

INDEX

MICROBIOLOGY

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preface

one of the most important factors contributing to success in college, and in microbiology courses, is the use of good study techniques. This material is organized to help you to study more efficiently. Thus this section briefly outline some practical study skills that help ensure success in microbiology and make your use of this material more productive. These suggestions are made in the hope that they may be useful to those who are unaware for studying other books.

A team of dedicated and highly committed members have prepared this illustrated material to create interest and provide a more simplified and interactive material so that it will be easy for any student to understand.

Our intention is to cater to the needs of the precisely planned perfect schedule. Our pattern has been modified in such a way that the finds maximum time to promote creative skills in the young minds.

By reading this material a student can reach maximum potential and it will enable the students to score maximum marks in the exams. We request you to wean the student away from the rote/cramming method of learning. With this new book, let us encourage our students to be more creative.

Finally your suggestions are always welcome to enhance the quality of this material.

Your suggestions are always welcome.

Wishing you all the best

Though we have made our best efforts present the book with out errors , some errors might have been committed. We are not liable or responsible for such errors legally. If any one brings them to our notice , we will correct them in the next edition.

B.Sc., MICROBIOLOGY(CBCS) SYLLABUS

FIRST YEAR-Semester 1

UNIT-I

History and mile stones in Microbiology- Meaning, definition and history of Microbiology. Contributions of Antonyvon Leeuwenhoek, Edward Jenner, Louis Pasteur, Robert Koch, Iwanowsky, Beijerinck, Winogradsky and Alexander Fleming. Importance and Applications of Microbiology. Virology- Basics of virology, history, milestones, taxonomy and Significance of virology.

UNIT-II

Classification of micro organisms-Hackel's three kingdom concept, whittaker's five kingdom concept and three domain concept of Carl Woese and phylogenetic trees. Basis of modern microbial classification and their concepts, nomenclature and taxonomic ranks. General characters of Fungi(Yeasts, Candida)- Algae(cyanobacteria, Chlorella)- protozoa(Entamoeba, Leishmania, Plasmodium). Isolation and identification of micro organisms-principles and types of stains(simple, differential, and negative stains); structural stains-spore, capsule, flagella, Hanging drop method.

UNIT-III

Sterilization and disinfection techniques principles and methods of sterilization. Physical methods- autoclave, Hot air oven, Pressure cooker, Laminar air flow, Filter sterilization, Radiation methods- UV rays, Gamma rays, ultrasonic methods. Chemical methods- use of alcohols, aldehydes, fumigants, phenols, halogens and hypochlorites. Phenol coefficient.

UNIT-IV

Isolation of pure culture techniques- Enrichment culturing, dilution-plating, streak plate, spread plate and micromanipulator. Preservation of microbial cultures- sub culturing, overlaying cultures with mineral oils, Lyophilization, sand cultures, storage at low temperature(ultra low temperature).

UNIT-V

Differentiation of prokaryotes and eukaryotes. General characteristics of bacteria, archaeobacteria, rickettsias, mycoplasmas, cyanobacteria and actinomycetes. Outline classification of bacteria as per the second edition of Bergey's manual of systematic bacteriology(up to order level). Ultra structure of a bacterial cell: invariant components-cell wall, cell membrane, ribosomes, nucleoid. Variant components-Capsule, flagella, fimbriae, endospore and storage granules. General characteristic and classification of viruses- animal, plant and microbial. Morphology, structure and replication of TMV, HIV and lambda bacteriophage. Eukaryotes- General characteristics and classification (up to the order level) of eukaryotic micro organisms- micro protozoa, micro algae, molds and yeasts.

LIST OF QUESTIONS

UNIT-I:

ESSAY QUESTIONS:

1. Describe briefly the contributions of scientists to the development of microbiology?
2. Define and describe meaning, definition and history of microbiology?
3. Describe the importance and applications of microbiology?
4. Write an account on taxonomy and significance of virology?

SHORT ANSWER QUESTIONS:

1. contributions of antonyvon leewenhoek
2. contributions of Edward jenner
3. contributions of Louis Pasteur
4. contributions of Robert Koch
5. contributions of Iwanowsky
6. contributions of Beijerinck
7. contributions of Winogradsky
8. Contributions of alexender fleming.

UNIT-II:

ESSAY QUESTIONS:

1. Write about classification proposed by Haeckel?
2. Give detailed account on the classification proposed by carl woese?
3. Write an account on the classification of microorganisms basing on whittaker's five kingdom concept
4. Describe the methods of isolation and identification of micro organisms?

SHORT ANSWER QUESTIONS:

1. Give general characters of yeasts?
2. Give general characters of candida?
3. Give general characters of cyanobacteria?
4. Give general characters of chlorella?
5. Give general characters of entameoba?
6. Give general characters of Leishmania?
7. Give general characters of plasmodium?
8. Write short note on differential staining?
9. Write short note on negative staining?
10. Write short note on hanging drop technique?

11. Write short note on spore staining?

UNIT-III:

ESSAY QUESTIONS:

1. Describe the methods of physical sterilization?
2. Describe the methods of chemical sterilization?

SHORT QUESTIONS:

1. Write short note on hot air oven?
2. Write short note on autoclave?
3. Write short note on pressure cooker?
4. Write short note on laminar air flow?
5. Write short note on gamma rays?
6. Write short note on U.V rays?
7. Write short note on filter sterilization?
8. Write short note on phenol coefficient?

UNIT-IV:

ESSAY QUESTIONS:

1. Explain the methods of isolation of pure cultures?
2. Describe the methods of preservation of microbial cultures?

SHORT QUESTIONS:

1. Write short note on enrichment culture technique?
2. Write short note on serial dilution?
3. Write short note on streak plate method?
4. Write short note on spread plate method?.
5. Write short note on lyophilization?

UNIT-V:

ESSAY QUESTIONS:

1. Describe the ultra structure of bacterial cell?
2. Differentiate prokaryotes and eukaryotes?
3. Give out line classification of bacteria as per the second edition of bergey's manual of systematic bacteriology?

SHORT ANSWER QUESTIONS:

1. Write general characteristics of bacteria?
2. Write general characteristics of archaebacteria?
3. Write general characteristics of mycoplasmas?

4. Write general characteristics of cyanobacteria?
5. Write general characteristics of actinomycetes?
6. Write short note on morphology and structure of TMV?
7. Write short note on morphology and structure of lambda bacteriophage?
8. Write short note on morphology and structure of HIV?
9. Give an account on the multiplication (or) replication in viruses?
10. Write general characteristics of microprotozoa?
11. Write general characteristics of microalgae?
12. Write general characteristics of molds?
13. Write general characteristics of yeasts?

UNIT-I

1) DESCRIBE BRIEFLY THE CONTRIBUTIONS OF SCIENTISTS TO THE DEVELOPMENT OF MICROBIOLOGY?

ANS: Microbiology is the study of organisms which are too small to be seen clearly by the naked eye. In other words study of microorganisms is called "micro biology". Organisms with diameter of 1mm or less are considered as Microorganisms. Algae, fungi, bacteria, virus, protozoa and some metazoan animals are examples of Microorganisms. These microorganisms are unknown till the invention of microscopes. Microscopes invented at the beginning of 17th century.

Contributions of antony vonleeuwenhoek:

Antony von leeuwenhoek a Dutch student of natural history whose hobby was making microscopes, is shown here with one of the more than 250 microscopes that he made. His best lenses were capable of magnification up to X270, and he was the first person to report descriptions of microorganisms in detail.

In early days there were only two types of microscopes. First was simple microscope with a single lens of very short focal length with high magnification. The second was compound microscope with a double lens system with an ocular.

Leeuwenhoek was a cloth maker, tailor and had little formal education and never attended a university. He used to examine fabrics with magnifying glasses. He made simple microscopes and observed microscopic organisms in drops of water. He transmitted his findings in a series of letters to the royal society in London. Among these letters the first stated letter was on sept 7 1674, in that he described the "very little animalcules" which we recognized now as free living protozoa. For almost 50 years leeuwenhoek transmitted his discoveries to the royal society in the form of long series of letters in Dutch. Most of these are translated and published in English in the proceedings of the royal society.

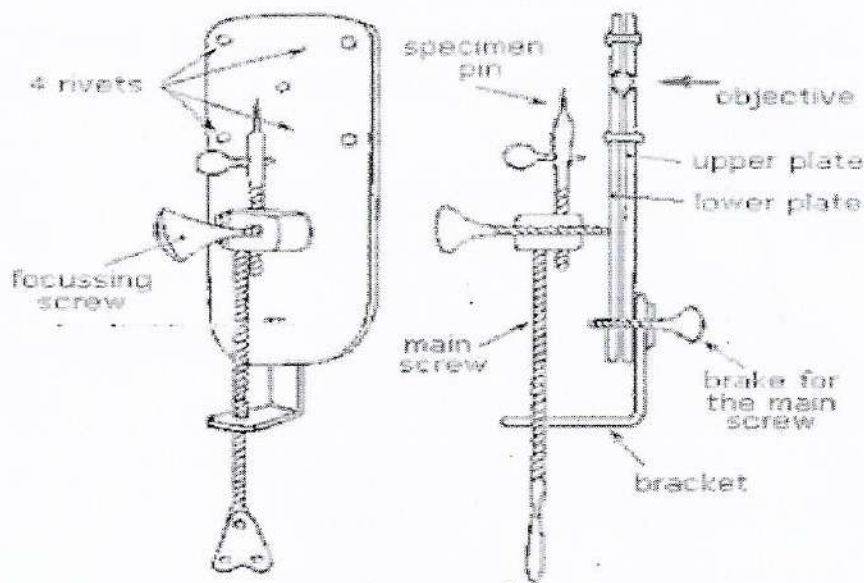


Figure 1 - Diagram of the microscope constructed by Antoni van Leeuwenhoek in the XVII century

He also constructed an aquatic microscope having a silver plate fitted with lens in front of its groove and a needle to amount the material on it. This aquatic microscope greatly aided (help) and enable the scientists to observe the flow of red blood cells through the capillaries of a fish's tail fins.

Leewenhoek also made many other contributions of biological significance. He observe number of seeds and embryos of plants, small invertebrate animals and so on. He discovered existence of spermatozoa and red blood cells. Leewenhoek also observed muscle fiber striations and nuclei of fish blood cells.

The fundamental observation in bacteriology started in fact from the innovation of microscope by Leewenhoek.

Contributions of Edward Jenner:

Edward Jenner was a doctor from England. The term "vaccine" was coined by Edward Jenner. A vaccine is a biological preparation that improves immunity to a particular disease. Ancient Greeks have belief that people who had recovered from plague, smallpox, yellow fever and various other diseases and rarely attack the diseases the first artificial immunizations were made in the late 18th century by Edward Jenner.

On May 14, 1796 Jenner first vaccinated an 8-year-old boy named James Phipps with material removed from a cowpox lesion on the hand of a milkmaid. Proof that inoculation gave protection against smallpox was obtained 6 weeks later, when the boy was inoculated with pus from a smallpox victim and did not develop the disease.

Jenner found that persons successfully inoculated with virus isolated from cowpox lesions developed a small scab at the site of applications, which dropped off after about 2 weeks leaving a single small scar. Before making his report he had successfully vaccinated 23 persons. The material he used came from cows (Latin, vacca); hence the term vaccination.

Today smallpox vaccine is made from virus grown on the skin of healthy calves or sheep or embryonated eggs, thus eliminating the possibility of transmitting other human diseases in the process. Jenner never saw the causative agent of smallpox; indeed his discovery and application came more than half a century before the establishment of the germ theory of disease.

Contribution of Louis Pasteur:

Louis Pasteur was a French chemist and physicist. He is called father of modern microbiology. Pasteur's contributions to science of microbiology are many and great. He discovered that fermentation of solutions was caused by microorganisms and not as most scientists then assumed. By a spontaneous generation by using "swan-necked flask experiment", strongly supported germ theory of disease by proving destructive silkworm disease due to the activities of protozoan parasites. He isolated the germ responsible for chicken cholera, germ responsible for rabies disease and rabies.

Louis Pasteur began his brilliant career as professor of chemistry at the University of Lille, France. A principal industry of France being the manufacture of wines and beer, Pasteur studied the methods and processes. He found the fermentation of fruits and grains resulting in alcohol, was brought about by microbes. By examining many batches of "ferment", he found microbes of different sorts (types). In good lots one type predominated and in the poor products another kind was present. Pasteur suggested that the undesirable types of microbes might be removed by heating not enough to hurt the flavor of the fruit juice, but enough to destroy a very high percentage of microbial population. He found that holding the juices at a temperature of 62.8°C for half

an hour did the job. Today PASTEURIZATION is widely used in fermentation industries, but we are more familiar with it in the dairy industry.

The discovery of anaerobic life:

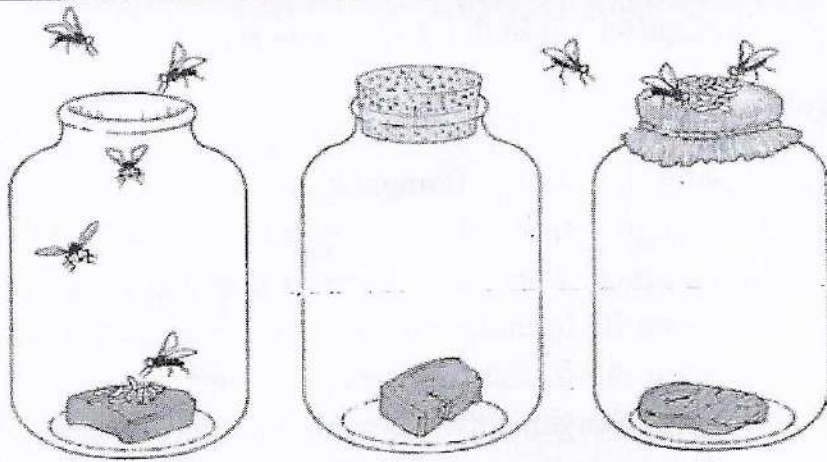
While studying the butyric fermentation Pasteur discovered another phenomenon that the existent forms can live only in the absence of free oxygen while examining microscopically Pasteur observed that the bacteria at the margin of a flattened drop immotile because which are in close contact with air where as the cells in centre are motile. This observation suggested that air had an inhibitory effect on the micro organisms. He passed air current through the fermenting fluid this retarded the butyric fermentation. He thus concluded that some micro organisms can live only in the absence of oxygen. He introduce the terms aerobic and anaerobic for life in the presence and absence of oxygen.

The conflict over spontaneous generation:

From earliest times, people believed that in spontaneous generation the living organisms could develop from non living matter. Even the great Aristotle thought some of the simple invertebrates could arise by spontaneous generation.

The view finally challenged by the Italian physician Francesco Redi who carried out a series of experiments on decaying meat and its ability to produce maggots spontaneously. Redi placed meat in three containers one was uncovered second one was covered with paper third one was covered with fine gauze (like muslin cloth). Flies laid their eggs on the uncovered meat and maggots developed. The other two species of meat did not produce maggots spontaneously. However flies were attracted towards the gauze covered container and flies laid their eggs on the gauze these eggs produced maggots.

Thus the generation of maggots on decaying meat resulted from the present of fly eggs and meat did not spontaneously generate maggots. as previously believed some proposed that micro organisms arose by spontaneous generation.



John Needham observed the appearance of organisms not present at the start of the experiment and concluded that the bacteria originated from the meat. About the same time, Lazzaro Spallanzani boiled beef broth for an hour and then sealed the flasks. No microbes appeared following incubation. But his results, confirmed in repeated experiments, failed to convince Needham, who insisted that air was essential to the spontaneous generation of microscopic beings and that it had been excluded from the flasks by sealing them. This argument was answered some 60 or 70 years later independently by two other investigators, Franz Schulze and Theodor Schwann. Schulze passed air through strong acid solutions into boiled infusions, whereas Schwann passed air into his flasks through red hot tubes. In neither case did microbes appear. In about 1850's H. Schroder and T. von Dusch performed a more convincing experiment by passing air through cotton into flasks containing heated broth. Thus the microbes were filtered out of the air by the cotton fibers so that growth did not occur, and a basic technique of plugging bacterial culture tubes with cotton stoppers was initiated.

Pasteur prepared a flask with a long narrow goose-necked opening. The nutrient solutions were heated in the flask and the air that was unfiltered could pass in or out. The germs settled in the goose neck. So, no microbes appeared in the solutions. Pasteur reported his results in 1864. He said that his flask would yield no sign of life and that he could only keep the germs away which float in the air.

Contribution of Robert Koch:

Robert Koch is a German physician who worked on Microorganisms in early days of his medical career. Koch is called as the father of modern BACTERIOLOGY. Robert Koch first demonstrated the role of bacteria

incausing disease. Robert Koch was a German physician. He established a relationship between "Bacillus anthracis" and "anthrax". He injected healthy mice with material from diseased animals and the mice became ill he transferred anthrax by inoculating through a series of 20 mice. He also incubated a piece of spleen containing the bacillus in beef spleen. The bacillus grew, reproduced and produced spores. They were isolated and injected into mice and observed anthrax.

Koch postulates are summarized as follows:

1. The microorganisms must be present in every case of the disease but absent from healthy organisms.
2. The suspected microorganism must be isolated and grow in a pure culture.
3. The same disease must result when the isolated microorganism is inoculated into a healthy host.
4. The same organism must be isolated again from the diseased host.

Koch cultured suspected bacterial pathogens on the sterile surfaces of cut, boiled potatoes but pathogens would not grow well on potatoes. He tried to solidify liquid medium by adding gelatin. When the gelatin hardened, bacteria produced separate colonies. Gelatin was digested by many bacteria and melted when the temperature raised above 28°C. Fannie Eilshemius, wife of Walther Hasse, who is a Koch's assistant suggests the agar as a solidifying agent. She had been using it for making jellies. Agar did not melt at 100°C and was not attacked by bacteria. Koch isolated pure forms of bacteria by using the petri dishes for solid culture media. Petri dish was developed by "Richard Petri" and he was one of the Koch's assistants.

Koch also developed nutrient broth and nutrient agar for growing bacteria isolated from body. Koch has used these techniques to isolate bacillus that caused tuberculosis. This followed a golden age of about 30 to 40 years in which most of the major bacterial pathogens were isolated.

Contribution of Dimitri Iwanosky:

Dimitri Iwanosky is a famous Russian pathologist. During middle of nineteenth century, mosaic disease of tobacco was causing serious damage to tobacco crop everywhere and especially in Holland and Germany. Regarding this disease a most remarkable discovery was made by Iwanosky.

In 1892, while doing an experiment to isolate the causative of tobacco mosaic disease, Dimitri Iwanowski filtered the sap of diseased tobacco plants through a porcelain filter which was designed to retain bacteria. He expected to find the microbe trapped in the filter, but he found the infectious agent passed through the micropores of the filter. When he injected healthy plants with the filtered fluid, they caused tobacco mosaic disease. He still believed the infectious agent was a bacterium that was small enough to pass through the filter. Iwanowski reported that a filtrate agent was responsible for the disease.

Contribution of Beijerinck:

M.W. Beijerinck, a Dutch microbiologist from Holland, established that viruses possess the property of replication that is common to all living things. This was demonstrated in TMV (Tobacco Mosaic Virus) and he concluded that it was not a pathogenic bacterium or toxin, but rather by some new type of pathogen which he called "CONTAGIUM VIVUM FLUIDUM" it is a contagious living fluid and referred to subsequently as a virus.

1. He developed a new technique for isolation of soil bacteria from a mixed population. Isolation requires knowledge about the growth requirements of particular organisms.

This technique is called Enrichment Culture. The enrichment culture helps in the isolation of bacteria and also in the study of physiological types of microorganisms. In this technique he prepared a culture medium in which one type of organism predominates, due to excessive growth.

2. He found that selectivity of enrichment culture depends not only on composition but also on other characters like, pH, Concentration, aeration, Temperature etc.

E. COLI can grow well at high temperature (45°C) and is not sensitive to bile salts. Enterobacter, which is also an enteric bacterium, favors low temperature and is sensitive to bile salts in the medium. In a medium having bile salts, at high temperature *Escherichia coli* overgrows then enterobacter.

3. He also established that in a carbohydrate-rich medium, yeasts and fermentative bacteria predominate, suppressing the other organisms.

4. He also informed that the spore-producing bacteria can be separated from non-spore producers by the process of PASTEURIZATION.

5. He isolated azatobacteria free living N_2 Fixing bacteria.
6. He also isolated root nodulating bacterium and named it as rhizobium.
7. Beijerinck in Holland discovered variety of metabolic pathways in different kinds of bacteria adapted to different ecological niches.
8. After iwanowsky, beijerinck also recognized that TMV are filterable viruses.

Contributions of Winogradsky:

Winogradsky a Russian microbiologist made several contributions to soil microbiology.

1. He discovered that soil bacteria can live autotrophically like photosynthetic bacteria, in the absence of light. They use chemical energy liberated during the oxidation of iron, Sulphur, ammonia etc in the incorporation of CO_2 into carbohydrate. These bacteria are called chemoautotrophic bacteria.
2. He also studied anaerobic nitrogen fixing soil bacteria. He isolated bacteria and studied their action on decomposition of cellulosic organic matter.
3. Winogradsky discovered carbohydrate metabolism pathways in bacteria towards the end of pasteurian era. He stated that carbohydrate metabolism pathways are similar in both microbes and mammals.

Contribution of Alexander Fleming:

He is born on August 6, 1881.

He is Scottish Bacteriologist best known for his discovery of penicillin.

His work on wound infection and lysozyme, an antibacterial enzyme found in tears and saliva. His discovery of penicillin in 1928 which started the antibiotic revolution.

2) DESCRIBE THE IMPORTANCE AND APPLICATIONS OF MICROBIOLOGY?

ANS: Applications of Microbiology:

Microorganisms are diverse and ubiquitous (present everywhere) and they influence man in several ways. They can proliferate in extreme conditions as well. Their diversity varies from causing deadly diseases in human, other animals and plants. They are used to the production of various useful

products, recovery of metals, increasing soil fertility. The modern development of sanitation and public health has resulted in the reduction of the incidence of many diseases.

1.Food and Dairy industry:

Food microbiology not only includes the study of those microbes that serve as food due to their high protein value but also those microbes that deteriorate the food by using it as a source of nutrient for their growth and multiplication.

2. Yeast may be useful or harmful in food. Yeast fermentations are usually involved in the manufacturing of foods such as breads, beer, wines, vinegar, and surface ripened cheese etc.

3. Pigmented bacteria cause changes in the surface color of foods, forming film over the surface of food, which results in undesirable cloudiness or sediment.

4.Industrial applications:

Many industrial products such as enzymes, amino acids, vitamins, antibiotics, organic acids and alcohols are commercially produced using the microorganisms.

5. Genetically engineered microorganisms are used industrially for the production of mammalian proteins such as insulin and human growth factors and vaccines.

6. Microorganisms play a significant role in recycling of bioelements. Cyanobacteria, heterotrophes and chemo lithotrophs use about 80% of the oxygen.

7. Many microorganisms live in water and ultimately affect its quality. The prevention and control of infectious diseases has been the greatest achievement of medical science.

8. Living cells infected with viruses produce viral proteins having a broad-spectrum antiviral action called interferon. Interferons have been shown a number of biological effects and cause inhibition of parasitic infections due to *Chlamydia*, rickettsias, protozoa and bacteria.

Microbes play an important role in agriculture as well. This type of associations would bring down the applications of chemical fertilizers that are expensive and energy intensive.

9. Microorganisms can help us generate clean energy in the form of ethanol and biogas.

10. Microbiology conversion of organic materials into methane gas through anaerobic digestion is being mass population in rural areas under the aegis (protection) of Ministry of Non-Conventional Energy Sources.

There are many more applications of microbiology. Consider any field and you can incorporate microbiology into it and use it for the welfare of man and his environment.

3) GIVE THE MEANING, DEFINITION, AND HISTORY OF MICROBIOLOGY?

ANS: Micro organisms are tiny creatures that are not easily seen by the naked eye. Their size is very small and hence the term "micro organisms" is assigned to them.

Microbiology is the branch of biology, concerned with study of micro organisms, a diverse group of simple life forms that include protozoa, algae, molds, bacteria, and viruses. Microbiology is concerned with the structure, function and classification of these organisms and with ways of controlling and using their activities.

History of microbiology:

Antonyvan leewenhoek is the father of microbiology. He assembled simple microscopes and in 1674 through his own microscope, he brought to light for the first time, the existence of a new world of micro organisms. He observed bacteria named them as animalcules.

Wirsbery described these micro organisms as in infusoria Linnaeus in 1767 classified these micro organisms as chaos infusoria.

Muller in 1773 and 1788 classified bacteria and coined the vibro and monas. In 1798, Edward Jenner introduced vaccination for the prevention of small pox through inoculation of cow pox.

Ehrenberg in 1829 established the genus bacterium, meaning staff in greek. He also discovered two more genera, namely spirillum and spirochete.

Theodore Schwann in 1836 demonstrated that yeast causes fermentation.

IN 1857 Nageli coined the term schizomycetes and he correlated bacteria to fungi.

Louis Pasteur (1822-1895) proposed the theory of spontaneous generation of life. He also stated that bacteria are the causative agents of some diseases. He found that fermentation of fruits and grains producing alcohol was brought about by microbes. Pasteur discovered another phenomenon that some forms can live only in the absence of free oxygen. He first introduced the terms aerobic and anaerobic lives. An accidental observation of Pasteur led the discovery of the development of live vaccines. Pasteur coined the term vaccine.

While studying the butyric fermentation Pasteur discovered another phenomenon that the existent forms can live only in the absence of free oxygen i.e, anaerobic life.

Robert Koch (1843-1910) discovered bacilli.

Robert Koch in 1882 isolated the TB bacillus and proved that it was the causative agent of tuberculosis.

Lord lister(1827-1912) employed practical applications of bacteriology in the field of surgery. For the first time, he used surgical and antiseptic dressings to save the surgical wounds from the entrance of bacteria.

Louis Pasteur in 1866 introduced the concept of pasteurization. He also discovered vaccine against rabies.

In 1879 Albert Neisser, discovered the gonococcus, the causative agent of gonorrhoea.

In 1884 gram introduced the gram staining technique for bacteria. Kark joseph eberth in 1880 isolated the typhoid bacillus.

In 1880 charies Louis alphonse laveran discovered the parasite of malaria.

In 1882 carl friendlander discovered the bacillus of klebsiellapneumoniae.

In 1888 Pasteur laid the foundation for Pasteur Institute in Paris, where microbiology was extensively studied.

Beijerinck in 1888 isolated and studied the root-nodule bacteria.

Winogradsky (1893, 1994) demonstrated that the aerobic bacterium *Clostridium* fixes atmospheric N_2 in the soil. He discovered that soil bacteria could oxidize iron, sulphur, and ammonia to obtain energy. Beijerinck (1901) isolated three aerobic nitrogen fixing bacteria, namely *Azotobacter*, *Chroococcum*.

Ivanowski in 1892 discovered tobacco mosaic virus (TMV). Edward Buchner in 1897 showed that extracts of yeast cells could produce alcoholic fermentation.

In 1894 Alexander Yersin discovered the plague bacillus. In 1898, Kiyoshi Shiga discovered the dysentery bacillus.

In 1898 Ronald Ross discovered malarial parasite in the salivary glands of the Anopheles mosquito.

Sir Alexander Fleming in 1929 discovered first antibiotic, penicillin. He was called the father of antibiotics. Waksman discovered antibiotic streptomycin.

Beadle and Tatum (1941) using *Neurospora*, showed that gene mutations were truly spontaneous and not directed by the environment. The genetics of lactose induction in *E. coli* was studied by J. Lederberg. Lederberg with Tatum discovered a genetic exchange between certain strains of *E. coli*.

In 1944, Avery, MacLeod and McCarty proved that DNA was the genetic material.

In 1966 cancer viruses were discovered by F.P. Rous.

In 1977 S.B. Prusiner discovered prions.

In 1970's new discoveries such as recombinant DNA technology and genetic engineering were also led to the development of microbiology which gave the service of microbial biotechnology.

4. WRITE AN ACCOUNT ON THE TAXONOMY AND SIGNIFICANCE OF VIRUSES?

ANS: Viruses (from the Latin, virus meaning toxin or poison) are microscopic, acellular infectious agents occurring on plants, animals, humans as

well as micro organisms. Viruses are unable to grow or reproduce outside a host cell. The first known virus, Tobacco mosaic virus (TMV) was discovered by Martinus Beijerinck in 1899. Now more than 5,000 viruses have been described. The study of viruses known as virology, and is a branch of Microbiology. Viruses possess the properties some of which are traditionally associated with organisms, while others are characteristics of non-living molecules.

Viruses multiply within the host cell and give rise to the same genetic types. They have genetic material (DNA or RNA) and can undergo mutation like living organisms. Viruses show specific intracellular parasitism. They can be easily transmitted from host to host. They are able to infect and cause disease to living beings. They react to heat, radiation and chemicals.

TAXONOMY OF VIRUSES:

Naming and placing of viruses into a taxonomic system is involved in virus classification. The pseudo living nature of viruses makes the virus classification a subject of on going debate and proposals. Virus classification is mainly based on phenotypic characteristics, including morphology, nucleic acid type and mode of replication, host organisms and the type of disease they cause.

The International committee on taxonomy of viruses (ICTV), established in 1966, authorizes and organizes the taxonomic classification of viruses. This committee was formed and is governed by the virology division of the International Union of Microbiological societies. The committee also operates an authoritative database, called ICTVdB, containing taxonomic information for over 82 families and 2083 viral species that infect human beings, animals and economically important plants. A minor point is that names of orders and families are italicized, as in the ICBN. Most notably, species names generally take form of (disease) virus. It only recognises the taxa below kingdom; these of order, family, sub family, genus, and species. Therefore, viral classification starts at the level of order and follows as thus, with the taxon suffixes given in italics.

Order-*virales*

Family-*viridae*

Sub family-*virinae*

Genus-virus.

Classification of viruses infecting vertebrates, invertebrates, fungi, bacteria and plants.

FAMILY	EXAMPLE
ds DNA VIRUSES	
<i>Myoviridae</i>	Coliphage T4
<i>Siphoviridae</i>	Coliphage-lambda
<i>Podoviridae</i>	Coliphage T7
<i>Tectiviridae</i>	Enterobacteria phage PRD1
<i>Corticoviridae</i>	Alteromonas phage PM2
<i>Plasmaviridae</i>	Acholeplasma phage L2
<i>Lipothrixviridae</i>	Thermoproteus phage 1
<i>Poxviridae</i>	Vaccinia virus, fowlpox virus, myxoma virus, swine-pox virus.
<i>Asfarviridae</i>	African swine fever virus
<i>Iridoviridae</i>	Chilo iridescent virus, Mosquito iridescent virus, frog virus 3, gold fish virus 1
<i>Phycodnaviridae</i>	Paramecium bursaria chlorella virus 1
<i>Baculoviridae</i>	Nuclear polyhedrosis virus, plodia interpunctella virus
<i>Herpesviridae</i>	Herpes simple virus 1, varicella-zoster virus, Human herpes virus 3, human herpes virus 4.
<i>Adenoviridae</i>	Human adeno virus, fowl adenovirus 1
<i>Polyomaviridae</i>	polyomavirus

<i>Papillomaviridae</i>	cottontail rabbit papillomavirus
<i>Polydnviridae</i>	compoletis sonorensis virus
ssDNA viruses	
Inoviridae	Coliphage fd, acholeplasma phage L51
Microviridae	Coliphage ØX174, Bdellovibrio phage MAC1, Chlamydia phage chip 1
Geminiviridae	Maize streak virus, beet curly top virus, tomato golden mosaic virus
Circoviridae	Chicken anemia virus
Parvoviridae	Human parvo virus B19, Adeno-associated virus2, Bombyx mori virus, aedes aegypti virus
DNA and RNA reverse transcribing viruses	
Hepadnaviridae	Hepatitis B virus, Duck hepatitis virus
Caulimoviridae	Cauliflower mosaic virus, soybean mottle virus
Retroviridae	Mouse mammary tumor virus, bovine leukemia virus, human immunodeficiency virus
ds RNA viruses	
<i>Cystoviridae</i>	Pseudomonas phage 16
<i>Reoviridae</i>	Reovirus3, simian rota virus SA11, Golden shiner virus, bombyx mori cytoplasmic polyhedrosis virus, wound tumor virus.

<i>Birnaviridae</i>	Infectious pancreatic necrosis virus, Drosophila X virus	
<i>Totiviridae</i>	Saccharomyces cerevisiae virus L-A, Giardia lamblia virus, leishmania brasiliensis virus 1-1	
<i>Partitiviridae</i>	Gaeumannomyces gramanis virus, White clover virus1, white clover cryptic virus 11.	
<i>chrysoviridae</i>	Penicillium chrysogenum virus	
Negative single strand RNA viruses		
Rhabdoviridae	Rabies virus, potato yellow dwarf virus	
Paramyxoviridae	Mumps virus, para influenza virus	
Bunyaviridae	Nairobi sheep disease virus, sand fly fever silican virus, tomato spotted wilt virus	
Positive single strand RNA viruses		
Leviviridae	Coliphage MS2, coliphage Q	
Astroviridae	Human astrovirus1	
Togaviridae	Rubella virus, tobacco mosaic virus, tobacco rattle virus, barley stripe mosaic virus	
barnaviridae	Mushroom bacilliform virus, maize rayado fino virus	
Sub viral agents: satellites, viroids, prions		
Taxon:undefined, un named	Satellites	Cucumber mosaicvirus satellite
Taxon: undefined, un named	Deltavirus	Hepatitis delta virus
Taxon:undefined, un named	Viroiods	Potato spindle tuber viriod
Taxon:undefined, un named	prions	Scarpie agent

SHORT ANSWER QUESTIONS:

Refer Q1, CONTRIBUTION OF SCIENTISTS,

- | | |
|---|-----------|
| 1. Contribution of Antonyvon leeuwenhoek? | -PAGE 6 |
| 2. Contribution of Edward jenner? | -PAGE 7 |
| 3. Contribution of Louis Pasteur? | -PAGE 8 |
| 4. Contribution of Robert Koch? | -PAGE 10 |
| 5. Contribution Of Iwanowsky? | -PAGE 11 |
| 6. Contribution of Beijerinck? | -PAGE 11 |
| 7. Contribution of winogradsky? | -PAGE 12 |
| 8. Contribution of Alexander fleming? | -PAGE 13. |

UNIT-II

1)WRITE ABOUT CLASSIFICATION OF ERNST HACKEL?

ANS: Ernst Hackel was an eminent(famous) german biologist, naturalist, and philosopher.

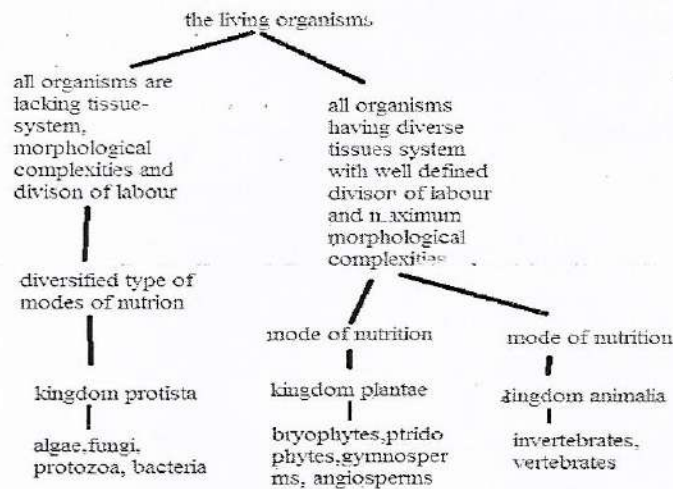
Haeckel discovered, described and named thousands of new species, mapped a genealogical tree relating to all life forms and coined many terms in biology including phylum,phylogeny,ecology and the kingdom protista.

Haeckel classified organisms into 3kingdoms On the basis of morphological complexities, tissue system and mode of nutrition.

1)Organisms lacking morphological complexities, tissue system and enjoying diversified type of modes of nutrition were placed under the kingdom protista (algae, fungi, protozoa and bacteria)

2)Organisms with autotrophic mode of nutrition were considered to be plants and were placed under kingdom plantae.

3)Those organisms having maximum morphological complexities,diverse tissue system and holophagic(phaprotrophic) mode of nutrition were considered to be animals and were placed under kingdom animalia.



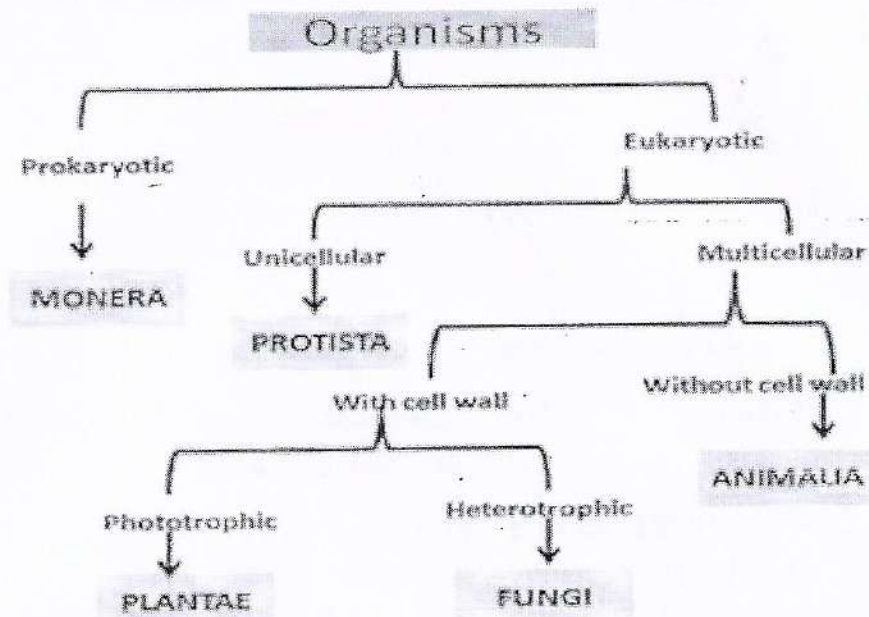
2)WRITE AN ACCOUNT ON THE CLASSIFICATION OF MICRO ORGANISMS BASING ON WHITTAKER'S FIVE KINGDOM CONCEPT?

ANS: Whittaker's five-kingdom concept:

A more recent and comprehensive system of classification, the five kingdom system, was proposed by R.H. Whittaker. This system of classification is based on three levels of cellular organization which evolved to accommodate three principle modes of nutrition: photosynthesis, absorption and ingestion.

1. The prokaryotes are included in the kingdom Monera. They lack the ingestive mode of nutrition.
2. Unicellular eukaryotic microorganisms are placed in the kingdom protista.
3. The unicellular and multicellular fungi are kept in the kingdom Fungi, based on the the absence of specialized tissues.
4. The multi cellular and multinucleate eukaryotic organisms are found in the kingdom plantae (photosynthetic nutrition, multicellular green plants and higher algae).
5. Kingdom animalia are in ingestion type nutrition. Eg: multicellular animals. Fungi nutritional mode was absorption. Eg: multinucleate higher fungi.

Robert H. Whittaker (1969)



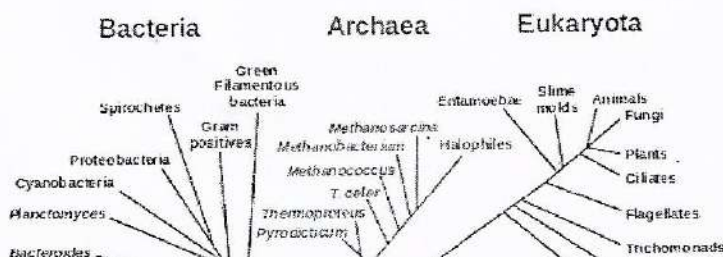
3)WRITE AN ACCOUNT ON THE CLASSIFICATION OF MICRO ORGANISMS BASING ON THE THREE DOMAIN CONCEPT OF CARLWOESE?

ANS: Carlwoese three-domain concept :

The studies of Carlwoese and his collaborates on r-RNA sequence in prokaryotic cells suggest that prokaryotes divides into two distinct groups very early on. A universal phylogenetic tree that reflects these views. The tree is divided into three majorbranches representing the three primary groups are bacteria, archea, eukarya. The primary groups are called "Domains".

1. Domain bacteria includes spirochetes, gram+ve bacteria, flavo bacteria, cyano bacteria, proto bacteria, aquifex, green non sulphur bacteria etc.
2. Domain archea includes pyrodictin, thermoproteaus, thermococcus, methanococcus, methanobacterium, halobacteria, thermoplasma etc.
3. Domain eukarya includes fungi, entamoeba, diplomonads, trichomonads, flagellates, ciliates, slimemolds, animals, plants etc.

Phylogenetic Tree of Life



4) DESCRIBE THE METHODS OF ISOLATION AND IDENTIFICATION OF MICROORGANISMS?

ANS: TYPES OF STAINING TECHNIQUES:

There are different types of staining techniques.

1. Simple staining
2. Differential staining
3. Special stains

1. SIMPLE STAINING:

AIM : To stain the bacterial cells with simple basic stain and observe the morphology.

REQUIREMENTS: 24 hours cold bacterial culture of bacillus, 1% methylene blue and glass slide.

PROCEDURE:

1. Take clean glass slide wash and dry them.
2. Place a loop full of broth of bacterial culture on the clean glass slide at the center.
3. Spread the bacterial suspension thinly on the slide with inoculating loop to prepare the smear.
4. Fix the smear by heating gently over the spirit lamp flame.
5. Stain the smear by adding few drops of methylene blue solution to the smear and allow it to stand for one to eight minutes.
6. Remove the stain and wash this smear gently with slowly running water tap.
7. Blot- dry the slide using blot paper but do not wipe the slide.

8. Examine the preparation under oil immersion objective of microscope.

2.DIFFERENTIAL STAINING:

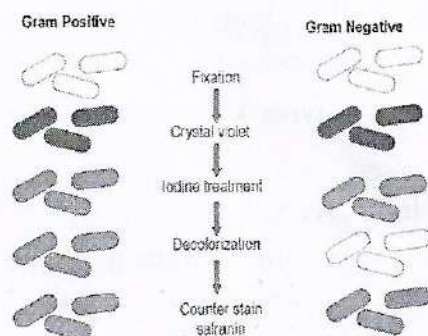
AIM: The staining differentiates bacteria into two classes. Gram positive bacteria and gram negative bacteria. In 1884 a Danish Physican Christian Gram developed a staining technique called "Grams Staining". Basing on cell wall.

REQUIREMENTS:

Glass slide, Crystal violet, Iodine, Acetone, Safranin, Water and Microscope.

PROCEDURE:

1. Take a clean grease free slide and a loop full of suspension is placed on the center of the slide.
2. Heat fix the smear by flame heating.
3. Stain the smear with basic dye like crystal violet for 30 sec. it acts as primary stain.
4. Rinse the slide with water for 2 sec.
5. It is followed by treatment with Iodine solution for 1 min it acts as moderant.
6. Rinse the smear with water.
7. The smear is decolorized by washing with ethanol or acetone. This step separates bacteria into gram positive and gram negative bacteria.
8. Gram positive bacteria retain crystal violet
9. Gram negative bacterialoss the crystal violet and become colourless.
10. Finally the smear is counter stained with basic dye called saffronin. Gram negative bacteria appears pink to red and gram positive bacteria appear purple colour.



NEGATIVE STAINING:

AIM: The size and shape of the bacterial cells can be determined by negative staining. Because it does not disturb the shape of its bacterial cells. The stain and the bacterial cells are negatively charged so there is repulsion action between the cells and dyes used are Negrocine or Indian ink.

REQUIREMENTS: Negrocine solution, sterile tooth pick, glass slide, marking pencil, inoculation loop, Bunsen burner.

PROCEDURE:

1. Take a clean and grease free slide.
2. Take a loop full of bacterial suspension and place at one end of the slide.
3. On to the bacterial suspension place a drop of Negrocine or Indian ink.
4. With the help of another glass slide spread out the dye and cells as a thin film.
5. This slide is air dried and observe under oil immersion objective. Bacteria appear brightly in black background.

1- Mix a drop of the sample with a drop of the stain.



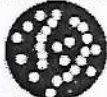
2- Spread the mixture with a clean slide.



3- Dry in air
Do not heat-fix



4- Examine under microscope



Dr. S.K. Singh

Negative staining

3.SPECIAL STAINS:

a)SPORE STAINING:

AIM: To stain the bacterial endospores.

PRINCIPLE: A number of gram positive bacteria can form a special resistant dormant structure called "endospores". Endospores develop within vegetative cells of several genera like Bacillus, Clostridium and Sporosarcina. Because spores are impermeable they are clearly visible. Endospores once stained strongly resist decolorization. This property is the basis for most spore staining methods.

REQUIREMENTS: Cultures of bacillus mycoides and E.Coli which are 48 hours old, 5% malachite green, 0.5% safranin, slide, inoculating needle, blotting paper, sprit lamp, microscope.

PROCEDURE:

1. Prepare the smear of bacteria on separate clean slides, air dry and heat fix the smear.
2. Place a drop of malachite green on smear and then heat the slides by steaming for 5 min add more stain to the smear from time to time.
3. Wash the slides under slow running tap water.
4. Air dry it to stay for 30 seconds and then wash it off.
5. Wash the stain with distilled water and dry with blotting paper.

OBSERVATION AND RESULTS:

Examine the slide under oil immersion objective of a microscope. For each preparation make drawings, indicate the position and size of spores in green and the vegetative cell in red.

b)CAPSULE STAINING:

Some bacteria have an outer layer called Capsule. Capsules are composed of polysaccharides. Capsules are clearly visible in light microscope by negative staining or by special capsule stains are employed.

PROCEDURE:

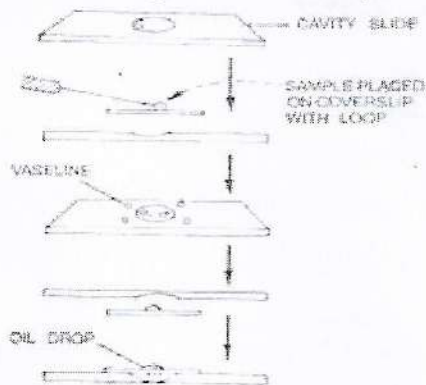
1. With the help of sterilized loop, transfer the organism on to a clean and grease free slide.
2. Air dry the suspension and flood the smear with 1% crystal violet.
3. After 5 minutes flood the smear with 20% CuSO₄ solution and air dry the slide and observe under oil immersion objective.
4. Cells appear dark purple while capsules are seen pale blue.

c)HANGING DROP TECHNIQUE:

Hanging drop technique provides information whether the bacterium is motile or not. This method is also called as "wet mount method". Hanging drop should be observed as quickly as possible.

PROCEDURE:

1. Apply Vaseline on the corners of the cover slip.
2. Place a small drop of suspension onto the center of the cover slip.
3. Take a clean cavity slide and quickly turn the coverslip into the cavity slide so that the culture drop was suspended in the cavity.
4. Vaseline forms a seal between coverslip and slide.
5. Observe motility under high power objective
6. In the hanging drop, we may see the size, shape and arrangement of the cells of microorganisms and their motility (if they are motile). At times bright refractive granules and spores may be seen with, proper light adjustment.



d) ACID FAST STAINING:

It is also a differential staining. Some bacteria like a mycobacterium do not bind simple stains readily. Cell wall of acid fast stained cells contain mycolic acid.

REQUIREMENTS: Mycobacterium species (TSA – Trypticase Soya Agar Medium) oil immersion (cedar wood oil) acid fast reagent set, staining rack, spirit lamp etc.

PROCEDURE:

1. Take a grease free clean slide and place a smear of bacteria.
2. Heat the slide gently with basic fuchsin and phenol.
3. Heating the slide helps the basic fuchsin to penetrate into the cells.
4. The cells are later treated with acid alcohol wash.
5. Acid fast cells do not colourise by acid alcohol wash.
6. Counter stain the cells by methylene blue.
7. Acid fast cells remained red and non-acid fast cells are stained as blue due to methylene blue.

SHORT ANSWER QUESTIONS:

1)WRITE GENERAL CHARACTERSTICS OF YEASTS?

ANS: General characterstics of yeasts:

1. Yeasts are unicellular fungi belonging to class Hemiascomycetes.
2. There are simplest eukaryotic organisms and have the capacity to ferment sugars into ethyl alcohol and carbon dioxide.
3. Habitat: Yeasts are widespread in their distribution and mostly live as saprophytes.
4. Morphology: Unlike other fungi the thallus of yeast is non-mycelial and consists of a single minute oval or spherical cell.
5. Cell structure: Yeast cell is surrounded by a cell wall and a cell membrane.
6. Mode of nutrition: Yeasts are heterotrophic in their mode of nutrition.
7. Reserve food materials: Glycogen granules, oil globules, protein particles are the reserve food products in yeast.
8. **Reproduction:** Yeast reproduces by means of asexual and sexual methods.
 - i. Asexual reproduction by budding, fission.
 - ii. Sexual reproduction in yeast is very simple type. It occurs during unfavorable conditions.
9. Life cycles in yeasts are haplontic, diplontic, haplodiplontic.

2)WRITE GENERAL CHARACTERSTICS OF CANDIDA?

ANS: General characteristics of candida:

1. Candida is a genus of yeasts and is the most common cause of fungal infections world wide.
2. candida albicans is after isolated from the warm-blooded animals, including humans, where it is part of the normal microbiota of mucus membranes.
3. Sometimes, this fungus may become pathogenic, causing candidiasis.
4. Candidiasis is a disease of the mucous membranes of the mouth, vagina and alimentary tract.
5. This makes fungus an opportunistic pathogen.
6. A common symptom among AIDS patient is oral thrush, where there is a huge over population of c.albicans on the back of the hosts tongue.

3)WRITE GENERAL CHARACTERSTICS OF CYANOBACTERIA?

ANS: GENERAL CHARACTERS OF CYANOBACTERIA

- 1.Previously scientists called them as blue green algae under the class cyanophyceae (or) myxophyceae.
- 2.In view of the cell structure and biochemistry the present day scientists treated them as prokaryotic bacteria.
- 3.Cyanobacteria differ from the normal bacteria due to the presence of chlorophyll-a pigment absence of flagella and in the liberation of ring photosynthesis.
- 4.Plant body is unicellular (or) mucilaginous, multicellular filament called trichomes. Some occur in colonies and appear as water blooms.
- 5.Most of the plants live in fresh water few live in marine water some lead terrestrial habitat.
- 6.The prokaryotic cell structure contains cell wall with peptidoglycans and outer mucilage.
- 7.In the absence of flagella-they show gliding movement.
- 8.cyanobacteria contains nitrogen fixing enzyme (Nitrogenase); so they are useful in nitrogen fixation by symbiotic (OR) non-symbiotic method.
- 9.Some are used as biofertilizers.
- 10.Some are used as SCP – due to the storage of food in the cells.
- 11.Nostoc commune; used as food.

4.WRITE GENERAL CHARACTERISTICS OF CHLORELLA?

ANS: General characteristics of chlorella:

- 1.chlorella is a genus of single cell green algae belonging into phylum chlorophyta.
- 2.It is spherical in shape about 2to 10 um in diameter
- 3.It is non motile with out flagella
- 4.It contains the green photosynthetic pigment chlorophyll a,b in its chloroplast
- 5.Many believe chlorella can serve as a potential source of food and energy because its photosynthetic efficiency.
- 6.Chlorella which has served as a useful tool in many investigation on photosynthesis.

5.WRITE GENERAL CHARACTERISTICS OF ENTAMEOBA?

ANS: General characteristics of Entameoba:

1. Entameobahistolytica belongs to phylum protozoa.
2. It is a histozoic, pathogenic, monogenetic and parasite that inhabits the large intestine.
3. It causes amoebic dysentery or amoebiasis in a man. The mode of infection through contaminated food and drinking water.
4. It is cosmopolitan in distribution but more common in tropical and subtropical regions of world.
5. The life cycle of E. Histolytica includes three stages trophozoite stage, precystic stage and cystic stage.
6. Trophozoite stage is the most active, motile, feeding and pathogenic stage. Cart wheel shaped nucleus is present in the endoplasm. It has an obligate anaerobic nature and mode of nutrition is holozoic (ingestion), presence of RBC in food vacuoles.
7. Precystic stage is the non-feeding and non-pathogenic stage and the cytoplasm of the precystic stage stores glycogen granules and chromatid bars which act as reserve food.
8. In the cystic stage encystation occurs and called tetra nucleate cyst or mature cyst which is the stage infective to man.
9. The trophozoites dissolve the mucosal lining by histolysin go deep into submucosa and cause ulcers.
10. Some people do not exhibit any symptoms such people are called carriers or asymptomatic cyst passers.
11. Infection of the parasite at the secondary site causes extra intestinal amoebiasis

7. WRITE GENERAL CHARACTERISTICS OF PLASMODIUM?

plasmodium:-

1. Plasmodium belongs to the phylum protozoa.

2. Plasmodium vivax is a cystozoic, pathogenic and digenetic parasite that lives in the liver cells and RBC of a man.

3. It is responsible for 43% of all malaria cases in world. It occurs in the liver cells and RBC of human.

4. Its primary host is the female anopheles mosquito and the secondary host is man and reservoir host is monkey.

5. The infective stage is sickle shaped sporozoite and the mode of infection is inoculation.

6. Four species of Plasmodium cause four types of malaria in man. The species are Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale and Plasmodium malariae.

7. Plasmodium vivax is the most common and most widely distributed malaria parasite.

8. Camillo Golgi observed Plasmodium vivax in the red blood cells of malaria patient.

10. Sir Ronald Ross a British Army doctor discovered cyst in the crop wall of female anopheles mosquito.

11. The infective stage of malaria parasite to human being is sporozoite. It was studied by Garnham and its body is covered with microtubules.

12. In man the Plasmodium reproduces asexual reproduction is called schizogony in liver cells it is called hepatic schizogony and in RBC it is called erythrocytic schizogony.

8. WRITE GENERAL CHARACTERISTICS OF LEISHMANIA?

ANS: General characteristics Leishmania Donovanii:

1. It causes Kalaazar or Dum Dum fever or Visceral Leishmaniasis.

2. The disease commonly occurs in India, Southern China, and Mediterranean countries, Africa, South America and Russia.

3. It is an intracellular parasite of reticulo endothelial system.

4.The parasite is oval measuring 1-20m in length and 2-3 m in breadth. The nucleus is central and basal granule is anterior in position. Reproduction occurs by binary fusion. After infection, the parasite migrates to the reticuloendothelial cells of liver, spleen bone marrow and visceral lymph nodes.

5.Ultimately it enters viscera and causes anemia, loss of weight, fever, hemorrhage etc.

6.Drugs that help are Sodium antimonyl gluconate, Glucontime Partostan.

Refer Q.4, METHODS OF ISOLATION AND IDENTIFICATION OF MICROORGANISMS

9.WRITE SHORT NOTE ON DIFFERENTIAL STAINING? -PAGE 25

10.WRITE SHORT NOTE ON NEGATIVE STAINING? -PAGE 26

11.WRITE SHORT NOTE ON SPORE STAINING? -PAGE27

12.WRITE SHORT NOTE ON HANGING DROP TECHNIQUE?-PAGE 28.

UNIT-III

1. DESCRIBE THE METHODS OF PHYSICAL STERILIZATION?

ANS: STERILIZATION:

The process of killing micro-organisms including their spores is known as "Sterilization"

Sterilization is of two types:

1. Physical Sterilization.
2. Chemical Sterilization.

Physical Sterilization : In this sterilization by using instruments and sunrays physically we kill the micro- organisms.

Types : 1.Sun light

2.Drying

3.Dry heat

- a)Flaming
- b)Incineration
- c)Hot air oven

4.Moist heat

- a)Pasteurization
- b)Boiling
- c)Steam under pressure

5.Filtration

- a)Candles
- b)Asbestos pads
- c)Membranes

6.Radiation

7.ultrasonic and sonic vibration

1).**Sun light** :

Sunlight possesses appreciable bactericidal activity and plays an important rôle in the spontaneous sterilization. The action is primarily due to Ultraviolet rays. Direct sunlight has an active germicidal effect due to the combine effect of Ultraviolet and heat rays. "sempleand Grieg" showed that in india typhoid bacilli exposed to the sun on piece of white drill cloth were killed in two hours. Bacteria suspended in wáter are readily destroyed by exposed of sunlight.

2).**Drying** :

Moisture is essential for the growth of bacteria. Four fifths of the weight of the bacterial cell is due to water. Drying in air has therefore, a deleterious effect on many bacteria .however this method is unreliable and is only of theoretical interest.Spores are unaffected by drying

3).**Dry heat** :

T.D.T [Thermal Death Time] : The Time required for sterilization is inversely proportional to the temperature of exposure and can be express as T.D.T.

a)**Flaming** : Inoculating loop or wire, the tip of forceps and searing spatulas are held in a Bunsen burner flame they become red hot. Inoculation loops carrying infective material may be dipped in a disinfectant before flaming to prevent spottering.

b)**Incineration** : This an excellent method for safety destroying materials such as contaminated cloth, animal carcasses and pathological material plastics such as pvc and polystyrene can dealt with similar but polystyrene materials emit cloud of dense black smoke and hence should be autoclave in Appropriate containers.

c)**Hot air oven** : Many objects are best sterilized in the absence of water by dry heat sterilization. Hot air oven is a device used for employing dry heat sterilization. Glassware should be perfectly dried and should be arranged in a manner that allows free circulation of hot air. Test tubes, flasks should be plugged with cotton wool. Other glassware such as Petri dishes and pipettes should be wrapped. Above 180°C cotton plugs would get charred. Some pharmaceutical products such as liquid paraffin, dusting powder, fats and greases are also sterilized in hot air oven. Hot air is a bad conductor of heat and its penetrating power is low when compared to moist heat. The items to be sterilized are packed in an oven at 160 to 170°C for 2 to 3 hours. Microbial death apparently result from the oxidation of cell constituents and denaturation of proteins. Although dry heat is less effective than moist heat – Clostridium botulinum spores are killed in 5 minutes at 121°C by moist heat only after 2 hours at 160°C with dry heat- it has some definite advantages. The oven is usually heated by electricity, with heating elements in the wall of the chamber. It is also fitted with a fan to ensure the equal distribution of air. It should not be overload and all the materials to be sterilized should be evenly distributed in the Hot air oven.

i. **Tyndalization**: In this process media has to sterilize when the media is exposure to 100°C for 20¹ on three successive days.

1st day- 100°C for 20 to kill vegetative cells

2nd day- 100°C for 20 remove all spores

3rd day- 100°C for 20 to kill all micro organisms.

It is also called fractional sterilization.

Temp Time
160 45
170 80
180 7.5
190 1.9

Autoclave : The principle of the auto-clave or steam sterilizer is that water boils when its vapour pressure equals that of the surrounding atmosphere.

Auto-clave is a cylindrical device made of steel or gunmetal. The vapour is equal to outside of the temperature. (The visible temp and inside vapour temp.) It kills the micro-organisms and vegetative spores at temp 121°C for 15 minutes at 15lbs pressure .

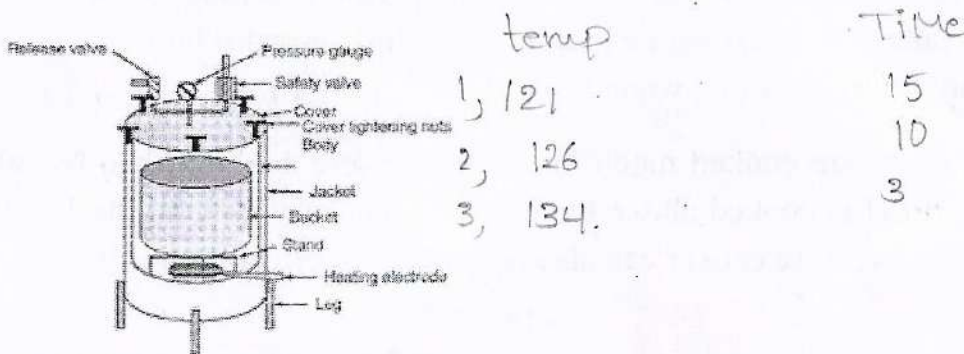


Fig. 25.2. A schematic diagram of laboratory autoclave.

Steam sterilization is carried out with an autoclave, a device like a fancy pressure cooker. Free flowing steam under pressure requires the use of an autoclave, a double walled metal vessel that allows steam to be pressurized in the outer jacket (fig.4.3). At a designed pressure, the saturated steam is released into the inner chamber. The steam under pressure in inner chamber is now capable of achieving temperatures above 100°C. The temperature is determined by the pounds of pressure per square inch(psi).

Water in the autoclave is first boiled to produces steam which is released through the jacket into the chamber. The air initially present in the chamber is forced out until the chamber is filled with saturated steam and now the outlets are closed. Hot, saturated steam continuous to enter until the chamber reaches the desired temperature and pressure, usually 121°C and 15lbs respectively. At this temperature saturated steam destroys all vegetative cells and endospores in a small volume of liquid with in 10to 12 minutes.

Pressure cooker : pressure cooking is a method of cooking in a sealed vessel that does not permit air or liquids to escape below a preset pressure. Because the boiling point of water increases as the pressure increases, the pressure built up inside the cooker allows the liquid in the vessel to rise to a higher temperature before boiling.

In the food industry, pressure cookers are often referred to as retorts. Pressure cookers are generally made from aluminum or stainless steel. Stainless steel has lower thermal conductivity. A gasket or sealing ring forms a gas-tight seal which does not allow air or steam to escape between the vessel and the lid. A safety valve is provided as a backup escape route for steam. The food to be cooked is placed in the pressure cooker, with a small amount of water.

Most pressure cookers have a working pressure setting of about one atmosphere (100 kPa or 15 psi gauge pressure). At this pressure boost relative to sea level atmospheric pressure, water boils at 125°C (257°F).

Advantages: foods are cooked much faster by pressure cooking than by other methods. The food is cooked above the boiling point of water, killing bacteria and viruses. The pressure cooker can also be used as an effective sterilizer.

Filtration:

An especially valuable technique for sterilizing heat sensitive materials is filtration. Filtration has been used since Pasteur's time to separate bacteria from media and to sterilize materials that would be destroyed by heat. As viruses pass through ordinary filters, filtration can be used to obtain bacteria free filtrates. Filter discs also help to concentrate bacteria from liquids. For example in testing water samples for cholera vibrios or typhoid bacilli. Bacterial toxins can be obtained by passing cultures through filters. The following types of filters have been used.

They are two types of filters:

1) Depth filters 2) Membrane filters

1) **Depth filters:** one of the oldest types used is the depth filters. On the depth filters micro organisms are retained either by electrostatic attraction or by absorption into the filter.

a) **Candle filters:** These are manufactured in different grades of porosity. These are widely used in purification of water for industrial and drinking purposes. These are two types unglazed ceramic filters and diatomous earth filters.

b) **Asbestos filters:** They are made from chrysotile type asbestos chemically composed of magnesium silicate. The filter is attached to a vacuum flask. The whole unit is wrapped and autoclaved.

c) Sintered glass filters: they are made of finely ground glass which are fused to make particles adhere. They are available with different pore sizes.

2) **Membrane filters:** the most common type of filter for sterilization in the field of microbiology is the membrane filter. Membrane filters are composed of polymers with high tensile strength such as cellulose esters. They can be manufactured with specific pore sizes from 25µm to less than 0.025µm.

HEPA Filters: High Efficiency Particulate Air filter (HEPA) can remove 99.7% of 0.3µm sized particles that is employed in **laminar air flow** cabinets. These cabinets force the air through HEPA filters, and then project a vertical flow of air across the opening of cabinet. A person uses this cabinet while working with pathogenic agents. They are also used in research labs and industries, such as pharmaceutical industry when a sterile working surface is needed for preparing media, examining tissue cultures etc. These filters are also used in ventilation systems of areas where microbial control is essential, such as operating room and burn units.

RADIATION: An effective way to sterilize or reduce the microbial burden in almost any substance is through the use of electromagnetic radiation. Radiation may be defined as the emission or propagation of energy through space or through a medium. The various types of radiations are separated on the basis of their wavelengths. There are two types of radiations used for sterilization: ionising and non ionising radiations. X-rays and gamma rays are major ionising radiations.

UV rays: UV light consists of light of wavelengths between 200 and 400nm. Sunlight can destroy microorganisms because it contains shorter wavelengths and high energy are more damaging to the living cells. Many lamps are available which emit a high concentration of UV rays. They are called as germicidal or sterilizing lamps.

UV light is of limited use because it does not penetrate glass, cloth, paper and most other materials. UV light can be turned on when the room is not in use because exposure to UV light can cause burns and can also damage the eyes. Years of exposure to UV light can cause skin cancers.

Gamma rays: gamma sterilization is carried out at specialized radiation plants. Gamma radiations are generated from the radio active isotope of cobalt. When

the isotope disintegrates, it emits two gamma rays in succession each of which has mean energy of 1.25Mev.

The gamma rays cause both excitation and ionization by targeting vital molecules present in the cell. The death of a cell takes place because of ionization of target molecules present either inside or outside the cell.

The unit of sterilizing dose of gamma rays is kilo Gray.(1Gray=100 radi) the sterilizing dose is between 18-25kgray and the exposure time is about few seconds.

Ultra sonic and sonic vibrations: organisms may be subjected to another type of radiation called acoustic radiation. Microorganisms not susceptible to normal sound. However, some are sensitive to ultrasonic waves of 20kilocycles/second.

The disruption of cells by sound waves called sonication. ultrasonic and sonic radiations are credited with bactericidal powers but the results have been variable.

2. DESCRIBE DETAILED ACCOUNTON CHEMICAL STERILIZATION?

A. Disinfection is the process of killing, inhibition or removal of micro organisms that may cause disease. The chemical agents that are used for this disinfection are called as chemical disinfectants. Disinfectants are effect and toxic in destroying pathogenic microorganisms but not necessarily in killing the resistant spores.

The major disinfectants that are commonly used are 1.phenol and phenolic compounds 2.alcohols 3.halogens 4.aldehydes 5.hypochlorites

1.phenol and phenolic compounds: phenol is the first disinfectant used by joseph lister, in 1880's. Phenol and phenolic compounds are very effective disinfectants. A 5% aqueous solution of phenol rapidly kills the vegetative cells of microorganisms.

Derivatives such as cresols, xylenols and orthophenyl phenol are used as disinfectant in laboratories and hospitals.

Lysol is a commercial disinfectant prepared by mixture of phenolics. Depending on the concentration in which they are used, phenolic substances are either bactericidal or bacteriostatic. Some phenols are high fungicidal.

The primary mode of action is to damage the cytoplasmic membrane followed by the leakage of constituents from the cell.

2.Alcohols: alcohols are one of the most widely used disinfectants and antiseptics. They are bactericidal and fungicidal but not sporicidal. The two most popular disinfectants are ethanol and isopropanol. They are effective when used in 70-80% concentration. They act by denaturing proteins and possibly by dissolving membrane lipids. Methyl alcohol is effective against fungal spores.

Alcohols are effective in reducing the microbial flora of skin and for the disinfection of clinical oral thermometers.

3.Halogens:The important halogens used as disinfectant agents are iodine and chlorine and their compounds. Iodine is one of the oldest and most effective disinfectants. At higher concentration it may even kill some spores. Iodine is usually applied in the form of tincture of iodine. It may cause skin disease. Iodophors are the preparatives of iodine complexed with some organic carriers. They are used in hospitals for preoperative skin degerming and in laboratories for disinfection.

Mode of action is an inactivation of essential metabolic compounds such as proteins with sulphohydral groups as they are highly oxidising agents.

4.Aldehydes: several aldehydes having low molecular weight are used as disinfectants. The two important and widely aldehydes are formaldehyde and glutaraldehyde. These are highly reactive molecules that combine with proteins and make them inactive. These are reactive molecules and act as microbicidal as well as sporicidal. Formaldehyde usually dissolved in water or alcohol before used. It is active against the amino group in the protein molecule.

Glutaraldehyde is an effective disinfectant. it used as 2% buffered solution. It causes less irritation than the formaldehyde. It inhibits wide spectrum of antimicrobial activity against bacteria, fungi, spores and viruses. Glutaraldehyde disinfect the object with in 10minutes of time. It is used for sterilizing urological instruments, lensed instruments, respiratory therapy equipment medical fields.

5.Hypochlorites: chlorine is the closely disinfectant for municipal water supplies and swimming pools. It is also employed in the dairy and food industries. It may be applied as chlorine gas, sodium hypochlorite, calcium hypochlorite and hypochlorous acid. But it most markedly used as

Hypochlorites which has a broad spectrum against all kinds of microorganisms. Chlorine gas is used to disinfect water and requires special equipment. It is applied to drinking water in a final concentration of 0.1 to 1 part per million.

Calcium and sodium hypochlorites are widely used for disinfection of dairy equipment and eating utensils in restaurants. The germicidal action is due to the formation of hypochlorous acid when free chlorine reacts with water.

SHORT ANSWER QUESTIONS:

REFER Q.1, PHYSICAL STERILIZATION

- | | |
|--|----------|
| 1. Write short note on hot air oven? | -PAGE 35 |
| 2. Write short note on autoclave? | -PAGE 36 |
| 3. Write short note on pressure cooker? | -PAGE 37 |
| 4. Write short note on laminar air flow? | -PAGE 38 |
| 5. Write short note on gamma rays? | -PAGE 39 |
| 6. Write short note on UV rays? | -PAGE 39 |
| 7. Write short note on filter sterilization? | -PAGE 38 |
| 8. write short note on phenol coefficient? | |

ANS: Phenol coefficient:

The best known disinfectant screening test is phenol coefficient test in which the potency of the disinfectant is compared with that of phenol.

The phenol coefficient is defined as the killing power of a disinfectant against the test organism as compared to that of phenol under ideal conditions.

A series of dilutions of phenol and similar dilutions of disinfectant are prepared.

Phenol coefficient is then calculated as the ratio of the highest dilution of the disinfectant killing organisms in 10 minutes to the corresponding dilution of phenol that will kill under the same conditions.

The higher the phenol coefficient, the more efficient will be the disinfectant. A value greater than 1 means that the disinfectant is more effective than phenol.

Eg; phenol- 1

Ethanol-0.4.

UNIT-IV

1.EXPLAIN THE METHODS OF ISOLATION OF PURE CULTURES?

ANS: Isolation of pure cultures:

By definition, a pure culture is a population of cells that arises from a single cell and is free from contamination with other species.

A pure culture of species can be prepared by isolating the particular species from a mixture of cells or species.

The pure culture technique is initiated and developed by the German bacteriologist "Robert Koch".

Importance of pure culture :-

Pure cultures are necessary and important for the following reasons.

1. Identification of the species.
2. Analysis of food, water, industrial products.
3. Sterility testing of products destined for human eye.
4. Assay of antibiotics and vitamins.
5. Determination of antibiotic sensitivities of pathogens isolated from patients.
6. Preparation of biological products or materials used for immunizations etc

Enrichment culturing:-

An enrichment culture is a particular micro organism that grows on a medium with specific and known qualities. The enrichment culture's environment will support the growth of selected microorganism, while inhibiting the growth of others. Martinus Beijerinck is credited with developing the first enrichment cultures. Winogradsky also experimented on bacteria using different cultures.

The principle of enrichment culture is to control the nutrients and culture conditions (temperature, air supply, light, pH, etc.,) in such a way that it suits only to the given species. The essence of this technique is to provide growth conditions that are very favorable for the organism of interest, and as unfavorable as possible for competing organisms.

For example, using the enrichment culture technique when trying to isolate a halophile, streak plates of varying high salt concentrations (an enriched medium) are used. The halophile will thrive on the salty media, while other organisms will not be able to tolerate it and will die out.

Isolation methods are of three types, they are:-

1. Plating technique
2. Serial dilution technique
3. Micro manipulator technique

1. Plating techniques: These are again divided into three types

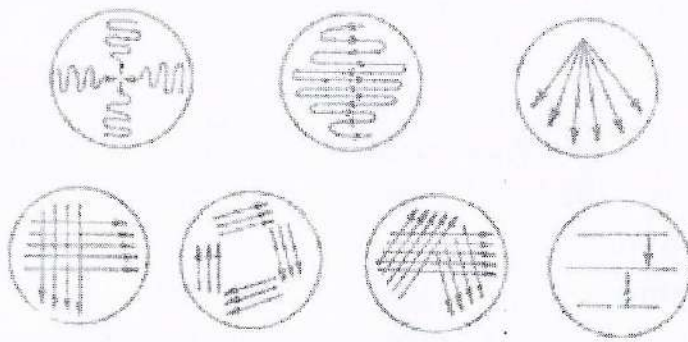
- a. Streak plate method
- b. Spread plate method
- c. Pour plate method

a. Streak plate method: This is the most practical method for the isolation of pure cultures. It was developed by two bacteriologists Foietrich and Loeffler in the laboratory of Robert Koch. The principle involved in this technique is thinning a dilution of inoculum along the successive streaks.

In this method a small amount of sample from a mixed inoculum is transferred to the surface of the suitable agar medium with the help of inoculating loop. Then the inoculum is streaked on the surface of agar medium and incubated. After incubation, isolation colonies appear on the agar surface at the end of the streaks.

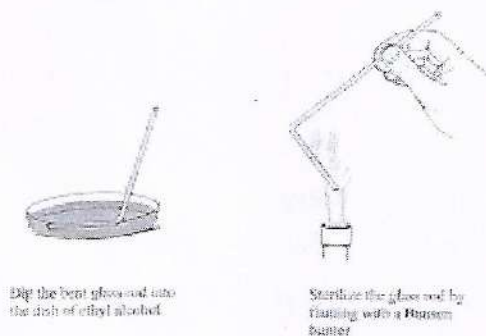
Depending on the mode of streaking there are different types of streak plate techniques. Some of them are:

- i. Clock plate technique
- ii. Radiant streak method
- iii. Spiral streak method
- iv. Zig-zag streak method



Of these methods, clock plate technique is the most common and widely used method.

b. Spread plate method: It is an easy and direct method. A loop full of technique containing a suspension of micro-organisms is placed on the centre of the agar plate and it is spread on the surface with the help of "L" shaped glass rod. The glass rod is sterilized by dipping in alcohol. When the suspension is spread over the plate of individual micro-organisms are separated by one another for this separation, it is necessary to dilute the suspension before application to prevent overcrowding growth of micro-organisms. After incubation colonies are picked up and streaked on to a fresh medium to ensure purity.



Dip the bent glass rod into the dish of ethyl alcohol.

Sterilize the glass rod by flaming with a Bunsen burner.

c. Pour plate method: This method developed by Robert Koch. In this method successive dilution ensures the development of separated colonies upon plating on agar surface. A small volume of diluted sample is mixed thoroughly with liquid agar medium which is cool about 45° C. Then the mixture is poured into sterile petri plates and allowed to solidify as the medium solidifies the microbial cells get fixed in position. These trapped cell develop into individual colonies on incubation growth of colonies occurs both at the surface and sub surface areas of medium. The pour plate technique is used for qualitative studies to enumerate the microbial cells in the sample.

Note: The main disadvantage of this method is the difficulty of removing sub surface colonies for further colonies.

2. Serial dilution technique:-

The purpose of serial dilution is to reduce the number of micro organisms So that isolated small amount is called the serial dilution. Serial dilution technique is used in water, soil, milk and other industrial purposes.

This method is based on the principle that material containing bacteria is cultured. Every viable bacteria develops into a visible colony on a nutrient agar medium the number of colonies is the number of micro organisms contained in the sample.

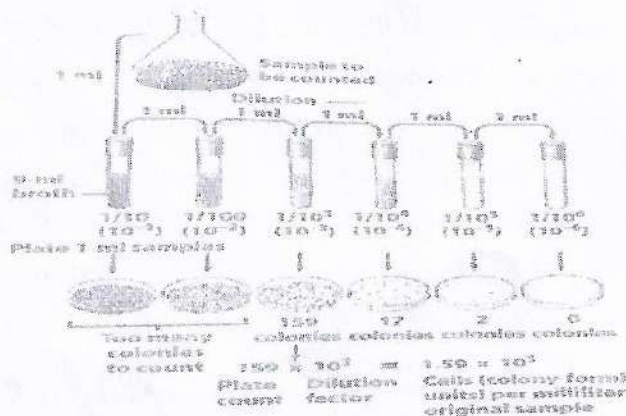


Fig. 5.2 Dilution Plating technique

Procedure:-

1. At first take some amount of inoculum in a sample test tube and take several test tubes with 9 ml of distilled water in each.
2. After that transfer 1ml of inoculum with a 1ml sterile pipette to the first test tube and is marked as 10^{-1} as an indication for dilution.
3. From the first dilution transfer 1 ml sample to the second test tube and marked as 10^{-2} diluting the original specimen to 100 times.
4. Repeat this dilution of the original sample of Upto 10^{-7} thus using each time a fresh pipette.
5. After appropriate dilution (10^{-6} , 10^{-7}) transfer the 0.1ml of diluted sample on to the petriplates and count for number of colonies as per the calculations. A single dilution calculated as follows

Dilution = volume of the sample / total volume of the sample and diluent

The number of micro organisms per ml calculated as follows

No. of cell/ml=no. of colonies/ dilution volume plated.

3. Micromanipulator technique:

This technique involves the isolation of a single cell from the mixed microbial population. Some times this method is also called as “single cell dilution” technique. For the isolation of a single cell by this method, a device namely micromanipulator is used. This method is more convenient to isolate large celled micro organisms such as algal and protozoa and harder to isolate bacteria.

2. DESCRIBE THE METHODS OF PRESERVATION OF MICROBIAL CULTURES?

ANS: Preservation of microbial structures

Microorganisms are usually grown or multiplied in laboratories.

By using artificial media to study various aspects of scientific and research interests. This multiplication process of microorganisms is known as “culturing” and the population of cells thus cultured is called as “culture”. The culture may be mixed culture, if it contains a population of more than one type of species. If a culture contains a population of single type of species it is called “pure culture”.

Pure culture of species is defined as a population of identical cells that arise from a single cell.

Sub culturing:

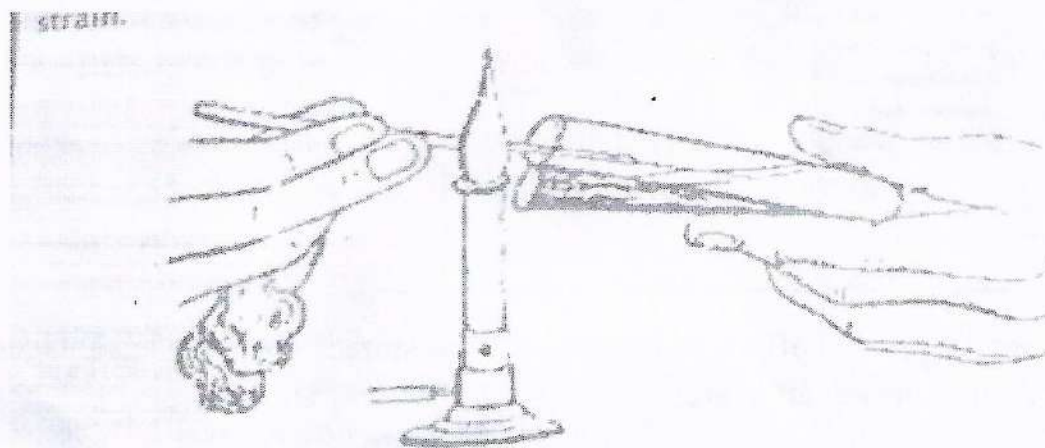


Fig : Method of Subculturing

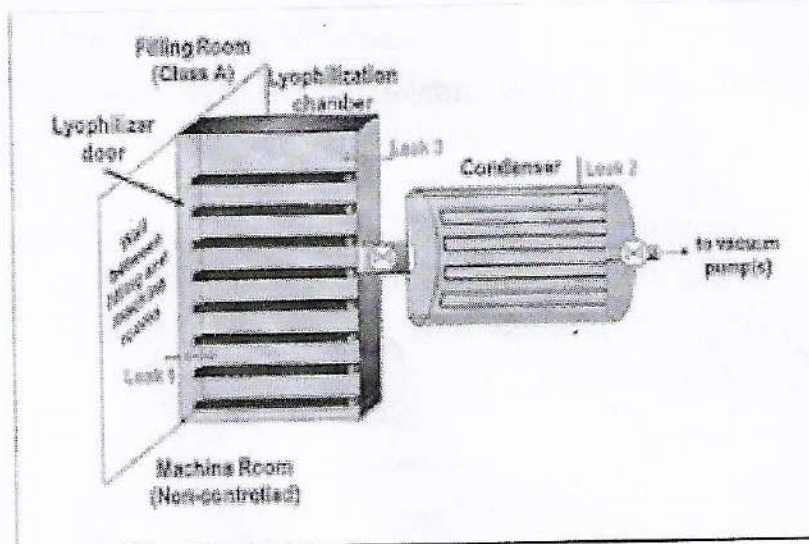
Storage under mineral oil:

This is a simple method for preserving the cultures of many bacteria and yeasts by storing them on agar slopes covered with sterile mineral oil. The oil used in medicinal grade liquids paraffin. In this way of protection, the interval between sub-culturing may after be extended for several years and upto 20 years for some fungi at the culture mineral substances on the highly preservation rates. -

Lyophilization:

In this sublimation process occurs solid to vapour state.

Many bacterial species that are killed by ordinary drying can be preserved even more than 30 years by following the freeze drying process known as lyophilization. It is a process of rapid freezing and rapid dehydration of biological specimens in a high vacuum state in this process a dense cell suspension is placed in small vials and then allowed to freeze at -60 to 78°C temperature. The vials are connected to a high vacuum line and frozen to -60 to 78°C . The ice in the frozen suspension sublimates under the vacuum. These results in dehydration of the bacteria with a minimum damage to delicate all structures.



The vials are then sealed off under the vacuum and stored. The main advantages of this method of preservation are:

- I. Occupation of less space for storage.
- II. Easy transportation to other places.

This method will not cause any changes in characteristics of the strain. However, loss of vacuum in the vials during storage leads to inactivation of cultures.

Storage at low temperature:

In this method, a dense cell suspension is frozen in the presence of protective agent such as glycerol or dimethyl sulfoxide in liquid nitrogen (-196°C). This method is mainly useful in the case of bacterial cultures that cannot be preserved by "lyophilization". However, this method is relatively expensive as it involves the use of liquid nitrogen.

Bacterial cultures can be viable for 10-30 years. When stored by this method.

Storage in sterile oil:

This method is widely used for preserving spore forming bacteria and fungi. Spore suspensions are added to sterile oil and the mixture is dried at room temperature. Then it is stored in a refrigerator. Bacterial cultures maintained by this procedure are found to be viable for 70 – 80 years.

Storage in silica gel: In this method, finely powdered heat sterilized and cooled silica powder is mixed with a thick suspension of cells and stored in a silica gel powder at low temperature for a period of 1 to 2 years.

Cryo preservation:

Helps survival of pure cultures for long storage times. In this method the microorganisms of culture are rapidly frozen in liquid nitrogen at -196°C . In the presence of stabilizing agents such as glycerol, that prevents the formation of ice crystals and promote cell survival.

SHORT ANSWER QUESTIONS:

Refer Q.1, methods of isolation of pure cultures

1. WRITE SHORT NOTE ON STREAK PLATING TECHNIQUE? –PAGE 44
2. WRITE SHORT NOTE ON SPREAD PLATING TECHNIQUE? –PAGE 44
3. WRITE SHORT NOTE ON SERIAL DILUTION PLATING TECHNIQUE?
-PAGE 45

Refer Q.2, preservation of microbial cultures

4. WRITE SHORT NOTE ON LYOPHILIZATION? –PAGE 47.

UNIT-V

1.DESCRIBE ULTRA STRUCTURE OF BACTERIAL CELL?

ANS: Microorganisms exist as either Prokaryotes or *Eukaryotes*. The prokaryotic cells are morphologically much simpler than eukaryotic cells. The prokaryotic cell lacks internal membrane bound organelles such as mitochondria and chloroplast etc. Though simple, structures are not found in every prokaryotic organism. A cell membrane lies below the cell wall and surrounds the inner cytoplasmic matrix. This cytoplasmic matrix includes unbounded *nucleoid*, *ribosomes* and *some inclusion bodies*. External to the cell wall there occurs extra components possessing different functions. The most typical example for prokaryotic cell organization is the bacterial cell.

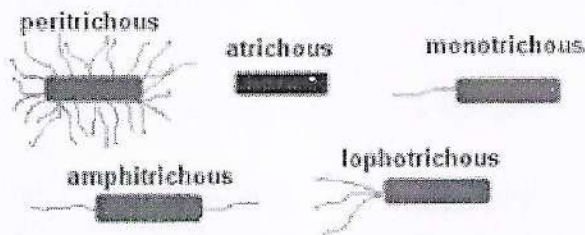
Structurally a bacterial cell consists of three categories of structures namely.

1. Structures external to the cell wall.
2. Cell wall
3. Structures internal to the cell wall

1. **Structures External to Cell Wall:** These include *flagella*, *fimbriae* and *pili* and *capsule* and *slime layers*.

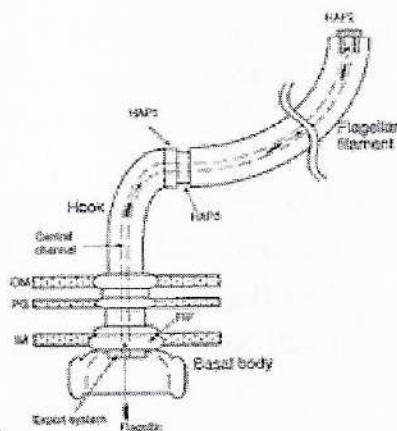
FLAGELLA: Majority of the motile bacteria move by the use of structures namely Flagella. The Flagella are thin, thread like, helical surface appendages that extend outward from the plasma membrane and cell wall. These appendages are *locomotory* in function and involve in motility of bacteria. Flagella are slender in nature with 0.01 to 0.02 μ m diameter and 15-20 μ m length.

The number and distribution of Flagella varies among the species of bacteria. Depending on the distribution of Flagella are of different types:



1. Polar Flagellation(mono trichous): The bacterial cell consists of flagellum located at one end or one pole e.g. *Pseudomonas*.
2. Amphitrichous flagellation: A single flagellum is located at each pole or end of the bacterial cell e.g. : *Aqua spirillum*
3. Lophotrichous Flagellation: A tuft or cluster of flagella are located at one or both ends of the cell e.g. : *spirillum, pseudomonas*.
4. Peritrichous flagellation: A number of flagella are spread fairly evenly over the surface of the cell. e.g. *Proteus, Salmonella typhi*.

Structurally, a flagellum is composed of 3 parts i.e, filament, hook and basal body.



Filament:

The filament is a fine, cylindrical hollow structure about 120-200 \AA in diameter. The outermost structure of the flagellum is the filament. Filament is a helical structure with 14nm diameter. It is composed of subunits or monomers of fibrin protein known as flagellin. The molecular weight of the flagellin is about 40,000daltons. The shape of the flagellum is dependent on flagellin. The flagellum grows at its tip rather than at the base as the newly synthesized flagellin monomers are added at the distal end of the filament.

Hook:

The hook is the part that lies between the filament and basal body of the flagellum. The hook is slightly wider than filament and is about 45nm in

length. It is made up of protein subunits. Which are antigenically different from that of flagella of filament.

Basal body:

Basal body is the most complex part of a flagellum. The basal body is located entirely within the cell envelope. The basal body consists a small central rod inserted into a system of rings. The system of rings, however, differs between gram+ve and gram -ve types of bacteria. The outer pair called 'L' and 'P' rings remain embedded in the lipopolysaccharide and the peptidoglycan layers. The inner pair namely 'S' and 'M' rings are located within the cytoplasmic membrane.

But in Gram +ve bacteria, the basal body bears only one pair of rings namely 'S' and 'M' rings. The 'L' and 'P' rings are absent.

Fimbriae and pili:

Fimbriae and Pili are thin, hair like non-flagellar surface appendages. Like that of flagella, they are also composed of protein subunits but do not involve in motility. They originate from cell membrane and extend outward through cell wall.

Fimbriae commonly occur among Gram-negative bacteria of Enterobacteriaceae and Pseudomonades. The number of fimbriae may range from very few to thousand. Fimbriae are considerably shorter than flagella. They do not involve in transfer of genetic material. They are believed to involve in the sticking of organisms to inert surfaces.

Pili are structurally similar to fimbriae but larger than fimbriae. The number of pili per cell range from one to few. The pili are made up of specific protein subunit known as pilin. There are 2 types of pili viz., ordinary pili and sex pili. Both are morphologically similar but the sex pili consists of different type of pili monomers. The function of ordinary pili is the adhesion to host surfaces which is an essential step in infection. The sex pili involve in mating process and transfer of genetic material.

Capsule and slime layers:

Many prokaryotic organisms, especially some bacteria secrete large amounts organic polymers may be slimy or gummy in nature and

generally called as “glycocalyx”. If the layer of glycocalyx is rigid, condensed, and closely surrounding the cell, it is called as Capsule. If the glycocalyx layer is loosely attached to cell wall, it is called as slimelayer. The main functions of capsule slime layer are:

- Involvement in attachment of pathogens to their hosts.
- Protection from desiccation.
- Protection for encapsulated bacteria from phagocytosis.

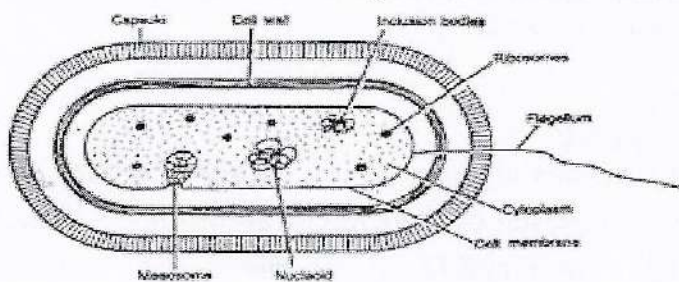


Fig. 12.2 Ultra structure of subbacterial cell

2.cell wall:

The cell wall is one of the most important layers of the prokaryotic cell. In bacteria, generally, the layer or layers of cell envelope laying between the inner cytoplasmic membrane and the capsule is called as cell wall. The main functions of the cell wall are:-

- It prevents the lysis or rupture of bacteria due to osmotic pressure.
- It gives shape and rigidity to the cell.
- It provides solid anchoring support to flagella.
- It contributes to the pathogenicity of many pathogens.
- It can protect a cell from toxic substances.
- It is the site of action of several antibiotics.

Basing on the difference in to gram's staining, reaction bacteria are divided into two groups namely gram +ve and gram -ve.

This difference is attributed to the difference in peptidoglycan layer and teichoic acids. The gram -ve cell wall is comparatively complex and consists of peptidoglycan outer membrane. Peptidoglycan layer is common in both gram+ve and gram -ve types of cell walls.

Peptidoglycan:-Peptidoglycan is called as murein. It is a complex polymer composed of various components. They are (1) back bone (2) tetra peptide side chains, and (3) peptide cross bridges.

1)Back bone:-

The back bone of the peptidoglycan layer is composed by two acetylated amino sugars namely N-acetylglucosamine and N-acetyl muramic acid arranged in alternate fashion.these are connected by β -1,4 glycosidic bond. These sugar derivatives are also known as murein saccules.

2)tetra peptide side chain:-

It is a short chain of peptides attaches to the carboxyl group of N-acetyl muramic acid. The chain is composed of 4 different amino acids namely L –alanine, D – glutamatic acid, L – lysine and D –alanine in that order of 1,2,3 and 4 positions. In some bacteria the L – lysine in the 3rd position is replaced by Diaminopimelic acid.

3) Peptide cross bridge:-

These cross bridges are the cross links between the adjacent tetrapeptide side chains projected from muramic acid. This cross links between the carboxyl group of terminal D- alanine of one side chain and the 3rd amino group of another side chain. In gram + ve cell wall the cross linking is by a peptide inter bridge which is usually formed by five molecules of glycine amino acid.

The peptidoglycon in gram+ve bacteria is many layers in thickness. The usually composed of about 40 sheets or chains of glycan. In gram -ve bacteria the peptidoglycon is very thin with only 1 or 2 sheets of glycan. The side chains and inter bridges contribute to the rigidity and strength of peptidoglycon layer.

Teichoic acid:The peptidoglycon matrix of gram + ve bacteria consists of special components called as teichoic acids. They are highly acidic, water soluble poly saccharide polymers. It is connected to either the peptidoglycon or plasma membrane lipids.teichoic acids confer negative charge to bacterial surface and also involve in maintenance of structure of the wall.

Outer membrane:-

This outer membrane occurs only in gram –ve bacteria. The layer that lies outside the peptidoglycon is called outer membrane. The outer membrane is strongly anchored to the underlying peptidoglycon.

The outer membrane is a phospholipid bilayer. The outer layer of phospholipids replaced by lipopolysaccharide molecules. Like the plasma membrane, the outer membrane is also fluid mosaic in nature with some specific proteins embedded in phospholipid matrix. The outer membrane consists of specific and non-specific porin proteins and non-porin proteins that play a role in permeability of different molecules through membrane. The lipopolysaccharide component of outer membrane consists of both lipid and carbohydrate distributed in 3 different parts. They are (1) lipid-A (2) core polysaccharide (3) o- side chain.

Functions of Lipopolysaccharides:-

1. It contributes to the negative charge on the bacterial surface.
2. It is extremely toxic to animals and often called as endotoxin.
3. The o-antigens of lipopolysaccharides can also serve as receptor for bacteriophage attachment.
4. Many serological properties of gram – ve bacteria are attributable to o-antigens of lipopolysaccharides.

3. Structures internal to cell wall:

The structures that present interior to the cell wall include cell membrane, mesosomes, cytoplasm, nuclear material, cytoplasmic inclusions, vacuoles etc.

Cell membrane:

The cell membrane is the limiting layer of the cytoplasm of the cell. This is also called as plasma membrane or cytoplasmic membrane. The cell membrane is a vital structure and critical barrier that separates the inside of the cell from the outer environment. Unlike the cell wall, it shrinks under high osmotic pressure. The most widely accepted current model for cell membrane structure is the fluid mosaic model proposed by Singer and Nicholson (1974).

Cell membrane is a composite, unit membrane which is trilaminar in nature and 7-8 nm in thickness. It is largely lipoprotein in nature. It is composed of 20-30% phospholipids and 60 to 70% proteins.

Plasma membrane –fluid –mosaic model:-

In 1974, Singer and Nicolson proposed the fluid mosaic model, which is now widely accepted as the best model for explaining the structure and properties of the plasma membrane. According to this model, the membrane is a bilayer of phospholipid molecules in which globular proteins are embedded. The proteins have been compared to icebergs floating in a sea of the phospholipid bilayer. Thus, biological membranes are considered to be quasifluid structures in which lipids and integral proteins are arranged in a mosaic manner.

Mesosomes:-

Plasma membrane of bacteria invaginates to form vesicles or tubular or lamellar structures called mesosomes. These invaginations are called as mesosomes.

The main functions of the mesosomes are:

1. They involve in wall formation during cell division.
2. They play a role in replication of chromosomes and distribution to daughter cells.
3. Mesosomes also involve in secretory processes.
4. The formation of mesosomes is a pre-requisite in sporulating bacteria.

Nuclear material:

The prokaryotic cell is strikingly different from eukaryotic cell in the lack of a well defined nucleus. The genetic material in prokaryotes is not well defined and it is not membrane bounded. The size of the prokaryotic DNA is smaller than eukaryotic DNA and is 5×10^6 Daltons in molecular weight.

In addition to this main DNA material, many bacteria possess other genetic material called as plasmids. The plasmid is non chromosomal genetic material. Plasmids are circular, double stranded DNA molecules with independent replicating capacity. They are smaller in size. They confer some properties to organisms such as drug resistance, special metabolic abilities etc.

Ribosome's:

The ribosomes distribute in cytoplasmic matrix. They are either free in cytoplasm or some loosely attach to the plasma membrane. The ribosome's are 70s type with 50s and 30s subunits. The ribosome's are the centers for protein synthesis.

Chromosomes:

Chromosomes are the pigments that are present in a series of cigar-shaped vesicles.

Carboxysomes or polyhedral bodies:

The photosynthetic bacteria such as cyanobacteria, certain purple bacteria and chemoautotrophic bacteria such as nitrifying bacteria and Thiobacilli contain structures known as polyhedral bodies.

Magnetosomes:

Magnetosomes are the magnetic power sensing organelles. The magnetosomes are the uniformly shaped structures enveloped with crystals of magnetite. They are found in a group of bacteria having the magnetotactic nature. These bacteria tends to move to one of the magnetic poles when placed in magnetic field.

Gas Vacuoles:

A number of cyanobacteria, purple and green photosynthetic bacteria and a few other aquatic forms such as Halo bacterium and Thiothrix contain a membranous structures and gas vacuoles. These organisms exhibit the floating nature called buoyancy due to the presence of these vacuoles. The vacuoles are the aggregates of a number of small, hollow, cylindrical structures called gas vesicles.

Granular cytoplasmic inclusions:

In prokaryotic cells, a Variety of cellular reserve materials are present. They usually occur as granular cytoplasmic inclusions. Eg: Poly-b-hydroxybutyric acid granules, Glycogen granules, Polyphosphate granules', Sulphur granules.

2.DIFFERNTIATEPROKARYOTES AND EUKARYOTES?

ANS:

Charazcters	Prokaryotes (cell)	Eukaryotes(cell)
1.cell size	Mostly 1-10 μm (small)	Mostly 10^4 - 10^5 μm (large)
2.Nucleus	Incipient nucleus is present	True nucleus is present
3.Nuclear membrane	Absent	Present
4.Nucleolus	Absent	Present
5.chromosome	Single circular chromosome is present.	Many linear chromosomes are present.
6.plasma membrane	Usually lacks sterols	Sterols are usually present.
7.Cell wall	Present in most but not in all cells	Present in plant and fungal cells only. Absent in animal cells.
8.zygote	Zygote nature is partially diploid	diploid
9.Cellular organelles:		
(i) Mitochondria	Absent	Present
(ii) Endoplasmic Reticulum	Absent	Present
(iii) Vacuoles	Absent	Present
(iv) Lysosomes	Absent	Present
(v) Chloroplasts	Absent	Present (only in plants)
(vi) Centrioles	Absent	Absent in higher plants
(vii) Ribosomes	Present (70S)	Present (80S)
(viii) Microtubules	Absent	Present
(ix) Flagellae	Simple structure composed of the protein flagellin	9+2 structure of tubulin and other protein
10.Respiratory system	Part of plasma membrane or mesosomes	Mitochondria
11.gas vesicles	Present in some	Absent
12.sexual reproduction	Fragmentary process, no meiosis, only portion of genetic material is usually	Regular process, meiosis occurs, reassortment of whole chromosome complement.

13.plasmids	reasserted. Commonly present	Rare.
14.genetic recombination	Unidirectional transfer of DNA by conjugation, transformation and transduction	Meiosis and fusion of gametes.
15.internal membranes	Relatively simple, and comprises of mesosomes	Complex, includes endoplasmic reticulum, golgi apparatus, etc

SHORT ANSWER QUESTIONS:

1.WRITE GENERAL CHARACTERSTICS OF BACTERIA?

ANS: General characterstics of bacteria:

1. Bacteria are typical Prokaryotic in Cell Organization.
2. They are distributed in almost all ecological niches and commonly found in soil, water, animal digestive tracts, food stuffs and many other environments.
3. Bacteria may be spherical or rod- shaped or spiral shape. Some are pleomorphic and some are filamentous.
4. They are very small in size and microscopic and unicellular.
5. Bacterial cells may remain together and form pairs, chains and clusters of various sizes and shapes.
6. Some forms are colonial or few are mycelia in nature.
7. Some bacteria are Gram+ve and some are Gram -ve towards Gram's staining reaction.
8. They may be Photoautotrophic, Photo heterotrophic.
9. They exhibit absorptive nutrition.
10. They may be aerobic, anaerobic, facultative anaerobic or microaerophilic.
11. Some are motile and some are non-motile.

2. WRITE GENERAL CHARACTERSTICS OF ARCHAEBACTERIA?

ANS: General characterstics of Archaeobacteria:

1. Archaeobacteria are now called "archaea"(Greek, archaios=ancient) because they use ancient energy metabolism.
2. They are prokaryotic organisms and are quite diverse , both in morphology and physiology.
3. Archaea are the first cells that originated on the earth.
4. They are found in aquatic, terrestrial habits and are often present in anaerobic, hyper saline, and high temperature environments.
5. Morphology they may be spherical, rod-shaped, spiral, lobed, plate shaped or pleomorphic.
6. They can stain either gram+ve or gram-ve.
7. Two types of lipid structures are found among the archaeobacterial plasma membranes, glycerol diphytanyl diethers and diglycerol diphytanyl tetraethers.
8. Mode of nutrition: autotrophy is widespread among the methanogens and extreme thermophiles.
9. Reproduction: archaeobacteria multiply either by binary fission, or budding, or by fragmentation.

3.WRITE GENERAL CHARACTERISTICS OF MYCOPLASMAS?

ANS: General characters of mycoplasmas

1. Mycoplasmas are a group of bacteria which are previously called as pleuro pneumonia-like organisms.
2. Mycoplasmas are the gram negative bacteria and simplest prokaryotes.
3. They are pleomorphic in nature and vary from spherical to pear-shape. Some are branched or helical filaments.
4. Normally their size range from 0.3 μ m to 0.8 μ m in diameter.
5. Most of them are non-motile but some show gliding movement on liquid-covered surfaces.
6. They exhibit self-reproduction capacity.
7. Mycoplasmas are flexible as they lack cell walls and cannot synthesize peptidoglycan precursors. The key wall components of bacteria i.e. muramic acid and diaminopimelic acid are absent.
8. Due to presence of sterols, mycoplasmas are osmotically more stable than eubacterial protoplasts.
9. Some mycoplasmas produce ATP by electron transport. some other species produce ATP by Embden-meyerhof-paranas (E.M.P) pathway and lactic acid fermentation.
10. They reproduce by binary fission.

11. Species of mycoplasma genus are parasites of animal mucous membranes and joints of animals or humans and cause diseases like arthritis, pneumonia and pleuropneumonia.

4. Write general characteristics of cyanobacteria?

ANS: General characters of cyanobacteria:

1. Previously scientists called them as blue green algae under the class cyanophyceae (or) myxophyceae.

2. In view of the cell structure and biochemistry the present day scientists treated them as prokaryotic bacteria.

3. Cyanobacteria differ from the normal bacteria due to the presence of chlorophyll-a pigment absence of flagella and in the liberation of ring photosynthesis.

4. Plant body is unicellular (or) mucilaginous, multicellular filament called trichomes. Some occur in colonies and appear as water blooms.

5. Most of the plants live in fresh water, few live in marine water, some live in terrestrial habitat.

6. The prokaryotic cell structure contains cell wall with peptidoglycans and outer mucilage.

7. In the absence of flagella they show gliding movement.

8. Cyanobacteria contains nitrogen fixing enzyme (Nitrogenase); so they are useful in nitrogen fixation by symbiotic (OR) non-symbiotic method.

9. Some are used as biofertilizers.

10. Some are used as SCP – due to the storage of food in the cells.

11. Nostoc commune; used as food.

5. WRITE GENERAL CHARACTERISTICS OF RICKETTSIAS?

Ans: General Characteristics of Rickettsias:

Rickettsias are microscopic, prokaryotic, nonmotile, nonspore-forming, gram-negative, pleomorphic, obligate intracellular parasites.

- 1) Morphology: Rickettsias vary in shape. They may be rod-shaped, coccoid, coccobacillus, or pleomorphic.
- 2) Nature of cell: prokaryotic nature.
- 3) Cell structure: Rickettsias possess both cell wall and cell membrane. Cell wall is chemically similar to that of Gram-ve bacteria. Cell membrane is trilayered lipoprotein membrane.
- 4) Cytoplasm contains 70S ribosomes, RNA, proteins, enzymes etc.

6. WRITE GENERAL CHARACTERS OF MYCOPLASMAS?

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Species of mycoplasma genus are parasites of animal mucous membranes and joints of animals or humans and cause diseases like arthritis, pneumonia and pleuropneumonia

7. WRITE GENERAL CHARACTERS OF ACTINOMYCETES?

ANS: General characters of actinomycetes:

1. Actinomycetes show filamentous body and hence called filamentous bacteria. Filamentous are branched and called hyphae. Members are chemoorganotrophs.
2. These are usually soil inhabitants and play important role in the decomposition organic matter. Their main ecological role, however, is in the decomposition of organic matter in the soil.
3. Hyphae is septate and cells are prokaryotic cell, containing many nucleoids.
4. Cell wall material varies greatly. Different sugars are present in the cell wall.
5. Most of the members are free living organisms. A few are parasitic and cause diseases on humans, plants and animals.
6. Actinomycetes reproduced by conidia or sporangia. Surface mycelium produce conidia on conidiophores and sporangia on sporangiophores.
7. Under unfavourable conditions, heat resistant spores are formed.
8. These bacteria are commercially very important. 85% of antibiotics are produced by these members.
9. Species of *Streptomyces* produce various antibiotics like streptomycin, erythromycin, tetracycline and chloramphenicol. Gentamycin antibiotic is produced by members of micromonospora.
10. Some members cause diseases in man, animals and plants.

8. WRITE GENERAL CHARACTERISTICS OF MICRO ALGAE?

ANS: General Characteristics Of Microalgae:

1. Distribution: Algae are found in a variety of habitats, such as water (fresh, marine or brackish), on moist rocks, wood, on or within the plant and animal body, on the surface of moist soil etc.
2. Thallus organization: The Vegetative body of algae is called a thallus. On the basis of thallus organization, algae are divided into Unicellular forms and Multicellular forms.
 - i. Unicellular forms: Unicellular forms are found in all groups of algae except *Charophyceae* and *Phaeophyceae*. Their plant body is represented by a single cell, and is of the following types:
 - a) Motile unicellular forms
 - b) Nonmotile unicellular forms.

ii. Multicellular forms: Multicellular forms arise due to repeated division of parental cell. They are of the following types:

- a) Colonial forms,
- b) Filamentous forms,
- c) Siphonaceous forms,
- d) Parenchymatous forms.

3. Cell structure: On the basis of cell organization, the algal cells may be differentiated into prokaryotic, mesokaryotic and eukaryotic types.

The prokaryotic cell nature is found in the members of Cyanophyceae. The mesokaryotic cell nature is found in the members of *Dinophyceae*. The eukaryotic cell nature is found in all other algae except Cyanophyceae and *Dinophyceae*.

4. Pigments of algae: Algal thallus has a characteristic of colour due to the presence of a combination of pigments, specific to each class. All eukaryotic algae possess pigments in a membrane-bound organelles known as plastids. They are of the following types:

- a) Leucoplasts
- b) Chromoplasts

The **Photosynthetic pigments** in algae are of three kinds, namely chlorophylls, carotenoids and phycobilins.

5. Mode of Nutrition: Algae are autotrophic with respect to their mode of nutrition. Reserve food: In algae, starch is the primary product of photosynthesis.

6. Locomotion: Majority of motile algae locomote with help of flagella.

9. WRITE GENERAL CHARACTERISTICS OF MOLDS?

ANS: General characteristics of molds:

1. Molds are microscopic, achlorophyllous, heterotrophic, eukaryotic sporebearing organisms.
2. They are usually filamentous, and their vegetative structure is typically surrounded by cell walls made up of chitin or cellulose or both.

3. Molds differ from bacteria in having eukaryotic cell structure and from other higher organisms in absorptive mode of nutrition.
4. Molds are a very large and divergent group of organisms, with more than 1,00,000 species distributed in almost every available habitats.
5. **Habitat:** Molds are universal in distribution and grow in almost every habitat on the earth where organic materials and moisture are available.
6. **Morphology:** The body or vegetative structure of the mould is called as Mycelium.
7. **Cell structure:** Molds have eukaryotic cell structure.
8. **Reproduction:** It has asexual reproduction and sexual reproduction.
Asexual reproduction by fragmentation, fission, budding, arthrospores or oidia, chlamydospores, sporangiospores, conidia.
Sexual reproduction by planogametic copulation, gametangial contact, gametangial copulation, spermatization, somatogamy.
9. Molds are found in all types of habitats and bring about a number of changes in the habitat due to their activities. Some of these changes are useful to man while some are harmful.
10. Useful aspects as food, acids, enzymes, antibiotics, vitamins, soil fertility and molds in biological control.

10. WRITE GENERAL CHARACTERISTICS OF YEASTS?

ANS: General characteristics of yeasts:

1. Yeasts are unicellular fungi belonging to class Hemiascomycetes.
2. There are simplest eukaryotic organisms and have the capacity to ferment sugars into ethyl alcohol and carbon dioxide.
3. Habitat: Yeasts are widespread in their distribution and mostly live as saprophytes.
4. Morphology: Unlike other fungi the thallus of yeast is non-mycelial and consists of a single minute oval or spherical cell.
5. Cell structure: Yeast cell is surrounded by a cell wall and a cell membrane.
6. Mode of nutrition: Yeasts are heterotrophic in their mode of nutrition.
7. Reserve food materials: Glycogen granules, oil globules, protein particles are the reserve food products in yeast.
8. **Reproduction:** Yeast reproduces by means of asexual and sexual methods.

- a. Asexual reproduction by budding, fission.
 - b. Sexual reproduction in yeast is very simple type. It occurs during unfavorable conditions.
9. Life cycles in yeasts are haplontic, diplontic, haplodiplontic.

11. WRITE GENERAL CHARACTERISTICS OF PROTOZOA?

ANS: general characteristics of Protozoa:

1. Protozoans (Greek, protos=first; zoon=animal) are the most primitive members of the animal kingdom.
2. They are microscopic, unicellular and eukaryotic protists.
3. Protozoans may be distinguished from other eukaryotic protists by their lack of cell wall and locomotion.
4. **Occurrence:** Protozoans are found in a wide variety of moist habitats.
5. **Morphology:** Protozoans are microscopic, unicellular eukaryotes.
6. **Cell structure:** The protozoan cell is surrounded by a plasma membrane. It is also known as plasma lemma and is lipoprotein in nature.
7. **Locomotion:** Protozoans move by one of the three major types of locomotory organelles namely pseudopodia, flagella or cilia.
8. **Nutrition:** Most of the protozoans are chemoheterotrophic in their mode of nutrition.
9. **Reproduction:** Protozoans reproduce both by asexual and sexual modes.

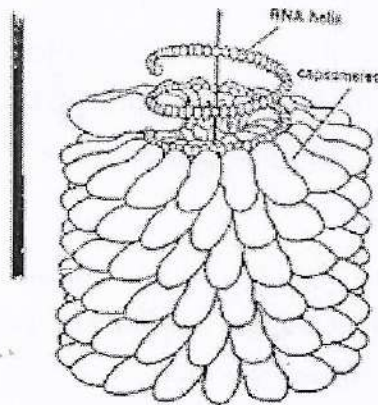
Asexual reproduction by binary fission, budding, schizogony, multiple fission.

Sexual reproduction is by conjugation, syngamy, autogamy.

12. DESCRIBE THE MORPHOLOGY AND STRUCTURE OF TMV?

ANS: The most thoroughly studied plant virus is the tobacco mosaic virus which is also called as TMV in short. For the first time w. stanley in 1937 crystalized the tobacco mosaic virus. It is the best and well studied example of helical capsid structure.

The structure of TMV has been described by franklin. It is rod shaped helical virus measuring about 300nm in length and 15-18nm diameter. Its molecular weight in 39×10^6 daltons.



The virus consists of two parts: a) capsid b) core

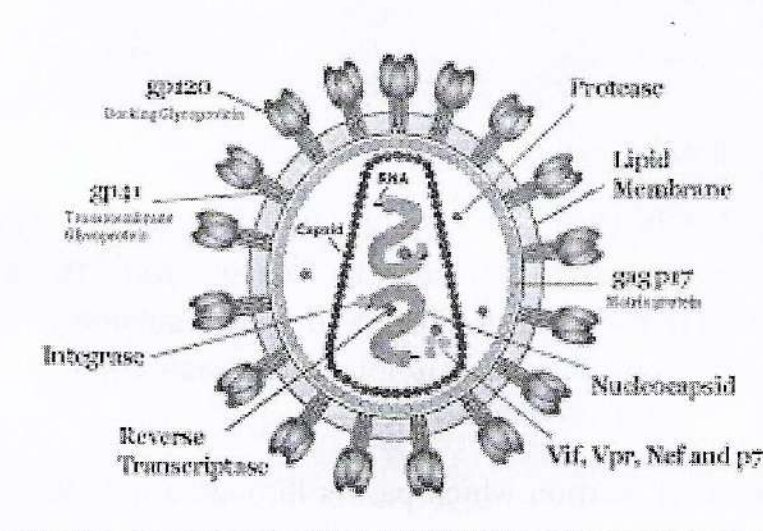
a) capsid or coat: the capsid is naked without any envelope. The helical capsid is made up of 2130 identical protein sub units called as protomers or capsomeres. Each sub unit of the capsid is of 158 amino acids in length with a molecular weight of 18000 daltons.

b) core or nucleic acid: this helical protein capsid encloses the genetic material RNA of the virion.

13. write an account on morphology and structure of HIV?

ANS: viruses are obligate intracellular parasites which can only be viewed with the aid of an electron microscope.

Structure of HIV: HIV is a spherical virus with a diameter of about 110nm.



HIV contains three parts: a) envelope b) capsid c) core.

a) envelope: three dimensional structure of helical envelope appears like a sphere made up from an assembly of 12 pentamers and 20 hexameres. Envelope is made up of lipid bilayer membrane has two glycoproteins gp41 and gp120 which located on the external surface. The surface antigen gp120 is very important for the viral infection.

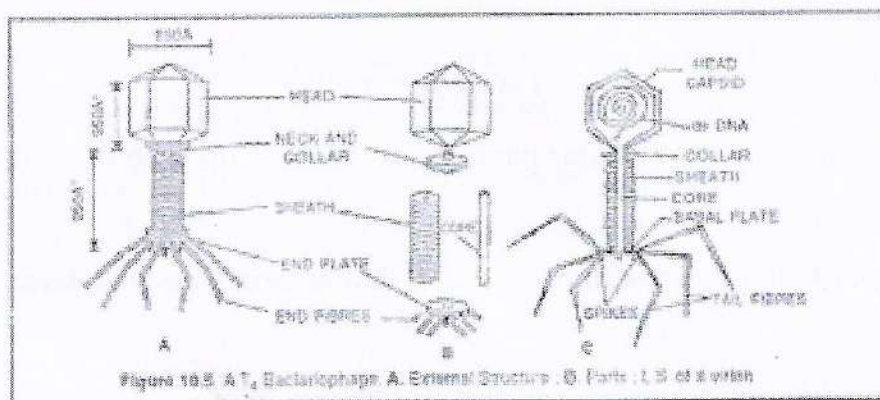
b) capsid: immediately under the envelope the proteins of the core surrounding the central mass also form a polygonal structure. The capsid is icosahedral enclosing RNA molecules.

c) core: the core of the HIV virus contains two copies of ssRNA genome and associated tRNA and several molecules of enzyme reverse transcriptase.

14. Describe structure and morphology of lambda bacteriophage?

ANS: The lambda phage is a virus that infects E. coli. it was described by Esther Lederberg.

The lambda phage particles are composed of 50% protein and 50% DNA.



Bacteriophage has two parts: a) head b) tail

a) Head: The head is icosahedral in shape, and about 55nm in diameter. The head consists of 300-600 capsomeres having molecular weights of 37,500 daltons. The capsomeres are arranged in clusters of 5 or 6 subunits, i.e. pentamers and hexameres, respectively. The head is joined to a non contractile tail by a head-tail connector.

b) Tail: tail consists of narrow neck portion which passes through a hole in the capsid and extends into a knob inside. The tail is flexible tube of about 180nm long and possesses a thin tail fibre at its end which recognizes the hosts. The tail also constitutes annuli.

15.GIVE AN ACCOUNT ON THE MULTIPLICATION (OR) REPLICATION IN VIRUSES?

ANS: Viruses having acellular, ultramicroscopic with nucleoprotein structure reproduces when they are parasites inside the hosts only.

This was mostly studied in the even number bacteriophages(T_2, T_4, T_6); these viruses infect bacteria having tadpole larva shape and also called phage viruses. Phage virus has a head with DNA and basal tubular tail and it ends with basal plate having six spikes and fibres.

This can be studied under 2 parts-

Lytic cycle – host bacteria shows lysis in the early stage

Lysogenic cycle – lysis occurs in the host bacteria after few generations.

Replication in lytic cycle:

1.adsorption: the basal plate of phage attach to the host cell wall with the help of spikes and fibres at some specific places and it is called adsorption.

2.penetration: lysozyme enzyme released by the phage dissolves the host cell wall and forms a hole. The capsid of the head contracts and results in the transport of DNA into the host cell through tail. This is called penetration and the protein coat of phage outside the bacterium is called “ghost”. The entry of phage DNA into the host results in the formation of bacterial DNA fragments.

3.latent period: in the early stage the phage DNA disappears for sometime and is called “ECLIPSE” period.

Later the viral DNA appears and takes control of the bacterium cell and synthesizes all the protein coats necessary for virion. Later the viral DNA replicates.

Now all the components are arranged to produce a complete new virion particles and this is called “maturation period”. Nearly 100-200 virions are produced.

4.lysis: at the end of latent period (maturation) the lysozyme enzyme produced in the host dissolves the cell wall, and the host cell gets ruptured to release the virion particle to the outside and it is called lysis of the host(or) “burst size”.

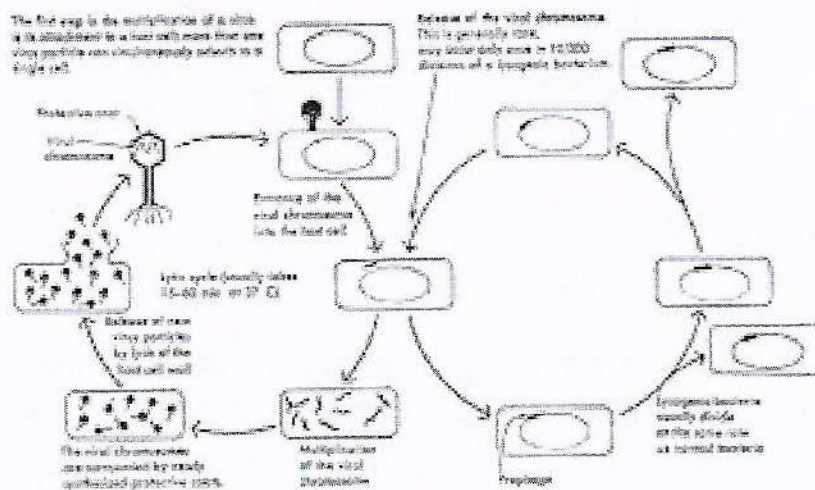
Replication in lysogenic cycle: coliphage-(lambda)

the phase particle after infecting the host do not cause lysis in the bacteria.

The phase DNA after entering into the host incorporated with the host DNA to form a new DNA complex. (prophase).

Host bacterium divides many times with the new DNA complex to get many generations of the host and it is called "temperate phages".

Later viral DNA takes control of the host bacterium and causes lysis to it as in the base of lytic cycle.



16. WRITE SHORT NOTE ON REPLICATION OF HIV?

ANS: 1. **BINDING**(also called attachment): HIV binds (attaches it self) to receptors on the surface of a CD4 cell.

2. **FUSION**: the HIV envelope and the CD4 cell membrane fuse(join together), which allows HIV to enter the CD4 cell.

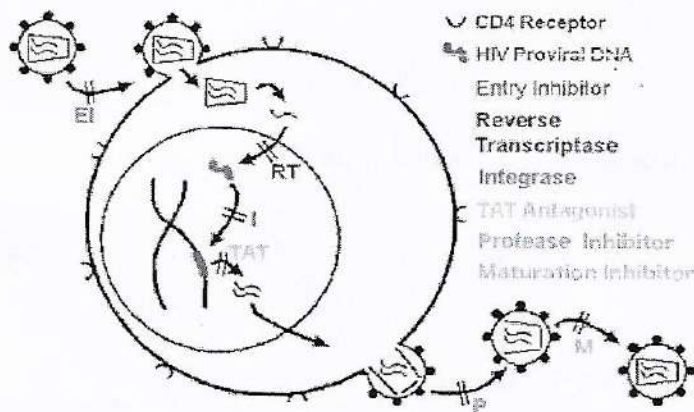
3. **REVERSE TRANSCRIPTION**: Inside the CD4 cell HIV releases and uses reverse transcriptase(an HIV enzyme) to converts its genetic material HIV RNA- HIV DNA. The conversion of HIV RNA to HIV DNA allows HIV to enter the CD4 cell nucleus and combine with the cell's genetic material-cell DNA.

4. **INTEGRATION**: Inside the CD4 cell nucleus, HIV releases integrase(an HIV enzyme) to insert its viral DNA in to the DNA of the CD4 cells.

5.REPLICATION:Once integrated in to the CD4 cell DNA, HIV begins to use the machinery of the CD4 cell to make long chains of HIV proteins. The protein chains are the building blocks for more HIV.

6.ASSEMBLY: New HIV proteins and HIV RNA move to the surface of the cell and assemble into immature(non infectious) HIV.

7.BUDDING: Newly formed immature(non infectious) HIV pushes it self out of the host CD4 cell. The new HIV releases proteases. Protease acts to break up the long protein chains that form the immature virus. The smaller HIV proteins combine to form mature (infectious)HIV.



MODEL PAPER

I-B.Sc DEGREE EXAMINATION, DEC-2015

SECTION-A

ANSWER ANY FIVE OF THE FOLLOWING

(5X5=25)

1. Contribution of antony von leeuwenhoek.
2. Spore staining.
3. Leishmania.
4. Gamma rays
5. Phenol coefficient
6. Lyophilization
7. Rickettsia
8. Replication of TMV

SECTION-B

Answer **FIVE** QUESTIONS

(5X10=50)

9. Describe the importance and applications of microbiology?

OR

10. Write an account on taxonomy and significance of virology?

11. Write an account on the classification of micro organisms basing on whittaker's five kingdom concept?

OR

12. Describe the methods of isolation and identification of micro organisms?

13. Describe the methods of sterilization?

OR

14. Write an account on halogens and Hypochlorites.

15. Describe the methods of preservation of microbial cultures?

OR

16. Explain the methods of isolation of pure cultures?

17. Differentiate prokaryotes and eukaryotes?

OR

18. Describe the ultra structure of bacterial cell?