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A Comprehensive Text Book for Microbiology

JV'n Ms. Anshika Kushwaha

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INDEX

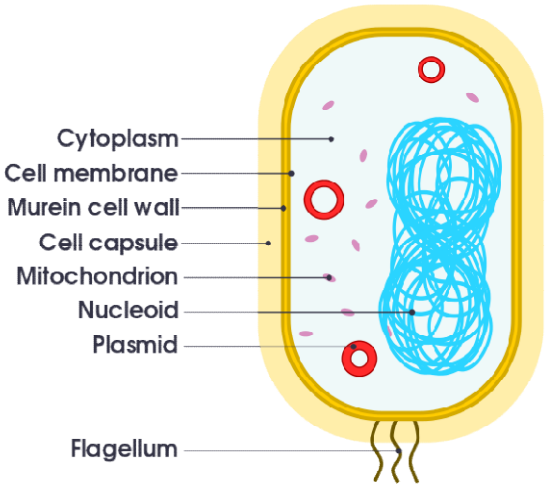
CHAPTER 1

Introduction to Microbiology

Microbiology, study of microorganisms, or microbes, a diverse group of generally minute, simple life-forms that include bacteria, archaea, algae, fungi, protozoa, and viruses. The field is concerned with the structure, function, and classification of such organisms and with ways of both exploiting and controlling their activities.

The 17th-century discovery of living forms existing invisible to the naked eye was a significant milestone in the history of science, for from the 13th century onward it had been postulated that "invisible" entities were responsible for decay and disease. The word microbe was coined in the last quarter of the 19th century to describe these organisms, all of which were thought to be related. As microbiology eventually developed into a specialized science, it was found that microbes are a very large group of extremely diverse organisms.

Daily life is interwoven inextricably with microorganisms. In addition to populating both the inner and outer surfaces of the human body, microbes abound in the soil, in the seas, and in the air. Abundant, although usually unnoticed, microorganisms provide ample evidence of their presence—sometimes unfavourably, as when they cause decay of materials or spread diseases, and sometimes favourably, as when they ferment sugar to wine and beer, cause bread to rise, flavour cheeses, and produce valued products such as antibiotics and insulin. Microorganisms are of incalculable value to Earth's ecology, disintegrating animal and plant remains and converting those to simpler substances that can be recycled in other organisms.



- **Diagram of Bacterial cell**

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  https://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5000494453

- **Competitive questions from today topic (2 questions Minimum)-**
  The unifying feature of the archaea that distinguishes them from the bacteria is
  (A) habitats which are extreme environments with regard to acidity
  (B) absence of a nuclear membrane temperature
  (C) presence of a cell wall containing a characteristic outer membrane
  (D) cytoplasmic ribosomes that are 70S

  A characteristic of protein synthesis in both the archaea and eukarya is
  (A) transcription and translation are coupled
  (B) translation is inhibited by diphtheria toxin
  (C) proteins are synthesized from D-, rather than L-, isomers of amino acids
  (D) the initiator tRNA is charged with N-formyl-methionine

- **Questions to check understanding level of students-**
  - Who discovered microorganism first?
  - What is the size of microorganism?

CHAPTER 2

Contributions of Antony van Leeuwenhoek

Antonie van Leeuwenhoek (1632–1723) was one of the first people to observe microorganisms, using a microscope of his own design, and made one of the most important contributions to biology. Robert Hooke was the first to use a microscope to observe living things. Hooke's 1665 book, *Micrographia,* contained descriptions of plant cells. Before Van Leeuwenhoek's discovery of microorganisms in 1675, it had been a mystery why grapes could be turned into wine, milk into cheese, or why food would spoil. Van Leeuwenhoek did not make the connection between these processes and microorganisms, but using a microscope, he did establish that there were forms of life that were not visible to the naked eye. Van Leeuwenhoek's discovery, along with subsequent observations by Spallanzani and Pasteur, ended the long-held belief that life spontaneously appeared from non-living substances during the process of spoilage.

In 1676, van Leeuwenhoek observed water closely and was surprised to see tiny organisms - the first bacteria observed by man. His letter announcing this discovery caused widespread doubt at the Royal Society but Robert Hooke later repeated the experiment and was able to confirm his discoveries.

As well as being the father of microbiology, van Leeuwenhoek laid the foundations of plant anatomy and became an expert on animal reproduction. He discovered blood cells and microscopic nematodes, and studied the structure of wood and crystals. He also made over 500 microscopes to view specific objects.
He also discovered sperm, which he considered one of the most important discoveries of his career, and described the spermatozoa from molluscs, fish, amphibians, birds and mammals, coming to the novel conclusion that fertilisation occurred when the spermatozoa penetrated the egg. Van Leeuwenhoek died on 30 August 1723.

Antonie van Leeuwenhoek

- **Leeuwenhoek** (1632 –1723) was a Dutch tradesman and scientist.
- He is commonly known as "the Father of Microbiology", and considered to be the first microbiologist.
- He is best known for his work on the improvement of the microscope and for his contributions towards the establishment of microbiology.
- Using his handcrafted microscopes, he was the first to observe and describe single-celled organisms.

- **Diagram of Antony van Leeuwenhoek.**
- **University Library Reference-**
  Microbiology by Pelczar- M.J.Chan ECS & Krieg NR-Tata Mcgraw Hill
  Online Reference Bergey's Manual of Systematic history of Bacteriology by John G

- **Ancient Indian Literature Reference - Rasa-]ala-Nidhi or Ocean of Indian chemistry andalchemy/vol.vIEd.1984/AvaniPrakashan,Ahmedabad,India;CharakSamhita https://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5000494453**

- **Competitive questions from today topic (2 questions Minimum)-**

Which of the following statements best describes Van Leeuwenhoek's educational background?

- He did not continue his studies past the elementary school level.
- He was tutored at home.
- He studied at some of Europe's most prestigious educational institutions.
- He studied informally under scientific giants like Hooke and Newton.

Which one of the following is widely considered Van Leeuwenhoek's most significant contribution to scientific knowledge?

- Discovery of mammalian sperm
- Discovery of microorganisms
- Discovery of the microscope
- Increasing the magnification power of the microscope

- **Questions to check understanding level of students-**
  - What is the major contribution of Antonie van Leeuwenhoek ?
  - Antonie van Leeuwenhoek died in which year?

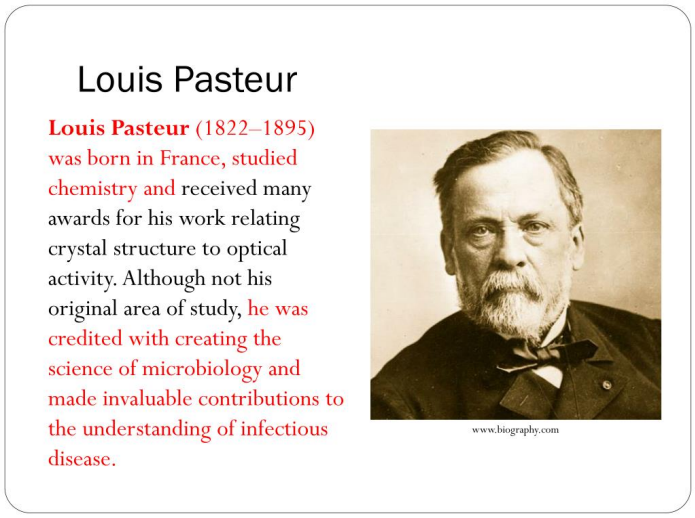
CHAPTER 3

Contributions of Louis Pasteur

Louis Pasteur (1822–1895) expanded upon Spallanzani's findings by exposing boiled broths to the air in vessels that contained a filter to prevent all particles from passing through to the growth medium. He also did this in vessels with no filter at all, with air being admitted via a curved tube that prevented dust particles from coming in contact with the broth. By boiling the broth beforehand, Pasteur ensured that no microorganisms survived within the broths at the beginning of his experiment. Nothing grew in the broths in the course of Pasteur's experiment. This meant that the living organisms that grew in such broths came from outside, as spores on dust, rather than spontaneously generated within the broth. Thus, Pasteur dealt the death blow to the theory of spontaneous generation and supported germ theory instead.

Ferdinand Julius Cohn (January 24, 1828 – June 25, 1898) was a German biologist. His classification of bacteria into four groups based on shape (sphericals, short rods, threads, and spirals) is still in use today. Among other things Cohn is remembered for being the first to show that *Bacillus* can change from a vegetative state to an endospore state when subjected to an environment deleterious to the vegetative state. His studies would lay the foundation for the classification of microbes and gave some of the first insights into the incredible complexity and diversity of microbial life.

In 1876, Robert Koch (1843–1910) established that microbes can cause disease. He found that the blood of cattle who were infected with anthrax always had large numbers of *Bacillus anthracis*. Koch found that he could transmit anthrax from one animal to another by taking a small sample of blood from the infected animal and injecting it into a healthy one, and this caused the healthy animal to become sick. He also found that he could grow the bacteria in a nutrient broth, then inject it into a healthy animal, and cause illness. Based on these experiments, he devised criteria for establishing a causal link between a microbe and a disease and these are now known as Koch's postulates. Although these postulates cannot be applied in all cases, they do retain historical importance to the development of scientific thought and are still being used today.



- **Diagram of Louis Pasteur.**

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  Online Reference Bergey's Manual of Systematic history of Bacteriology by John G

- **Ancient Indian Literature Reference - AvS'5/23/5;Medicine in the Veda Ikenneth Zysk'1. https://archive.org/stream/in.ernet.dli.2015.201547/2015.201547.Medicine-In_djvu.txt**

- **Competitive questions from today topic (2 questions Minimum)-**

French microbiologist Louis Pasteur is renowned for discoveries of principles of?

- Vaccination.
- microbial fermentation
- Pasteurization.
- all of above

Disease for which Louis Pasteur created first vaccines was

- anthrax and rabies
- polio
- measles
- chicken pox

- **Questions to check understanding level of students-**
  - What is the major contribution of Louis Pasteur ?
  - What is the discovery of Louis Pasteur?

CHAPTER 4

Microscope

A compound microscope is an instrument that is used to view magnified images of small objects on a glass slide. It can achieve higher levels of magnification than stereo or other low power microscopes and reduce chromatic aberration. It achieves this through the use of two or more lenses in the objective and the eyepiece. The objective lens or objectives located on the nosepiece have a short focal length and are close to the target object where it collects light and focuses the image of the object into the microscope. The second lens, in the eyepiece, has a longer focal length and further enlarges the image.

The characteristics of a compound microscope

- Two or more convex lenses
- Typical magnification range between 40x and 1000x
- One objective is used at a time
- Two-dimensional images
- Available in monocular, binocular and trinocular configurations

Parts of a Compound Microscope

Each part of the compound microscope serves its own unique function, with each being important to the function of the scope as a whole. The individual **parts of a compound microscope** can vary heavily depending on the configuration & applications that the scope is being used for. Common compound microscope parts include:

OPTICAL COMPONENTS

There are two optical systems in a compound microscope: Eyepiece Lenses and Objective Lenses:

Eyepiece or Ocular is what you look through at the top of the microscope. Typically, standard eyepieces have a magnifying power of 10x. Optional eyepieces of varying powers are available, typically from 5x-30x.

Eyepiece Tube holds the eyepieces in place above the objective lens. Binocular microscope heads typically incorporate a diopter adjustment ring that allows for the possible inconsistencies of our eyesight in one or both eyes. The monocular (single eye usage) microscope does not need a diopter. Binocular microscopes also swivel (Interpupillary Adjustment) to allow for different distances between the eyes of different individuals.

Objective Lenses are the primary optical lenses on a microscope. They range from 4x-100x and typically, include, three, four or five on lens on most microscopes. Objectives can be forward or rear-facing.

Nosepiece houses the objectives. The objectives are exposed and are mounted on a rotating turret so that different objectives can be conveniently selected. Standard objectives include 4x, 10x, 40x and 100x although different power objectives are available.

Coarse and Fine Focus knobs are used to focus the microscope. Increasingly, they are coaxial knobs - that is to say they are built on the same axis with the fine focus knob on the outside. Coaxial focus knobs are more convenient since the viewer does not have to grope for a different knob.

Stage is where the specimen to be viewed is placed. A mechanical stage is used when working at higher magnifications where delicate movements of the specimen slide are required.

Stage Clips are used when there is no mechanical stage. The viewer is required to move the slide manually to view different sections of the specimen.

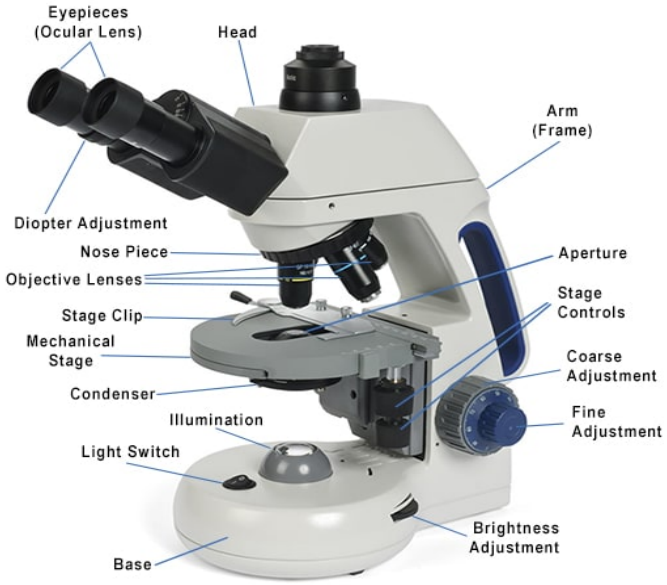
Aperture is the hole in the stage through which the base (transmitted) light reaches the stage.

Illuminator is the light source for a microscope, typically located in the base of the microscope. Most light microscopes use low voltage, halogen bulbs with continuous variable lighting control located within the base.

Condenser is used to collect and focus the light from the illuminator on to the specimen. It is located under the stage often in conjunction with an iris diaphragm.

Iris Diaphragm controls the amount of light reaching the specimen. It is located above the condenser and below the stage. Most high quality microscopes include an Abbe condenser with an iris diaphragm. Combined, they control both the focus and quantity of light applied to the specimen.

Condenser Focus Knob moves the condenser up or down to control the lighting focus on the specimen.



- **Diagram of Compound Microscope.**

- **University Library Reference-**
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  Online Reference Bergey's Manual of Systematic history of Bacteriology by John G

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- **Competitive questions from today topic (2 questions Minimum)-**

Total Magnification is obtained by_____

- Magnifying power of the objective lens.
- Magnifying power of eyepiece
- Magnifying power of condenser lens.
- Magnifying power of both the objective lens and eyepiece

Which part of the light microscope controls the intensity of light entering the viewing area?

- Coarse adjustment screw
- Fine adjustment screw
- Diaphragm
- Condenser lens

- **Questions to check understanding level of students-**
  - What is the structure and function of microscope?
  - Explain the different types of microscope?

Dark field microscope

Darkfield microscopy the condenser is designed to form a hollow cone of light (see illustration below), as opposed to brightfield microscopy that illuminates the sample with a full cone of light. In darkfield microscopy, the objective lens sits in the dark hollow of this cone and light travels around the objective lens, but does not enter the cone shaped area. The entire field of view appears dark when there is no sample on the microscope stage. However, when a sample is placed on the stage it appears bright against a dark background. It is similar to back-lighting an object that may be the same color as the background it sits against - in order to make it stand out.

Darkfield Microscope Applications

- Viewing blood cells (biological darkfield microscope, combined with phase contrast)
- Viewing bacteria (biological darkfield microscope, often combined with phase contrast)
- Viewing different types of algae (biological darkfield microscope)
- Viewing hairline metal fractures (metallurgical darkfield microscope)
- Viewing diamonds and other precious stones (gemological microscope or stereo darkfield microscope)
- Viewing shrimp or other invertebrates (stereo darkfield microscope)

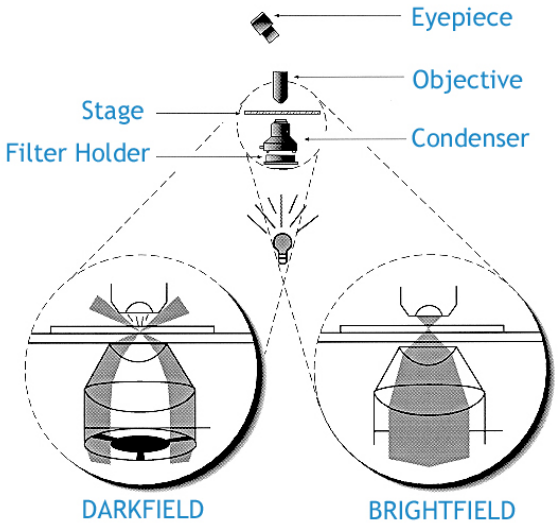

Diagram of Dark field microscope.

- **University Library Reference-**

  Microbiology by Pelczar- M.J.Chan ECS & Krieg NR-Tata Mcgraw Hill
  Textbook of Microbiology - Ananthanarayan And Paniker
  A textbook of microbiology by RC dubey

- **Ancient Indian Literature Reference - AvS'5/23/5; Medicine in the Veda Ikenneth Zysk'1. https://archive.org/stream/in.ernet.dli.2015.201547/2015.201547.Medicine-In_djvu.txt**

- **Competitive questions from today topic (2 questions Minimum)-**

Which of the following is the correct reason why liquid media is favoured for culturing thermophilic archaea?

- Liquid media can be heated to higher temperatures.
- Liquid media is easier to store.
- Solid media is usually unstable at optimum growing temperatures.
- Solid media becomes glass-like at high temperatures.

Which of the following microscopy techniques relies on the specimen interfering with the wavelength of light to produce a high contrast image without the need for dyes or any damage to the sample?

- Conventional bright field light microscopy
- Phase contrast microscopy
- Electron microscopy
- Fluorescence microscopy

- **Questions to check understanding level of students-**
  - What is the structure and function of Dark field microscope?
  - Explain the difference between dark field and bright field microscope?

Phase contrast microscope

Phase contrast microscopy, first described in 1934 by Dutch physicist Frits Zernike, is a contrast-enhancing optical technique that can be utilized to produce high-contrast images of transparent specimens, such as living cells (usually in culture), microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and sub cellular particles (including nuclei and other organelles).

In effect, the phase contrast technique employs an optical mechanism to translate minute variations in phase into corresponding changes in amplitude, which can be visualized as differences in image contrast. One of the major advantages of phase contrast microscopy is that living cells can be examined in their natural state without previously being killed, fixed, and stained. As a result, the dynamics of ongoing biological processes can be observed and recorded in high contrast with sharp clarity of minute specimen detail.
Presented in is a cut-away diagram of a modern upright phase contrast microscope, including a schematic illustration of the phase contrast optical train. Partially coherent illumination produced by the tungsten-halogen lamp is directed through a collector lens and focused on a specialized annulus (labeled **condenser annulus**) positioned in the sub stage condenser front focal plane. Wave fronts passing through the annulus illuminate the specimen and either pass through undeviated or are diffracted and retarded in phase by structures and phase gradients present in the specimen. Undeviated and diffracted light collected by the objective is segregated at the rear focal plane by a **phase plate** and focused at the intermediate image plane to form the final phase contrast image observed in the eyepieces.

Prior to the invention of phase contrast techniques, transmitted brightfield illumination was one of the most commonly utilized observation modes in optical microscopy, especially for fixed, stained specimens or other types of samples having high natural absorption of visible light. Collectively, specimens readily imaged with brightfield illumination are termed **amplitude objects** (or specimens) because the amplitude or intensity of the illuminating wavefronts is reduced when light passes through the specimen.

The addition of phase contrast optical accessories to a standard brightfield microscope can be employed as a technique to render a contrast-enhancing effect in transparent specimens that is reminiscent of optical staining. Light waves that are diffracted and shifted in phase by the specimen (termed a **phase object**) can be transformed by phase contrast into amplitude differences that are observable in the eyepieces. Large, extended specimens are also easily visualized with phase contrast optics due to diffraction and scattering phenomena that occur at the edges of these objects. The performance of modern phase contrast microscopes is so refined that it enables specimens containing

very small internal structures, or even just a few protein molecules, to be detected when the technology is coupled to electronic enhancement and post-acquisition image processing.

Presented in is a comparison of living cells in culture imaged in both brightfield and phase contrast illumination. The cells are human glial brain tissue grown in monolayer culture bathed with a nutrient medium containing amino acids, vitamins, mineral salts, and fetal calf serum. In brightfield illumination ,the cells appear semi-transparent with only highly refractive regions, such as the membrane, nucleus, and unattached cells (rounded or spherical), being visible. When observed using phase contrast optical accessories, the same field of view reveals significantly more structural detail. Cellular attachments become discernable, as does much of the internal structure. In addition, the contrast range is dramatically improved.

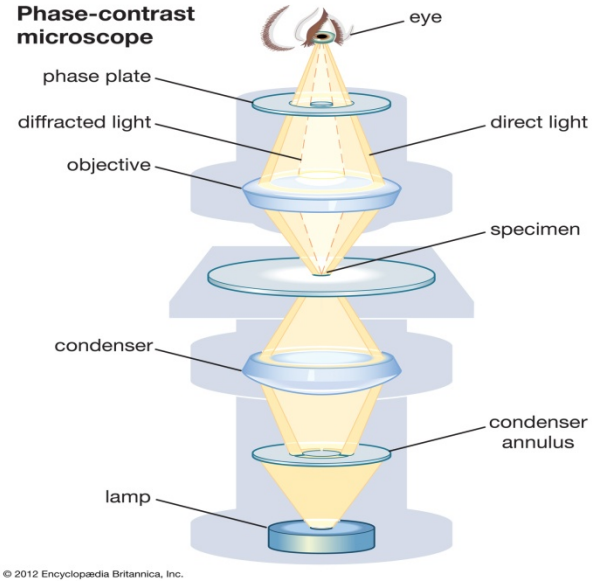

Diagram of Phase contrast microscope.

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- **Competitive questions from today topic (2 questions Minimum)-**

A light microscope is also referred to as a?

- Electron microscope.
- Compound microscope.
- Scanning problem microscope.
- X-ray.

Which of the following is not a type of light microscope?

- Phase contrast microscope
- Bright field microscope
- Electron microscope
- Stereo microscope

- **Questions to check understanding level of students-**
  - What is the structure and function of Phase contrast microscope?
  - Who discovered phase contrast microscope?

Fluorescence and Electron (Scanning and Transmission) microscope

Scanning electron microscope (SEM):

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The electron beam is scanned in a raster scan pattern, and the position of the beam is combined with the intensity of the detected signal to produce an image. In the most common SEM mode, secondary electrons emitted by atoms excited by the electron beam are detected using a secondary electron detector (Everhart-Thornley detector). The number of secondary electrons that can be detected, and thus the signal intensity, depends,

among other things, on specimen topography. SEM can achieve resolution better than 1 nanometer.

Transmission Electron Microscopy:

(**TEM**, an abbreviation which can also stand for the instrument, a **transmission electron microscope**) is a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image. The specimen is most often an ultrathin section less than 100 nm thick or a suspension on a grid. An image is formed from the interaction of the electrons with the sample as the beam is transmitted through the specimen. The image is then magnified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor such as a scintillator attached to a charge-coupled device.

Transmission electron microscopes are capable of imaging at a significantly higher resolution than light microscopes, owing to the smaller de Broglie wavelength of electrons. This enables the instrument to capture fine detail—even as small as a single column of atoms, which is thousands of times smaller than a resolvable object seen in a light microscope. Transmission electron microscopy is a major analytical method in the physical, chemical and biological sciences. TEMs find application in cancer research, virology, and materials science as well as pollution, nanotechnology and semiconductor research, but also in other fields such as paleontology and palynology.

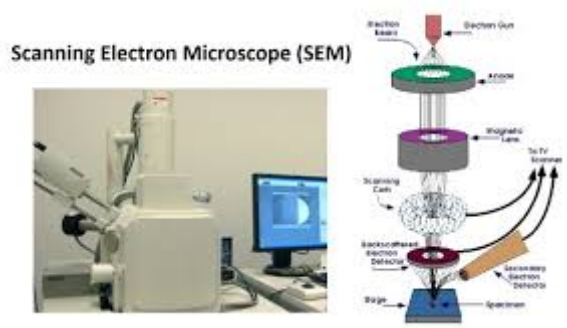
Working Principle of Scanning Electron Microscopes and Transmission Electron Microscopes

- An electron source;
- A series of electromagnetic and electrostatic lenses to control the shape and trajectory of the electron beam;
- Electron apertures.

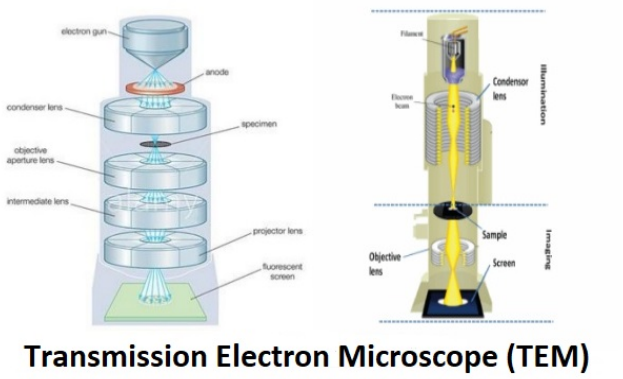
All of these components live inside a chamber which is under high vacuum.

Now over to the differences. SEMs use a specific set of coils to scan the beam in a raster-like pattern and collect the scattered electrons. The transmission electron microscopy (TEM) principle, as the name suggests, is to use the transmitted electrons; the electrons which are passing through the sample before they are collected. As a result, TEM offers invaluable information on the inner structure of the sample, such as crystal structure, morphology and stress state information, while SEM provides information on the sample's surface and its composition.

Moreover, one of the most pronounced differences between the two methods is the optimal spatial resolution that they can achieve; SEM resolution is limited to ~0.5 nm, while with the recent development in aberration-corrected TEMs, images with spatial resolution of even less than 50 pm have been reported.



- **Diagram of scanning electron microscope.**



- **Diagram of scanning microscope.**

- **University Library Reference-**

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  Microbial Physiology 4th ed. By Alber G.Moat & John W.Foster Wileyliss.

  Online Reference Becker's World of the Cell, 9th Edition

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- **Review of Literature-** Giepmans BNG, Adams SR, Ellisman MH, Tsien RY (2006) The fluorescent toolbox for assessing protein location and function. Science 312: 217–224.

- **Competitive questions from today topic (2 questions Minimum)-**

Which among the following helps us in getting a three-dimensional picture of the specimen?

- Transmission Electron Microscope
- Scanning Electron Microscope
- Compound Microscope
- Simple Microscope

Where do we obtain the magnified image of the specimen in SEM?

- cathode ray tube
- phosphorescent screen
- anode
- scanning generator

- **Questions to check understanding level of students-**
  - Function of cathode ray tube is to_?
  - Difference between SEM and TEM.

CHAPTER 5
STERILIZATION

Sterilization: Principles and Applications of Physical Methods

Sterilization:

Sterilization refers to any process that eliminates, removes, kills, or deactivates all forms of life (in particular referring to microorganisms such as fungi, bacteria, viruses, spores, unicellular eukaryotic organisms such as Plasmodium, etc.) and other biological agents like prions present in a specific surface, object or fluid, for example food or biological culture media. Sterilization can be achieved through various means, including heat, chemicals, irradiation, high pressure, and filtration. Sterilization is distinct from disinfection, sanitization, and pasteurization, in that those methods reduce rather than eliminate all forms of life and biological agents present. After sterilization, an object is referred to as being sterile or aseptic.

Physical methods of sterilization include the following:

a. Sunlight

b. Heat

c. Filtration

d. Radiation

e. Sound (sonic) waves

- **Sunlight**

Direct sunlight is a natural method of sterilization of water in tanks, rivers, and lakes. Direct sunlight has an active germicidal effect due to its content of ultraviolet and heat rays. Bacteria present in natural water sources are rapidly destroyed by exposure to sunlight.

- **Heat**

Heat is the most dependable method of sterilization and is usually the method of choice unless contraindicated. As a rule, higher temperatures (exceeding the maximum) are ***microbicidal***, whereas lower temperatures (below the minimum) tend to have inhibitory or ***microbistatic*** effects. Two types of physical heat are used in sterilization—moist and dry heat.

Flaming: Sterilization of inoculating loop or wire, the tipof forceps, searing spatulas, etc., is carried out by holding them in the flame of the Bunsen burner till they become red hot. Glass slides, scalpels, and mouths of culture tubes are sterilized by passing them through the Bunsen flame without allowing them to become red hot.

Incineration: Incineration is an excellent method for safely destroying infective materials by burning them to ashes. It has many uses:

Incinerators are used to carry out this process and are regularly employed in hospitals and research labs to destroy hospital and laboratory wastes.

The method is used for complete destruction and disposal of infectious material, such as syringes, needles, culture material, dressings, bandages, bedding, animal carcasses, and pathology samples.

Hot-air oven: The hot-air oven provides another means of dry heat sterilization and is the most widely used method. The hot-air oven is electrically heated and is fitted with a fan to ensure adequate and even distribution of hot air in the chamber. It is also fitted with a thermostat that ensures circulation of hot air of desired temperature in the chamber. Heated, circulated air transfers its heat to the materials inside the chamber. While sterilizing by hot-air oven, it should be ensured that the oven is not overloaded. The materials should be dry and arranged in a manner which allows free circulation of air inside the chamber. It is essential to fit the test tubes, flasks, etc., with cotton plugs and to wrap Petri dishes and pipettes in a paper. Sterilization by hot-air oven requires exposure to 160–180°C for 2 hours and 30 minutes

- **Filtration**

Filtration is an excellent way to reduce the microbial population in solutions of heat-labile material by use of a variety of filters. Filters are used to sterilize these heat-labile solutions.

Filters simply remove contaminating microorganisms from solutions rather than directly destroying them. The filters are of two types: (*a*) depth filters and (*b*) membrane filters.

TABLE 3-2 Types and uses of radiation for sterilization

Types	Uses	Comments
Ionizing radiation administered using Cobalt-60-based instruments	For sterilization of pharmaceuticals like antibiotics, hormones, sutures; and prepacked disposable items, such as syringes, infusion sets, catheters, etc.	Though expensive and fraught with safety risks, it is very effective due to better penetration power
Nonionizing radiation administered through UV lamps	Only for disinfection of clear surfaces in OTs, laminar flow hoods, etc.	Hazardous and not as effective as ionizing radiation

- **Sound (sonic) waves**

High-frequency sound (*sonic*) waves beyond the sensitivity of the human ear are known to disrupt cells. Sonication transmits vibrations through a water-filled chamber (sonicator) to induce pres-sure changes and create intense points of turbulence that can stress and burst cells in the vicinity. Sonication also forcefully dislodges foreign matter from objects. Heat generated by the sonic waves (up to 80°C) also appears to contribute to the antimicrobial action.

- **University Library Reference-**

  General Microbiology by Brock.
  Microbial Physiology 4th ed. By Alber G.Moat & John W.Foster Wileyliss.

  Online Reference Becker's World of the Cell, 9th Edition

- **Ancient Indian Literature Reference - Rasa-Jala-Nidhi or Ocean of Indian chemistry and alchemy/vol.vIEd.1984/AvaniPrakashan,Ahmedabad,India;Charak Samhita** https://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5000494453

- **Review of Literature-** Giepmans BNG, Adams SR, Ellisman MH, Tsien RY (2006) The fluorescent toolbox for assessing protein location and function. Science 312: 217–224.

- **Competitive questions from today topic (2 questions Minimum)-**

Which among the following helps us in getting a three-dimensional picture of the specimen?

- Transmission Electron Microscope
- Scanning Electron Microscope
- Compound Microscope
- Simple Microscope

Where do we obtain the magnified image of the specimen in SEM?

- cathode ray tube
- phosphorescent screen
- anode
- scanning generator

- **Questions to check understanding level of students-**
  - What is the function of stains?
  - Give some name of stains.

CHAPTER 6
STAINING

Stains and staining techniques: Principles of staining.

Staining:

Staining is a technique used to enhance contrast in samples, generally at the microscopic level. Stains and dyes are frequently used in histology (the study of tissue under the microscope) and in the medical fields of histopathology, hematology, and cytopathology that focus on the study and diagnoses disease at a microscopic level. Stains may be used to define biological tissues (highlighting, for example, muscle fibers or connective tissue), cell populations (classifying different blood cells), or organelles within individual cells.

In biochemistry it involves adding a class-specific (DNA, proteins, lipids, carbohydrates) dye to a substrate to qualify or quantify the presence of a specific compound. Staining and fluorescent tagging can serve similar purposes. Biological staining is also used to mark cells in flow cytometry, and to flag proteins or nucleic acids in gel electrophoresis. Staining is not limited to biological materials, it can also be used to study the structure of other materials for example the lamellar structures of semi-crystalline polymers or the domain structures of block copolymers.

Simple stains can define as the **basic dyes**, which are the alcoholic or aqueous solution, diluted up to 1-2%. These can easily release $\mathbf{OH^-}$ and accepts $\mathbf{H^+}$ ion, and hence the simple stains are positively charged. As the simple stains are **positively charged,** they usually refer to as "Positive or Cationic dyes".

It is commonly used to colour most of the bacteria. As the simple stain carry a positive charge, that's why they firmly adhere to a negative bacterial cell by which organism appears coloured with a colourless background.

Preparation: The preparatory steps involved depend on the type of analysis planned; some or all of the following procedures may be required.

Fixation–which may itself consist of several steps–aims to preserve the shape of the cells or tissue involved as much as possible. Sometimes heat fixation is used to kill, adhere, and alter the specimen so it accepts stains. Most chemical fixatives (chemicals causing fixation) generate chemical bonds between proteins and other substances within the sample, increasing their rigidity. Common fixatives include formaldehyde, ethanol, methanol, and/or picric acid. Pieces of tissue may be embedded in paraffin wax to increase their mechanical strength and stability and to make them easier to cut into thin slices.

Mordant: These are chemical agents which have power of making dyes to stain materials which otherwise are unstainable Mordants are classified into two categories:

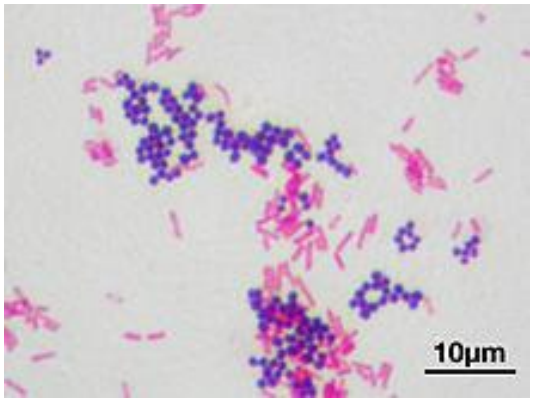
a) Basic Mordant: React with acidic dyes e.g. alum, ferrous sulfate, cetylpyridinium chloride etc.

b) Acidic Mordant: React with basic dyes e.g. picric acid, tannic acid etc.

Direct Staining: Carried out without mordant.

Indirect Staining: Staining brought by the aid of a mordant.

Gram Stain



- **Figure. Bacteria stained with Gram stain.**

In 1884, physician Hans Christian Gram was studying the etiology (cause) of respiratory diseases such as pneumonia. He developed a staining procedure that allowed him to identify a bacterium in lung tissue taken from deceased patients as the etiologic agent of a fatal type of pneumonia.

Although it did little in the way of treatment for the disease, the Gram stain method made it much easier to diagnose the cause of a person's death at autopsy. Today we use Gram's staining techniques to aid in the identification of bacteria, beginning with a preliminary classification into one of two groups: **Gram positive** or **Gram negative**.

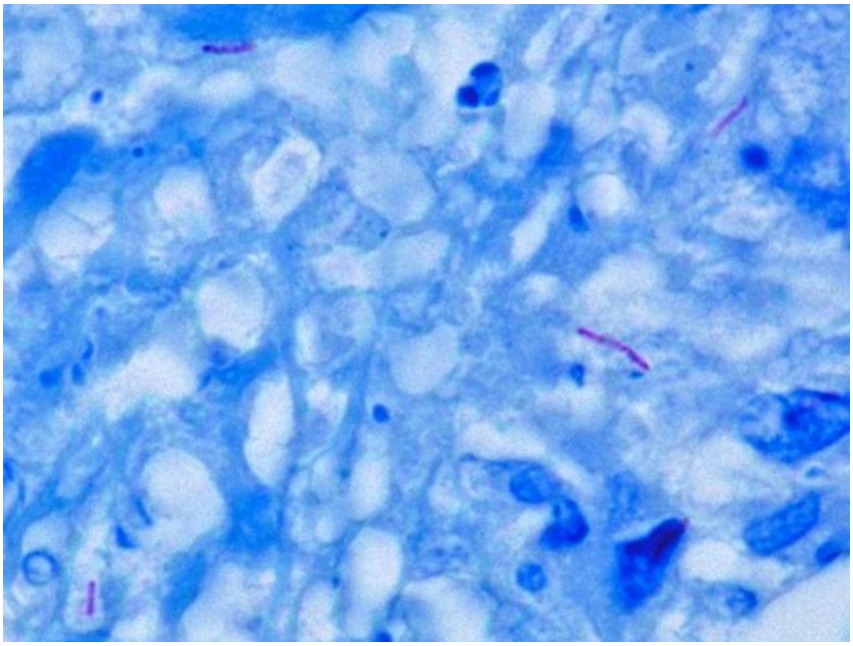
The differential nature of the Gram stain is based on the ability of some bacterial cells to retain a primary stain (crystal violet) by resisting a decolorization process. Gram staining involves four steps. First cells are stained with crystal violet, followed by the addition of a setting agent for the stain (iodine). Then alcohol is applied, which selectively removes the stain from only the Gram negative cells. Finally, a secondary stain, safranin, is added, which counter stains the decolorized cells pink.

Although Gram didn't know it at the time, the main difference between these two types of bacterial cells is their cell walls. Gram negative cell walls have an outer membrane (also called the envelope) that dissolves during the alcohol wash. This permits the crystal violet dye to escape. Only the decolorized cells take up the pink dye safranin, which explains the difference in color between the two types of cells. At the conclusion of the Gram stain procedure, Gram positive cells appear purple, and Gram negative cells appear pink.

When you interpret a Gram stained smear, you should also describe the morphology (shape) of the cells, and their arrangement. In Figure 5, there are two distinct types of bacteria, distinguishable by Gram stain reaction, and also by their shape and arrangement.

Acid Fast Stain

Some bacteria produce the waxy substance **mycolic acid** when they construct their cell walls. Mycolic acid acts as a barrier, protecting the cells from dehydrating, as well as from phagocytosis by immune system cells in a host. This waxy barrier also prevents stains from penetrating the cell, which is why the Gram stain does not work with mycobacterium such as *Mycobacterium*, which are pathogens of humans and animals. For these bacteria, the **acid–fast staining** technique is used.



- **Figure. Acid-fast bacilli in sputum**

To perform the acid-fast stain, a heat-fixed smear is flooded with the primary stain carbol fuchsin, while the slide is heated over a steaming water bath. The heat "melts" the waxy cell wall and permits the absorption of the dye by the cells. Then the slide is allowed to cool and a solution of acid and alcohol is added as a decolorizer. Cells that are "acid-fast" because of the mycolic acid in their cell wall resist decolorization and retain the primary stain. All other cell types will be decolorized. Methylene blue is then used as a counter stain. In the end, acid-fast bacteria (AFB) will be stained a bright pink color, and all other cell types will appear blue.

Endospore Stain

Endospores are dormant forms of living bacteria and should not be confused with reproductive spores produced by fungi. These structures are produced by a few genera of Gram-positive bacteria, almost all bacilli, in response to adverse environmental conditions. Two common bacteria that produce endospores are *Bacillus* or *Clostridum*. Both live primarily in soil and as symbionts of plants and animals, and produce endospores to survive in an environment that change rapidly and often.

The process of **endosporulation** (the formation of endospores) involves several stages. After the bacterial cell replicates its DNA, layers of peptidoglycan and protein are produced to surround the genetic material. Once fully formed, the endospore is released from the cell and may sit dormant for days, weeks, or years. When more favorable environmental conditions prevail, endospores **germinate** and return to active duty as vegetative cells.

Mature endospores are highly resistant to environmental conditions such as heat and chemicals and this permits survival of the bacterial species for very long periods. Endospores formed millions of years ago have been successfully brought back to life, simply by providing them with water and food.

Because the endospore coat is highly resistant to staining, a special method was developed to make them easier to see with a brightfield microscope. This method, called the **endospore stain**, uses either heat or long exposure time to entice the endospores to take up the primary stain, usually a water soluble dye such as malachite green since endospores are permeable to water. Following a decolorization step which removes the dye from the vegetative cells in the smear, the counterstain safranin is applied to provide color and contrast. When stained by this method, the endospores are green, and the vegetative cells stain pink.

- **University Library Reference-**

  Microbiology by Pelczar- M.J.Chan ECS & Krieg NR-Tata Mcgraw Hill
  Textbook of Microbiology - Ananthanarayan And Paniker

- **Ancient Indian Literature Reference - Rasa-]ala-Nidhi or Ocean of Indian chemistry and alchemy/vol.vIEd.1984/AvaniPrakashan,Ahmedabad,India;CharakSamhitahttps://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5000494453**

- **Review of Literature-** Pramod K. Raghav & MituSaini**(2017)** Antimicrobial Properties of Tulsi (*Ocimum sanctum*) in Relation to Shelf Life Enhancement of Fruits & Vegetables. International Journal of Green and Herbal Chemistry, 7(1):020-032, E-ISSN:2278-3229

- **Competitive questions from today topic (2 questions Minimum)-**

Which of the following statements is NOT true for eukaryotic DNA replication?

- It has multiple origins
- It is synchronized to phases of cell cycle
- It does not involve Okazaki fragment
- It requires licencing of Pre-replicative complex

Hygromycin B, generally used as a selection marker in plant transformation protocols is

- an aminocyclitol antibiotic produced by Streptomyces hygroscopicus
- an aminoglycoside bacteriocidal antibiotic isolated from the bacterium Streptomyces kanamyceticus
- a beta-lactam antibiotic that is part of the amino-penicillin family and is roughly equivalent to amoxicillin in terms of activity
- an ammonium butanoate antibody produced by Streptomyces hygroscopicus

Questions to check understanding level of students-

- What is simple stain?
- Give some name of simple stains.

Types of stains? Simple stains.

Simple Staining:

Simple staining is one of the conventional methods of staining techniques. As from the name, it is quite clear that it is very simple and **direct staining method** which makes the use of a **single stain** only. The microorganism is invisible to the naked eye, therefore to make it visible, the staining is performed, which gives **divergence** to a microscopic image. Simple staining makes the use of basic dyes like methylene blue, safranin, crystal violet, malachite green etc. which refers as "**Simple** or **Direct stains**".

The basic stains are having a positive **auxochrome** which charges the chromogen particle of the stain to bind with the specimen. The **chromophore** group of the stain imparts colour to the microscopic image that has to study.

As the basic stain carries a positive charge, it also refers as **Positive** or **Cationic stain**. The purpose of simple staining is to add **contrast** to the specimen by directly stain the bacterial cell with a **colourless background**.

Therefore simple stain not only able us to observe the organism but also helps us to examine the organism's shape, size and arrangement, which is necessary to distinguish a particular group of organism. The process generally involves **three sequential steps** like smear preparation, heat fixing and staining of the bacteria.

Definition of Simple Staining

Simple staining can define as one of the ordinaries yet popular method which is used to elucidate the bacterial size, shape and arrangement to differentiate the group of bacteria. It stains the bacterial cell **uniformly** and thus increases the visibility of an organism.

Simple staining sometimes interchangeable with the names like direct, positive or monochrome staining. Now let us understand why simple staining is called by such alternative names.

Refers as Direct staining: Because it is a direct method that directly stains the bacterial cell with a colourless background.

Refers as Positive staining: Because it makes the use of basic dyes which are positively charged and binds with the negatively charged bacterial cell.

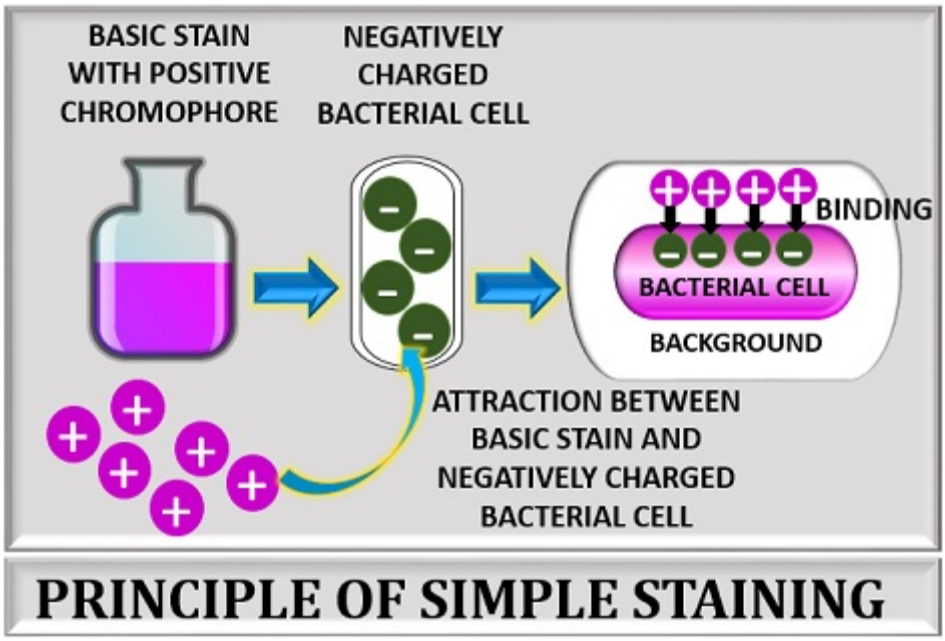
Simple Stains

Simple stains can define as the **basic dyes**, which are the alcoholic or aqueous solution, diluted up to 1-2%. These can easily release $\mathbf{OH^-}$ and accepts $\mathbf{H^+}$ ion, and hence the simple stains are positively charged. As the simple stains are **positively charged,** they usually refer to as "Positive or Cationic dyes".

It is commonly used to colour most of the bacteria. As the simple stain carry a positive charge, that's why they firmly adhere to a negative bacterial cell by which organism appears coloured with a colourless background.

Examples of simple stain include safranin, methylene blue, crystal violet etc.

The basic stains have different **exposure time** to penetrate and stain the bacterial cell



Advantages

- Simple staining is a very **simple** method to perform which stains the organism by a single reagent.
- It is a **rapid** method which reduces the performance time by taking only 3-5 minutes.
- Simple staining helps to examine or elucidate the bacterial shape, size and arrangement.
- It also helps us to differentiate the bacterial cells from the non-living structures.
- Simple staining can be useful in the **preliminary study** of the morphological characters of the bacteria.

Disadvantages

- It does not give much information rather than the morphological characteristics of bacteria.
- Through simple staining, we cannot classify a particular type of organism.

- **University Library Reference-**

  Microbiology by Pelczar- M.J.Chan ECS & Krieg NR-Tata Mcgraw Hill
  Textbook of Microbiology - Ananthanarayan And Paniker

- **Ancient Indian Literature Reference - Rasa-]ala-Nidhi or Ocean of Indian chemistry and alchemy/vol.vIEd.1984/AvaniPrakashan,Ahmedabad,India;CharakSamhitahttps://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5000494453**

- **Review of Literature-** Pallavi Singh, Pramod K. Raghav & Mansi Pareek **(2019)** Development of Ragi-Wheat Composite Value-Added Product Enriched with *Moringaolifera* for Reproductive Age Women. Pramana Research Journal**,** 9(3):614-621, ISSN: 2249-297 **UGC Approved Journal**

Competitive questions from today topic (2 questions Minimum)-

The natural reservoir of Ebola virus is

- Fruit bat
- Dog
- Sheep
- Pig

Drug resistance among bacteria involved in hospital infections is commonly due to

- Multi drug therapy
- Probiotic bacteria
- Transfer of resistance genes
- Mutation in target genes

- **Suggestions to secure good marks to answer in exam-**
  - Give answer with complete labeled diagrams.
  - Explain answer with key point answers

Types of stains? Differential stains.

Differential staining:

Differential Staining is a staining process which uses more than one chemical stain. Using multiple stains can better differentiate between different microorganisms or structures/cellular components of a single organism.

Differential staining is used to detect abnormalities in the proportion of different white blood cells in the blood. The process or results are called a WBC differential. This test is useful because many diseases alter the proportion of certain white blood cells. By analyzing these differences in combination with a clinical exam and other lab tests, medical professionals can

diagnose disease. One commonly recognizable use of differential staining is the Gram stain. Gram staining uses two dyes: Crystal violet and Fuchsin or Safranin (the counterstain) to differentiate between Gram-positive bacteria (large Peptidoglycan layer on outer surface of cell) and Gram-negative bacteria. Acid-fast Stains are also differential stains.

Differential Staining Techniques

In microbiology, differential staining techniques are used more often than simple stains as a means of gathering information about bacteria. Differential staining methods, which typically require more than one stain and several steps, are referred to as such because they permit the differentiation of cell types or cell structures. The most important of these is the Gram stain. Other differential staining methods include the endospore stain (to identify endospore-forming bacteria), the acid-fast stain (to discriminate *Mycobacterium* species from other bacteria), a metachromatic stain to identify phosphate storage granules, and the capsule stain (to identify encapsulated bacteria). We will be performing the Gram stain and endospore staining procedures in lab, and view prepared slides that highlight some of the other cellular structures present in some bacteria.

Negative Staining Technique

In a **negative staining technique**, an acidic, anionic dye is mixed with a cell sample. The dye changes the color of the background, not the cells, causing the cells to stand out. This process can be considered the opposite of simple staining. An **anion** is a negatively charged ion, therefore an anionic dye has a negative charge. When the negatively charged dye is added to the negatively charged cells, the two repel each other, meaning they push apart. When the mixture is placed on a slide and air dried, what results is a darkly dyed background, surrounding clear, unstained cells. The transparent cells are now highly visible but are unaffected by direct contact with the dye and distortion from heat fixing, which is not needed in a negative stain.

India ink is the classic example of a negative stain. It will turn the background a dark brown to black, leaving the clear, bright cells unstained and highly visible. Below are cells of the fungal pathogen Cryptococcus. The India ink has colored the background brown, leaving the cells their natural color.

- **University Library Reference-**

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  Textbook of Microbiology - Ananthanarayan And Paniker

- **Ancient Indian Literature Reference - Rasa-]ala-Nidhi or Ocean of Indian chemistry and alchemy/vol.vIEd.1984/AvaniPrakashan,Ahmedabad,India;CharakSamhita**[**https://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5000494453**](https://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5000494453)

- **Review of Literature-** Pallavi Singh, Pramod K. Raghav & Mansi Pareek**(2019)** Development of Ragi-Wheat Composite Value-Added Product Enriched with *Moringaolifera* for Reproductive Age Women. Pramana Research Journal**,** 9(3):614-621, ISSN: 2249-297 **UGC Approved Journal**

- **Competitive questions from today topic (2 questions Minimum)-**

Vaccine is available for all except one of the following pathogens

- Bordetella pertussis
- Haemophilus influenzae type b
- Clostridium tetani
- Helicobacter pylori

Drug resistance among bacteria involved in hospital infections is commonly due to

- Multi drug therapy
- Probiotic bacteria
- Transfer of resistance genes
- Mutation in target genes

- **Questions to check understanding level of students-**
  - What is Negative staining technique?
  - Give some name of Differential stains.

CHAPTER 7
MICROBIAL DIVERSITY AND TAXONOMY

General Introduction: Microbial diversity and taxonomy.

Microbial Diversity:

Microbial diversity' considers the vast array of microorganisms—the smallest forms of life—which exist everywhere. The three primary groups of microorganisms are bacteria, archaea, and eukaryotes. Bacteria and archaea are prokaryotes with their genetic material held in a single chromosome. In eukaryotes, most of the genome is held in multiple chromosomes. Over 11,000 species of bacteria have been identified using microscopic identification of cell shape and metabolic activity, Gram-staining techniques, and genetic identification of RNA and DNA sequences. There are 500 named species of archaea, divided into two phyla: the euryarchaeota and the crenarchaeota. There are eight supergroupings of eukaryotes, all of them include single-celled organisms, and five are entirely microbial.

Microbial taxonomy:

Microbial taxonomy is a means by which **microorganisms** can be grouped together. Organisms having similarities with respect to the criteria used are in the same group, and are separated from the other groups of microorganisms that have different characteristics.

There are a number of taxonomic criteria that can be used. For example, numerical taxonomy differentiates microorganisms, typically **bacteria**, on their phenotypic characteristics. Phenotypes are the appearance of the microbes or the manifestation of the genetic character of the microbes. Examples of phenotypic characteristics include the Gram stain reaction, shape of the bacterium, size of the bacterium, where or not the bacterium can propel itself along, the capability of the microbes to grow in the presence or absence of oxygen, types of nutrients used, chemistry of the surface of the bacterium, and the reaction of the **immune system** to the bacterium.

Numerical taxonomy typically invokes a number of these criteria at once. The reason for this is that if only one criterion was invoked at a time there would be a huge number of taxonomic groups, each consisting of only one of a few microorganisms. The purpose of grouping would be lost. By invoking several criteria at a time, fewer groups consisting of larger number of microorganisms result.

The groupings result from the similarities of the members with respect to the various criteria. A so-called similarity coefficient can be calculated. At some imposed threshold value, microorganisms are placed in the same group.

A well-known example of taxonomic characterization is the kingdom, division, class, family, genus, species and strain divisions. Such a "classical" bacterial organization, which is typified by the Bergey's Manual of Determinative Bacteriology, is based on metabolic, immunological, and structural characteristics. Strains, for example, are all descended from the same organism, but differ in an aspect such as the antigenic character of a surface molecule.

Microbial taxonomy can create much order from the plethora of microorganisms. For example, the **American Type Culture Collection** maintains the following, which are based on taxonomic characterization (the numbers in brackets indicate the number of individual organisms in the particular category): algae (120), bacteria (14400), **fungi** (20200), **yeast** (4300), **protozoa** (1090), animal **viruses** (1350), **plant viruses** (590), and bacterial viruses (400). The actual number of microorganisms in each category will continue to change as new microbes are isolated and classified. The general structure, however, of this classical, so-called phenetic system will remain the same.

- **University Library Reference-**

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- **Competitive questions from today topic (2 questions Minimum)-**

BCG vaccine

- is an attenuated M. tuberculosis strain.
- reduces the incidence of tubercular meningitis.
- induces protective CMI response against atypical mycobacteria
- protects against pulmonary tuberculosis

Why catalase is induced in microbes during exposure to the pollutants?

- Because it involve in biotransformation of that pollutant.
- Because of oxidative stress produced due to exposure of pollutant.
- Pollutants are general inducers of catalase
- Because catalase in involved in the metabolism of metabolite generated from pollutants.

- **Questions to check understanding level of students-**
  - What is Microbial diversity?
  - Explain microbial taxonomy.

Types of microorganisms. Classification: Haeckel's three kingdom concept.

Haeckel's Three kingdom System of Classification:

- Classification is the arrangement of organisms into taxonomic groups known as taxa on the basis of similarities or relationships.
- Closely related organisms (i.e., organisms having similar characteristics) are placed into the same taxon.
- Organisms are categorized into larger groups based on their similarities and differences.
- The classification of living organisms is a complex and controversial subject because of which different taxonomic classification schemes existed at different times.

- In his classification scheme, Linnaeus recognized only two kingdoms of living things: Animalia and Plantae.
- At the time, microscopic organisms had not been studied in detail. Either they were placed in a separate category called Chaos or, in some cases, they were classified with plants or animals.
- As the knowledge of the properties of various groups of microbial life exploded, it became apparent that at this level of biological knowledge a division of the living world into two kingdoms cannot really be maintained on a logical and consistent ground.

Then in the 1860s, the German investigator Ernst Haeckel proposed a three-kingdom system of classification.

 Three kingdom classification system was put forward by Haeckel in order to overcome the objections and limitations of the Two Kingdom System of Classification.

 Haeckel suggested that the inconsistencies of the two-kingdom system could be avoided by the recognition of a third kingdom, and he proposed Protista as a new kingdom to accommodate organisms exhibiting characters either common to both plants and animals, or unique to their own.

 Haeckel's three kingdoms were Animalia, Plantae, and Protista.

- The arrangement of kingdoms was done on the basis of morphological complexities and tissue system, the division of labor, and mode of nutrition.
- Unicellular animals, algae and fungi were separated from other organisms on the basis of lack of tissue differentiation.
- The new group was called the kingdom Protista.
- Organisms lacking morphological complexities, tissue system, the division of labor, and enjoying the diversified type of modes of nutrition were segregated and put under the kingdom Protista.
- Members of the kingdom Protista included the protozoa, fungi, bacteria, and other microorganisms.
- Later fungi and multicellular algae were taken out from the group.
- Organisms having diverse tissue-system with well- defined division of labour and maximum morphological complexities in their body remained segregated from protists and were

bifurcated into two categories: those enjoying autotrophic mode of nutrition were considered to be plants and put under kingdom Plantae, and those that have entirely holophagic (phagotrophic) mode of nutrition were considered to be animals and put under kingdom Animalia.

- According to this system, all known microorganisms came to be recognised as protists; neither plants nor animals.

 Haeckel's system was not widely accepted, however, and microorganisms continued to be classified as plants (for example, bacteria and fungi) or animals (for example, protozoa).
 Nucleated and anucleated organisms were kept together in protists.

 Heterotrophic bacteria and fungi placed along with autotrophic algae.

- **University Library Reference-**

  Microbiology by Pelczar- M.J.Chan ECS & Krieg NR-Tata Mcgraw Hill.
  Microbial Physiology 4th ed. By Alber G.Moat & John W.Foster Wileyliss

- **Ancient Indian Literature Reference - Rasa-]ala-Nidhi or Ocean of Indian chemistry and alchemy/vol.vIEd.1984/AvaniPrakashan,Ahmedabad,India;CharakSamhitahttps://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5000494453**

- **Review of Literature-** Engelkirk, P. G., Duben-Engelkirk, J. L., & Burton, G. R. W. (2011). Burton's microbiology for the health sciences. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

- **Competitive questions from today topic (2 questions Minimum)-**

Which one of the following DNA viruses has part of its life cycle involving Reverse Transcriptase enzyme, which is a hallmark of Retroviruses

- Epstein-Barr Virus
- Herpes Simplex Virus
- Hepatitis B Virus
  - Hepatitis C Virus

Which of the following enzymes is required to release the tension imposed by uncoiling of strands?

- Endonuclease
- DNA ligase
- DNA gyrase
- DNA helicase

- **Questions to check understanding level of students-**
  - Who discovered three kingdom classification?
  - What kingdom classification?

Basic Concept in Taxonomy Characteristics and structure of microbes: Algae, fungi.

What Are Algae?

Algae are a diverse group of aquatic organisms that have the ability to conduct photosynthesis. Certain algae are familiar to most people; for instance, seaweeds (such as kelp or phytoplankton), pond scum or the algal blooms in lakes. However, there exists a vast and varied world of algae that are not only helpful to us, but are critical to our existence. The classification of living organisms is a complex and controversial subject because of which different taxonomic classification schemes existed at different times.

Definition

The term "algae" covers many different organisms capable of producing oxygen through photosynthesis (the process of harvesting light energy from the sun to generate carbohydrates). These organisms are not necessarily closely related. However, certain features unite them, while distinguishing them from the other major group of photosynthetic organisms: the land plants.

Primarily, algae are not highly differentiated in the way that plants are, according to the authors of "Algae: Anatomy, Biochemistry, and Biotechnology, 2nd Ed." (CRC Press, 2014). That is to say, they lack true roots, stems and leaves, and a vascular system to circulate water and nutrients throughout their bodies. Second, many algae are unicellular, according to a 2014 articlepublished in the journal Current Biology. They also occur in a variety of forms and sizes. They can exist as single, microscopic cells; they can be macroscopic and multicellular; live in colonies; or take on a leafy appearance as in the case of seaweeds such as giant kelp. Picoplankton are between 0.2 to 2 micrometers in diameter, while the fronds of giant kelp are as large as 60 meters in length. Lastly, algae are found in a range of aquatic habitats, both freshwater and saltwater.

By virtue of these characteristics, the general term "algae" includes prokaryotic organisms — cyanobacteria, also known as blue-green algae — as well as eukaryotic organisms (all other algal

species). "Since "algae" do not form a natural group that has descended from a common ancestor, including cyanobacteria into the informal group "algae" is common," said Linda Graham, a professor of botany at the University of Wisconsin-Madison. "The term 'eukaryotic algae' excludes cyanobacteria." It is also interesting to note that chloroplasts, which are the site of photosynthesis in land plants, are adapted forms of cyanobacteria.

General characteristics

Habitat

The majority of algae live in aquatic habitats (Current Biology, 2014). Yet, the word "aquatic" is almost limited in its ability to encompass the diversity of these habitats. These organisms can thrive in freshwater lakes or in saltwater oceans. They can also endure a range of temperatures, oxygen or carbon dioxide concentrations, acidity and turbidity. For example, giant kelp are found more than 200 meters below the polar ice sheets, according to "Algae," while the unicellular green algal species *Dunaliella salina* is found in very salty, or hypersaline, environments such as the Dead Sea, according to a 2005 review article published in the journal Saline Systems. Free-floating, mostly unicellular algae that live within illuminated regions of water are known as planktonic. Those that adhere to surfaces are known as benthic algae. Such algae grow on mud, stones, other algae and plants, or animals, according to "Algae."

Algae are also able to survive on land. Some unexpected places where they grow are tree trunks, animal fur, snow banks, hot springs (according to "Algae") and in soil, including desert crusts (Current Biology, 2014).

Mostly, algae live independently in their various growth forms (single cells, colonies, etc.), but they can also form symbiotic relationships with a variety of non-photosynthetic organisms including ciliates, sponges, mollusks and fungi (as lichens). One of the benefits of such relationships is that they enable algae to broaden the horizons of their habitats.

Nutrition

As a general rule, algae are capable of photosynthesis and produce their own nourishment by using light energy from the sun and carbon dioxide in order to generate carbohydrates and oxygen. In other words, most algae are autotrophs or more specifically, photoautotrophs (reflecting their use of light energy to generate nutrients).

However, there exist certain algal species that need to obtain their nutrition solely from outside sources; that is, they are heterotrophic. Such species apply a variety of heterotrophic strategies to acquire nutrients from organic materials (carbon containing compounds such as carbohydrates, proteins and fats). Osmotrophy is the absorption of dissolved substances, and phagotrophy involves engulfing bacteria or other such prey. Other algae, known as auxotrophs, need to only acquire essential vitamins such as the B_{12}complex or fatty acids (according to "Algae").

According to the authors of "Algae," it is widely accepted that the nutritional strategies of algae exist on a spectrum combining photoautotrophy and heterotrophy. This ability is known as mixotrophy.

Reproduction

Algae are capable of reproducing through asexual or vegetative methods and via sexual reproduction.

According to the authors of "Algae," asexual reproduction involves the production of a motile spore, while vegetative methods include simple cell division (mitosis) to produce identical offspring and the fragmentation of a colony. Sexual reproduction involves the union of gametes (produced individually in each parent through meiosis).

Classification

Cyanobacteria

These are also referred to as blue-green algae. Though they are capable of conducting oxygen-producing photosynthesis and live in many of the same environments as eukaryotic algae, cyanobacteria are gram-negative bacteria, and therefore are prokaryotes. They are also capable of independently conducting nitrogen fixation, the process of converting atmospheric nitrogen to usable forms of the element such as ammonia.

The prefix "cyano" means blue. These bacteria have pigments that absorb specific wavelengths of light and give them their characteristic colors. Many cyanobacteria have the blue pigment phycocyanin, a light-harvesting pigment (it absorbs red wavelengths of light). Cyanobacteria all have some form of the green pigment chlorophyll, which is responsible for harvesting light energy during the photosynthetic process (Current Biology, 2014). Some others also have the red pigment phycoerythrin, which absorbs light with the green region and bestows the bacteria with a pink or red color.

Eukaryotic algae

The eukaryotic algae are polyphyletic, meaning that they did not evolve from a single common ancestor. This is clearly demonstrated in our current understanding of the tree of life — a family tree of all living organisms organized by their various evolutionary relationships. Eukaryotic algae are found distributed among many different groups, or major branches of the tree.

In a 2014 review article published in the journal Cold Spring Harbor Perspectives in Biology, author Fabien Burkilists five supergroups of eukaryotic organisms: Ophiskontha, Amoebozoa, Excavata, Archaeplastida and SAR (which comprises three groups, Stramenopiles, Alveolata and Rhizaria).

Archaeplastida includes plants and a variety of photosynthetic algal species such as the chlorophytes (a subset of green algae), charophytes (mainly freshwater green algae) and

glaucocystophytes (unicellular freshwater algae). Chlorophytes are the green algae that commonly form lichen partnerships with fungi.

Dinoflagellates are found within Alveolata. These are primarily unicellular marine and freshwater organisms. Many dinoflagellates have lost their plastids — the site of photosynthesis — through the course of evolution and are phagotropic or live as parasites. Still other algal species are found distributed amongst Alveolata, Excavata, Rhizaria and Chromista (Current Biology, 2014).

Importance

Probably the most important contribution of algae to our environment and well-being is the generation of oxygen through photosynthesis. "Algae are indispensable because they produce about half the oxygen in Earth's atmosphere," Graham told LiveScience.

According to a 2010 review article published in the journal Biofuels, petroleum is partially derived from ancient algae deposits. "Some very old oil deposits are attributed to cyanobacteria, though the identity of the producers is still uncertain," Graham said. "Younger oil deposits probably arose from eukaryotic marine green algae, coccolithophorids, and other microscopic marine phytoplankton." These oil deposits are a limited resource and are slowly dwindling with human use. As a result, researchers are looking into renewable alternatives.

Algal biofuels are a promising replacement for fossil fuels. All algae have the ability to produce energy-rich oils and several microalgal species naturally accumulate high levels of oil in their dry mass. Moreover, algae are found in diverse habitats and can reproduce quickly. They also efficiently use carbon dioxide. "Algae help to keep atmospheric carbon dioxide levels stable by storing [the gas] in organic materials that include oil deposits and inorganic carbonate rocks," Graham said. Green algae, diatoms and cyanobacteria are just some of the microalgal species that are considered good candidates for the production of biofuel (Biofuels, 2010).

Algal blooms

Algae, in the form of algal blooms, get a bad rap for creating toxic conditions in oceans and lakes. "Algal blooms" refers to the rampant growth of certain microalgae, which in turn leads to the production of toxins, disruption of the natural aquatic ecosystems and increases the costs of water treatments, according to the Environmental Protection Agency (EPA). The blooms take on the colors of the algae contained within them. Graham states that the main toxin producers in oceans are certain dinoflagellates and diatoms. In freshwaters, cyanobacteria are the main toxin producers, though some eukaryotic algae also cause problems. Under natural conditions, Graham notes that algae use the toxins to protect themselves from being eaten by small animals and only need a small amount to protect themselves.

The main cause of algal blooms is a phenomenon called nutrient pollution. With nutrient pollution, there is an excess of nitrogen and phosphorus, which can push algae toward unrestrained growth. The phenomenon is caused by a variety of human activities. The fertilizers

we use in agriculture and animal manures are rich in nitrogen, while improperly treated wastewater is high in both nitrogen and phosphorus, according to the EPA.

"It is a common societal perception that algae are noxious and should be eliminated at every opportunity. But that perception is wrong, because algae make oxygen, fish [they are a major source of food for aquatic organisms], oil, and many other useful materials," Graham told LiveScience. "Only a few species cause problems, and the worst of these is *Homo sapiens*."

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- **Ancient Indian Literature Reference - Rasa-]ala-Nidhi or Ocean of Indian chemistry and alchemy/vol.vIEd.1984/AvaniPrakashan,Ahmedabad,India;CharakSamhitahttps://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5000494453**

- **Review of Literature-** Trivedi P.C., Pandey S, and Bhadauria S. (2010). Textbook of Microbiology. Pointer Publishers; First edition.

- **Competitive questions from today topic (2 questions Minimum)-**

Usually intracellular pathogens avoid their transport to lysosome for their survival in the host cell. But which of the following intracellular pathogens survives in the lysosomes?

- Legionella
- Salmonella
- Mycobacterium
- Leishmania

When Hfr strain of E. coli is crossed with F- strain, recombinant obtained are

- always F+ ease
- always HFr+
- rarely F+
- rarely HFr+

- **Questions to check understanding level of students-**
    - Give five algae name?
    - Explain classification of algae and fungi?

Basic Concept in Taxonomy Characteristics and structure of microbes: Mycoplasma, viruses.

Mycoplasma:

Mycoplasma is a genus of bacteria that lack a cell wall around their cell membranes. This characteristic makes them naturally resistant to antibiotics that target cell wall synthesis (like the beta-lactam antibiotics). They can be parasitic or saprotrophic. Several species are pathogenic in humans, including *M. pneumoniae*, which is an important cause of "walking" pneumonia and other respiratory disorders, and *M. genitalium*, which is believed to be involved in pelvic inflammatory diseases. *Mycoplasma* species are the smallest bacterial cells yet discovered, can survive without oxygen, and come in various shapes. For example, *M. genitalium* is flask-shaped (about 300 x 600 nm), while *M. pneumoniae* is more elongated (about 100 x 1000 nm). Hundreds of mycoplasma species infect animals.

Etymology

The term *mycoplasma*, from the Greek μύκης, *mykes* (fungus) and πλάσμα, *plasma* (formed), was first used by Albert Bernhard Frank in 1889 to describe an altered state of plant cell cytoplasm resulting from infiltration by fungus-like microorganisms.Julian Nowak later proposed the genus name *Mycoplasma* for certain filamentous microorganisms imagined to have both cellular and acellular stages in their lifecycles, which could explain how they were visible with a microscope, but passed through filters impermeable to other bacteria.

Later, the name for *Mycoplasma* was **pleuropneumonia-like organisms** (PPLO), broadly referring to organisms similar in colonial morphology and filterability to the causative agent (a mycoplasma) of contagious bovine pleuropneumonia.

Characteristics

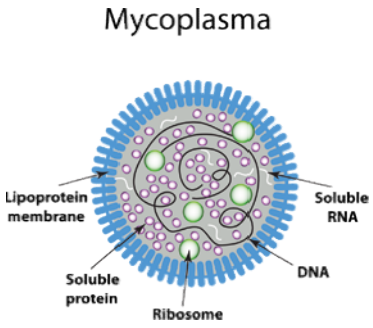
Over 100 species have been included in the genus *Mycoplasma*. Microbes of the class Mollicutes, to which Mycoplasma belongs, are parasites or commensals of humans, animals, and plants. The genus *Mycoplasma* uses vertebrate and arthropod hosts. Dietary nitrogen availability has been shown to alter codon bias and genome evolution in *Mycoplasma* and *Phytoplasma*.

Mycoplasmal bacteria are also known as mollicutes. They are the simplest and the smallest free-living prokaryotes.

Mycoplasmal bacteria have been found in the pleural cavities of cattle suffering from pleuropneumonia. These organisms are often called MLO (mycoplasma-like organisms) or PPLO (pleuropneumonia-like organisms).

Important characteristics of mycoplasmal bacteria

1. Cell wall is absent and plasma membrane forms the outer boundary of the cell.
2. Due to the absence of cell wall these organisms can change their shape and are pleomorphic.
3. Lack of nucleus and other membrane-bound organelles.
4. Genetic material is a single DNA duplex and is naked.
5. Ribosomes are 70S type.
6. Possess a replicating disc at one end which assist replication process and also the separation of the genetic materials.
7. Heterotrophic nutrition. Some live as saprophytes but the majority are parasites of plants and animals. The parasitic nature is due to the inability of mycoplasmal bacteria to synthesise the required growth factor.



- Diagram: Mycoplasma

Virus:

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

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

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

The origins of viruses in the evolutionary history of life are unclear: some may have evolved from plasmids—pieces of DNA that can move between cells—while others may have evolved from bacteria. In evolution, viruses are an important means of horizontal gene transfer, which increases genetic diversity in a way analogous to sexual reproduction. Viruses are considered by some to be a life form, because they carry genetic material, reproduce, and evolve through natural selection, although they lack key characteristics (such as cell structure) that are generally considered necessary to count as life. Because they possess some but not all such qualities, viruses have been described as "organisms at the edge of life", and as replicators.

Viruses spread in many ways. One transmission pathway is through disease-bearing organisms known as vectors: for example, viruses are often transmitted from plant to plant by insects that feed on plant sap, such as aphids; and viruses in animals can be carried by blood-sucking insects. Influenza viruses are spread by coughing and sneezing. Norovirus and rotavirus, common causes of viral gastroenteritis, are transmitted by the faecal–oral route, passed by contact and entering the body in food or water. HIV is one of several viruses transmitted through sexual contact and by exposure to infected blood. The variety of host cells that a virus can infect is called its "host range". This can be narrow, meaning a virus is capable of infecting few species, or broad, meaning it is capable of infecting many.

Viral infections in animals provoke an immune response that usually eliminates the infecting virus. Immune responses can also be produced by vaccines, which confer an artificially acquired immunity to the specific viral infection. Some viruses, including those that cause AIDS, HPV, and viral hepatitis, evade these immune responses and result in chronic infections. Several antiviral drugs have been developed.

Microbiology

Life properties

Opinions differ on whether viruses are a form of life, or organic structures that interact with living organisms. They have been described as "organisms at the edge of life", since they resemble organisms in that they possess genes, evolve by natural selection, and reproduce by creating multiple copies of themselves through self-assembly. Although they have genes, they do not have a cellular structure, which is often seen as the basic unit of life. Viruses do not have their own metabolism, and require a host cell to make new products. They therefore cannot naturally reproduce outside a host cell—although bacterial species such as rickettsia and chlamydia are considered living organisms despite the same limitation. Accepted forms of life use cell division to reproduce, whereas viruses spontaneously assemble within cells. They differ from autonomous growth of crystals as they inherit genetic mutations while being subject to natural selection. Virus self-assembly within host cells has implications for the study of the origin of life, as it lends further credence to the hypothesis that life could have started as self-assembling organic molecules.

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- **Review of Literature-** Tortora, Gerard J., Funke, Berdell R.Case, Christine L.. (2013) Microbiology :an introduction Boston : Pearson,

- **Competitive questions from today topic (2 questions Minimum)-**

Archea is considered as a separate group from bacteria and eukaryotes, based on

- genome sequence.
- 16S rRNA gene sequence.
- 23S rRNA gene sequence.
- EFTu sequence.

When Hfr strain of E. coli is crossed with F- strain, recombinant obtained are

- always F+ ease
- always HFr+
- rarely F+
- rarely HFr+

- **Questions to check understanding level of students-**
  - Give five mycoplasma name?
  - Explain classification of virus?

Basic Concept in Taxonomy Characteristics and structure of microbes: Protozoan's, bacteria.

Protozoa:

Protozoa (also **protozoan**, plural **protozoans**) is an informal term for single-celled eukaryotes, either free-living or parasitic, which feed on organic matter such as other microorganisms or organic tissues and debris. Historically, the protozoa were regarded as "one-celled animals", because they often possess animal-like behaviors, such as motility and predation, and lack a cell wall, as found in plants and many algae. Although the traditional practice of grouping protozoa with animals is no longer considered valid, the term continues to be used in a loose way to identify single-celled organisms that can move independently and feed by heterotrophy.

In some systems of biological classification, **Protozoa** is a high-level taxonomic group. When first introduced in 1818, Protozoa was erected as a taxonomic class, but in later classification schemes it was elevated to a variety of higher ranks, including phylum, subkingdom and kingdom. In a series of classifications proposed by Thomas Cavalier-Smith and his collaborators since 1981, Protozoa has been ranked as a kingdom. The seven-kingdom scheme presented by Ruggiero et al. in 2015, places eight phyla under Kingdom Protozoa: Euglenozoa, Amoebozoa, Metamonada, Choanozoa *sensu* Cavalier-Smith, Loukozoa, Percolozoa, Microsporidia and Sulcozoa. Notably, this kingdom excludes several major groups of organisms traditionally placed among the protozoa, including the ciliates, dinoflagellates, foraminifera, and the parasitic apicomplexans, all of which are classified under Kingdom Chromista. Kingdom Protozoa, as defined in this scheme, does not form a natural group or clade, but a paraphyletic group or evolutionary grade, within which the members of Fungi, Animalia and Chromista are thought to have evolved.

Characteristics

Size

Protozoa, as traditionally defined, range in size from as little as 1 micrometre to several millimetres, or more. Among the largest are the deep-sea–dwelling xenophyophores, single-celled foraminifera whose shells can reach 20 cm in diameter.

Habitat

Free-living protozoans are common and often abundant in fresh, brackish and salt water, as well as other moist environments, such as soils and mosses. Some species thrive in extreme environments such as hot springsand hypersaline lakes and lagoons. All protozoa require a moist habitat; however, some can survive for long periods of time in dry environments, by forming resting cysts which enable them to remain dormant until conditions improve.

Parasitic and symbiotic protozoa live on or within other organisms, including vertebrates and invertebrates, as well as plants and other single-celled organisms. Some are harmless or

beneficial to their host organisms; others may be significant causes of diseases, such as babesia, malaria and toxoplasmosis.

Feeding

All protozoans are heterotrophic, deriving nutrients from other organisms, either by ingesting them whole or consuming their organic remains and waste-products. Some protozoans take in food by phagocytosis, engulfing organic particles with pseudopodia (as amoebae do), or taking in food through a specialized mouth-like aperture called a cytostome. Others take in food by osmotrophy, absorbing dissolved nutrients through their cell membranes.

Parasitic protozoans use a wide variety of feeding strategies, and some may change methods of feeding in different phases of their life cycle. For instance, the malaria parasite *Plasmodium* feeds by pinocytosis during its immature trophozoite stage of life (ring phase), but develops a dedicated feeding organelle (cytostome) as it matures within a host's red blood cell.

Motility

Organisms traditionally classified as protozoa are abundant in aqueous environments and soil, occupying a range of trophic levels. The group includes flagellates (which move with the help of whip-like structures called flagella), ciliates (which move by using hair-like structures called cilia) and amoebae (which move by the use of foot-like structures called pseudopodia). Some protozoa are sessile, and do not move at all.

Classification

Further information: wikispecies: Protozoa

Historically, the Protozoa were classified as "unicellular animals", as distinct from the Protophyta, single-celled photosynthetic organisms (algae) which were considered primitive plants. Both groups were commonly given the rank of phylum, under the kingdom Protista. In older systems of classification, the phylum Protozoa was commonly divided into several sub-groups, reflecting the means of locomotion. Classification schemes differed, but throughout much of the 20th century the major groups of Protozoa included:

- Flagellates, or Mastigophora (motile cells equipped with whiplike organelles of locomotion, e.g., *Giardia lamblia*)
- Amoebae or Sarcodina (cells that move by extending pseudopodia or lamellipodia, e.g., *Entamoeba histolytica*)
- Sporozoans, or Sporozoa (parasitic, spore-producing cells, whose adult form lacks organs of motility, e.g., *Plasmodium knowlesi*)
    - Apicomplexa (now in Alveolata)
    - Microsporidia (now in Fungi)
    - Ascetosporea (now in Rhizaria)
    - Myxosporidia (now in Cnidaria)

- Ciliates, or Ciliophora (cells equipped with large numbers of short hairlike organs of locomotion, e.g., *Balantidium coli*

What Are Bacteria?

Bacteria are microscopic, single-celled organisms that thrive in diverse environments. These organisms can live in soil, the ocean and inside the human gut.

Humans' relationship with bacteria is complex. Sometimes bacteria lend us a helping hand, such as by curdling milk into yogurt or helping with our digestion. In other cases, bacteria are destructive, causing diseases like pneumonia and methicillin-resistant *Staphylococcus aureus*

Structure

Bacteria (singular: bacterium) are classified as prokaryotes, which are single-celled organisms with a simple internal structure that lacks a nucleus, and contains DNA that either floats freely in a twisted, thread-like mass called the nucleoid, or in separate, circular pieces called plasmids. Ribosome are the spherical units in the bacterial cell where proteins are assembled from individual amino acids using the information encoded in ribosomal RNA.

Bacterial cells are generally surrounded by two protective coverings: an outer cell wall and an inner cell membrane. Certain bacteria, like the mycoplasmas, do not have a cell wall at all. Some bacteria may even have a third, outermost protective layer called the capsule. Whip-like extensions often cover the surfaces of bacteria — long ones called flagella or short ones called pili — that help bacteria to move around and attach to a host.

Classification

A few different criteria are used to classify bacteria. The organisms can be distinguished by the nature of their cell walls, by their shape, or by differences in their genetic makeup.

The Gram stain is a test used to identify bacteria by the composition of their cell walls, named for Hans Christian Gram, who developed the technique in 1884. The test stains Gram-positive bacteria, or bacteria that do not have an outer membrane. Gram-negative bacteria don't pick up the stain. For example, *Streptococcus pneumoniae* (*S. pneumoniae*), which causes pneumonia, is a Gram-positive bacterium, but *Escherichia coli* (*E. coli*) and *Vibrio cholerae*, which causes cholera, are Gram-negative bacteria.

There are three basic bacterial shapes: Round bacteria called cocci (singular: coccus), cylindrical, capsule-shaped ones known as bacilli (singular: bacillus); and spiral bacteria, aptly called spirilla (singular: spirillum). The shapes and configurations of bacteria are often reflected in their names. For example, the milk-curdling *Lactobacillus acidophilus* are bacilli, and pneumonia-causing *S. pneumoniae* are a chain of cocci. Some bacteria take other shapes, such as stalked, square or star.

Reproduction

Most bacteria multiply by a process called binary fission, according to the Cornell University College of Agriculture and Life Sciences. In this process, a single bacterial cell, called the "parent," makes a copy of its DNA and grows larger by doubling its cellular content. The cell then splits apart, pushing the duplicated material out and creating two identical "daughter" cells.

Some bacterial species, such as cyanobacteria and firmicutes, reproduce via budding. In this case, the daughter cell grows as an offshoot of the parent. It starts off as a small nub, grows until it is the same size as its parent, and splits off.

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- **Review of Literature-** Tortora, Gerard J., Funke, Berdell R.Case, Christine L.. (2013) Microbiology :an introduction Boston : Pearson,

- **Competitive questions from today topic (2 questions Minimum)-**

Measles, Mumps, Rubella-MMR combined vaccine represents which one of following vaccine categories?

- Inactivated/killed.
- Live, attenuated.
- Subunit.
- Toxoid (inactivated toxin).

Electrophoresis of a purified protein in SDS-PAGE in the presence of 2- mercaptoethanol yields two bands of 35 kDa and 45 kDa. However, in a gel filtration chromatography, the same protein elutes as 80 kDa. What conclusion will you draw from the results?

- The protein is not purified to homogeneity.
- Two bands generated in SDS-PAGE due to degradation.
- The protein is a homodimer

- The protein is a heterodimer

- **Questions to check understanding level of students-**
  - Give five Bacteria name?
  - Explain classification of protozoan?

Reproduction of fungi.

Fungi Reproduction:

Fungi can reproduce asexually by fragmentation, budding, or producing spores, or sexually with homothallic or heterothallic mycelia.

Reproduction

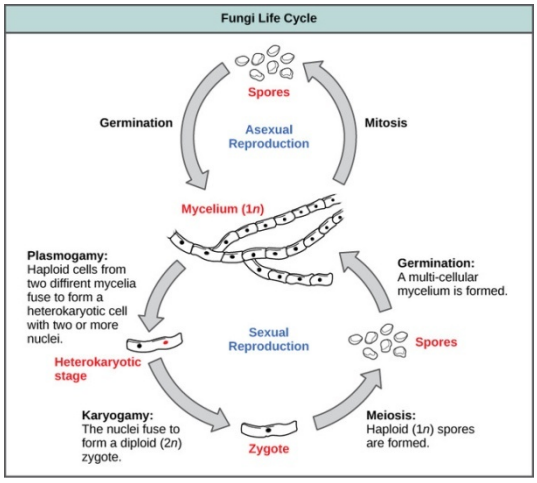
Fungi reproduce sexually and/or asexually. Perfect fungi reproduce both sexually and asexually, while imperfect fungi reproduce only asexually (by mitosis).

In both sexual and asexual reproduction, fungi produce spores that disperse from the parent organism by either floating on the wind or hitching a ride on an animal. Fungal spores are smaller and lighter than plant seeds. The giant puffball mushroom bursts open and releases trillions of spores. The huge number of spores released increases the likelihood of landing in an environment that will support growth.

Asexual Reproduction

Fungi reproduce asexually by fragmentation, budding, or producing spores. Fragments of hyphae can grow new colonies. Mycelial fragmentation occurs when a fungal mycelium separates into pieces with each component growing into a separate mycelium. Somatic cells in yeast form buds. During budding (a type of cytokinesis), a bulge forms on the side of the cell, the nucleus divides mitotically, and the bud ultimately detaches itself from the mother cell.

The most common mode of asexual reproduction is through the formation of asexual spores, which are produced by one parent only (through mitosis) and are genetically identical to that parent. Spores allow fungi to expand their distribution and colonize new environments. They may be released from the parent thallus, either outside or within a special reproductive sac called a sporangium.



- Diagram: fungi reproduction

Sexual Reproduction

Sexual reproduction introduces genetic variation into a population of fungi. In fungi, sexual reproduction often occurs in response to adverse environmental conditions. Two mating types are produced. When both mating types are present in the same mycelium, it is called homothallic, or self-fertile. Heterothallic mycelia require two different, but compatible, mycelia to reproduce sexually.

Although there are many variations in fungal sexual reproduction, all include the following three stages. First, during plasmogamy (literally, "marriage or union of cytoplasm"), two haploid cells fuse, leading to a dikaryotic stage where two haploid nuclei coexist in a single cell. During karyogamy ("nuclear marriage"), the haploid nuclei fuse to form a diploid zygote nucleus. Finally, meiosis takes place in the gametangia (singular, gametangium) organs, in which gametes of different mating types are generated. At this stage, spores are disseminated into the environment.

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- **Ancient Indian Literature Reference - Rasa-]ala-Nidhi or Ocean of Indian chemistry and alchemy/vol.vIEd.1984/AvaniPrakashan,Ahmedabad,India;CharakSamhitahttps://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5000494453**

- **Review of Literature-** Engelkirk, P. G., Duben-Engelkirk, J. L., & Burton, G. R. W. (2011). Burton's microbiology for the health sciences. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

- **Competitive questions from today topic (2 questions Minimum)-**

Which of the following organisms does NOT require sunlight to live?

- Trees photosynthetic.
- bacteria.
- algae.
- chemosynthetic bacteria.

Agar-Agar is derived from

- A fungi
- B algae
- C bryophytes
- gymnosperm

- **Questions to check understanding level of students-**
  - Types of reproduction in fungi?
  - Difference between asexual and sexual reproduction.

Reproduction of mycoplasma.

Reproduction of mycoplasma:

The cell reproduction cycle of parasitic wall-free bacteria, mycoplasma, is reviewed. DNA replication of *Mycoplasma capricolum* starts at a fixed site neighboring the *dnaA* gene and proceeds to both directions after a short arrest in one direction. The initiation frequency fits to the slow speed of replication fork and DNA content is set constant. The replicated chromosomes migrate to one and three quarters of cell length before cell division to ensure delivery of the replicated DNA to daughter cells. The cell reproduction is based on binary fission but a branch is formed when DNA replication is inhibited. *Mycoplasma pneumoniae* has a terminal structure, designated as an attachment organelle, responsible for both host cell adhesion and gliding motility. Behavior of the organelle in a cell implies coupling of organelle formation to the cell reproduction cycle. Several proteins coded in three operons are delivered sequentially to a position neighboring the previous organelle and a nascent one is formed. One of the duplicated attachment organelles migrates to the opposite pole of the cell before cell division. It is becoming clear that mycoplasmas have specialized cell reproduction cycles adapted to the limited genome information and parasitic life.

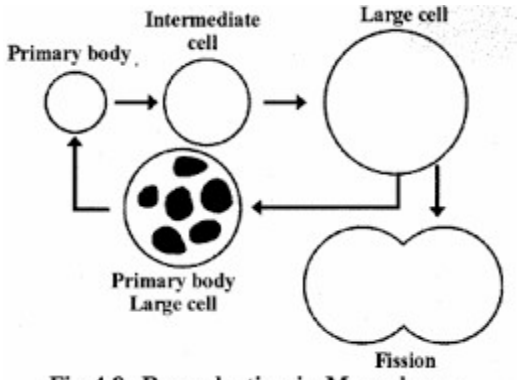

Fig 4.8: Reproduction in Mycoplasma

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  Microbial Physiology 4th ed. By Alber G.Moat & John W.Foster Wileyliss

- **Ancient Indian Literature Reference - Rasa-]ala-Nidhi or Ocean of Indian chemistry and alchemy/vol.vIEd.1984/AvaniPrakashan,Ahmedabad,India;CharakSamhitahttps://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.5000494453**

- **Review of Literature-** Engelkirk, P. G., Duben-Engelkirk, J. L., & Burton, G. R. W. (2011). Burton's microbiology for the health sciences. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

- **Competitive questions from today topic (2 questions Minimum)-**

Cyanobacteria name has been given to

- Mycoplasma.
- Myxophyceae
- Myxomycete.
- Schizomycetes.

Which one among the following is considered as the connecting link between non-living and living according to the theory of evolution of life?

- Viruses
- Bacteria
- Phaeophyceae members
- Green algae

- **Questions to check understanding level of students-**
  - Types of reproduction in mycoplasma?
  - Difference between asexual and sexual reproduction.

Reproduction of algae.

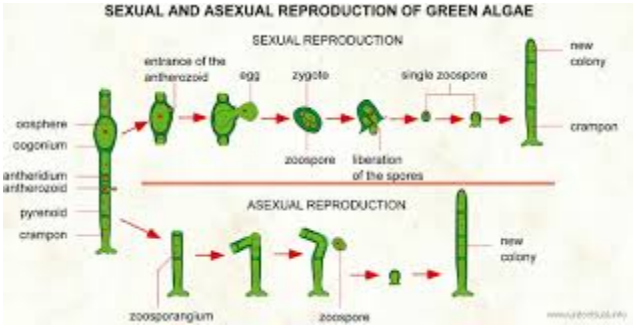
Reproduction and life histories:

Asexual reproduction is the production of progeny without the union of cells or nuclear material. Many small algae reproduce asexually by ordinary cell division or by fragmentation, whereas larger algae reproduce by spores. Some red algae produce monospores (walled, nonflagellate, spherical cells) that are carried by water currents and upon germination produce a new organism. Some green algae produce nonmotile spores called aplanospores, while others produce zoospores, which lack true cell walls and bear one or more flagella. These flagella allow zoospores to swim to a favourable environment, whereas monospores and aplanospores have to rely on passive transport by water currents.

Sexual reproduction is characterized by the process of meiosis, in which progeny cells receive half of their genetic information from each parent cell. Sexual reproduction is usually regulated by environmental events. In many species, when temperature, salinity, inorganic nutrients (e.g., phosphorus, nitrogen, and magnesium), or day length become unfavourable, sexual reproduction

is induced. A sexually reproducing organism typically has two phases in its life cycle. In the first stage, each cell has a single set of chromosomes and is called haploid, whereas in the second stage each cell has two sets of chromosomes and is called diploid. When one haploid gamete fuses with another haploid gamete during fertilization, the resulting combination, with two sets of chromosomes, is called a zygote. Either immediately or at some later time, a diploid cell directly or indirectly undergoes a special reductive cell-division process (meiosis). Diploid cells in this stage are called saprophytes because they produce spores. During meiosis the chromosome number of a diploid sporophyte is halved, and the resulting daughter cells are haploid. At some time, immediately or later, haploid cells act directly as gametes. In algae, as in plants, haploid cells in this stage are called gametophytes because they produce gametes.

The life cycles of sexually reproducing algae vary; in some, the dominant stage is the sporophyte, in others it is the gametophyte. For example, (class Phaeophyceae) has a diploid (sporophyte) body, and the haploid phase is represented by gametes. *Ectocarpus* (class Phaeophyceae) has alternating diploid and haploid vegetative stages, whereas (class Charophyceae) has a haploid vegetative stage, and the zygote is the only diploid cell.



- **University Library Reference-**

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- **Competitive questions from today topic (2 questions Minimum)-**

Which one of the following algae is a source of iodine ?

- Polysiphonia.
- Laminaria
- Nostoc.
- Diatoms.

True nucleus is absent in which one of the following?

- Green algae
- Bacteria
- Lichen
- Fungi

- **Questions to check understanding level of students-**
  - Types of reproduction in algae?
  - Difference between asexual and sexual reproduction.

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