particular organism.

The growth of bacteria with varying oxygen requirements in thioglycolate tubes is illustrated in Figure2. In tube A, all the growth is seen at the top of the tube. The bacteria are **obligate (strict) aerobes** that cannot grow without an abundant supply of oxygen. Tube B looks like the opposite of tube A. Bacteria grow at the bottom of tube B. Those are **obligate anaerobes**, which are killed by oxygen. Tube C shows heavy growth at the top of the tube and growth throughout the tube, a typical result with **facultative anaerobes**. Facultative anaerobes are organisms that thrive in the presence of oxygen but also grow in its absence by relying on fermentation or anaerobic respiration, if there is a suitable electron acceptor other than oxygen and the organism is able to perform anaerobic respiration. The **aerotolerant anaerobes** in tube D are indifferent to the presence of oxygen. They do not use oxygen because they usually have a fermentative metabolism, but they are not harmed by the presence of oxygen as obligate anaerobes are. Tube E on the right shows a "Goldilocks" culture. The oxygen level has to be just right for growth, not too much and not too little. These microaerophiles are bacteria that require a minimum level of oxygen for growth, about 1%–10%, well below the 21% found in the atmosphere.

Examples of obligate aerobes are ***Mycobacterium tuberculosis***, the causative agent of tuberculosis and ***Micrococcus luteus***, a gram-positive bacterium that colonizes the skin. ***Neisseria meningitidis***, the causative agent of severe bacterial meningitis**,** and *N. gonorrheae*, the causative agent of sexually transmitted gonorrhea**,** are also obligate aerobes.

Fig: different types of bacteria according to oxygen requirement (Distribution in

Thio gycolate tubes).Source Sciencedirect.com

6.3.3. pH : The **optimum growth pH** is the most favorable pH for the growth of an organism. The lowest pH value that an organism can tolerate is called the minimum growth pH and the highest pH is the maximum growth pH**.** These values can cover a wide range, which is important for the preservation of food and to microorganisms' survival in the stomach. For example, the optimum growth pH of *Salmonella* spp. is 7.0–7.5, but the minimum growth pH is closer to 4.2.

Most bacteria areneutrophiles**,** meaning they grow optimally at a pH within one or two pH units of the neutral pH of 7 (see Figure2). Most familiar bacteria, like*Escherichia coli*, *Staphylococci,* and*Salmonella*spp. are neutrophiles and do not fare well in the acidic pH of the stomach. However, there are pathogenic strains of*E. coli, S. typhi,* and other species of intestinal pathogens that are much more resistant to stomach acid. In comparison, fungi thrive at slightly acidic pH values of 5.0–6.0.

Microorganisms that grow optimally at pH less than 5.55 are called**acidophiles**. For example, the sulfur-oxidizing ***Sulfolobus*** spp. isolated from sulfur mud fields and hot springs in Yellowstone National Park are extreme acidophiles. These archaea survive at pH values of 2.5–3.5. Species of the archaean genus***Ferroplasma*** live in acid mine drainage at pH values of 0–2.9.***Lactobacillus*** bacteria, which are an important part of the normal microbiota of the vagina, can tolerate acidic environments at pH values 3.5–6.8 and also contribute to the acidity of the vagina (pH of 4, except at the onset of menstruation) through their metabolic production of lactic acid. The vagina's acidity plays an important role in inhibiting other microbes that are less tolerant of acidity. Acidophilic microorganisms display a number of adaptations to survive in strong acidic environments. For example, proteins show increased negative surface charge that stabilizes them at low pH. Pumps actively eject H^+ions out of the cells. The changes in the composition of membrane phospholipids probably reflect the need to maintain membrane fluidity at low pH.

Fig: Different category of Microorganism based on pH

Source: Science Direct. Com

? Neutrophiles grow best around neutral pH (7)

? Acidophiles grow best at pH < 7

? Alkophiles grow best at pH > 7

? Acidotolerant grow best at pH 7 but can also grow at lower pH

? Alkotolerant grow best at pH 7 but can also grow at higher pH.

6.3.4. Water Activity: Liquid water is essential for life.Aqueous solutions actually have different amounts of water available,

depending on how many solutes are dissolved in it. As a very simple model, consider two glasses, one full of pure water, the other containing the same amount of water plus a sponge. Which one would be easier to drink? On a much smaller scale, dissolved solutes act like a sponge, making less water available. Water activity (a_w) can be decreased by the addition of any soluble molecule although salt (NaCl) and sugars are probably the most common.Microbes that requires a high water activity (near or at 1) are termed **nonhalophiles**. (Halophile = salt-loving) Some bacteria require salt to grow and are called **halophiles** . If a very high concentration of salt is required (around saturation), the organisms are termed **extreme halophiles**. A nonhalophile that can grows best with almost no salt but can still grow with low levels of salt (~ 7%) is called **halotolerant**. In general, fungi are more tolerant of low water activity. (That's why your jelly is more likely to get contaminated by fungi than bacteria.)

6.3.5. Nutritional Requirements:

Every organism must find in its environment all of the substances required for energy generation and cellular biosynthesis. The chemicals and elements of this environment that are utilized for bacterial growth are referred to asnutrientsornutritional requirements. Many bacteria can be grown the laboratory inculture mediawhich are designed to provide all the essential nutrients in solution for bacterial growth. Bacteria that are symbionts or obligate intracellular parasites of other cells, usually eucaryotic cells, are (not unexpectedly) difficult to grow outside of their natural host cells. Whether the microbe is a mutualist or parasite, the host cell must ultimately provide the nutritional requirements of its resident.

The Major Elements

At an elementary level, the nutritional requirements of a bacterium such as*E. coli*are revealed by the cell's elemental composition, which consists of C, H, O, N, S. P, K, Mg, Fe, Ca, Mn, and traces of Zn, Co, Cu, and Mo. These elements are found in the form of water, inorganic ions, small molecules, and macromolecules which serve either a structural or functional role in the cells. The general physiological functions of the elements are outlined in Table 1.

6.4. Nutritional diversity :

Different organisms require the same nutrients but may require different forms of the nutrients

Table : Major elements, their sources and functions in bacterial cell.

Element	Percentage dry weight	Sou rces	Function

Carbon	**50**	**organic compounds or CO_2**	Main constituent of cellular Material
Oxygen	**20**	**H_2O, organic compounds, CO_2, and O_2**	Constituent of cell material and cell water; O_2 is electron acceptor in aerobic respiration
Nitrogen	**14**	**NH_3, NO_3, organic compounds, N_2**	Constituent of amino acids, nucleic acids nucleotides, and coenzymes

Hyderogen	**8**	**H_2O, organic compounds, H_2**	Main constituent of organic compounds and cell water
Phosphorus	**3**	**inorganic phosphates (PO_4)**	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
Sulphur	**1**	**SO_4, H_2S, S^o, organic sulfur compounds**	Constituent of cysteine, methionine, glutathione, several coenzymes

Potassium	1	Pot assi um salt s	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	**0.5**	**Ma gne siu m Salt**	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	**0.5**	**Cal ciu m Salt**	Inorganic cellular cation, cofactor for certain enzymes and a component of endospores
Iron	**0.2**	**Iron Salt**	Component of cytochromes and certain nonheme iron-proteins and a cofactor for some enzymatic reactions

6.4.1.Trace Elements

Above Table ignores the occurrence of trace elements in bacterial nutrition. **Trace elements** are metal ions required by certain cells in such small amounts that it is difficult to detect (measure) them, and it is not necessary to add them to culture media as nutrients. Trace elements are required in such small amounts that they are present as "contaminants" of the water or other media components. As metal ions, the trace elements usually act as cofactors for essential enzymatic reactions in the cell. One organism's trace element may be another's required element and vice-versa, but the usual cations that qualify as trace elements in bacterial nutrition are Mn, Co, Zn, Cu, and Mo.

6.5.Carbon and Energy Sources for Bacterial Growth

In order to grow in nature or in the laboratory, a bacterium must have an energy source, a source of carbon and other required nutrients, and a permissive range of physical conditions such as O_2 concentration, temperature, and pH. Sometimes bacteria are referred to as individuals or groups based on their patterns of growth under various chemical (nutritional) or physical conditions. For example, phototrophs are organisms that use light as an energy source; anaerobes are organisms that grow without oxygen; thermophiles are organisms that grow at high temperatures. All living organisms require a source of energy. Organisms that use

radiant energy (light) are called **phototrophs**. Organisms that use (oxidize) an organic form of carbon are called **heterotrophs** or **(chemo)heterotrophs**. Organisms that oxidize inorganic compounds are called **lithotrophs**. The carbon requirements of organisms must be met by organic carbon (a chemical compound with a carbon-hydrogen bond) or by CO_2. Organisms that use organic carbon are **heterotrophs** and organisms that use CO_2 as a sole source of carbon for growth are called **autotrophs**.

Thus, on the basis of carbon and energy sources for growth four major nutritional types of procaryotes may be defined (Table given below).

Table: Nutritional Type of Bacteria

Nutritional Type	Energy source	Carbon Source	Exampe
Photoautotrophs	Light	Co_2	Cyanobacteria, some Purple and Green Bacteria
Photohetrotrophs	Light	Organic compound	Some Purple and Green Bacteria
Chemooutotrophs/Lithotrophs/Lithoautotrophs	Inorganic compounds, e.g. H_2, NH_3, NO_2, H_2S	Co_2	A few Bacteria and many Archaea
Chemoheterotrophs or Heterotrophs	Organic co mpounds	Organic compound	Most Bacteria, some Archaea

Almost all eucaryotes are either photoautotrophic (e.g. plants and algae) or heterotrophic (e.g. animals, protozoa, fungi). Lithotrophy is unique to procaryotes and photoheterotrophy, common in the Purple and Green Bacteria, occurs only in a very few eucaryotic algae. Phototrophy has not been found in the Archaea, except for nonphotosynthetic light-driven ATP synthesis in the extreme halophiles.

6.6. Growth Factors: Some bacteria cannot synthesize some cell constituents**,** These must be added to growth environment referred to as growth factors. Organisms can display wide variety of factor requirements Some need very few while others require many These termed fastidious **organism.** Typical molecules Amino acids Nucleotide bases Enzymatic cofactors or "vitamins"

6.7. Characteristics of Media :

A **growth medium** or **culture medium** is a solid, liquid or semi-solid designed to support the growth of microorganisms or cells, or

small plants like the moss Physcomitrella patens. Different types of media are used for growing different types of cells.

The two major types of growth media are those used for cell culture, which use specific cell types derived from plants or animals, and microbiological culture, which are used for growing microorganisms, such as bacteria or fungi. The most common growth media for microorganisms are nutrient broths and agar plates; specialized media are sometimes required for microorganism and cell culture growth. Some organisms, termed fastidious organisms, require specialized environments due to complex nutritional requirements. Viruses, for example, are obligate intracellular parasites and require a growth medium containing living cells.

6.7.1. Culture media[

Culture media contain all the elements that most bacteria need for growth and are not selective, so they are used for the general cultivation and maintenance of bacteria kept in laboratory culture collections.

An undefined medium (also known as a basal or complex medium) contains:

- a carbon source such as Glucose
- water
- various salts
- a source of amino acids and nitrogen (e.g., beef, yeast extract)
    - This is an undefined medium because the amino-acid source contains a variety of compounds with the exact composition being unknown.

A defined medium (also known as chemically defined medium or synthetic medium) is a medium in which

- all the chemicals used are known
- no yeast, animal, or plant tissue is present

Some examples of nutrient media include:

- Plate count agar
- Nutrient agar
- Trypticase soy agar

6.7.2. Minimal media.

A defined medium that has just enough ingredients to support growth is called a **Minimal Medium.** The number of ingredients that must be added to a minimal medium varies enormously depending on which microorganism is being grown .Minimal media are those that contain the minimum nutrients possible for colony growth, generally without the presence of amino acids, and are often used by microbiologists and geneticists to grow "wild-type" microorganisms. Minimal media can also be used to select for or against recombinants or exconjugants.

Minimal medium typically contains:

- a carbon source, which may be a sugar such as glucose, or a less energy-rich source such as succinate

- various salts, which may vary among bacteria species and growing conditions; these generally provide essential elements such as magnesium, nitrogen, phosphorus, and sulfur to allow the bacteria to synthesize protein and nucleic acids
- water

Supplementary minimal media are minimal media that also contains a single selected agent, usually an amino acid or a sugar. This supplementation allows for the culturing of specific lines of auxotrophic recombinants.

6.7.3.Selective Media

Selective media are used for the growth of only selected microorganisms. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium to prevent other cells, which do not possess the resistance, from growing. Media lacking an amino acid such as proline in conjunction with *E. coli* unable to synthesize it were commonly used by geneticists before the emergence of genomics to map bacterial chromosomes.

Selective growth media are also used in cell culture to ensure the survival or proliferation of cells with certain properties, such as antibiotic resistance or the ability to synthesize a certain metabolite. Normally, the presence of a specific gene or an allele of a gene confers upon the cell the ability to grow in the selective medium. In such cases, the gene is termed a marker.

Selective growth media for eukaryotic cells commonly contain neomycin to select cells that have been successfully transfected with a plasmid carrying the neomycin resistance gene as a marker. Gancyclovir is an exception to the rule, as it is used to specifically kill cells that carry its respective marker, the Herpes simplex virus thymidine kinase (HSV TK).

Examples of selective media include:

- Eosin methylene blue contains dyes that are toxic for Gram-positive bacteria. It is the selective and differential medium for coliforms.
- YM (yeast extract, malt extract agar) has a low pH, deterring bacterial growth.
- MacConkey agar is for Gram-negative bacteria.
- Hektoen enteric agar is selective for Gram-negative bacteria.
- HIS-selective medium is a type cell culture medium that lacks the amino acid histidine.
- Mannitol salt agar is selective for Gram-positive bacteria and differential for mannitol.
- Xylose lysine deoxycholate is selective for Gram-negative bacteria.
- Buffered charcoal yeast extract agar is selective for certain Gram-negative bacteria, especially *Legionella pneumophila*.
- Baird–Parker agar is for Gram-positive staphylococci.
- Sabouraud's agar is selective to certain fungi due to its low pH(5.6) and high glucose concentration(3-4%)

6.7.4.Differential Media:

Differential or indicator media distinguish one microorganism type from another growing on the same medium. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin y, or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism. These media are used for the detection of microorganisms and by molecular biologists to detect recombinant strains of bacteria.

Examples of differential media include:

- Blood agar (used in strep tests) contains bovine heart blood that becomes transparent in the presence of β-hemolytic organisms such as *Streptococcus pyogenes* and *Staphylococcus aureus*.
- Eosin methylene blue is differential for lactose fermentation.
- Granada medium is selective and differential for *Streptococcus agalactiae* (group B streptococcus) which grows as distinctive red colonies in this medium.
- MacConkey agar is differential for lactose fermentation.
- Mannitol salt agar is differential for mannitol fermentation.
- X-gal plates are differential for lac operon mutants.

6.7.5.Transport Media:

Transport media should fulfill these criteria:

- Temporary storage of specimens being transported to the laboratory for cultivation
- Maintain the viability of all organisms in the specimen without altering their concentration
- Contain only buffers and salt
- Lack of carbon, nitrogen, and organic growth factors so as to prevent microbial multiplication
- Transport media used in the isolation of anaerobes must be free of molecular oxygen.

Examples of transport media include:

- Thioglycolate broth is for strict anaerobes.
- Stuart transport medium is a non-nutrient soft agar gel containing a reducing agent to prevent oxidation, and charcoal to neutralize.
- Certain bacterial inhibitors are used for gonococci, and buffered glycerol saline for enteric bacilli.

- Venkataraman Ramakrishna (VR) medium is used for *V. cholerae*

6.7.6.Enriched Media:

- Enriched media contain the nutrients required to support the growth of a wide variety of organisms, including some of the more fastidious ones. They are commonly used to harvest as many different types of microbes as are present in the specimen. Blood agar is an enriched medium in which nutritionally rich whole blood supplements the basic nutrients. Chocolate agar is enriched with heat-treated blood (40–45°C), which turns brown and gives the medium the color for which it is named.

6.8. CONTROL OF MICROBIAL GROWTH BY PHYSICAL METHODS

Mode of Actions of Microbial Control Agents

Two possible antimicrobial effects include:

6.8.1 Alteration of membrane permeability

The susceptibility of the plasma membrane is due to its lipid and protein components.

Certain chemical control agents damage the plasma membrane by altering its permeability.

6.8.2. Damage to proteins and nucleic acids

Some microbial control agents damage cellular proteins by breaking hydrogen bonds and covalent bonds.

Other agents interfere with DNA and RNA replication and protein synthesis.

6.8.2.1.Several factors influence the effectiveness of antimicrobial treatment

6.8.2.1.1.Number of Microbes: The more microbes present, the more time it takes to eliminate population.

6.8.2.1.2.Type of Microbes: Endospores are very difficult to destroy. Vegetative pathogens vary widely in susceptibility to different methods of microbial control.

6.8.2.1.3.Environmental influences: Presence of organic material (blood, feces, saliva) tends to inhibit antimicrobials, pH etc.

6.8.2.1.4.Time of Exposure: Chemical antimicrobials and radiation treatments are more effective at longer times. In heat treatments, longer exposure compensates for lower temperatures.

6.8.2.1.5. Physical Method to control microbial growth.

The control methods can be broadly divided into two categories physical and chemical methods

Physical method can be listed as follows

A.Heat: Moist and Dry Heat

B. Filtration

C. Low Temperature: Refrigeration, Deep freezing, Lyophilization

D. Desiccation

E. Osmotic pressure

F. Radiation: Ionizing and Non-Ionizing

A.Heat: Heat is frequently used to eliminate microorganisms. Moist heat kills microbes by denaturing proteins (enzymes). Dry heat kills organisms by oxidation. For sterilization one must consider the type of heat, and most importantly, the time of application and temperature to ensure destruction of all microorganisms. Endospores of bacteria are considered the most thermoduric of all cells so their destruction guarantees sterility.

Thermal Death Point (TDP) is the lowest temperature at which all the microbes in a liquid culture will be killed in 10 minutes. Thermal Death Time (TDT) is the length of time required to kill all bacterial in a liquid culture at a given temperature. Decimal Reduction Time (DRT) is the length of time required to kill 90% of a bacterial population at a given temperature; D value. Z value is an increase in temperature required to reduce D by 1/10. F value is time in minutes at a specific temperature needed to kill a population of cells or spores.

Moist heat

Moist heat is thought to kill microorganisms by causing denaturation of essential proteins. Death rate is directly proportional to the concentration of microorganisms at any given time. Increasing the temperature decreases TDT, and lowering the temperature increases TDT. Processes conducted at high temperatures for short periods of time are preferred over lower temperatures for longer times.

Environmental conditions also influence TDT. Increased heat causes increased toxicity of metabolic products and toxins. TDT decreases with pronounced acidic or basic pH. However, fats and oils slow heat penetration and increase TDT. It must be remembered that thermal death times are not precise values; they measure the effectiveness and rapidity of a sterilization process. Autoclaving 121°C/15 psi for 15 minutes exceeds the thermal death time for most organisms except some extraordinary spore formers.

Common Examples of methods based on moist heat are:

a).Boiling

It involves heating at 100°C for 30 minutes. This method kills everything except some endospores. To kill endospores, and therefore sterilize a solution, very long (> 6 hours) boiling or intermittent boiling is required.

b)Autoclaving

Autoclaving is the most effective and most efficient means of sterilization. All autoclaves operate on a time/temperature relationship. These two variables are extremely important. Higher temperatures ensure more rapid killing. The usual standard temperature/pressure employed is 121ºC/15 psi for 15 minutes). Longer times are needed for larger loads, large volumes of liquid, and more dense materials. Autoclaving is ideal for sterilizing biohazardous waste, surgical dressings, glassware, many type of microbiologic media, liquids, and many other things. However, certain items, such as plastics and certain medical instruments (e.g. fiber-optic endoscopes), cannot withstand autoclaving and should be sterilized with chemical or gas sterilants. When proper conditions and time are employed, no living organisms will survive a trip through an autoclave. The autoclave is a large pressure cooker; it operates by using steam under pressure as the sterilizing agent .High pressures enable steam to reach high temperatures, thus increasing its heat content and killing power. Most of the heating power of steam comes from its latent heat of vaporization. This is the amount of heat required to convert boiling water to steam. This amount of heat is large compared to that required to make water hot. For example, it takes 80 calories to make 1 liter of water boil, but 540 calories to convert that boiling water to steam. Therefore, steam at 100ºC has almost seven times more heat than boiling water.

C. Pasteurization:

This heat treatment developed by famous microbiologist Louis Pasteur is used to destroy mostly pathogenic bacteria present in liquid medium e.g. milk and wine. Pasteurization is the use of mild heat to reduce the number of microorganisms in a product or food. In the case of pasteurization of milk (Fig. 19.4), the time and temperature depend on killing potential pathogens that are transmitted in milk, i.e. staphylococci, streptococci, Brucella abortus and Mycobacterium tuberculosis. But pasteurization kills many spoilage organisms, as well, and therefore increases the shelf life of milk especially at refrigeration temperatures (2°C).,Milk is usually pasteurized by heating, typically at 63°C for 30 min (batch method) or at 71°C for 15 s (flash method), to kill bacteria and extend the milk's usable life. The process kills pathogens but leaves relatively benign microorganisms that can sour improperly stored milk. Various time-temperature combinations used for pasteurization are given in Table (19.3).

Dryheat

a) Hot air oven

Basically in the cooking oven the rules of relating time and temperature apply, but dry heat is not as effective as moist heat

(i.e. higher temperatures are needed for longer periods of time). For example 160°/2h or 170°/1h is necessary for sterilization. The dry heat oven is used for glassware, metal, and objects that won't melt; used on substances that would be damaged by moist heat sterilization e.g. gauzes, dressings or powders.

b) Incineration

Burns organisms and physically destroys them (Fig. 19.5b). Used for needles, inoculating wires, glassware, etc. and objects not destroyed in the incineration process.

B. Filtration

Filtration is the passage of a liquid or gas through a screen like material with pores small enough to retain microorganisms. A vacuum that is created in the receiving flask aids gravity in pulling the liquid through the filter. Some operating theaters occupied by burn patients receive filtered air. High efficiency particulate air (HEPA) filter remove almost all microorganisms larger than 0.3 µm in diameters. Filtration is especially important for sterilization of solutions which would be denatured by heat (e.g. antibiotics, injectable drugs, amino acids, vitamins, etc.).

C. Low temperature

Low temperature (refrigeration and freezing): Most organisms grow very little or not at all at 0oC. Perishable foods are stored at low temperatures to slow rate of growth and consequent spoilage (e.g. milk). Low temperatures are not bactericidal. Psychrotrophs, rather than true psychrophiles, are the usual cause of food spoilage in refrigerated foods. Although a few microbes will grow in supercooled solutions as low as minus 20°C, most foods are preserved against microbial growth in the household freezer. The effectiveness of low temperatures depends on the particular microorganism and the intensity of the application. Most microorganisms do not reproduce at ordinary refrigerator temperatures (0-7°C); bacteriostatic. Many microbes survive (but do not grow) at the subzero temperatures used to store foods.

D. Freeze drying

Microbes are placed in a suspending medium and frozen quickly at temperatures between -52 and 95°C. Water is removed by vacuum (sublimation) lyophilization. Powder-like product can be reconstituted to bring culture back to viable conditions.

Desiccation

Drying (removal of H2O): Most microorganisms cannot grow at reduced water activity (aw < 0.90). Drying is often used to preserve foods (e.g. fruits, grains, etc.). Methods involve removal of water from product by heat, evaporation, freeze-drying, and addition of salt or sugar.

F.Osmotic Pressure: The use of high concentrations of salts and sugars in foods is used to increase the osmotic pressure and create a hypertonic environment.

Plasmolysis: As water leaves the cell, plasma membrane shrinks away from cell wall. Cell may not die, but usually stops growing. Yeasts and molds: More resistant to high osmotic pressures. Staphylococci spp. that live on skin are fairly resistant to high osmotic pressure.

E. Irradiation

Irradiation (UV, X-ray, Gamma radiation): The effects of radiation depend on its wavelength, intensity, and duration. Ionizing radiation (Gamma rays, X-rays, and high-energy electron beams) has a high degree of penetration and exerts its effect primarily by ionizing water and forming highly reactive hydroxyl radicals. Ultraviolet (UV) radiation, a form of non-ionizing radiation, has a low degree of penetration and causes cell damage by making thymine dimers in DNA that interfere with DNA replication (Fig. 19.8). The most effective germicidal wavelength is 260 nm.

Microwaveradiation

Wavelength ranges from 1 mm to 1 m. Heat is absorbed by water molecules. It may kill vegetative cells in moist foods. Bacterial endospores, which do not contain water, are not damaged by microwave radiation. Solid foods are unevenly penetrated by microwaves.

Gamma radiation and electron beam radiation

These radiations are formed of ionizing radiation used primarily in the health care industry. Gamma rays, emitted from cobalt-60, are similar in many ways to microwaves and x-rays. Gamma rays delivered during sterilization break chemical bonds by interacting with the electrons of atomic constituents. Gamma rays are highly effective in killing microorganisms and do not leave residues or have sufficient energy to impart radioactivity.

Electron beam (e-beam) radiation, a form of ionizing energy, is generally characterized by low penetration and high-dose rates. E-beam irradiation is similar to gamma radiation in that it alters various chemical and molecular bonds on contact. Beams produced for e-beam sterilization are concentrated, highly-charged streams of electrons generated by the acceleration and conversion of electricity. E-beam and Gamma radiation are for sterilization of items ranging from syringes to cardiothoracic devices.

7. CONTROL OF MICROBIAL GROWTH BY CHEMICAL METHODS

Introduction

Chemical agents are the disinfectants that kill microorganisms, but not necessarily their spores, but are not safe for application to living tissues; they are used on inanimate objects such as tables, floors, utensils, etc. e.g. hypochlorites, chlorine

compounds, lye, copper sulfate, quaternary ammonium compounds, formaldehyde and phenolic compounds

Phenol and phenolics

Phenolics exert their action by injuring the lipid-containing plasma membrane which results in leakage of cellular contents. Mycobacteria are susceptible to phenolics due to their rich lipid content e.g. Cresols (O-phenylphenol, main ingredient in Lysol), bisphenols (Hexachlorophene, used in pHisoHex, effective against Gram positive cocci), triclosan (soap, toothpaste, plastics kitchenware; Gram positive and fungi).

Biguanides

Chlorohexedine damages plasma membranes of vegetative cells and is broad spectrum. These are commonly used for surgical hand scrubs. These are effective against most vegetative bacteria and fungi. Mycobateria, endospores, and protozoan cysts are not affected.

Halogens

Some halogens (iodine and chlorine) are used alone or as components of inorganic or organic solutions. Iodine may combine with certain amino acids to inactivate enzymes and other cellular proteins. Iodine is available as a tincture (in solution with alcohol) or as an iodophor (combined with an organic molecule) like in Betadine. The germicidal action of chlorine is based on the formation of hypochlorous acid when chlorine is added to water. It is an excellent oxidizing agent. Chlorine is used as a disinfectant in gaseous form (Cl2) or in the form of a compound, such as calcium hypochlorite, sodium hypochlorite, sodium dichloroisocyanurate, and chloramines.

Alcohol

Alcohols exert their action by denaturing proteins and dissolving lipids. In tinctures, they enhance the effectiveness of other antimicrobial agents. Aqueous ethanol (60-90%) and isopropanol are used as disinfectants. Not effective against spores or non-enveloped viruses.

Heavy metals and their compounds

Silver, mercury, copper, and zinc are used as germicidals. They exert their antimicrobial action through oligodynamic action. When heavy metal ions combine with sulfhydryl (-SH) groups, proteins are denatured. Examples are 1% Silver nitrate solution, mercuric chloride, copper sulfate (algicide).

Surface-active agents-soaps and acid anionic detergents

The agents decrease the surface tension among molecules of a liquid; soaps and detergents are examples. Soaps have limited germicidal action but assist in the removal of microorganisms through scrubbing. Acid-anionic detergents are used to clean

dairy equipment.

Quaternary Ammonium Compounds: These are cationic detergents attached to NH4+. By disrupting the plasma membranes, they allow cytoplasmic constituents to leak out of the cell Quats are most effective against Gram-positive bacteria. They do not kill endospores or mycobacteria. Examples include Zephiran (benzalkonium chloride) and Cepacol (cetylpyridinium chloride). Pseudomonads are highly resistant, can even live in quats.

Organic acids and derivatives

This class of organic compounds is commonly used as food preservatives. These are effective mostly against mold as they interfere with mold metabolism or the integrity of the plasma membrane.

Nitrates

It can be found in some cheeses, adds flavor, maintains pink color in cured meats and prevents botulism in canned foods. Can cause adverse reactions in children, and potentially carcinogenic.

Sulfur dioxide and sulfites

These are used as preservatives and to prevent browning in alcoholic beverages, fruit juices, soft drinks, dried fruits and vegetables. Sulfites prevent yeast growth and also retard bacterial growth in wine. Sulfites may cause asthma and hyperactivity. They also destroy vitamins.

Benzoic acid and sodium benzoate

These are used to preserve oyster sauce, fish sauce, ketchup, non-alcoholic beverages, fruit juices, margarine, salads, confections, baked goods, cheeses, jams and pickled products. They have also been found to cause hyperactivity.

Propionic acid and propionates: These are used in bread, chocolate products, and cheese for lasting freshness.

Sorbic acid and sorbates: It prevents mold formation in cheese and flour confectioneries

Aldehydes

Aldehydes such as formaldehyde and glutaraldehyde (Gidex) exert their antimicrobial effect by inactivating proteins. They are among the most effective chemical disinfectants.

Gaseous chemosterilants

This class of chemosterilants includes chemicals that sterilize in a closed chamber. Chemicals used for sterilization include the gases ethylene oxide and formaldehyde, and liquids such as glutaraldehyde. Ozone, hydrogen peroxide and peracetic acid are also examples of chemical sterilization techniques are based on oxidative capabilities of the chemical.

Ethylene oxide (ETO)

It is the most commonly used form of chemical sterilization. Due to its low boiling point of 10.4°C at atmospheric pressure,

ETO behaves as a gas at room temperature. ETO chemically reacts with amino acids, proteins, and DNA to prevent microbial reproduction. The sterilization process is carried out in a specialized gas chamber. After sterilization, products are transferred to an aeration cell, where they remain until the gas disperses and the product is safe to handle. ETO is used for cellulose and plastics irradiation, usually in hermetically sealed packages. Ethylene oxide can be used with a wide range of plastics (e.g. petri dishes, pipettes, syringes, medical devices, etc.) and other materials without affecting their integrity).

Ozone sterilization has been recently approved for use in the U.S. It uses oxygen that is subjected to an intense electrical field that separates oxygen molecules into atomic oxygen, which then combines with other oxygen molecules to form ozone. Ozone is used as a disinfectant for water and food. It is used in both gas and liquid forms as an antimicrobial agent in the treatment, storage and processing of foods, including meat, poultry and eggs. Many municipalities use ozone technology to purify their water and sewage.

Low temperature gas plasma (LTGP)

It is used as an alternative to ethylene oxide. It uses a small amount of liquid hydrogen peroxide (H_2O_2), which is energized with radio frequency waves into gas plasma. This leads to the generation of free radicals and other chemical species, which destroy organisms.

The End

Chapter-8

BACTERIAL GENETICS

Bacterial genetics: It is the field of genetics to the study the bacterial genetic system and reproduction. As we know the Prokaryotic Bacterial genetics are different from eukaryotic genetics. Bacteria still serve as a good model for animal genetic

studies. One of the major difference between bacterial and eukaryotic genetics system is that the bacteria's lack membrane-boundorganelles. This necessitating protein synthesis occur in thecytoplasm for all prokaryotic system. Like any other organisms, bacteria also breed true and maintain their characteristics from generation to generation; They also exhibit variations among their progeny although in small quantity. Heritability and variations in bacteria had been noticed from the early days of bacteriology,

ut it was not realised then that bacteria too obey the laws of genetics. At that time the existence of a bacterial nucleus was a subject of controversy. The differences in morphology and other properties .i.e. bacterial pleomorphism were attributed by Nageli in 1877, was which postulated the existence of a single, a few species of bacteria, which possessed a protein capacity for a variation. With the development and application of precise methods of pure culture, it became apparent that different types of bacteria retained constant form and function through successive generations. This led to the concept of monomorphism.

To know the bacterial genetics, following Structrurs need to be understand

7.1 **DNA STRUCTURE**

Deoxyribonucleic acid (DNA)

Deoxyribonucleic acid (DNA) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms with the exception of some viruses. The main role of DNA molecules is the long-term storage of information. DNA is often compared to a se of blueprints, like a recipe or a code, since it contains the instructions needed to construct other components of cells, such as proteins and RNA molecules. The DNA segments that carry this genetic information are called genes, but other DNA sequences have structural purposes, or are involved in regulating the use of this genetic information. Within cells, DNA is organized into long structures called chromosomes. These chromosomes are duplicated before cells divide, in a process called DNA replication. Eukaryotic organisms (animals, plants, fungi, and protists) store most of their DNA inside the cell nucleus and some of their DNA in organelles, such as mitochondria or chloroplasts. In contrast, prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm.The information in DNA is made up of four bases which combine to form chains. These bases include two purines (Adenine and Guanine) and two pyrimidines (Cytosine and Thymine). These are commonly referred to as A, G, C and T respectively

7.2. Chargaff's Rules

These state that DNA from any cell of all organisms should have a 1:1 ratio of pyrimidine and purine bases and, more specifically, that the amount of guanine is equal tocytosine and the amount of adenine is equal to thymine. This Pattern is found in both strands of the DNA. They were discovered by Austrian chemist Erwin Chargaff.

DNA bases pair up with each other, A with T and C with G, to form units called base pairs. Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix. The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder's rungs and the sugar and phosphate molecules forming the vertical sidepieces of the ladder.

7.3.Structure

In 1953 Watson and Crick postulated a three dimensional model of DNA structure. It consists of two helical DNA chains

wound around the same axis to form a right handed double helix. The hydrophilic backbones of alternating deoxyribose and phosphate groups are on the outside of the double helix, facing the surrounding water. The purine and pyrimidine bases of both strands are stacked inside the double helix, with their hydrophobic and nearly planar ring structures very close together and perpendicular to the long axis. The offset pairing of the two strands creates a major groove and minor groove on the surface of the duplex. Each nucleotide base of one strand is paired in the same plane with a base of the other strand. Watson and Crick found that the hydrogen-bonded base pairs, G with C and A with T, are those that fit best within the structure, providing a rationale for Chargaff's rule. It is important to note that three hydrogen bonds can form between G and C, but only two can form between A and T. This is one reason for the finding that separation of paired DNA strands is more difficult the higher the ratio of GC to AT base pairs. Other pairings of bases tend (to varying degrees) to destabilize the double-helical structure. When Watson and Crick constructed their model, they had to decide at the outset whether the strands of DNA should be parallel or antiparallel whether their 5'-3' phosphodiester bonds should run in the same or opposite directions. An antiparallel orientation produced the most convincing model, and later work with DNA polymerases provided experimental evidence that the strands are indeed antiparallel, a finding ultimately confirmed by x-ray analysis

Structure of DNA Watson and Crick

The sugars in the backbone

The backbone of DNA is based on a repeated pattern of a sugar group and a phosphate group. The full name of DNA,

deoxyribonucleic acid, gives you the name of the sugar present - deoxyribose. Deoxyribose is a modified form of another sugar called ribose. Ribose is the sugar in the backbone of RNA, ribonucleic acid

Ribose

This diagram misses out the carbon atoms in the ring for clarity. Each of the four corners where there isn't an atom shown has a carbon atom. Deoxyribose, as the name might suggest, is ribose which has lost an oxygen atom – 'de-oxy'

Deoxyribose

The only other thing you need to know about deoxyribose (or ribose, for that matter) is how the carbon atoms in the ring are numbered. The carbon atom to the right of the oxygen as we have drawn the ring is given the number 1, and then you work around to the carbon on the CH2OH side group which is number 5

Fig. Deoxyribose with carbon numbering

You will notice that each of the numbers has a small dash by it - 3' or 5', for example. If you just had ribose or deoxyribose on its own, that wouldn't be necessary, but in DNA and RNA these sugars are attached to other ring compounds. The carbons in the sugars are given the little dashes so that they can be distinguished from any numbers given to atoms in the other rings. You read 3' or 5' as '3-prime' or '5-prime'.

Attaching a phosphate group

The other repeating part of the DNA backbone is a phosphate group. A phosphate group is attached to the sugar molecule in place of the -OH group on the 5' carbon

Attaching a base and making a nucleotide

The final piece that we need to add to this structure before we can build a DNA strand is one of four complicated organic bases. In DNA, these bases are cytosine (C), thymine (T), adenine (A) and guanine (G).These bases attach in place of the -OH group on the 1' carbon atom in the sugar ring .The nitrogen and hydrogen atoms shown in blue on each molecule show that where these molecules join on to the deoxyribose. In each case, the hydrogen is lost together with the -OH group on the 1' carbon atom of the sugar. This is a condensation reaction - two molecules joining together with the loss of a small one (not

necessarily water).

Joining the nucleotides into a DNA strand.

A DNA strand is simply a string of nucleotides joined together. The phosphate group on one nucleotide links to the 3' carbon atom on the sugar of another one. In the process, a molecule of water is lost - another condensation reaction.

DNA chain

If the top of this segment was the end of the chain, then the phosphate group would have an -OH group attached to the spare bond rather than another sugar ring. Similarly, if the bottom of this segment of chain was the end, then the spare bond at the bottom would also be to an -OH group on the deoxyribose ring.

Joining the two DNA chains together

If you look at the diagram carefully, you will see that an adenine on one chain is always paired with a thymine on the second chain. And a guanine on one chain is always paired with a cytosine on the other one. The first thing to notice is that a smaller base is always paired with a bigger one. The effect of this is to keep the two chains at a fixed distance from each other all the way along But, more than this, the pairing has to be exactly:

Adenine (A) pairs with thymine (T);

Guanine (G) pairs with cytosine (C).

That is because these particular pairs fit exactly to form very effective hydrogen bonds with each other. It is these hydrogen bonds which hold the two chains together

The base pairs fit together as follows.

The A-T base pair:

Fig : DNA pairing

The bond between Aand T is double bond and Cand T is triple bond.These type of bond have special features for the bacterial genetics that will be dealt in another chapter.

The End

Chapter -9

DNA REPLICATION, TRANSCRIPTION, TRANSLATION

Introduction

It is the process that can duplicate the DNA of a cell. The next step is the cell to duplicate. Every cell (of eukaryotes or prokaryotes) has one or more DNA (or RNA) polymer molecules that need to duplicate in order the cell duplication to take place. This is what DNA replication or DNA synthesis succeeds. In the eukaryotes (organisms with cell that have nucleus) the DNA is formed in two strands, each composed of units called nucleotides. The two strands look like two chains that form the DNA double helix. The DNA replication process is capable of opening the double helix and separating the two strands. Then the two strands are copied. As a result two new DNA molecules are created. The next step is the cell division. After that a daughter cell is created. In its nucleus lies a copy of the parental DNA.

Fig: Double helical model of DNA

DNA Replication Models

The process of DNA Replication was hiding many secrets. One of the most important was how the two daughter strands are created. In order the hereditary phenomenon to be explained, these strands should be accurately copied and transmitted from the parental cell to the daughter ones.

These are three possible models that describe the accurate creation of the daughter chains.

A. Semiconservative replication

According to this model, DNA Replication would create two molecules. Each of them would be a complex of an old (parental and a daughter strand).

B. Conservative replication

According to this model, the DNA Replication process would create a brand new DNA double helix made of two daughter strands while the parental chains would stay together.

C. Dispersive replication

According to this model the replication process would create two DNA double-chains, each ofthem with parts of both parent and daughter molecules.

Steps in DNA Replication

1) The first major step in DNA Replication to take place is the breaking of hydrogen bonds between bases of the two anti-parallel strands. The un-wounding of the two strands is the starting point. The splitting happens in places of the chains which are rich in A-T. That is because there are only two bonds between Adenine and Thymine (there are three hydrogen bonds between Cytosine and Guanine). Helicase is the enzyme that splits the two strands. The initiation point where the splitting starts is called 'origin of replication'.The structure that is created is known as '**Replication Fork.**

Fig: Replication fork

2) One of the most important steps of DNA Replication is the binding of RNA Primase in the the initiation point of the 3'-5' parent chain. RNA Primase can attract RNA nucleotides which bind to the DNA nucleotides of the 3'-5' strand due to the hydrogen bonds between the bases. RNA nucleotides are the primers (starters) for the binding of DNA nucleotides.

3) **The elongation process is different for the 5'-3' and 3'-5' template.**

a) 5'-3' Template: The 3'-5' proceeding daughter strand -that uses a 5'-3' template- is called leading strand because DNA Polymerase A can read the template and continuously adds nucleotides (complementary to the nucleotides of the template, for example Adenine opposite to Thymine etc.)

b) 3'-5'Template: The 3'-5' template cannot be read by DNA Polymerase. The replication of this template is complicated and the new strand is called lagging strand. In the lagging strand the RNA primase adds more RNA primers. DNA polymerase A reads the template and lengthens the bursts. The gap between two RNA primers is called 'Okazaki Fragments'. The RNA primers are necessary for DNA polymerase A to bind nucleotides to the 3' end of them. The daughter strand is elongated with the binding of more DNA nucleotides.

4) In the lagging strand the DNA Pol I - exonuclease reads the fragments and removes the RNA primers. The gaps are closed with the action of DNA Polymerase (adds complementary nucleotides to the gaps) and DNA Ligase (adds phosphate in the remaining gaps of the phosphate - sugar backbone).Each new double helix is consisted of one old and one new chain.

This is what we call semi conservative replication

5) The last step of DNA replication is the termination. This process happens when the DNA Polymerase reaches to an end of the strands We can easily understand that in the last section of the lagging strand, when the RNA primer is removed, it is not possible for the DNA Polymerase to seal the gap (because there is no primer). So, the end of the parental strand where the last primer binds isn't replicated.

6) The DNA Replication is not completed before a mechanism of repair fixes possible errors caused during the replication. Enzymes like nucleases remove the wrong nucleotides and the DNA polymerase fills the gaps.

Fig: DNA replication

Source: Wilkepedia

8.1 Transcription

Transcription is the mechanism by which a template strand of DNA is utilized by specific RNA polymerases to generate one of the three different classifications of RNA.

These 3 RNA classes are:

Messenger RNAs (mRNAs): This class of RNAs is the genetic coding templates used by the translational machinery to determine the order of amino acids incorporated into an elongating polypeptide in the process of translation.

Fig :Transcription

Transfer RNAs (tRNAs): This class of small RNAs form covalent attachments to individual amino acids and recognize the encoded sequences of the mRNAs to allow correct insertion of amino acids into the elongating polypeptide chain.

Ribosomal RNAs (rRNAs): This class of RNAs is assembled, together with numerous ribosomal proteins, to form the ribosomes. Ribosomes engage the mRNAs and form a catalytic domain into which the tRNAs enter with their attached amino acids. The proteins of the ribosomes catalyze all of the functions of polypeptide synthesis.

All RNA polymerases are dependent upon a DNA template in order to synthesize RNA. The resultant RNA is, therefore, complimentary to the template strand of the DNA duplex and identical to the non-template strand. The non-template strand is called the coding strand because its' sequences are identical to those of the mRNA. However, in RNA, U is substituted for T.

8.2 Translation

Translation is the RNA directed synthesis of polypeptides. This process requires all three classes of RNA. Although the chemistry of peptide bond formation is relatively simple, the processes leading to the ability to form a peptide bond are exceedingly complex. The template for correct addition of individual amino acids is the mRNA, yet both tRNAs and rRNAs are involved in the process. The tRNAs carry activated amino acids into the ribosome which is composed of rRNA and ribosomal proteins. The ribosome is associated with the mRNA ensuring correct access of activated tRNAs and containing the necessary enzymatic activities to catalyze peptide bond formation.

The genetic code

Shown below are the triplets that are used for each of the 20 amino acids found in eukaryotic proteins. The row on the left side indicates the first nucleotide of each triplet and the row across the top represents the second nucleotide. The wobble position nucleotides are indicatedbelow. The three stop codons are highlighted in yellow

Fig: Genetic code: Wobble at third position

Order of events in translation

The ability to begin to identify the roles of the various ribosomal proteins in the processes of ribosome assembly and translation was aided by the discovery that the ribosomal subunits will self assemble in vitro from their constituent parts

Fig: Elongation of Chain on Ribosome

Following assembly of both the small and large subunits onto the mRNA, and given the presence of charged tRNAs, protein synthesis can take place. To reiterate the process of protein synthesis:

Fig: Synthesis of Polypeptide chain

Synthesis proceeds from the N-terminus to the C-terminus of the protein.

The ribosomes read the mRNA in the 5' to 3' direction.

Active translation occurs on polyribosomes (also termed polysomes). This means that more than one ribosome can be bound to and translate a given mRNA at any one time.

Chain elongation occurs by sequential addition of amino acids to the C-terminal end of the ribosome bound polypeptide.

Translation proceeds in an ordered process. First accurate and efficient initiation occurs, then chain elongation, and finally accurate and efficient **termination** must occur. All three of these processes require specific proteins, some of which are ribosome associated and some of which are separate from the ribosome, but may be temporarily associated with it.
Initiation

Fig: Termination of Polypeptide Chain

Source: Wilkipedia

First the 70s ribosome must separate into 50s and 30s subunits.The 3 initiation factors IF-1, IF-2, and IF-3 then bind to the 30S ribosomal subunit.Then the specific initiator tRNA carrying the N-formylmethionine and the mRNA join the complex (remember base pairing via Shine-Dalgarno sequence). The initiator tRNA is specifically recognized by IF-2. At this point in the process we have what is termed a 30s preinitiation complex. The 50S subunit binds, triggering the release of the IFs.Thus, formation of the 70S initiation complex is completed and it is ready for the next phase, elongation

Fig: Overall process of Transcription and Translation.

Overall Mechanism of Translation: Ribosome moves along the mRNA molecule to the third codon, releasing the first tRNA molecule, as only two tRNA molecules can be brought together by a single ribosome at one time. The next complementary tRNA with the correct anticodon complementary to the third codon is selected for, delivering the next amino acid to the ribosome which is covalently joined to the growing polypeptide chain. This process continues with the ribosome moving along the mRNA molecule adding up to 15 amino acids per second to the polypeptide chain. Behind the first ribosome, up to 50 additional ribosomes can bind to the mRNA molecule and synthesise an identical polypeptide - this enables simultaneous synthesis of multiple identical polypeptide chains. Termination of the growing polypeptide chain occurs when the ribosome encounters a stop codon (UAA, UAG, or UGA) in the mRNA molecule. When this happens, no tRNA can recognise it and arelease factorinduces the release of the polypeptide chain.

Protein Folding: The primary structure of protein get folded into secondary and teririary and the finally quaternary protein structure that made then active protein model which can be seen as below:

Fig Protein Folding

Source: Wilkepedia.org.in

Once synthesis of the polypeptide chain iscompleted by the ribosome, the polypeptide chain released and folds to adopt a specific structure which enables the protein to carry out its functions. The basic form ofprotein structureis known as theprimary structure, which is simply the polypeptide chain i.e. a sequence of covalently bonded amino acids. The primary structure of a protein is encoded by a gene. Therefore, any changes to the sequence of the gene can alter the primary structure of the protein and all subsequent levels of protein structure ultimately changing the overall structure and function.(Wilkepedia.org.in)

The primary structure of a protein (the polypeptide chain) can then fold or coil to form the secondary structure of the protein. The most common types of secondary structure are known as an alpha helix or beta sheet, these are small structures produced by hydrogen bonds forming within the polypeptide chain. This secondary structure then folds to produce the tertiary structure of the protein, this is its overall 3D structure which is made of different secondary structures folding together. In the tertiary structure, key protein features e.g. the active site, are folded and formed enabling the protein to function. Finally, whilst most proteins are made of a single polypeptide chain, however, some proteins are composed of multiple polypeptide chains (known as subunits) which fold and interact to form the quaternary structure.

Protein Synthesis in Disease

Many diseases are caused by mutations in a gene due to the direct connection between the genetic code of the gene and the amino acid sequence of the protein it encodes. Changes to the primary structure can result in the protein mis-folding and unable to function. Single mutations in genes have been identified as a cause of multiple diseases including sickle cell disease and cystic fibrosis.

Sickle cell disease:

Sickle cell disease is a group of diseases, the most dangerous is known as sickle cell anaemia. Sickle cell anaemia is the most common homozygous recessive single gene disorder, meaning the individual must carry the mutation in both copies of the gene to suffer from the disease. The mutation occurs in the haemoglobin B gene. Haemoglobin is an essential component of red blood cells and is composed of four subunits - two A subunits and two B subunits.[13]. Patients suffering from sickle cell anaemia have a missense mutation in the haemoglobin B subunit gene. A missense mutation means the nucleotide mutation alters the overall codon triplet such that a different amino acid is paired with the new codon. In the case of sickle cell anaemia, there is a single nucleotide mutation from thymine to adenine in the haemoglobin B subunit genewhich changes codon 6 from glutamic acid to valine

The change in the primary structure of the haemoglobin B subunit alters the functionality of the haemoglobin multi-subunit complex in low oxygen conditions.

Cystic fibrosis:

Post-translational Modifications

Completion of protein folding into the mature, functional 3D state is not necessarily the end of

- Cleavage
- Addition of chemical groups
- Addition of complex molecules
- Addition of peptide

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THE END

Chapter-10

BACTERIAL RECOMBINATION

Introduction : Bacteria can exchange or transfer DNA between other bacteria in three different ways. In every case the source cells of the DNA are called the DONORS and the cells that receive the DNA are called the RECIPIENTS.

I n each case the donor DNA is incorporated into the recipients cell's DNA by recombination exchange (Fig. 1). If the exchange involves an allele of the recipient's gene, the recipient's genome and phenotype will have changed. The three forms of bacterial DNA exchange are (1) TRANSFORMATION, (2) CONJUGATION and (3) TRANSDUCTION

Fig: General scheme of bacterial exchange of DNA. DNA from a donor cell is transferred to a recipient cell where it undergoes recombinational exchange, replacing one or more of the recipient's genes with those from the donor.

PLASMIDS

- Plasmid are MINI-CHROMOSOMES. Plasmids are composed of DNA which usually exists as a CIRCULAR MOLECULE, only much SMALLER than the genomic DNA.
- Plasmids vary in size, but most are between 1,000 to 25,000 base pairs vs. 4,000,000 bp in the genome. Plasmids REPLICATE AUTONOMOUSLY from the genomic chromosome.
- Often there are many plasmid copies present in one cell. Further, a cell may contain several different plasmids or it may contain NO PLASMIDS at all. Plasmids generally carry genes that are not essential for a cell's survival except under special circumstances. For example, many plasmids carry genes for antibiotic resistance

When these plasmids are present in a cell, it is unaffected by the appropriate antibiotic, but if the plasmid and its antibiotic resistant gene is lost, the host cell becomes sensitive to a given antibiotic.

Some plasmids carry resistance genes to several antibiotics, making them very dangerous pathogens. In other cases plasmids,

called VIRULENCE-PLASMIDS, carry VIRULENCE GENES that enhance a host's ability to cause a disease.

That is, a bacterium carrying a plasmid containing the *virulence gene* is able to CAUSE A DISEASE, but when the plasmid is missing that same bacterium is unable to produce that disease. One such plasmid-based disease of recent concern is the strain of *E. coli* O157:H7 that produces a severe food-borne disease.

Other plasmids carry genes for protecting a cell against deleterious substances like mercury, copper or they may carry genes that make it possible for a cell to metabolize an unusual substrate, such as gasoline, as a nutrient or energy source.

The question naturally arises as to the purpose of these plasmids in the evolutionary scheme. The current explanation is that plasmids constitute an extra pool of gene alleles and thus enlarge the effective gene pool of the population. Remember that the genome of prokaryotes carries only enough information for between 1,000 to 5,000 genes. But, as we've already learned, the more variety the better a species' chances of survival are in a fickle universe.

The phenomenon of Antibiotic resistance is a case in point. Antibiotics, being natural products of certain organisms, are never-the-less unlikely to be encountered very often in quantities that endanger susceptible sensitive strains, so there is no need to carry resistance genes against the hundreds of antibiotics that lurk in the nooks 'n crannies of the environment. Indeed, to do so would likely tie up all your genes just for this one purpose; clearly not a survival plus.

Plasmids in a bacterial host cell. A cell may contain no plasmids, one plasmid or many copies of a plasmid. A single host may contain a number of different plasmids (green, blue & pink

- However, random mutation has produced antibiotic resistance genes that clearly can prove useful under the right circumstances, but how do they remain available, without tying up huge quantities of limited resources? The answer is

PLASMIDS, of course

- A RARE PLASMID, randomly carrying a RARE ANTIBIOTIC RESISTANT GENE to, for example, penicillin, happens to be in a patient suffering from an infection which is treated by a shot in the *you-bloody-well-know-where*.
- All the *resistant bacteria's* mates, lacking the resistance plasmid, are quickly killed, but the lucky bacterium with its penicillin-resistant-plasmid survives and reproduces while swimming in a sea of penicillin. Naturally, all the subsequent daughter cells carry the resistance plasmid, because if they didn't they'd die very quickly. This is a classical example of SURVIVAL OF THE FITTEST & of evolution in action.

Fig: Selection of antibiotic resistant bacteria

Selection of Antibiotic-Resistant Mutants. If an antibiotic-sensitive bacterium is grown in a culture, occasionally a random mutation occurs that renders a bacterium resistant to a given antibiotic. To detect the presence of such a mutation one plates the culture on a medium containing a lethal dose of the antibiotic in question. Any cells that grow on the left plate must be resistant (red colonies) to the antibiotic. All the cells (green & red) will grow on a medium lacking the antibiotic

TRANSFORMATION

- The discovery of transformation was previous. Since its initial discovery transformation has been shown to occur throughout the bacterial world and it has become the most commonly used artificial way of moving genes from one bacterium to another. The basic procedure involves:

1. Breaking open the donor cells and removing DNA from them so as to obtain a CELL-FREE, usually purified, form of DNA (NAKED DNA).

Fig: Isolation of CELL-FREE or NAKED DNA.

The cells are broken and the DNA released. The cell-free DNA is subsequently isolated and collected.

2. Mixing of Donor DNA with Recipient Competent Cells. The naked donor DNA is incubated with the competent recipient cells to which it binds

3.The recipient cells take the donor DNA into their cytoplasm where it may EXCHANGE into the recipient's DNA or if it is a plasmid, it will replicate.

1. Uptake and Recombination of Donor DNA. Donor DNA binds to competent recipient cells, following which it enters the recipient cells. Portions of the donor DNA align, at random, with genes on the recipient DNA and segments of the two DNA's are exchanged. The exchange inserts Donor genes into the recipient cell"s DNA. 5.Transformation is used to move DNA between bacteria, plants and animals. In each case the methods used to get the DNA into the recipient cells are slightly different. In bacteria COMPETENCY is an empirical matter; that is it can not be predicted what conditions will produce competency in a given strain of bacteria. However, the following treatment often induces competency in G- bacteria:

6. Young cells are incubated with a CALCIUM CHLORIDE SOLUTION for approximately 30 min on ice. In some cases magnesium is also present.The cells are concentrated and suspended as a thick suspension in the calcium solution. The cells may be mixed with reagents like glycerol and stored at -80°C for later use or they may be used immediately. Cell-free DNA is then mixed with these competent cells on ice for approximately 30 min followed by a brief mild heating.

8. The transformed cells are incubated in a rich medium for approximately 1 to 1.5 hr. and then plated on medium containing materials that will detect the presence of the transformed genes. A variety of other transformation techniques are used for eukaryotic cells. These include mixing certain salts with DNA. These salts bind the DNA and the salt-DNA-complex is then taken into the eukaryotic cells where the DNA is subsequently incorporated into the recipient cell's DNA. Plant cells are often covered with a thick cell wall that is difficult to penetrate.

9. To get DNA into these cells tiny metal beads coated with the donor DNA are "shot" into the cytoplasm of the recipient cells using a "gas gun". A strong jolt of electricity is also used to drive the DNA into recipient cells. Because of the similar chemical nature of DNA, DNA from any living form can, in theory, function in any other life form. Animals or plants that have been transformed with DNA from other species are called TRANSGENIC organisms.

10.For example, we have transgenic pigs and cows containing functional "human genes". Transgenic plants containing "bacterial genes" that make a protein toxic to certain insect pathogens are currently growing around the world.

Griffith's experiment:

Griffith's experiment, conducted in 1928 by Frederick Griffith, was one of the first experiments suggesting that bacteria are capable of transferring genetic information through a process known as transformation. Griffith used two strains of *Pneumococcus* (which infects mice), a type III-S (smooth) and type II-R (rough) strain. The III-S strain covers itself with a polysaccharide capsule that protects it from the host's immune system, resulting in the death of the host, while the II-R strain doesn't have that protective capsule and is defeated by the host's immune system. A German bacteriologist, Fred Neufeld, had discovered the three pneumococcal types (Types I, II, and III) and discovered the Quellung reaction to identify them in vitro Until Griffith's experiment, bacteriologists believed that the types were fixed and unchangeable, from one generation to another.

Fig: Griffith Experiment

Source:Wilkipedia

In this experiment, bacteria from the III-S strain were killed by heat, and their remains were added to II-R strain bacteria. While neither alone harmed the mice, the combination was able to kill its host. Griffith was also able to isolate both live II-R and live III-S strains of *pneumococcus* from the blood of these dead mice. Griffith concluded that the type II-R had been "transformed" into the lethal III-S strain by a "transforming principle" that was somehow part of the dead III-S strain bacteria.Today, we know that the "transforming principle" Griffith observed was the DNA of the III-S strain bacteria. While the bacteria had been killed, the DNA had survived the heating process and was taken up by the II-R strain bacteria.The III-S strain DNA contains the genes that form the protective polysaccharide capsule. Equipped with this gene, the former II-R strain bacteria were now protected from the host's immune system and could kill the host. The exact nature of the transforming principle (DNA) was verified in the experiments done by Avery, McLeod and McCarty and by Hershey and Chase.

CONJUGATION:

The discovery of conjugation, the ability of bacterial cells to transfer dna between cells that are in physical contact, as a form of dna exchange between bacteria in the 1950s stunned scientists and lay people alike.Its obvious anthropomorphic similarly to mammalian gene exchange amused some and shocked others. Since its discovery, conjugational exchange of DNA has been shown to be more common and promiscuous than first thought possible. Initially, conjugation was thought to occur only between the SAME or CLOSELY RELATED SPECIES, but data has accumulated which shows that conjugation between bacteria crosses prokaryotic species lines and even occurs between bacteria and some eukaryotic cells. How pervasive this latter situation is remains to be determined

The basic conditions for conjugation are:

1.Donor cells carry a unique plasmid that contain a set of genes that make conjugation possible. These PLASMIDS are called FERTILITY or SEX PLASMIDS. Cells that contain the fertility plasmids are called F+ or MALE cells, whereas cells, lacking the sex plasmids are said to be FEMALE or F-.

2. The sex plasmid genes are responsible for the synthesis of special pili called sex pili. Sex pili are thin long, hollow protein tubes that have "sticky" RECEPTORS on their ends that bind firmly to molecules (ligands) on recipient cell walls.

3. Following the ATTACHMENT of the two cells by the pili, they become united through a "CONJUGATION BRIDGE". The nature of this union is unclear as is the exact role the sex pili plays in its formation. But what is clear is that a GATE IS OPENED between the donor and recipient cells through which DNA can pass.

4. A special enzyme cuts one strand of the donor's dna at a unique site and a newly synthesized stand of dna passes through the conjugation bridge into the recipient cell.

5.This newly synthesized strand of donor DNA is converted to a double stranded form

which is able to exchange into the host's DNA by recombination.

- The most common form of conjugation involves the transfer of plasmids from one cell to another. this process is very efficient, as the recipient cells that receive the f-plasmids, themselves become f+ (they literally change sex) and quickly begin to mate with f- cells. certain f+ plasmids are able to fuse with the genomic dna of the host cell and upon mating the entire donor's genome can be transferred into a recipient cell. under these conditions any of the donor's genes can end up exchanged into the recipient's genome.

- HFR strain hfr cell (also called hfr strain) is a bacterium with a conjugative plasmid (often the F-factor) integrated into its Genomic DNA.

- Hfr is the abbreviation for *high frequency recombination*, which was first characterized by Luca Cavalli-Sforza. Unlike a normal F+ cell, hfr strains will, upon conjugation with a F- cell, attempt to transfer their *entire* DNA through the mating bridge, not to be confused with the pilus.

- This occurs because the F factor has integrated itself via an insertion point in the bacterial chromosome. Due to the F factor's inherent nature to transfer itself during conjugation, the rest of the bacterial genome is dragged along with it, thus making such cells very useful and interesting in terms of studying gene linkage and recombination.

- Because the genome's rate of transfer through the pilus is constant, molecular biologists and geneticists can use Hfr strain of bacteria (often *E. coli*) to study genetic linkage and map the chromosome. The procedure commonly used for this is called intrupted mating . A bacterium may undergo conjugation.

- During this process, genetic material is transferred to another bacterium through the mating bridge. Pili are often incorrectly believed to transfer DNA, but these structures are simply used to bring mating bacteria close enough to form a mating bridge. To form pili, an F plasmid is required.

- The F plasmid consists of 28 genes which are mostly required for the production of the pilus. F+ denotes cells that contain the F plasmid, while F- cells do not. The F plasmid is considered to be an episome which may become integrated into the main chromosome.

- When the F genes become integrated into the chromosome, the cell is said to be Hfr (high frequency of recombination). An Hfr cell may transfer F genes to an F- cell. During this transfer of genetic material, the F episome may take chromosomal DNA with it.

The donor cell does not lose any genetic material as anything transferred is also replicated concurrently. It is extremely rare that an Hfr cell's chromosome is transferred in its entirety. Homologous recombination occurs when the newly acquired DNA crosses over with the homologous region of its own chromosome.

- A structure as fragile as a mating bridge will, however, likely break, and so the transfer is rarely complete. Thus, the F

- cell uses only part of the genomic DNA of the Hfr cell for recombination. Though there is some debate on the issue, the pili themselves are not the structures through which the actual exchange of DNA takes place; rather, a Type IV secretion system is used to transfer DNA between the bacteria.

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Fig: Mecanism of gene transfer

Source: text book of Microbiology(Pleczar)

Transfer of a functional gene for tryptophan biosynthesis from a wild-type bacterium (genotype described as *trp+*) to a recipient that lacks a functional copy of this gene (*trp-*). The recipient is called a tryptophan auxotroph (the word used to describe a mutant bacterium that can survive only if provided with a nutrient - in this case, tryptophan - not required by the wild type.After transfer, two crossovers (shown as green crosses) are needed to integrate the transferred gene into the recipient cell's chromosome, converting the recipient from *trp-* to *trp+*. (B) During conjugation, DNA is transferred from donor to recipient in the same way that a string is pulled through a tube. The relative positions of markers on the DNA molecule can therefore be mapped by determining the times at which the markers appear in the recipient cell. In the example shown, markers *A*, *B* and *C* are transferred 8, 20 and 30 minutes after the beginning of conjugation, respectively. The entire *Escherichia coli* chromosome takes approximately 100 minutes to transfer. (C) To be co-transferred during transduction and transformation, two or more markers must be closely linked, because these processes usually result in less than 50 kb of DNA being passed from donor to recipient.

- Transduction and transformation mapping are used to determine the relative positions of markers that are too close together to be mapped precisely by conjugation analysis.

SUPERBACTERIA

- The conjugative transfer of antibiotic resistant plasmids between bacteria is a major problem facing the medical profession today (that means you and me folks!). In the case of some important pathogens we currently have only **ONE EFFECTIVE ANTIBIOTIC** (Vancomycin) remaining to use against them and resistant strains against that one are being reported around the world.
- That is, these **SUPERBACTERIA** contain plasmids carrying resistant genes to **ALL** of the other available antibiotics.
- Since people infected with pathogens are CONCENTRATED in hospitals and since antibiotics of all kinds are extensively used to treat these infections, the chances are high that antibiotic resistant plasmids will be selected and that they will be passed on to other bacteria. Further, the odds that a bacterium receiving a resistance plasmid will already have other antibiotic resistant plasmids will be statistically high.
- Other activities like the feeding of antibiotics to farm animals to increase their weight gain, or the rinsing of chickens in antibiotics to increase their shelf-life in stores contribute to the selection and subsequent spread of antibiotic resistant plasmids. The increase of resistant bacteria over time

TRANSDUCTION

The third way of transporting DNA between organisms involves the mediation of viruses. As you will learn when viruses are covered, bacteria also have viruses that attack them. Bacterial viruses are called BACTERIOPHAGE or just plain PHAGE by most microbiologists. Two scientists, F. Twort and F. d'Herrelle discovered bacteriophage independently in 1915 & 1917. The term "phage" means "eater". Phage show themselves in two ways. On agar plates holes lacking any bacteria can be seen on a bacterial "lawn'.These holes, called PLAQUES, vary in size from 1 to 3 mm in diameter, depending on the phage and the growth conditions, But under standard conditions the PLAQUE SIZE is a DEFINING CHARACTERISTIC of each phage.

Evidence of Tranduction

- The ability of phage to carry bacterial DNA between bacteria was discovered in 1952.
- Briefly scientists, who were studying conjugation found that if they separated two genetically unique bacteria by a membrane that PREVENTED CONTACT between them they could still detect GENE TRANSFER between them. As this transfer was *not prevented by DNase*, it was not Transformation.
- They reasoned that the DNA must be protected from the DNase by something. This "something" turned out to a bacteriophage infecting one of the strains being used in the experiment. Phage are composed of genetic material surrounded by a protein cover that does indeed protect them from DNase. This process of phage-mediated DNA transfer is known as TRANSDUCTION.In liquid cultures the addition of phage often causes the bacteria to

DISAPPEAR or "CLEAR" within a few minutes and appear as if it was a sterile, tube of medium.

- The killing of bacteria suggested to many that they might be used to treat bacterial infections, however this turned out to be ineffective because of the immune response of the body to the phage--more about this in the section on IMMUNITY.
- However, during the early attempts to use phage therapeutically, it was discovered that they are VERY SPECIFIC as to the bacterial hosts they will attack and kill. In fact they are so specific, that phage are used to IDENTIFY strains of bacteria that cause infections so that the source of the infection can be accurately traced.
- **Mechanism of Transduction**
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Fig: Mechanism of transduction

- A phage infects a susceptible bacterium and injects its DNA into the host.
- The phage DNA proceeds to TAKE OVER the host's entire metabolism, turning it into a PHAGE-FACTORY.
- It then directs the synthesis of PHAGE PARTS or COMPONENTS, including phage DNA.
- As a final step in the phage life-cycle, the VARIOUS COMPONENT PARTS in the cytoplasm are ASSEMBLED into COMPLETE PHAGE and the cell is broken open or LYSED, releasing the newly made phage particles.
- During the assembly process, occasional RARE MISTAKES are made and bits of the host's bacterial DNA become incorporated INTO THE NEW PHAGE.
- Since there is NO ROOM FOR PHAGE DNA in these situations DEFECTIVE PHAGE are made.
- However, these defective phage still have the ability to subsequently bind and INSERT their DNA into a host bacterial cell. But, since these defective phage contain only bacterial DNA, an infection DOES NOT OCCUR.

- Recombination however can take place between the previous host's DNA and the new host's DNA and any alleles carried into the host by the defective phage can become incorporated into the host's DNA, thereby Changing its genotype
- To detect this process the new hosts are grown on medium that will somehow DEMONSTRATE the presence of a particular allele from the previous host.
- For example, if transducing phage are first grown on a bacterial host that carries a gene Conferring resistance to the antibiotic penicillin, a few rare defective phage will **I**ncorporate the penicillin resistance gene. If the phage from this culture are subsequently used to infect a Penicillin-sensitive host and the survivors are plated on medium containing Penicillin, only those rare bacteria which have both received the penicillin-resistant gene from a defective phage and exchanged it into their genome, will produce colonies on the medium.

Chapter-11

INTRODUCTION TO FUNGI AND CLASSIFICATION

The word fungus comes from the Latin word for mushrooms. Indeed, the familiar mushroom is a reproductive structure used by

many types of fungi. However, there are also many fungi species that don't produce mushrooms at all. Being eukaryotes, a typical fungal cell contains a true nucleus and many membrane-bound organelles. The kingdom Fungi includes an enormous variety of living organisms collectively referred to as Ascomycota, or true Fungi. While scientists have identified about 100,000 species of fungi, this is only a fraction of the 1.5 million species of fungus probably present on earth. Edible mushrooms, yeasts, black mold, and the producer of the antibiotic penicillin,*Penicilliumnotatum*, are all members of the kingdom Fungi, which belongs to the domain Eukarya.

Key Point anout Fungi:

1. Some aquatic, some terrestrial and some are air borne. Many are plant parasite, animal of human parasite.
2. Plant body branched, and filamentous hyphal form,form a net like structure called as Myceilium.Hypha are aseptate (coenocytic) or septate or separate in uni,bi,or multinucleate.
3. Septate with simple or dolipore septum in Basidiomycetes.
4. Cell wall chitinious or cellulosic,also contain glucan or manan,
5. Devoid of chlorophyll but caretionod may present**.**
6. Heterotrophic, some are saprophyte or parasite or symbiont.

Cell Organisation

The cell wall of fungi is mainly made up of chitin and cellulose. Chitin is a polymer of N-acetyl glucosamine. On the other hand, cellulose is nothing but a polymer of d-glucose. Besides, the cell wall may be made up of cellulose-glycogen, cellulose-chitin or polygalactosamine-galactan.

Nutrition

The fungi are achlorophyllous organisms. Hence, they cannot prepare their food. They live as heterotrophs*i.e.*, as parasites and saprophytes. Some forms live symbiotically with other green forms.

- **Parasites:** They usually obtain their food from a living host. A parasite could be facultative or obligate. The obligate parasites survive and settle on a living host throughout their life. The facultative parasites are saprophytes that have turned parasitic.
- **Saprophytes:** These organisms procure their nutrition from dead and decaying organic matter. The saprophytes are either obligate or facultative. An obligate saprophyte remains saprophytic during its entire lifetime. While a facultative saprophyte is nothing but a parasite that has secondarily become saprophytic.

- **Symbionts:** Some fungi develop in symbiotic association with the green or blue-green algae. These constitute the lichen. Here the algal component is photosynthetic. While the fungal component plays the reproductive part.

Reproduction

The fungi either reproduces vegetatively, asexually or sexually:

- **Vegetative Reproduction**
  - **Fragmentation:** Some forms belonging to Ascomycotina and Basidiomycotina multiply by breakage of the mycelium.
  - **Budding:** Some unicelled forms multiply by budding. A bud arises as a papilla on the parent cell and then after its enlargement separates into a completely independent entity.
  - **Fission:** A few unicelled forms like yeasts and slime moulds multiply by this process.
- **Asexual Reproduction**
  - **Sporangiospores:** These are thin-walled, non-motile spores formed in a sporangium. They may be uni-or multinucleate. On account of their structure, they are also called as aplanospores.
  - **Zoospores:** They are thin-walled, motile spores formed in a zoosporangium.
  - **Conidia:** In some fungi, the spores are not formed inside a sporangium. They are born freely on the tips of special branches called conidiophores. Thus, these spores are conidia.
- **Sexual reproduction:** With the exception of Deuteromycotina (Fungi imperfecti), we find sexual reproduction in all groups of fungi. During sexual reproduction, the compatible nuclei show a specific behaviour which is responsible for the onset of three distinct mycelial phases. The three phases of nuclear behaviour are as under:
  - **Plasmogamy:** Fusion of two protoplasts.
  - **Karyogamy:** Fusion of two nuclei.

Meiosis: The reduction division.

Classification of Fungi

Phycomycetes

We can find these in aquatic habitats and on decaying wood in moist and damp places. The mycelium is aseptate and coenocytic. Asexual reproduction takes place by zoospores (motile) or by aplanospores (non-motile).

Rhizopus/Mucor

They are the cosmopolitan and saprophytic fungus, living on the dead organic matter.Rhizopus stolonifera occurs very frequently on moist bread. Hence, they are black bread mould.

Albugo

Albugo is a member of Phycomycetes.It is an obligate parasite and grows in the intercellular spaces of host tissues. It is parasitic mainly on the members of families Cruciferae, Compositae, Amaranthaceae and Convolvulaceae. The disease caused by this fungus iswhite rust or white blisters. The most common and well-known species is Albugo candida. It attacks the embers of the mustard family (Cruciferae).

Ascomycetes

They are saprophytic, decomposers, parasitic or coprophilous (growing on dung). Some examples are Aspergillus, Claviceps and Neurospora. Neurospora is used extensively in biochemical and genetic work.

Yeast

Antony Von Leeuwenhoek in 1680 first described yeast.Yeast is nonmycelial or unicellular, which is very small and either spherical or oval in shape. Individual cells are colourless but the colonies may appear white, red, brown, creamy or yellow. Yeast reproduces by vegetative or asexual and sexual methods.

Basidiomycetes

The most common forms of basidiomycetes are puffballs, mushrooms and bracket fungi. They grow in soil, on logs and tree stumps and in living plant bodies as parasites, e.g., rusts and smuts. They havebranched and septate mycelium. These organisms do not have sex organs. But, plasmogamy takes place by fusion of two vegetative or somatic cells of different strains or genotypes.

Deuteromycetes.

They are imperfect fungi because we only know about the asexual or vegetative phases of these fungi. The Deuteromycetes reproduce only by asexual spores. These spores are conidia. Some members are saprophytes or parasites. However, a large number of them are decomposers of litter. They are very helpful in mineral cycling.Examples**:**Alternaria, Colletotrichum and Trichoderma.

Fungi are classified according to their structure and method of reproduction.

The four main groups of fungi are:

1. Common molds (Zygomycota)
2. Sac fungi (Ascomycota)
3. Club fungi (Basidiomycota)
4. Imperfect fungi (Deuteromycota

Subkingdom Amastigomycota

- Terrestrial inhabitants including those of medical importance:

1. **Zygomycota** – zygospores; sporangiospores and some conidia
2. **Ascomycota** – ascospores; conidia
3. **Basidiomycota** – basidiospores; conidia
4. **Deuteromycota** – majority are yeasts and molds; no sexual spores known; conidia

The Common Molds

Familiar molds that grow on meat, cheese, and bread are members of the phylum **Zygomycota**.

Zygomycetes have life cycles that include a **zygospore**.

- A **zygospore** is a resting spore that contains zygotes ***formed during the sexual phase*** of the mold's life cycle.

Black bread mold, *Rhizopus stolonifer*, is a zygomycete.

Black bread mold has two types of hyphae:

- **Rhizoids** are rootlike hyphae that penetrate the bread's surface.
- **Stolons** are stemlike hyphae that run along the surface of the bread.

Life Cycle of a Black Bread Mold

Haploid (N) gametes produced in the gametangia fuse with gametes of the opposite mating type to form diploid (2N) zygotes.

- Zygotes develop into thick-walled zygospores.

Asexual Reproduction

The Sac Fungi :

The phylum Ascomycota is named for the **ascus**, a reproductive structure that contains spores.

The life cycle of an ascomycete usually includes both asexual and sexual reproduction.

Life Cycle of Sac Fungi

In asexual reproduction, spores called **conidia** form at tips of ***conidiophores***.

- **Conidiophores** are specialized hyphae.

During sexual reproduction, haploid hyphae of two different mating types (+ and -) grow close together.

The N + N hyphae then produce a fruiting body in which sexual reproduction continues.

The **Ascus** forms within the fruiting body.

- Within the ascus, two nuclei of different mating types fuse to form a diploid zygote (2N).

The zygote divides by meiosis, producing four haploid cells.

- In most ascomycetes, meiosis is followed by mitosis, so that eight cells called **ascospores** are produced.
- An ascospore can germinate and grow into a haploid mycelium.

Yeasts

- Yeasts are unicellular fungi.
- Yeasts reproduce asexually by budding.
- Dry granules of yeast contain ascospores, which become active in a moist environment.

Life cycle of club fungi: eg.Agaricus (Mushroom) :

- Sexual spores is known as basidiospores
- Formed on a club shaped basidium

Diploid nuclei formed four basidiospores that germinate into individual plant body

Importance of Fungi:

Fungi are one of the most important groups of organisms on the planet. This is easy to overlook, given their largely hidden, unseen actions and growth. They are important in an enormous variety of way

1.Recycling

Fungi, together with bacteria, are responsible for most of the recycling which returns dead material to the soil in a form in which it can be reused. Without fungi, these recycling activities would be seriously reduced. We would effectively be lost under piles many metres thick, of dead plant and animal remains.

2.Mycorrhizae and plant growth

Fungi are vitally important for the good growth of most plants, including crops, through the development of mycorrhizal associations. As plants are at the base of most food chains, if their growth was limited, all animal life, including human, would be seriously reduced through starvation.

3.Food

Fungi are also important directly as food for humans. Many mushrooms are edible and different species are cultivated for sale worldwide. While this is a very small proportion of the actual food that we eat, fungi are also widely used in the production of many foods and drinks. These include cheeses, beer and wine, bread, some cakes, and some soya beanproducts.While a great many wild fungi are edible, it can be difficult to correctly identify them. Some mushrooms are deadly if they are eaten. Fungi with names such as 'Destroying Angel' and 'Death Cap' give us some indication that it would not be a terribly good idea to eat them! In some countries, collecting wild mushrooms to eat is a popular activity. It is always wise to be totally sure that what you have collected is edible and not a poisonous look-a-like.

4.Medicines

Penicillin, perhaps the most famous of all antibiotic drugs, is derived from a common fungus called *Penicillium.* Many other fungi also produce antibiotic substances, which are now widely used to control diseases in human and animal populations. The discovery of antibiotics revolutionized health care worldwide.

Some fungi which parasitise caterpillars have also been traditionally used as medicines. The Chinese have used a particular caterpillar fungus as a tonic for hundreds of years. Certain chemical compounds isolated from the fungus may prove to be useful treatments for certain types of cancer.A fungus which parasitises .Rye crops causes a disease known as Ergot. The fungus can occur on a variety of grasses. It produces small hard structures, known as sclerotia. These sclerotia can cause poisoning in humans and animals which have eaten infected material. However, these same sclerotia are also the source of a powerful and important drug which has uses in childbirth.

5.CropDiseases

Fungal parasites may be useful in biocontrol, but they can also have enormous negative consequences for crop production. Some fungi are parasites of plants. Most of our common crop plants are susceptible to fungal attack of one kind or another. Spore production and dispersal is enormously efficient in fungi and plants of the same species crowded together in fields are ripe for attack. Fungal diseases can on occasion result in the loss of entire crops if they are not treated with antifungal agents.

BioControl agent :Fungi such as the Chinese caterpillar fungus, which parasitise insects, can be extremely useful for controlling insect pests of crops.The spores of the fungi are sprayed on the crop pests. Fungi have been used to control Colorado potato beetles, which can devastate potato crops. Spittlebugs, leaf hoppers and citrus rust mites are some of the other insect pests which have been controlled using fungi. This method is generally cheaper and less damaging to the environment than using chemical pesticides.

6.AnimalDisease

Fungi can also parasitise domestic animals causing diseases, but this is not usually a major economic problem. A wide range of fungi also live on and in humans, but most coexist harmlessly. Athletes foot and Candida infections are examples of human fungal infections.

7.Food Spoilage: Fungihas been seen as a method of food spoilage, causing only an undesirable appearance to food, however, there has been significant evidence of various fungi being a cause of death of many people spanning across hundreds of years in many places through the world. Fungi are caused by acidifying, fermenting, discoloring and disintegrating processes and can create fuzz, powder and slimes of many different colors, including black, white, red, brown and green. Moldis a type of fungus, but the two terms are not reciprocal of each other; they have their own defining features and perform their own tasks.Very well known types of mold are Aspergillus and Penicillium, and, like regular fungi, create a fuzz, powder and slime of various colors. Yeastis also a type of fungus that grows vegetatively via single cells that either bud or divide by way of fission, allowing for yeast to multiply in liquid environments favoring the dissemination of single celled microorganisms. Yeast forms mainly in liquid environments and anaerobic conditions, but being single celled, it oftentimes cannot spread on or into solid surfaces where other fungus flourish. Yeast also produces at a slower rate than bacteria, therefore being at a disadvantage in environments where bacteria are. Yeasts can be responsible for the decomposition of food with a high sugar content. The same effect is useful in the production of various types of food and beverages, such as bread, yogurt, cider, and alcoholic beverages.

THE END

Chapter -12

STRUCTURE OF VIRUS AND CLASSIFICATION

A **virus** is a small infectious agent that replicates only inside the living cells of an organism. Viruses can infect all types of life forms, from animals and plants to microorganisms, including bacteria and archaea.

Since Dmitri Ivanovsky's 1892 article describing a non-bacterial pathogen infecting tobacco plants, and the discovery of the tobacco mosaic virus by Martinus Beijerinck in 1898, about 5,000 virus species have been described in detail, although there are millions of types. Viruses are found in almost every ecosystem on Earth and are the most numerous type of biological entity. The study of viruses is known as virology, a sub-speciality of microbiology.

While not inside an infected cell or in the process of infecting a cell, viruses exist in the form of independent particles, or *virions*, consisting of: (i) the genetic material, i.e. long molecules of DNA or RNA that encode the structure of the proteins by which the virus acts; (ii) a protein coat, the *capsid*, which surrounds and protects the genetic material; and in some cases (iii) an outside envelope of lipids. The shapes of these virus particles range from simple helical and icosahedral forms for some species to more complex structures for others. Most virus species have virions too small to be seen with an optical microscope, about one hundredth the size of most bacteria.

History

Louis Pasteur was unable to find a causative agent for rabies and speculated about a pathogen too small to be detected by microscopes.In 1884, the French microbiologist Charles Chamberland invented the Chamberland filter (or Pasteur-Chamberland filter) with pores small enough to remove all bacteria from a solution passed through it.In 1892, the Russian biologist Dmitri Ivanovsky used this filter to study what is now known as the tobacco mosaic virus: crushed leaf extracts from infected tobacco plants remained infectious even after filtration to remove bacteria. Ivanovsky suggested the infection might be caused by a toxin produced by bacteria, but did not pursue the idea. At the time it was thought that all infectious agents could be retained by filters and grown on a nutrient medium—this was part of the germ theory of disease.In 1898, the Dutch microbiologist Martinus Beijerinck repeated the experiments and became convinced that the filtered solution contained a new form of infectious agent.[24] He observed that the agent multiplied only in cells that were dividing, but as his experiments did not show that it was made of particles, he called it a *contagium vivum fluidum* (soluble living germ) and re-introduced the word *virus*. Beijerinck maintained that viruses were liquid in nature, a theory later discredited by Wendell Stanley, who proved they were particulate.In the same year Friedrich Loeffler and Paul Frosch passed the first animal virus through a similar filter: aphthovirus, the agent of foot-and-mouth disease.

In the early 20th century, the English bacteriologist Frederick Twort discovered a group of viruses that infect bacteria, now called bacteriophages (or commonly *phages*), and the French-Canadian microbiologist Félix d'Herelle described viruses that, when added to bacteria on an agar plate, would produce areas of dead bacteria. He accurately diluted a suspension of these viruses and discovered that the highest dilutions (lowest virus concentrations), rather than killing all the bacteria, formed discrete areas of dead organisms. Counting these areas and multiplying by the dilution factor allowed him to calculate the number of viruses in the original suspension.Phages were heralded as a potential treatment for diseases such as typhoid and cholera, but their promise was forgotten with the development of penicillin. The development of bacterial resistance to antibiotics has renewed interest in the therapeutic use of bacteriophages.

Virus structure

Viruses display a wide diversity of shapes and sizes, called *morphologies*. In general, viruses are much smaller than bacteria. Most viruses that have been studied have a diameter between 20 and 300 nanometres. Some filoviruses have a total length of up to 1400nm; their diameters are only about 80nm.Most viruses cannot be seen with an optical microscope, so scanning and transmission electron microscopes are used to visualise them.To increase the contrast between viruses and the background,

electron-dense "stains" are used. These are solutions of salts of heavy metals, such as tungsten, that scatter the electrons from regions covered with the stain. When virions are coated with stain (positive staining), fine detail is obscured. Negative staining overcomes this problem by staining the background only.

A complete virus particle, known as a virion, consists of nucleic acid surrounded by a protective coat of protein called a capsid. These are formed from identical protein subunits called capsomeres. Viruses can have a lipid "envelope" derived from the host cell membrane. The capsid is made from proteins encoded by the viral genome and its shape serves as the basis for morphological distinction. Virally-coded protein subunits will self-assemble to form a capsid, in general requiring the presence of the virus genome. Complex viruses code for proteins that assist in the construction of their capsid. Proteins associated with nucleic acid are known as nucleoproteins, and the association of viral capsid proteins with viral nucleic acid is called a nucleocapsid. The capsid and entire virus structure can be mechanically (physically) probed through atomic force microscopy. In general, there are four main morphological virus types:

Helical

These viruses are composed of a single type of capsomere stacked around a central axis to form a helical structure, which may have a central cavity, or tube. This arrangement results in rod-shaped or filamentous virions which can be short and highly rigid, or long and very flexible. The genetic material (typically single-stranded RNA, but ssDNA in some cases) is bound into the protein helix by interactions between the negatively charged nucleic acid and positive charges on the protein. Overall, the length of a helical capsid is related to the length of the nucleic acid contained within it, and the diameter is dependent on the size and arrangement of capsomeres. The well-studied tobacco mosaic virus is an example of a helical virus.

Icosahedral

Most animal viruses are icosahedral or near-spherical with chiral icosahedral symmetry. A regular icosahedron is the optimum way of forming a closed shell from identical sub-units. The minimum number of identical capsomeres required for each triangular face is 3, which gives 60 for the icosahedron. Many viruses, such as rotavirus, have more than 60 capsomers and appear spherical but they retain this symmetry. To achieve this, the capsomeres at the apices are surrounded by five other capsomeres and are called pentons. Capsomeres on the triangular faces are surrounded by six others and are called hexons .Hexons are in essence flat and pentons, which form the 12 vertices, are curved. The same protein may act as the subunit of both the pentamers and hexamers or they may be composed of different proteins.

Prolate

This is an icosahedron elongated along the fivefold axis and is a common arrangement of the heads of bacteriophages. This structure is composed of a cylinder with a cap at either end.

Envelope

Some species of virus envelop themselves in a modified form of one of the cell membranes, either the outer membrane surrounding an infected host cell or internal membranes such as nuclear membrane or endoplasmic reticulum, thus gaining an outer lipid bilayer known as a viral envelope. This membrane is studded with proteins coded for by the viral genome and host

genome; the lipid membrane itself and any carbohydrates present originate entirely from the host. The influenza virus and HIV use this strategy. Most enveloped viruses are dependent on the envelope for their infectivity.

Complex

These viruses possess a capsid that is neither purely helical nor purely icosahedral, and that may possess extra structures such as protein tails or a complex outer wall. Some bacteriophages, such as Enterobacteria phage T4, have a complex structure consisting of an icosahedral head bound to a helical tail, which may have a hexagonal base plate with protruding protein tail fibres. This tail structure acts like a molecular syringe, attaching to the bacterial host and then injecting the viral genome into the cell. The poxviruses are large, complex viruses that have an unusual morphology. The viral genome is associated with proteins within a central disc structure known as a nucleoid. The nucleoid is surrounded by a membrane and two lateral bodies of unknown function. The virus has an outer envelope with a thick layer of protein studded over its surface. The whole virion is slightly pleiomorphic, ranging from ovoid to brick-shaped.

Giant viruses

Mimivirus is one of the largest characterised viruses, with a capsid diameter of 400nm. Protein filaments measuring 100nm project from the surface. The capsid appears hexagonal under an electron microscope, therefore the capsid is probably icosahedral. In 2011, researchers discovered the largest then known virus in samples of water collected from the ocean floor off the coast of Las Cruces, Chile. Provisionally named *Megavirus chilensis*, it can be seen with a basic optical microscope. In 2013, the Pandoravirus genus was discovered in Chile and Australia, and has genomes about twice as large as Megavirus and Mimivirus. All giant viruses have dsDNA genomes and they are classified into several families: *Mimiviridae, Pithoviridae, Pandoraviridae, Phycodnaviridae,* and the Mollivirus genus.

Some viruses that infect Archaea have complex structures that are unrelated to any other form of virus, with a wide variety of unusual shapes, ranging from spindle-shaped structures, to viruses that resemble hooked rods, teardrops or even bottles. Other archaeal viruses resemble the tailed bacteriophages, and can have multiple tail structures.

An enormous variety of genomic structures can be seen among viral species; as a group, they contain more structural genomic diversity than plants, animals, archaea, or bacteria. There are millions of different types of viruses, although only about 5,000 types have been described in detail. As of September 2015, the NCBI Virus genome database has more than 75,000 complete genome sequences, but there are doubtlessly many more to be discovered.

Fig: Some structure of Virus

Genomic Structure :

A virus has either a DNA or an RNA genome and is called a DNA virus or an RNA virus, respectively. The vast majority of viruses have RNA genomes. Plant viruses tend to have single-stranded RNA genomes and bacteriophages tend to have double-stranded DNA genomes.

Viral genomes are *circular*, as in the polyomaviruses, or *linear*, as in the adenoviruses. The type of nucleic acid is irrelevant to the shape of the genome. Among RNA viruses and certain DNA viruses, the genome is often divided up into separate parts, in which case it is called segmented. For RNA viruses, each segment often codes for only one protein and they are usually found together in one capsid. All segments are not required to be in the same virion for the virus to be infectious, as demonstrated by brome mosaic virus and several other plant viruses. A viral genome, irrespective of nucleic acid type, is almost always either *single-stranded* or *double-stranded*. Single-stranded genomes consist of an unpaired nucleic acid, analogous to one-half of a ladder split down the middle. Double-stranded genomes consist of two complementary paired nucleic acids, analogous to a ladder. The virus particles of some virus families, such as those belonging to the *Hepadnaviridae*, contain a genome that is partially double-stranded and partially single-stranded.

For most viruses with RNA genomes and some with single-stranded DNA genomes, the single strands are said to be either positive-sense (called the *plus-strand*) or negative-sense (called the *minus-strand*), depending on if they are complementary to the

viral messenger RNA (mRNA). Positive-sense viral RNA is in the same sense as viral mRNA and thus at least a part of it can be immediately translated by the host cell. Negative-sense viral RNA is complementary to mRNA and thus must be converted to positive-sense RNA by an RNA-dependent RNA polymerase before translation. DNA nomenclature for viruses with single-sense genomic ssDNA is similar to RNA nomenclature, in that positive-strand viral ssDNA is identical in sequence to the viral mRNA and is thus a coding strand, while negative-strand viral ssDNA is complementary to the viral mRNA and is thus a template strand. Several types of ssDNA and ssRNA viruses have genomes that are ambisense in that transcription can occur off both strands in a double-stranded replicative intermediate. Examples include geminiviruses, which are ssDNA plant viruses and arenaviruses, which are ssRNA viruses of animals.

Genome size

Genome size varies greatly between species. The smallest viral genomes—the ssDNA circoviruses, family *Circoviridae*—code for only two proteins and have a genome size of only two kilobases;the largest—the pandoraviruses—have genome sizes of around two megabases which code for about 2500 proteins. Virus genes rarely have introns and often are arranged in the genome so that they overlap.

Table: Genetic Diversity among Viruses:

Property	Parameter
Nucleic Acid	• DNA • RNA Both DNA and RNA (at different stages in the life cycle)
Size	• Linear • Circular • Segmented
Strand of DNA	• Single-stranded • Double-stranded • Double-stranded with regions of single-strand
Sense	• Positive sense (+) • Negative sense (-) Ambisense (+/-)

In general, RNA viruses have smaller genome sizes than DNA viruses because of a higher error-rate when replicating, and have a maximum upper size limit.Beyond this limit, errors in the genome when replicating render the virus useless or uncompetitive. To compensate for this, RNA viruses often have segmented genomes—the genome is split into smaller molecules—thus reducing the chance that an error in a single-component genome will incapacitate the entire genome. In contrast, DNA viruses generally have larger genomes because of the high fidelity of their replication enzymes.Single-strand DNA viruses are an exception to this rule, as mutation rates for these genomes can approach the extreme of the ssRNA virus case.

Life Cycle of Virus:

The life cycle of viruses differs greatly between species but there are six *basic* stages in the life cycle of viruses:

Attachment: It is a specific binding between viral capsid proteins and specific receptors on the host cellular surface. This specificity determines the host range of a virus. For example, HIV infects a limited range of human leucocytes. This is because its surface protein, gp120, specifically interacts with the CD4 molecule—a chemokine receptor—which is most commonly found on the surface of CD4+T-Cells. This mechanism has evolved to favour those viruses that infect only cells in which they are capable of replication. Attachment to the receptor can induce the viral envelope protein to undergo changes that result in the fusion of viral and cellular membranes, or changes of non-enveloped virus surface proteins that allow the virus to enter.

Penetration follows attachment: Virions enter the host cell through receptor-mediated endocytosis or membrane fusion. This is often called viral entry. The infection of plant and fungal cells is different from that of animal cells. Plants have a rigid cell wall made of cellulose, and fungi one of chitin, so most viruses can get inside these cells only after trauma to the cell wall.Nearly all plant viruses (such as tobacco mosaic virus) can also move directly from cell to cell, in the form of single-stranded nucleoprotein complexes, through pores called plasmodesmata.Bacteria, like plants, have strong cell walls that a virus must breach to infect the cell. Given that bacterial cell walls are much thinner than plant cell walls due to their much smaller size, some viruses have evolved mechanisms that inject their genome into the bacterial cell across the cell wall, while the viral capsid remains outside.

Uncoating: it is a process in which the viral capsid is removed: This may be by degradation by viral enzymes or host enzymes or by simple dissociation; the end-result is the releasing of the viral genomic nucleic acid.

Replication: Replication of viruses involves primarily multiplication of the genome. Replication involves synthesis of viral messenger RNA (mRNA) from "early" genes (with exceptions for positive sense RNA viruses), viral protein synthesis, possible assembly of viral proteins, then viral genome replication mediated by early or regulatory protein expression. This may be followed, for complex viruses with larger genomes, by one or more further rounds of mRNA synthesis: "late" gene expression is, in general, of structural or virion proteins.

Assembly – Following the structure-mediated self-*assembly* of the virus particles, some modification of the proteins often occurs. In viruses such as HIV, this modification (sometimes called maturation) occurs *after* the virus has been released from the

host cell.

Release – Viruses can be *released* from the host cell by lysis, a process that kills the cell by bursting its membrane and cell wall if present: This is a feature of many bacterial and some animal viruses. Some viruses undergo a lysogenic cycle where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosome. The viral genome is then known as a "provirus" or, in the case of bacteriophages a "prophage". Whenever the host divides, the viral genome is also replicated. The viral genome is mostly silent within the host. At some point, the provirus or prophage may give rise to active virus, which may lyse the host cells. Enveloped viruses (e.g., HIV) typically are released from the host cell by budding. During this process the virus acquires its envelope, which is a modified piece of the host's plasma or other, internal membrane.

Variations in the reproductive cycles of viruses

Many var: 'dons are present in the basic mechanism of reproductive cycle of animal and plant viruses:

(a) Animals viruses

1. Viruses without envelop: Its example is phage virus. Phage virus attack on bacteria E. coli. It has simple mechanisr of penetration and replication. But it has two cycles of infection'

(a) Lytic cycle: In this case, bacteria burst arid release a large number of phage.

(b) Lysogenic cycle: In this case, bacteria do not burst. Viral DNA incorporates in the bacterial DNA as **prophage.** It remains their in inactive form and replicates with the replication of bacterial DNA. Sometimes, it comes out during process of **induction** and start lytic cycle.

2. Viruses with envelopes: Several viruses have external envelopes. These envelopes are derived from the host cells. The envelops have **glycoprotein spikes.** These viruses attach on their host receptors with the help of these spikes. Viral envelope fuse with plasma membrane of host and capsid and viral genome enters into the host cells. After that replication start. New viruses develop new nucleic acids, capsids and spike. They stimulate the

Figure: Lysogenic and Lytic Cycle of Virus

Image from http://www.accessexcellence.org/AB/GG/retrovirus.html.

Retroviruses

Retroviruses use RNA instead of DNA as their nucleic acid core. They also contain the enzyme reverse transcriptase, which will detranscribe the RNA sequence into a DNA strand.. Once the retroviral RNA and reverse transcriptase are inside the host cell, as shown in Figure 9, the enzyme reverses transcription by making a single stranded DNA from the retroviral RNA. Viral DNA can be integrated into the host DNA. It remains in the genome and is replicated whenever the host DNA replicates. If viral DNA is transcribed, new viruses are produced by biosynthesis, maturation, and release by budding. Retroviruses include HIV and also cause certain forms of cancer.

Figure . Replication of a retrovirus. Image from http://www.accessexcellence.org/AB/GG/retrovirus.html.

Viruses and Diseases: Viruses cause a variety of diseases among all groups of living things. Viral plant diseases can be controlled solely by burning those plants that show symptoms of disease. Viral diseases in humans are controlled by preventing transmission, administering vaccines, and only recently by the administration of antiviral drugs. Virally caused human diseases include the flu, common cold, herpes, measles, chicken pox, small pox, and encephalitis. Antibiotics are not effective against viruses. Vaccination offers protection for uninfected individuals. Frequent hand washing and condom use may help prevent transmission. Vaccines are substances that stimulate an immune response **without** causing the illness. Commonly used virus vaccines include polio, measles, and mumps.

Antibiotics do not cure viral infections because viruses use enzymes produced by the host cell, rather than produce not their own. A few antiviral drugs are available that interfere with viral replication without interfering with host metabolism in cells free of the virus. Antivirals include acyclovir for herpes and AZT for AIDS. Despite recent successes with antiviral drugs, vaccination and the prevention of exposure remain the most effective ways to deal with viral infections.

Smallpox was effectively elimated as a scourge on humanity by worldwide vaccination programs. In fact the chances of getting smallpox from a bad vaccine were greater than that of ever encountering the virus in nature. The last U.S. case of smallpox occurred in 1949, with the world's last naturally occurring case was from Somalia in 1977. For this reason, in 1972 routine U.S. smallpox vaccinations were discontinued. However, recent (September 2001) concerns about bioweapons and bioterrorism have led the U.S. government to consider plans to vaccinate the population against smallpox.

Hepatitis A, B, and C are all viral diseases that can cause liver damage. Like any viral disease, the major treatment efforts focus

on treatment of symptoms, not removal of the viral cause.

Hepatitis A is usually a mild malady indicated by a sudden fever, malaise, nausea, anorexia, and abdominal discomfort. The virus causing Hepatitis A is primarilly transmitted by fecal contamination, although contaminated food and water also can promote transmission.

A rare disease in the United States, hepatitis B is endemic in parts of Asia where hundreds of millions of individuals are possibly infected. The Hepatitis B virus (HBV) may be transmitted by blood and blood products as well as sexual contact. The blood supply in developed countries has been screened for the virus that causes this disease for many years, and transmission by blood transfusion is rare. The risk of HBV infection is high among promiscuous homosexual men although it is also transmitted hetereosexually. Correct use of condoms is thought to reduce or eliminate the risk of transmission. Effective vaccines are available for the prevention of Hepatitis B infection. Some individuals with chronic hepatitis B may develop cirrhosis of the liver. Individuals with chronic hepatitis B are at an increased risk of developing primary liver cancer. Although this type of cancer is relatively rare in the United States, it is the leading cause of cancer death in the world, primarily because the virus causing it is endemic in eastern Asia.

Hepatitis C affects approximately 170 million people worldwide and 4 million in the United States. The virus is transmitted primarily by blood and blood products. Most infected individuals have either received blood transfusions prior to 1990 (when screening of the blood supply for the Hepatitis C virus began) or have used intravenous drugs. Sexual transmission can occur between monogamous couples (rare) but infection is far more common in those who are promiscuous. In rare cases, Hepatitis C causes acute disease and even liver failure. About twenty percent of individuals with Hepatitis C who develop cirrhosis of the liver will also develop severe liver disease. Cirrhosis caused by Hepatitis C is presently the leading cause of the need for liver transplants in the United States. Individuals with cirrhosis from Hepatitis C also bear increased chances of developing primary liver cancer. All current treatments for Hepatitis C employ of various preparations of the potent antiviral interferon alpha. However, not all patients who have the disease are good candidates for treatment, so infected individuals are urged to regularly consult their physician.

Vaccination

Vaccination is a term derived from the Latin *vacca* (cow, after the cowpox material used by British physician Edward Jenner (1749-1823) in the first vaccination in 1796). A vaccine stimulates the antibody production and formation of memory cells without causing of the disease. Vaccines are made from killed pathogens or weakened strains that cause antibody production but not the disease. Recombinant DNA techniques can now be used to develop even safer vaccines.

The immune system can develop long-term immunity to some diseases. Man can use this to develop vaccines, which produce induced immunity. Active immunity develops after an illness or vaccine. Vaccines are weakened (or killed) viruses or bacteria that prompt the development of antibodies. Application of biotechnology allows development of vaccines that are the protein (antigen) which in no way can cause the disease. Passive immunity is the type of immunity when the individual is given antibodies

to combat a specific disease. Passive immunity is short-lived.

Emergent Viruses

Viruses are usually quite specific as to their hosts and even to the types of cells they infect in a multicellular host. Recently, some viruses appear to have shifted their host: HIV, hantavirus, and ebola appear to be either viruses shifting to a new (human) host or else viruses whose existence and effects are just now being realized by scientists and the general public. Recently in the Month of January a New Corona Virus killed more than 1000 peoples in the Wuhan city of China is reported as a deadly Virus. The Cause of spread of this virus is thought due to consumption of a wild animal like bat and snakes. Scientist are trying to develop vaccines for the virus but no vaccine haveben developed till date and death rate is still increasing day by day.

Viroids and Prions

Viruses would appear to be the simplest form of infectious particle. The discovery ofviroids, nucleic acid without a protein capsule, andprions, infectious proteins, subtracts another level of complexity. Both viroids and prions can cause diseases, the most famous of which is mad cow disease (caused by a prion).

The END

Chapter-13

MICROBIOLOGY OF WATER

Human activities generate a tremendous volume of sewage and wastewater that require treatment before discharge into waterways. Often this wastewater contains excessive amounts of nitrogen, phosphorus, and metal compounds, as well as organic pollutants that would overwhelm waterways with an unreasonable burden. Wastewater also contains chemical wastes that are not biodegradable, as well as pathogenic microorganisms that can cause infectious disease.Some of the infectious agents are as follow:

Waterborne microbial pathogens: Microbes in water include:

Bacteria

Virus

Protozoa

A few microbes (*pathogens*) are capable of causing disease, and may be transmitted by water. Some common water born pathogens are

Salmonella typhi

Escherichia coli

Vibrio cholera

Pseudomonas aeruginosa

Shigella spp.

Cryptosporidium

Giardia lamblia

Norwalkvirus

Indicator bacteria for water

Indicator microorganisms are used to indicate an increased risk of pathogen contamination due to fecal contamination.Indicator microbes should be:

Always present when feces/sewage is present

Always absent when feces/sewage is absent

Survives longer in water than any of the pathogenic species

***Coliform* bacteria (*E. coli*-like) are the most often used indicator bacteria for water quality assessment in the laboratory.**

Characteristics of coliforms:

Aerobic or facultative,

Gram-negative,

Non-spore forming,

Bacilli

ferment lactose to form acid and/or gas within 48 hours at 35 °C.

Coliforms are often found naturally in soil, water, plants, etc.

Fecal coliforms are a more specific coliforms that usually come from feces.

E.coli is the most specific indicator for faecal coliform

Fig: E.coli

Methods for enumerating coliforms:

EPA guidelines for coliforms in drinking water are < 1 CFU/100 ml.

Tests used to isolate and enumerate coliforms in water include:

- m-Endo media
- m-FC media
- MUG media

Other indicators for drinking and recreational Water

Limitation of Total coliform as drinking water microbial indicator

Ubiquitous.

Less resistant to traditional disinfection.

Proliferate in the biofilms of water distribution systems

Limitation of Fecal coliforms

Klebsiella pneumoniae

Less resistant to traditional disinfection.

Limitation of *E. coli*

Less resistant to traditional disinfection.

Microbial Indicators for Recreational Water

Total coliform?

Fecal coliform?

Average 200 MPN/100 ml

E. Coli (fresh water)?

Average 126 MPN/100 ml.

Enterococci (salt water)

Average 35 MPN/100 ml.

Water samples are first tested for Coliform Bacteria. This group of bacteria, normally found in the environment, is used as an indicator, to indicate the possible presence of Pathogens (disease causing organisms). Generally, when pathogens are present in drinking water, coliforms are present as well The **coliform** group is comprised of Gram-negative, nonspore-forming, rods, which ferment lactose to acid and gas organisms in this group include *E. coli*, theonly true fecal which is found only in fecal material from warm-blooded animals The presence of this organism in a water is evidence of fecal contaminationit impossible to test each water sample for each pathogensit is easier to test for the presence of nonpathogenic intestinal organisms such as *E. coli*.

Three test are proformed to indicate the presence of *E.coli* in water

Presumptive,

confirmed and

completed tests

The Presumptive Test

In the presumptive test, a lactose broth tubes are inoculated with

Amounts of the water sample to be tested.

Presumptive test

Material:

1. 3 tubes of lactose broth

2. 10, 1.0 and 0.1 ml pipets

3. Water samples

Procedure: (work in groups of four)

Presumptive Test

1. Take a water sample (dilute) and inoculate three

tubes of lactose broth with 10 ml, 1.0 ml and

0.1 ml.

Incubate all tubes at 37oC for **24 hours..**

Observe the number of tubes at each dilution that show acid, gas production in24 hrs. Record results.

Reincubate for an additional **24 hours** at 37°C.

The Confirmed Test

If any of the tubes inoculated with the water sample produce gas,

it is necessary to inoculate EMB (eosin methylene blue) agar plates

from a positive presumptive tube. The methylene blue in EMB agar inhibits Grampositive organisms and allows the Gram-negative coliforms to grow

E. coli colonies are small and have a green metallic

Material:

EMB agar plates

Procedure:

1. Inoculate an EMB plate with material from a tube containing acid or gas.
2. Invert and incubate the plate at 37°C for **24 hours**

Observe EMB agar plates.

A positive confirmed test is indicated by small

colonies green metallic sheen (*E. coli*). Record results

The Completed Test

The completed test is using the organisms which grew on the **confirmed** test media

These organisms are used to inoculate a tube of lactose broth After 24 hours at 37°C

the lactose broth is checked for the production of gas.

Material:

1. Lactose broth tubes

Procedure:

1. Inoculate a lactose broth tube with organisms from the

EMB plate.Incubate the broth tube at 37°C for **24 hours**.

in the lactosebroth tube Check for gas production.

Make a Gram stain from the organisms **3.** Record results

MPN method (Most probable number/100 ml of water)

TheMPNindex is determined by comparing the pattern of positive results (the number of tubes showing growth at each dilution) with statistical tables. The tabulated value is reported asMPNper 100 ml of sample. There are a number of variants to the multiple fermentation tubetechnique.

MPN: Most Probable Number (MPN) is a method used to estimate the concentration of viable microorganisms in a sample by means of replicate liquid broth growth in ten-fold dilutions. It is commonly used in estimating microbial populations in soils, waters, agricultural products and is particularly useful with samples that contain particulate material that interferes with**plate count enumeration methods.**

Importance of MPN Method

MPN is most commonly applied for quality testing of water i.eto ensure whether the water is safe or not in terms of bacteria present in it.

The presence of very few faecal coliform bacteria would indicate that a water probably contains no disease causing organisms.

while the presence of large numbers of faecal coliform bacteria would indicate a very high probability that the water could contain disease producing organisms making the water unsafe for consumption.

Principle*:* Water to be tested is diluted serially and inoculated in lactose broth, coliforms if present in water utilize the lactose present in the medium to produce acid and gas.The presence of acid is indicated by color change of the medium and the presence of gas is detected as gas bubbles collected in the inverted durham tube present in the medium. The number of total coliforms is determined by counting the number of tubes giving positive reaction(i.e both color change and gas production)and comparing the pattern of positive results(the number of tubes showing growth at each dilution)with standard statistical tables.

MPN test is performed in 3 steps

1. Presumptive test
2. Confirmatory test
3. Completed test

1. Presumptive test:

The presumptive test, is a screening test to sample water for the presence of coliform organisms.

If the presumptive test is negative, no further testing is performed, and the water source is considered microbiologically safe. If, however, any tube in the series shows acid and gas, the water is considered unsafe and the confirmed test is performed on the tube displaying a positive reaction.

The method of presumptive test varies for treated and untreated water.

Requirements :

Medium: Lactose broth or Mac Conkey Broth or Lauryl tryptose (lactose) broth

Glasswares: Test tubes of various capacities (20ml, 10ml, 5ml), Durham tube

Others: Sterile pipettes

Preparation of the Medium

Prepare medium (either mac conkey broth or Lactose broth)in single and double strength concentration.

For untreated or polluted water :

Dispense the double strength medium in 10 tubes (10ml in each tube) and single strength medium in 5 tubes (10 ml in each tube)and add an durham tube in inverted position.

For treated water:

Dispense the double strength medium in 5 tubes (10ml in each tube) and 50 ml single strength medium in 1 bottle and add an durham tube in inverted position.

Examine the tubes to make sure that the inner vial is full of liquid with no air bubbles.

Sterilize by**autoclaving at 15 lbs pressure (121°C) for 15 minutes**.

Procedure of the test: Untreated Water:

- Take 5 tubes of doublestrength and 10 tubes of singlestrength for each water sample to be tested.
- Using a sterile pipette add 10 ml of water to 5 tubes containing 10 ml double strengthmedium.

- Similarly add 1 ml of water to 5 tubes containing 10 ml double strength strength medium and 0.1 ml water to remaining 5 tubes containing 10 ml double strength medium.

- Incubate all the tubes at 37°C for 24 hrs. If no tubes appear positive re-incubate up to 48 hrs.

- Compare the number of tubes giving positive reaction to a standard chart and record the number of bacteria present in it.

  For example: a water sample tested shows a result of 3–2–1 (3 × 10 ml positive, 2 × 1 ml positive, 1 × 0.1 ml positive) gives an MPN value of 17, i.e. the water sample contains an estimated 17 coliforms per 100 ml .

Fig: MPN Procedure to estimate *E.coli* in Water(Microbe online)

Fig:MPN Table (Microbeonline)

B. For untreated water

1. Take 1 tube of single strength (50ml) and 5 tubes of double strength (10ml) for each water sample to be tested.
2. Using a sterile pipette add 50 ml of water to the tubes containing 50 ml single strength medium.
3. Similarly add 10 ml of water to 5 tubes containing 10 ml double strength medium Incubate the tubes at 37°C for 24 hrs. If no tubes appear positive re-incubate up to 48 hrs.
4. Compare the number of tube giving positive reaction to a standard chart and record the number of bacteria present in it. *For example: a water sample tested shows a result of 1-4 (1 × 50 ml positive, 4 × 10 ml positive) gives an MPN value of 16, i.e. the water sample contains an estimated 16 coliforms per 100 ml*

Fig: MPN table and Microbes number (Microbes.online)

2. Confirmed test:

Some microrganisms other than coliforms also produce acid and gas from lactose fermentation. In order to confirm the presence of coliform, confirmatory test is done.

From each of the fermentation tubes with positive results transfer one loopful of medium to:

1. 3 ml lactose-broth or brilliant green lactose fermentation tube,
2. to an agar slant and
3. 3 ml tryptone water.

Incubate the inoculated lactose-broth fermentation tubes at 37°C and inspect gas formation after 24 ± 2 hours. If no gas production is seen,further incubate up to maximum of 48 ±3 hours to check gas production.

The agar slants should be incubated at 37°C for 24± 2 hours and**Gram-stained preparations**made from the slants should be examined microscopically.

The formation of gas in lactose broth and the demonstration of Gram negative, non-spore-forming bacilli in the corresponding agar indicates thepresence of**a member of the coliform group**in the sample examined.

The absence of gas formation in lactose broth or the failure to demonstrate Gram-negative, non-spore-forming bacilli in the corresponding agar slant constitutes a negative test*(absence of coliforms in the tested sample)*.

Tryptone water Test

1. Incubate the tryptone water at (44.5 ±0.2°C) for 18-24 hours
2. Following incubation, add approximately 0.1ml of Kovacs reagent and mix gently.
3. The**presence of indole**is indicated by a red colour in the Kovacs reagent, forming a film over the aqueous phase of the medium.

a. Confirmatory tests positive for indole, growth, and gas production show the presence of thermotolerant*E. coli.*

b. Growth and gas production in the absence of indole confirm thermotolerant coliforms.

3. Completed test:

Since some of the positive results from the confirmatory test may be false, it is desirable to do completed tests. For this inoculum from each positive tube of the confirmatory test is streaked on a plate of EMB or Endo agar.

In this process, a loopful of sample from each positive BGLB tubesis streaked onto selective medium like**Eosin Methylene Blue agar**or Endo's medium. One plate each is incubated at 37°C and another at 44.5± 0.2°C for 24 hours.

High temperature incubation (44.5 ±0.2) is for detection of thermotolerantE.coli.

Following incubation,all plates are examined for presence of typical colonies.

Coliforms produce colonies with greenish metallic sheen which differentiates it from non-coliform colonies (show no sheen). Presence of typical colonies on high temperature (44.5 ±0.2) indicate presence of thermotolerant***E.coli.***

Advantages of MPN :

Ease of interpretation, either by observation or gas emission
Sample toxins are diluted
Effective method of analyzing highly turbid samples such as sediments, sludge, mud, etc.
that cannot be analysed by membrane filtration.

Disadvantages of MPN:

It takes a long time to get the results
Results are not very accurate
Requires more hardware (glassware) and media
Probability of false positives

THE END

Chapter-14

SAFETY MEASURS IN MICROBIOLOGY METHODS (LABORATORY RULES)

Laboratory Rules: There are some rules which must be observed for the successful completion of the laboratory exercise, personal safety and convenience of others working in the laboratory:

1. Wash your hands with disinfectant soap when you arrive at the lab and again before you leave.

2. Absolutely no food, drinks, chewing gum, or smoking is allowed in the laboratory. Do not put anything in your mouth such as pencils, pens, labels, or fingers. Do not store food in areas where microorganisms are stored.

3. Avoid loose fitting items of clothing. Wear appropriate shoes (sandals are not allowed) in the laboratory.

4. Keep your workspace free of all unnecessary materials. Backpacks, purses, and coats should be placed in the cubbyholes by the front door of the lab. Place needed items on the floor near your feet, but not in the aisle.

5. Disinfect work areas before and after use with 70% ethanol or fresh 10% bleach. Laboratory equipment and work surfaces

should be decontaminated with an appropriate disinfectant on a routine basis, and especially after spills, splashes, or other contamination.

6. Label everything clearly.

7. Replace caps on reagents, solution bottles, and bacterial cultures. Do not open Petri dishes in the lab unless absolutely necessary.

8. Inoculating loops and needles should be flame sterilized in a Bunsen burner before you lay them down.

9. Always wear a laboratory coat or apron before entering a laboratory for protecting clothes from contamination or accidental discoloration by standing solutions like long sleeved shirt that buttons or snaps closed. This garment must cover your arms and be able to be removed without pulling it over your head. Leave protective clothing in the lab and do not wear it to other non-lab areas.

10. Turn off Bunsen burners when not is use. Long hair must be tied off if Bunsen burners are in use.

11. When you flame sterilize with alcohol, be sure that you do not have any papers under you.

12. Treat all microorganisms as potential pathogens. Use appropriate care and do not take cultures out of the laboratory.

13. Wear disposable gloves when working with potentially infectious microbes or samples (e.g., sewage). If you are working with a sample that may contain a pathogen, then be extremely careful to use good bacteriological technique.

14. Sterilize equipment and materials.

15. Never pipette by mouth. Use a pipetting aid or adjustable volume pipettors. This practice has been known to result in many laboratory-acquired infections. With the availability of mechanical pipetting devices, mouth pipetting is strictly prohibited.

16. Consider everything a biohazard. Do not pour anything down the sink. Autoclave liquids and broth cultures to sterilize them before discarding.

17. Dispose of all solid waste material in a biohazard bag and autoclave it before discarding in the regular trash.

18. Familiarize yourself with the location of safety equipment in the lab (e.g., eye-wash station, shower, sinks, fire extinguisher, biological safety cabinet, first aid kit, emergency gas valve).

19. Dispose of broken glass in the broken glass container.

20. Dispose of razor blades, syringe needles, and sharp metal objects in the “sharps” container.

21. Report spills and accidents immediately to your instructor. Clean small spills with care. Seek help for large spills.

22. Report all injuries or accidents immediately to the instructor, no matter how small they seem.

23. Before and after each laboratory period, clean your work bench with a disinfectant like Lysol(1:500),phenol(1:100) or 90% ethanol.

24. Keep your laboratory bench clean of everything (eg.books, purses, papers, etc.) except your laboratory equipment and notebook.

25. Never smoke, drink, or eat in the laboratory.

26. Never place pencils, labels or any other material in your mouth.

27. If a live culture is spilled, cover the area with a disinfectant such as mercuric chloride for 15 min and then clean it.

28. In a event of personal injury such as cut or burn, inform your instructor immediately as bacteria love open wound.

29. Long hair should be tied back to minimize contamination of cultures and fire hazards.

30. Be careful of laboratory burners and turn-off when not on use.

31. All microbial cultures should be handled as being potentially pathogenic.

32. The waste papers and contaminated glassware should be kept in receptacles.

33. Wash your hands with soap and water before and after finishing work in the laboratory.

35. Broth culture must never be pipetted with mouth and must be filled with use of pipette aid and operated in such a way so as to avoid creating aerosol.

36. Aseptic technique must rigorously observe at all time.

37. Always keep culture tubes in upright position in a rack or basket.

38. You must familiarize yourself in advance with the exercise to be performed.

39. Label all the plates, tubes in an upright position in a rack or basket.

40. Material such stain and reagent bottles, petriplates, pipettes must be returned to their original location

41. Clean the laboratory desktop with a damp cloth at the close of each experiment.

42. Always clean microscope stage, eyepiece, lenses and objectives, before and after use. All the lenses must be wiped with the lens paper.

43. Keep the laboratory doors and window closed when experiment are in progress. Precaution must be taken while removing the closures from shaken culture tubes and plunging of contaminated loops into a flame to avoid inhalation of an infectious microbes..

44. As you perform your exercise, record your data in ink and make sketches and labels in pencils.

45. Make your manual note book regularly.

The End