D.K.M. COLLEGE FOR WOMEN

(AUTONOMOUS), VELLORE

eRESOURCES

Digital Learning

E CONTENT TITLE : GENERAL MICROBIOLOGY

DEPARTMENT : MICROBIOLOGY

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CORE I

GENERAL MICROBIOLOGY

Unit-I: History and Microscopy (20 Hrs)

Definition, branches and scope of microbiology – History – Contributions of Antony Van Leewenhoek, Louis Pasteur, Robert Koch, Joseph Lister, Beijerinck, Winogradsky, Alexander Fleming Selman Waksman, Lazarro Spallanzani and John Tyndall. Spontaneous generation and biogenesis. Branches of Microbiology – Industrial, medical and Environmental microbiology. Microscopy – Simple, Compound – Light, Dark, Phase contrast and Fluorescent microsopes. Electron microscopy – Transmission and scanning electron microscopes.

Unit-II: Microbial Classification and Taxonomy (18 Hrs)

Microbial kingdoms: Woose classification - Bacteria, Eubacteria, Archaebacteria, Hackels three kingdom classification and Whittaker's five kingdom classification. General characteristics of algae, fungi & viruses. Anatomy of Prokaryotes – Ultra structure and function of cell wall, flagella, slime layer, capsule, pili, cytoplasmic membrane, cytoplasmic inclusions and endospore. Mechanism of spore germination and sporulation.

Unit-III: Sterilization and Antimicrobial chemotherapy (17 Hrs)

Definitions: Sterilization, flaming, incineration, disinfection and antisepsis. Sterilization principles & techniques – dry heat, moist heat, pasteurization, tyndallization, radiation and filtration. Disinfection methods. Antimicrobial chemotherapy: Classification & mode of action of antibiotics [Cellwall synthesis inhibitors, antibiotic causes damage to cell membrane, inhibitors of protein & nucleic acid synthesis and inhibitors of specific enzyme system]: Testing methods – disc diffusion and dilution susceptibility [MIC] test.

Unit-IV: Culture and Staining techniques (17 Hrs)

Culture media and its types – basal, enriched, enrichment, selective, differential, transport & anaerobic media. Preservation of cultures – Lyophillization – aerobic and anaerobic culture techniques. Stains and staining techniques – Simple staining, differential staining [Gram staining & Acid fast staining], special staining [capsule staining & endospore staining].

Unit-V: Bacterial growth and metabolism (18 Hrs)

Microbial growth – factors affecting microbial growth, techniques for quantifying microbial growth. Nutritional requirements – Growth factors - Growth curve. Microbial metabolism: Respiration – aerobic respiration [ETC, TCA Cycle], Anaerobic respiraton [Glycolysis]; fermentation and Photosynthesis [Cyclic & Non-cyclic]. Photosynthetic bacteria.

UNIT – I

DEFINITION & SCOPE OF MICROBIOLOGY

MICROBIOLOGY:

Microbiology is the science that deals with microorganisms, a vast multitude of living organisms whose existence cannot even be suspected with an unaided eye. Microbes include bacteria,viruses, fungi, algae, protozoa and helminths, recently prions [``infectious proteins"]

SCOPE OF MICROBIOLOGY

Scope of microbiology includes different field of science. There are many branches of study in microbiology which includes:

BACTERIOLOGY: Study of bacteria

MYCOLOGY : Study of fungi and yeast

VIROLOGY : Study of viruses

PARASITOLOGY : Study of parasitic protozoans and helminthes

IMMUNOLOGY : Study of the humoral and cellular immune response to disease agents and allergens

BRANCHES IN MICROBIOLOGY:

Epidemiology and public health microbiology which deals with distribution and spread of diseases and their control and prevention.

FOOD MICROBIOLOGY:

- Use of microbes in the production of food products and drinks.

AGRICULTURAL AND VETERINARY MICROBIOLOGY:

- Use of microbes to increase crop and livestock yield and control of plant pest and animal diseases.

ENVIRONMENTAL MICROBIOLOGY:

- Study of the beneficial and harmful effects of microbes on the environment.

BIOTECHNOLOGY:

- Use of genetic engineering and recombinant technology of the microorganism.

GENETIC ENGINEERING AND RECOMBINANT DNA TECHNOLOGY:

- Microorganisms can be used to manufacture large amount of human hormones and medical substances.

IMMUNOLOGY:

- Infectious diseases and its allergic materials were studied by the diagnosis.

HISTORY OF MICROBIOLOGY

In the early days of the development of the study of microorganism, the branch of science was dominated by the medical profession.

The science of bacteriology had its birth with man's growing enthusiasm in grinding pieces of glass and then combining them to produce enlargement of various structures which could be seen through then.

ANTON VON LEEUWENHOEK:

With such crude microscope he discovered in 1674 a hither to unknown type of life in drops of saliva and water from various sources.He viewed through his microscope bacteria, protozoa, yeast, spermatozoa, RBC and muscle nerve tissues.He described and depicted these tiny living creatures, wriggling and darting across the field of his little microscope.He named these micro organism ``dierkens" also spelled ``diertgens" which was translated to animalcules by the Royal society.

SPONTANEOUS GENERATION VS BIOGENESIS:

Aristotle [384-322BC] thought simple invertebrates from spontaneous generation.Francesco redi [1626-1697] series of experiment on decaying meat and it ability to produce maggots spontaneously.

JOHN NEEDHAM (1713-1781):

He boiled mutton broth and tightly stoppered the flask.Many flasks became cloudy and contain microorganism.He thought organic matter is vital force to confer the properties of life on non-living matter.

LAZZARO SPALLANZANI(1729-1799):

He improved needham experiment by sealing the flask and placed in boiling water for ¾ of an hour, no growth to place.He proposed air carried germs to the culture medium but others said air is external force required for the growth of animals.

THEODORE SCHWANN [1810-1882]:

He allowed the air to enter the flask but after passing in red hot tube. Broth remains sterile

FRIEDRICH SCHRODER AND THEODER VON DUSCH:

He allowed the air to enter a flask after passing it in cotton wool, no growth occurred.

LOUIS PASTEUR[1822-1895]

He settled the matter once and for all he filtered the air through cotton and found objects and trapped. If placed in sterile medium after air filter through it, microbial growth occurred

He placed a sterile nutrient solution and heated their neck in flame and drew them to variety of curves and boiled for few minutes. no growth, dust and germs trapped in walls of curved neck.[1861]he resolved the controversy.

JOHN TYNDALL [1820-1893] deal a final blow to abiogenesis 1877 that dust carry germs, if dust is absent broth remain sterile.

He also provide evidence for heat – resistant forms of bacteria.

BEIJERINCK, MARTINUS W.: (1851-1931) Dutch microbiologist who was the first person to use the term "virus" for the invisible disease-causing material that he showed to be self-replicating.

He originated selective culture techniques, also known as enrichment culturing, and was the first to isolate a wide range of microorganisms.

In studying tobacco mosaic disease, Beijerinck concluded that the filterable pathogen was a "contagium vivum fluidum," a term he coined to convey his concept of a living infectious agent in a fluid (noncellular) form -- a revolutionary idea at a time when life and cellularity were thought to be inextricably connected.

SERGEI WINOGRADSKY 1856 – 1953.founded microbial ecology and he was a founding father of microbiology. In an unusually long scientific career, he:

- Discovered *chemosynthesis* – an entirely new mode of life, in which the energy to build organic molecules comes from chemical reactions rather than from sunlight in the more familiar *photosynthesis*.
- Invented the Winogradsky column.
- Discovered and isolated nitrogen fixing bacteria in soil that make nitrates available to green plants.
- Founded microbial ecology, where the interactions of microbes in cycles with their natural environments are studied holistically.

SELMAN ABRAHAM WAKSMAN (July 22, 1888 – August 16, 1973) was a Ukrainian-born, Jewish-American inventor, biochemist and microbiologist whose research into the decomposition of organisms that live in soil enabled the discovery of streptomycin and several other antibiotics. He discovered a number of antibiotics and he introduced procedures that have led to the development of many others. In 1952, he was awarded the Nobel Prize in Physiology or Medicine for "ingenious, systematic and successful studies of the soil microbes that led to the discovery of streptomycin."

FERBINAND COHN

He discovered heat resistance bacterial endospores. He discovered dust free chamber ``Incubator" for growth. Later on he introduced tyndallization process that is intermittent heating techniques. Spores survive above 100˚. So heated the broth and till the spores are completely become vegetative cells.

JOSEPH LISTER (1827-1912)

After discovery of germ theory of diseases he worked on prevention of wound infections. (carbolic acid). Antiseptic surgery was designed to prevent cross infection. Instruments were heat sterilized and phenol was used on surgical dressing and sprayed over the surgical area.

LOUIS PASTEUR

He is rightly called father of microbiology,

MAIN CONTRIBUTIONS

Tartaric acid- two types of crystals which could be separated microscopically.Discovery of microbial fermentation- grape juice and yeast-wine –heating the juice-yeast killed-applying again-reappears. Disproving spontaneous generation:Discovery of aerobicand anaerobic microorganisms-19th century butyric acid fermentation. Absence of oxygen-survive micro organisms-anerobes. Silk worm disease-pebrine-protozoan microbe is responsible. He demonstrated eggs free from microbes for breeding could control the disease, significant for germ theory of disease.

CHICKEN CHOLERAE VACCINE, CHICKEN DEMONSTRATION

Anthrax vaccine attenuated culture increases immunity. Rabies vaccine from rabbit brain.Pasteurization-process of partial killing of micro organisms.

ROBERT KOCH (1843-1910)

He studied on anthrax bacilli and explained the germ theory of disease- which is microbes are responsible for diseases. He grown the bacilli in broth and transferred to humans and observed multiplication and spore formation and he transferred to fresh humans and it germinates and finally to mice and it causes disease. ``Jacob Henle" discovered the *Bacillus anthrax.*

Koch postulates

Microorganisms must be present in every cases of the disease but absence from healthy organisms. The suspected microorganisms must be isolated and grown in pure culture. The same disease must produce when isolated microorganisms inoculate into a healthy host (lab animals). Same organisms must be isolated again from diseased host (lab animals)

Discovery of tuberculosis bacilli*; M.tuberculosis.* Method of making smears of bacteria on glass slide and staining them with aniline dyes. Plate method for isolating pure cultures, Koch used, gelatin for solidifying agent. Dr. Walter Hesse and his wife Fanny used agar instead of gelatin.

Richard petri- petri dish.

Alexander flemming – blue green mould *Penicilliumnotatum* contaminates clothes, shoes, fruits, bread and cheese looking like broom "Penicillin" antibiotics. Iwanowsky– causative agent of TM disease. Edward jenner Cow pox to small pox vaccine Pustules developed in boy while injecting cow pox fluid. Several weeks later injected with small pox fluid no disease occur. Boy got the immunity toward small pox variola ,vaccinae virus. ElieMetchikoff Phagocytosis mechanisms Emil vonbehring (1854-1917) and shibasaburokitasato, inactivated toxin into rabbits inducing them to produce an anti-toxin.

MICROSCOPY

INTRODUCTION :-

- Earlier microscopes was **singlelens magnifying glasses** with limited magnifications.
- **Antonie van Leeuwenhoek (1623 – 1723)** developed a very high magnifications in simple microscope.
- " Microscope is an instrument used to produce a large and clear image of the object."
- Clarity refers to Resolving Power.
- Magnification obtained in Magnifying × Enlarging the image.
- They are in

SIMPLE:

Single convex lens (or) more combinations of cells.

M = 250/f +1, 3x, 20x

COMPOUND:

- Higher magnification and better resolution.
- It has most common types compound microscopes they are,
- LIGHT :- Bright, Dark field phase, Fluorescence, UV, Interference.
- ELECRONIC:- Transmission, Scanning.
- ATOMIC FORCE MICROSCOPY:- Confocal , 3D.
- SCANNING PROBE (or) SCANNING TUNNELLING.

PARTS OF MICROSCOPE:-

MECHANICAL PARTS: Base, Pillar, Inclination joint, Arm & Body tube, Resolving Nose piece, Adjustment knobs, Stage (illumination), Substage condenser, Iris diaphragm.

OPTICAL PARTS: Eye pieces (ocular) – 5x, 10x, 15x

Objective lens – 10x (low), 40x & 60x (high), 100x (oil immersion). Achromatic, Apochromatic and fluorite lens, Which reduces the chromatic abbreviation.

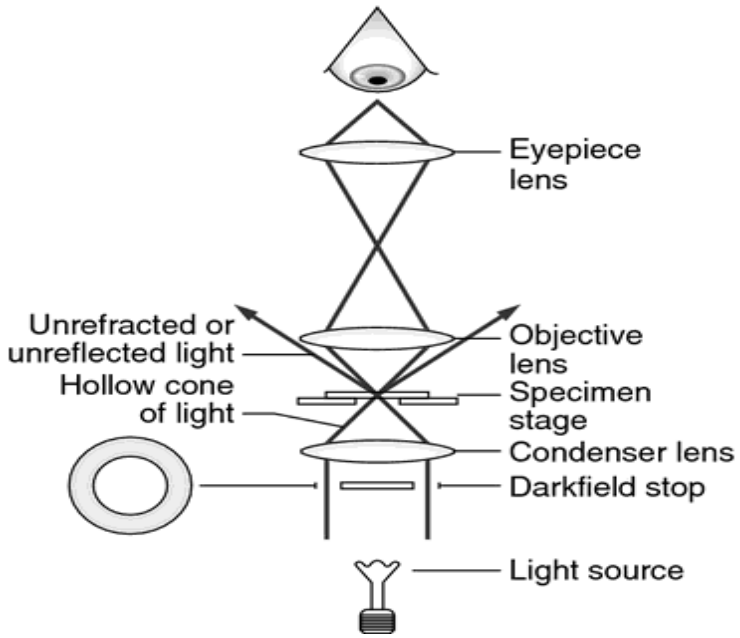
Abbe equation 2 = 0.5x/n sinσ

It is minimal distance (d) between two objects that reveals them as separates the entities depends on the wavelength of light (λ). NA= n Sinϕ.

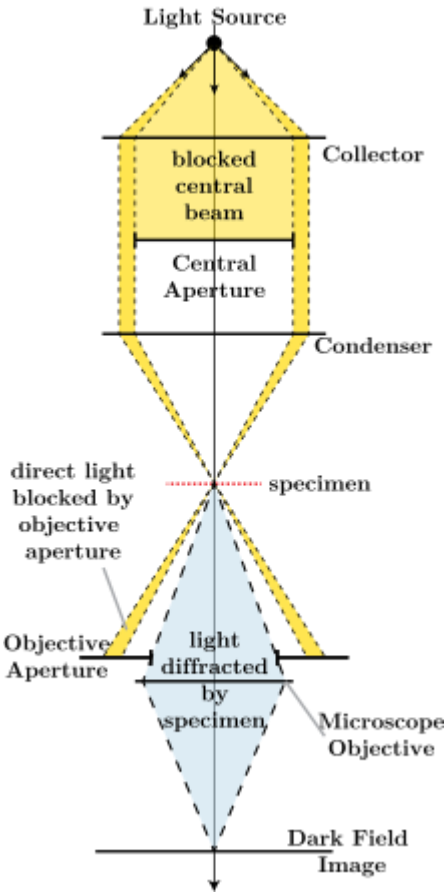
Refractive index for air is 1.00. R.I. of orl is 1.5.

BRIGHT FIELD MICROSCOPE:

- Simple, Image is dark around a bright background.
- Light sense is by mirror and electric illuminator.
- Same parts.
- Advanced form binocular.



DARK FIELD MICROSCOPE:



- Used for utilizing the unstained cells and organisms.
- A hollow cone of light is focused on the specimen in which unreflected / Unrefracted light rays do not enter the objective.
- Only light that has been reflected / refracted forms a image.
- Here field is dark and specimen will be bright.
- Especially for *Treponema pallidum.*
- Eg: In dark (room dust particles) in air are visible as bright particles in light. Dark field when light rays passes.

TYPES OF CONDENSOR:

- ABBE'S CONDENSOR: Simple, 2 lenses but they don't correct the any chromate (or) spherical aberration. Useful for low power.
- APALANTIC CONDENSOR: 2+1 third lens to correct the spherical aberration it gives good image in mono chromate light.
- ACHROMATIC CONDENSOR: Focuses on incident light of object, very little to escape and produce glare.
- It corrects both sphere and chromatic aberration.

ABERRATION IN OBJECTIVE:

- Aberrations are defects in the images because of the variation in refraction and focus by the peripheral parts of objectives.
- 2 types:- Spherical and Chromate
- SPHERICAL: Light rays passing through periphery of lenses which cannot bring to same focus leads to image disorted shape.
- CHROMATIC: Splitting of light in to its individual component colours as in prism. Result is hazy image not clear.

FLUORESCENCE MICROSCOPE

- Fluorescence microscope involves staining of specimen with special fluorescent dyes and is an indispensible instrument.
- The difference between ordinary microscope.
- Light source- mercury vapour lamp.
- Dark filed condenser-normal abbe condenser.
- Three filters-light rays-reach the eye.
- George. G. stokes- describes fluorescence microscope.

PRINCIPLE OF FLUORESCENCE

- Light energy and waves are transmitted.

- Waves of light encounters any obstruction-reaches molecule of substance, electrons of the mile at the outer orbit get existed and oscillated in resonance with the light waves.
- When light is transmitted by the energized (existed) molecules it is said to be photoluminescence.
- Time lapse between adsorption and emission always take place.
- Time lapse greater $>10^{-4}$ seconds- phosphorescence.
- Time lapse less $< 10^{-4}$ seconds – fluorescence.
- Fluorescent dye it absorbs a wave length of light (excitation wave length) and emits light in different wave length.
- Eg: fluorescein iso thiocyanate is illuminated with blue light and emits green light.
- Other dyes-Auramine o, Aeridine orange, fluorescein,etc….

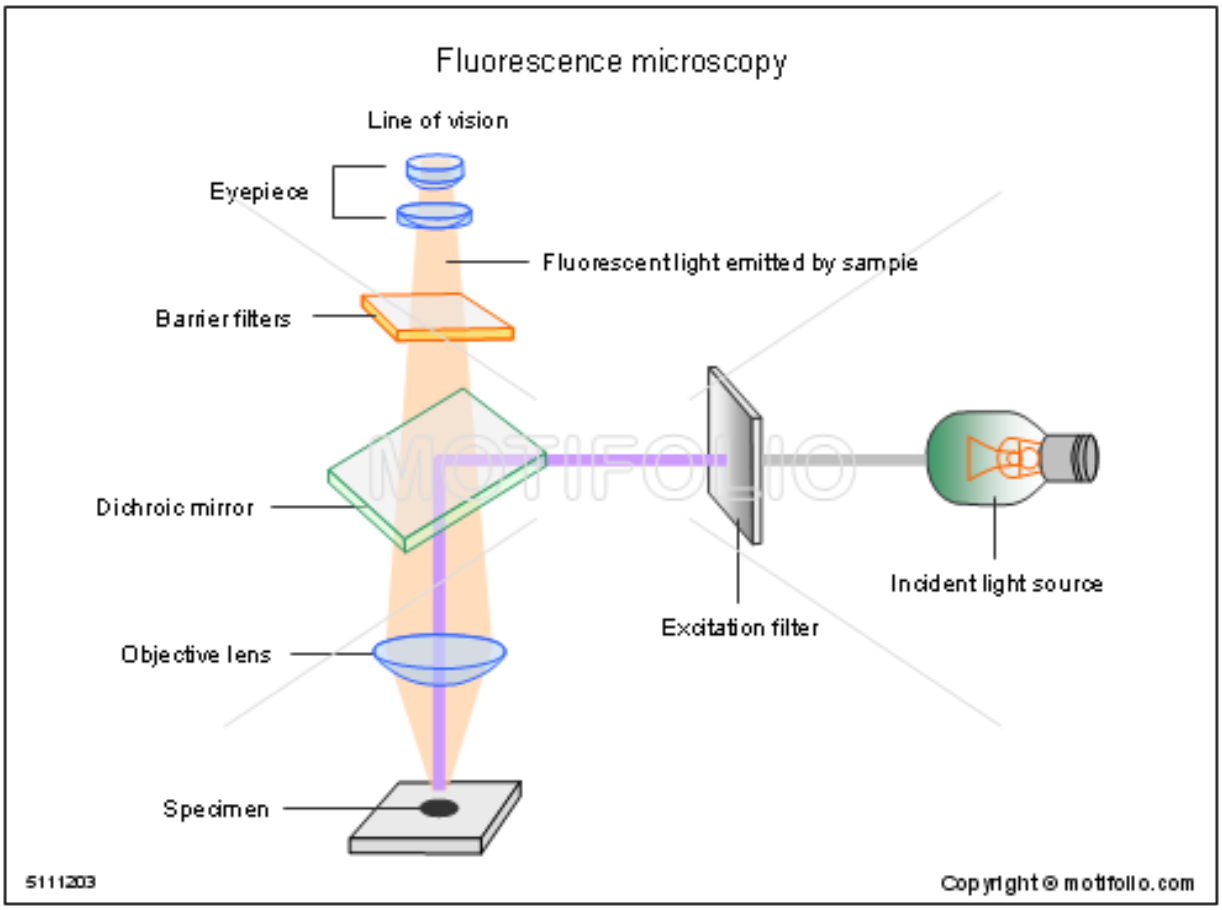
USING OF MICROSCOPE

- Warming up- illumination is good when it is warmed after 30 to 45 mins.
- Check of mercury lamp-life of mercury lamp is 400hrs log book is maintained. Check main 200hrs of usage.
- Selection of filter- combination of barrier and excitation, BG12, OG1.
- Examination – use it property to examine.

PARTS OF MICROSCOPE

- Light source- mercury vapour arc lamp- UV, violet and blue light rays of 200-400nm and visible rays above 700nm, eyes should not directly exposed.
- Heat filter- infra red rays produced by lamp consider heat. To reduce heat between reaching the condenser.
- Exciter filter- light cooled down by het filter and pass excite and absorbs all. It allows only green, blue, violet or UV rays to pass through.

- Condenser- dark field condenser, it is deflect major UV rays protecting eyes. NA should up to at 0.05 less than that of condenser.
- Barrier filter- situated in body tube. It removes the exciting light and allows only visible light. It is yellow or deep orange in colour.



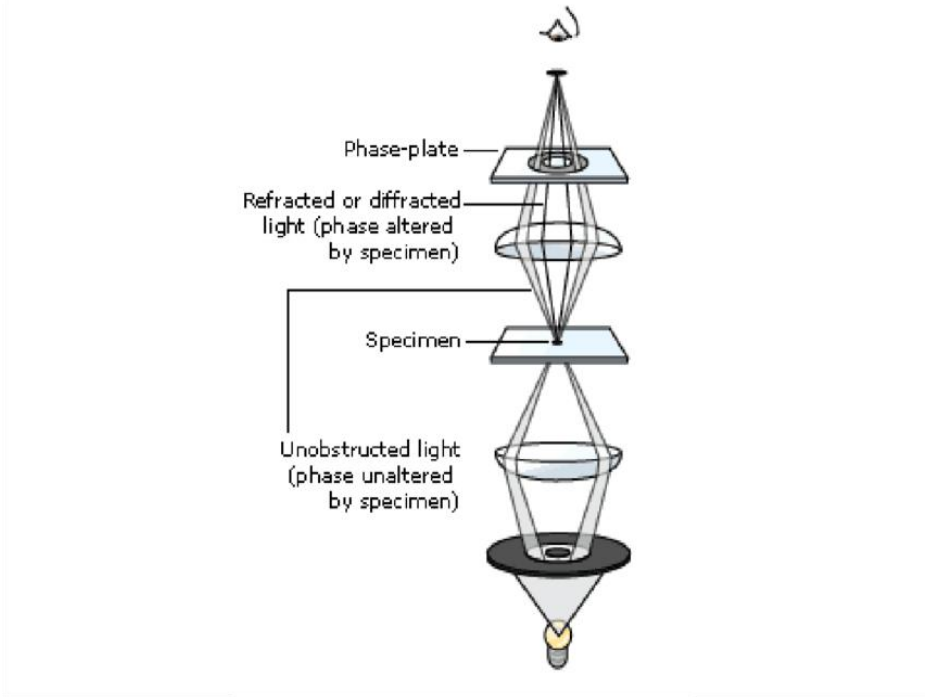
PHASE CONTRAST MICROSCOPE

- Frederick Zernike(1933)hence –Zernike microscope.
- Ideal for observing living protozoans and other transparent microbes without staining.
- Transparent protoplasmic contents can be differentiated.

PRINCIPLE

- Objects in a microscope field can be categorized into two.

- Amplified objects and phase objects.
- Amplified-shows dark objects under microscopic view due to reduction in intensity of rays.
- Phase-transparent, light rays without any reduction of intensity can pass through.
- Light rays pass though a transparent objects will have retardation by about one quarter wave length. This retardation is called phase shift.
- Phase shift is utilized in the phase contrast microscope to create image contrast.
- **LIGHT RAYS**
    - Direct-rays pass through straight line.
    - Diffracted- rays bent or slowed down due to different in density.
- In any specimen or cell there will be different in thickness between the structures or components.
- When the light rays pass through these there will be variable refraction of the rays and phase changes are converted into visible different of light intensity(due to the phase ring).
- Change result in interference of light waves resulting in high contract image.



ELECTRON MICROSCOPY

- The electron microscopy is a remarkable research tool of the 20th century. It power runs 100000X or a resolving power of 10^{9}cms.
- This microscopic inventors are Gerd binning and Heinrich Rohner shared the Nobel prize 1986, in physics for their work together with Ernst Ruska the designer of 1st transmission Electron microscopy.
- Microscopic resolution ranges from 100µm to 10Å.Hence epithelial cells to aminoacids can be viewed through this microscope.
- Light microscopy ranges from 0.2µm(bacterial cells) to 100µm(normal cells).

PRINCIPLE OF WORKING:

- A beam of electron is used as a source of illumination instead of a beam of light as in light microscopy.

- Electrons are negatively charged, sub atomic particles having mass of 9.1×10^{-28}gms.
- Metal like tungsten is heated by applying a high voltage current, electrons comes out in a continuous stream and this can be directed to form a high velocity electron beam.

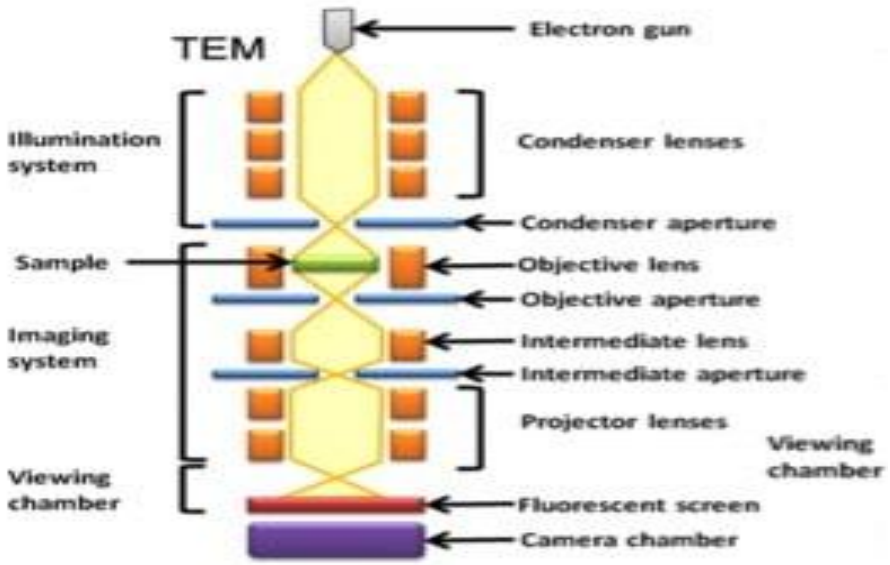
BASIC CONSTRUCTION OF AN ELECTRON MICROSCOPE:

- Electron generator (gun) - Source of illumination
- Electromagnetic coils (lenses) - Condenser lens which corrects the aberration
- Screen (for viewing) - Final image
- Vaccum pump - To avoid scattering of electrons.

TYPES OF ELECTRON MICROSCOPE:

- TEM - Transmission Electron Microscopy
- SEM - Scanning Electron Microscopy

TEM



TEM:

- In a TEM, the beam of electrons passes through the specimen forming image of the detailed structure of the specimen.

  1) Source of illumination is a beam of electrons [not visible light]

  2) There are a series (usually 3 sets) of electromagnets for focusing the beam of electrons. These are called magnetic lenses.

- TEM is for studying the structural details of the various components present in a cell or microbe.

PREPARATION OF SPECIMEN FOR TEM:

- Biological specimens to be examined have to be specially prepared in order to obtain proper structural details with high magnification.
- Artifacts (appearance of something in image due to optical mistake or preparation mistake) should be avoided.

Following steps:

1) DEHYDRATION AND FIXATION:

- In light microscope, the specimen containing water or solution can be viewed. But in electron microscope it should be absolutely dry. Otherwise, it will boil, disintegrates the structural organelles (in vacuum) ,by keeping higher and higher concentration of acetone or alcohol for dehydration is necessary.
- Fixation is done by keeping in dilute solution of plastic embedded media and polymerized by heating in an oven.

2) ULTRA SECTIONING:

- Normally they are cut into thin sections using a ultra microtome. Microbes embedded in plastic resins helps in facilitate sectioning.

3) SECTIONING:

- Specimens are stained with heavy metals containing compounds like phosphotungstic acid. Positive and Negative staining is their. In Negative staining, background is darker and specimens are visualised.

4) FREEZE ETCHING:

- This is used to reveal the detailed structure of microbes.
- In this technique instead of using fixative, specimens are rapidly frozen and biological cells became harder. So this sections are sliced by knife blade.
- At this temperature (freezing) harder leads to crack along the moving lines. Fractured specimen is then etched (i.e. the water is allowed to evaporate from the surface layer).
- Freeze etched specimen is kept in platinum vapour at 45° to produce a shadow effect which produces replica of image.

- Surface details of organelles can be clearly visualized by the freeze etching techniques.

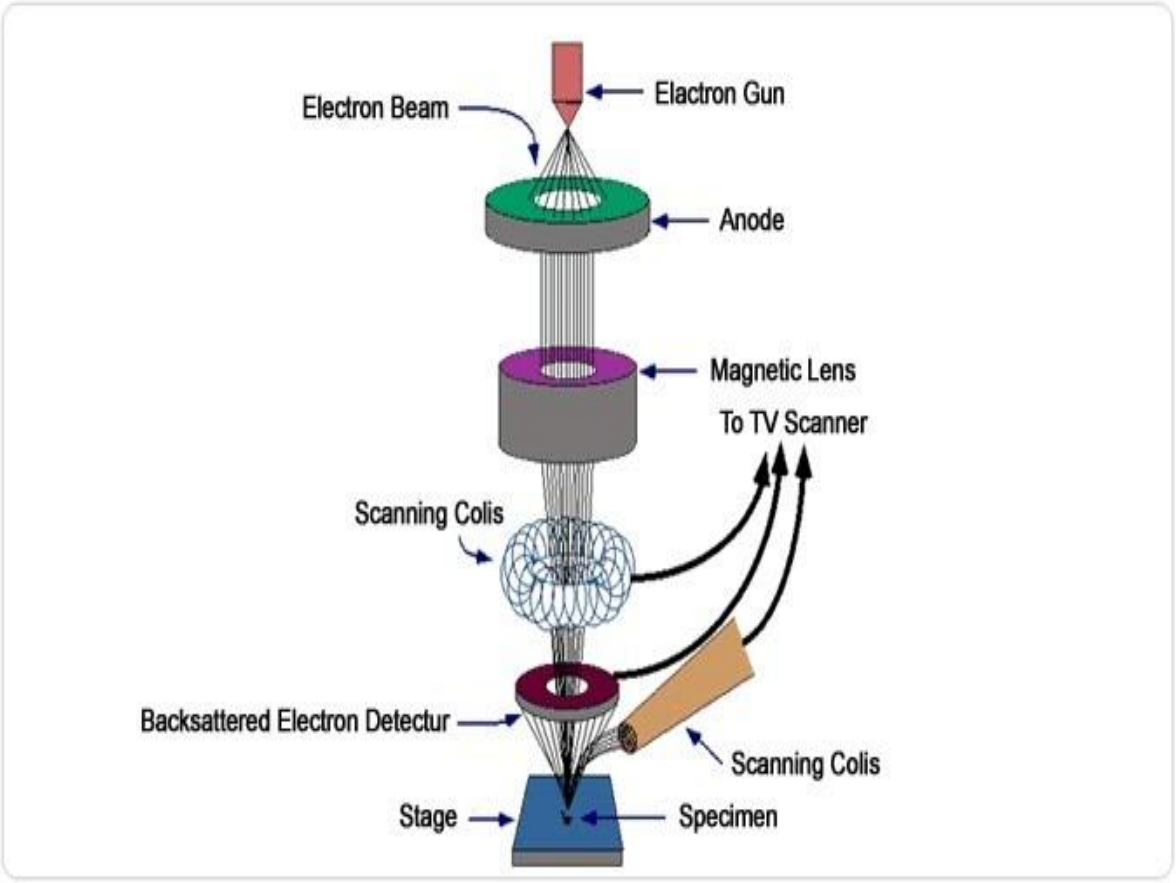
APPLICATIONS OF TEM:

- TEM provides topographical, morphological, composed and crystalline information.
- To analyze the structure and texture of crystals and metals.
- In production of computer and silicon chips.

SCANNING ELECTRON MICROSCOPY:

- SEM is used for visualizing the surface architecture of the specimen (pollen grains, hairs, membranes, etc.,) rather than internal details.

SEM DIAGRAM: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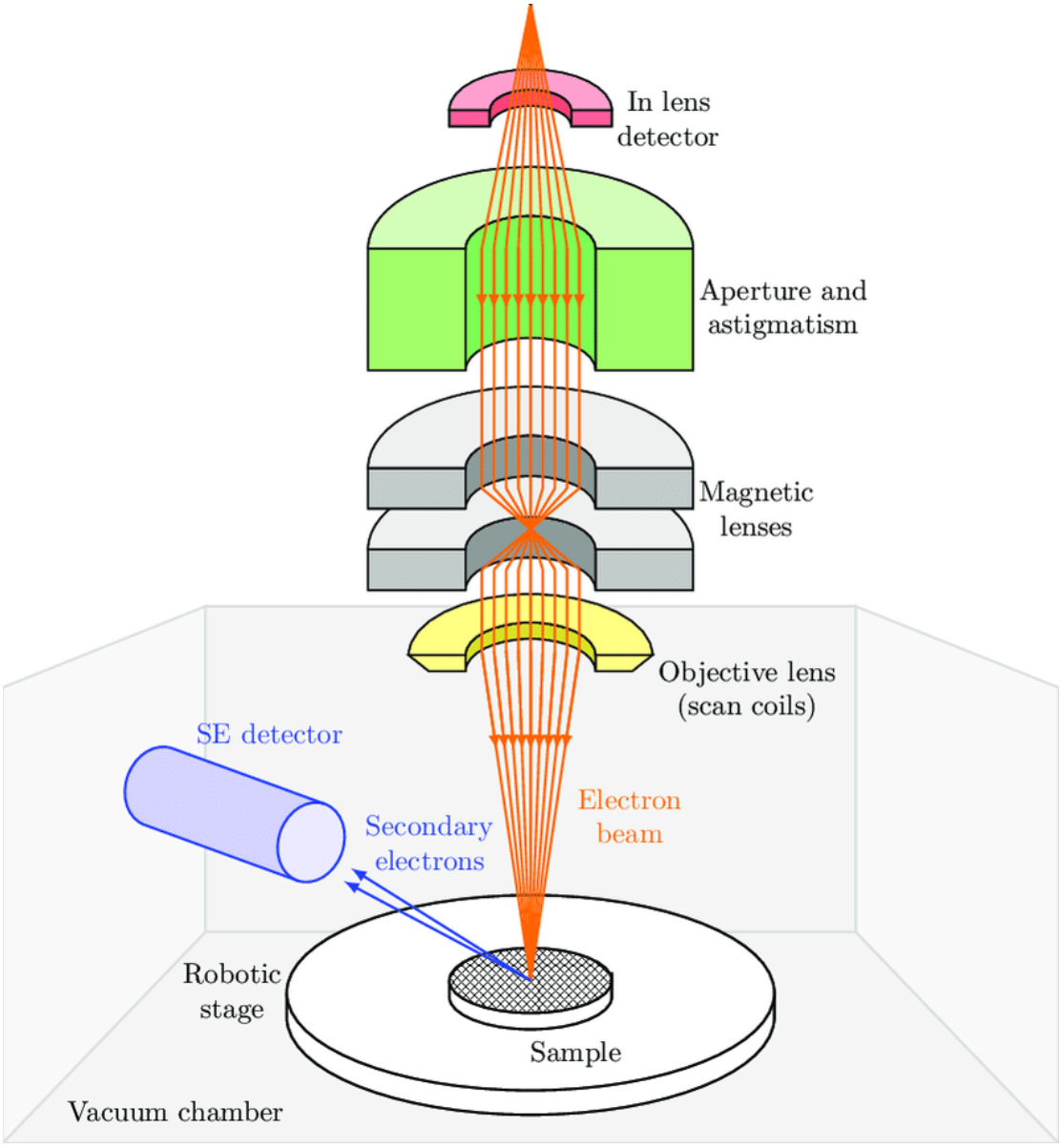

WORKING PRINCIPLE:

- In SEM, an accelerated beam of electrons is produced from the electron gun and is focussed on the specimen by the condensor lens.
- Primary electron beams as it strikes the specimen, forces out electron from the surface
- This forms the secondary electron that is transmitted to a collector.

- The electron then transmitted from the collector to a detector which has substrates that emits light when struck by electrons.
- The light so emitted is converted to an electrical current which is used to control the brightness of an image on a CRT (cathode ray tube) screen.
- The image on the CRT screen will be 3D.
- Scanning tunnelling microscope ranges from 0.1nm (1Å) to 100µm

PREPARATION OF SPECIMEN:

1) DEHYDRATION:

- As in TEM, the specimen should be dried without moisture content as it leads to boiling of specimen and disintegrates the structural organelles.

2) SHADOW CASTING:

- In this technique, specimen is coated with an extremely thin layer of gold, gold-palladium, or platinum at an oblique angle.
- The object produces a shadow on the uncoated side.
- It produces 3D topographic image of the specimen, coating is done with sputher coater.

3) SURFACE REPLICA (FREEZE ETCHING):

- The cells are rapidly frozen in liquid nitrogen and armed to -100°c in vacuum chamber.
- Knife was precooled (16°c), it fractures the frozen cells which are very brittle and break along lines.
- The specimen was kept at high vacuum leads to sublimation and uncover the structure.
- Finally exposed to the layers of platinum vapours and carbon vapours to form a replica.

- This replica provides a detailed 3D view of intracellular structures.

Cooled knife

↓

Fracture faces

↓

Sublimation

↓

Shadowing with platinum and carbon

↓

Replica was observed

DIFFERENCE BETWEEN LIGHT AND ELECTRON MICROSCOPE

LIGHT MICROSCOPE	ELECTRON MICROSCOPE
● Simple to use	● Users requires technical skills
● Can view both live and dead specimen	● Views only dead specimen
● Poor surface view	● Good surface and internal details view
● Uses light rays	● Beam of electrons
● Glass lens	● Electroabsorption modulator integrated laser (EMR) lens
● Low resolution 0.30µm	● High resolution 0.0001µm
● Low magnification 1500X	● High magnification 1000000X
● Eye piece	● Photographic plate
● Cheap	● Costlier
● Specimen must be very thin	● Very thin sections in TEM and Image surfaces in SEM
● 2D	● 2D & 3D
● Wavelength of radiation	● Effective wavelength
● 400-700nm	● <1nm

REFERENCE

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Electron microbiology: Textbook of Microbiology by Tortora

UNIT- II

MICROBIAL CLASSIFICATION AND TAXONOMY

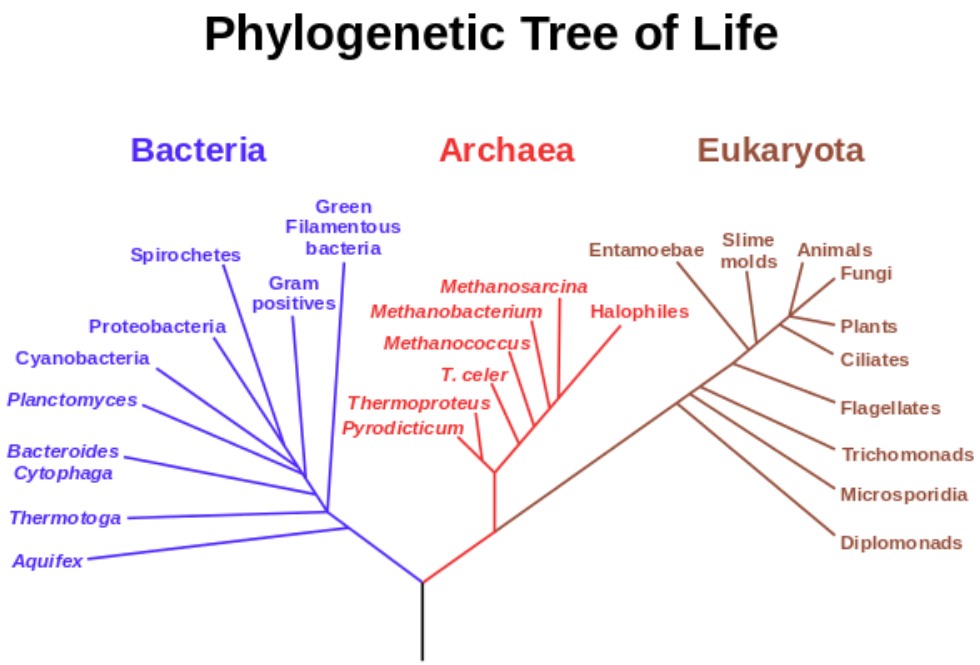
MICROBIAL KINGDOM

WOOSE CLASSIFICATION:

The Earth is 4.6 billion years old and microbial life is thought to have first appeared between 3.8 and 3.9 billion years ago; in fact, 80% of Earth's history was exclusively microbial life. Microbial life is still the dominant life form on Earth. It has been estimated that the total number of microbial cells on Earth on the order of 2.5 X 10^{30} cells, making it the major fraction of biomass on the planet.

Phylogeny refers to the evolutionary relationships between organisms. The Three Domain System, proposed by Woose and others, is an evolutionary model of phylogeny based on differences in the sequences of nucleotides in the cell's ribosomal RNAs (rRNA), as well as the cell's membrane lipid structure and its sensitivity to antibiotics. Comparing rRNA structure is especially useful. Because rRNA molecules throughout nature carry out the same function, their structure changes very little over time. Therefore similarities and dissimilarities in rRNA nucleotide sequences are a good indication of how related or unrelated different cells and organisms are.

There are various hypotheses as to the origin of prokaryotic and eukaryotic cells. Because all cells are similar in nature, it is generally thought that all cells came from a common ancestor cell termed the **last universal common ancestor (LUCA)**. These LUCAs eventually evolved into three different cell types, each representing a domain. The three domains are the *Archaea*, the *Bacteria*, and the *Eukarya*.



More recently various fusion hypotheses have begun to dominate the literature. One proposes that the diploid or 2N nature of the eukaryotic genome occurred after the fusion of two haploid or 1N prokaryotic cells. Others propose that the domains *Archaea* and *Eukarya* emerged from a common archaeal-eukaryotic ancestor that itself emerged from a member of the domain *Bacteria*.

Some of the evidence behind this hypothesis is based on a "superphylum" of bacteria called PVC, members of which share some characteristics with both archaea and eukaryotes. There is growing evidence that eukaryotes may have originated within a subset of archaea.

In any event, it is accepted today that there are three distinct domains of organisms in nature: *Bacteria, Archaea*, and *Eukarya*. A description of the three domains follows.

INTERMEDIARIES BETWEEN BACTERIA, ARCHAEAE, AND EUKARYA DOMAINS?

There is a "super phylum" of bacteria called PVC, referring to the three members of that super phylum: the Planctomycetes, the Verrucomicrobia, and the Chlamydia. Members of the PVC, while belonging to the domain *Bacteria*, show some features of the domains *Archaea* and *Eukarya*.

Some of these bacteria show cell compartmentalization wherein membranes surround portions of the cell interior, such as groups of ribosomes or DNA, similar to eukaryotic cells. Some divide by budding or contain sterols in their membranes, again similar to eukaryotes.

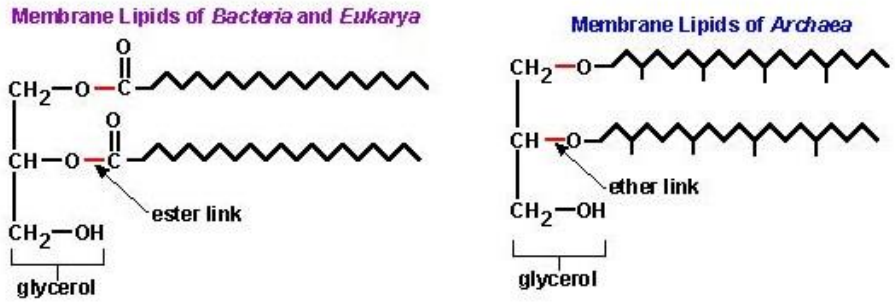
Some lack peptidoglycan, similar to eukaryotes and archaea. It has been surmised that these bacteria might be an intermediate step between an ancestor that emerged from a bacterium (domain *Bacteria*) and an archael-eukaryotic ancestor prior to its split into the domains *Archaea* and *Eukarya*.

The Archaea (archaebacteria)

The *Archaea* possess the following characteristics:

a. *Archaea* are prokaryotic cells.
b. Unlike the *Bacteria* and the *Eukarya*, the *Archaea* have membranes composed of branched hydrocarbon chains (many also containing rings within the hydrocarbon chains) attached to glycerol by ether linkages (Figure 1.3.31.3.3).
c. The cell walls of *Archaea* contain no peptidoglycan.
d. *Archaea* are not sensitive to some antibiotics that affect the *Bacteria*, but are sensitive to some antibiotics that affect the *Eukarya*.

e. *Archaea* contain rRNA that is unique to the *Archaea* as indicated by the presence molecular regions distinctly different from the rRNA of *Bacteria* and *Eukarya*.



Membrane Lipids of Archaea, Bacteria, and Eukarya. The Bacteria and the Eukarya have membranes composed of unbranched fatty acid chains attached to glycerol by ester linkages. The Archaea have membranes composed of branched hydrocarbon chains attached to glycerol by ether linkages.

Archaea often live in extreme environments and include methanogens, extreme halophiles, and hyperthermophiles. One reason for this is that the ether-containing linkages in the *Archaea* membranes is more stabile than the ester-containing linkages in the *Bacteria* and *Eukarya* and are better able to withstand higher temperatures and stronger acid concentrations.

The *Bacteria* (eubacteria)

Bacteria (also known as eubacteria or "true bacteria") are prokaryotic cells that are common in human daily life, encounter many more times than the archaebacteria. Eubacteria can be found almost everywhere and kill thousands upon thousands of people each year, but also serve as antibiotics producers and food digesters in our stomachs. The *Bacteria* possess the following characteristics:

a. *Bacteria* are prokaryotic cells.

b. Like the *Eukarya*, they have membranes composed of unbranched fatty acid chains attached to glycerol by ester linkages (Figure 1.3.31.3.3).
c. The cell walls of *Bacteria*, unlike the *Archaea* and the Eukarya, contain peptidoglycan.
d. *Bacteria* are sensitive to traditional antibacterial antibiotics but are resistant to most antibiotics that affect *Eukarya*.
e. *Bacteria* contain rRNA that is unique to the *Bacteria* as indicated by the presence molecular regions distinctly different from the rRNA of *Archaea* and *Eukarya*.

Bacteria include mycoplasmas, cyanobacteria, Gram-positive bacteria, and Gram-negative bacteria.

The *Eukarya* (eukaryotes)

The *Eukarya* (also spelled *Eucarya*) possess the following characteristics:

a. *Eukarya* have eukaryotic cells.
b. Like the *Bacteria*, they have membranes composed of unbranched fatty acid chains attached to glycerol by ester linkages (Figure 1.3.31.3.3).
c. Not all *Eukarya* possess cells with a cell wall, but for those *Eukarya* having a cell wall, that wall contains no peptidoglycan.
d. *Eukarya* are resistant to traditional antibacterial antibiotics but are sensitive to most antibiotics that affect eukaryotic cells.
e. *Eukarya* contain rRNA that is unique to the *Eukarya* as indicated by the presence molecular regions distinctly different from the rRNA of *Archaea* and *Bacteria*.

The *Eukarya* are subdivided into the following four kingdoms:

1. **Protista Kingdom**: Protista are simple, predominately unicellular eukaryotic organisms. Examples include slime molds, euglenoids, algae, and protozoans.
2. **Fungi Kingdom**: Fungi are unicellular or multicellular organisms with eukaryotic cell types. The cells have cell walls but are not organized into tissues. They do not carry out photosynthesis and obtain nutrients through absorption. Examples include sac fungi, club fungi, yeasts, and molds.
3. **Plantae Kingdom**: Plants are multicellular organisms composed of eukaryotic cells. The cells are organized into tissues and have cell walls. They obtain nutrients by photosynthesis and absorption. Examples include mosses, ferns, conifers, and flowering plants.
4. **Animalia Kingdom**: Animals are multicellular organisms composed of eukaryotic cells. The cells are organized into tissues and lack cell walls. They do not carry out photosynthesis and obtain nutrients primarily by ingestion. Examples include sponges, worms, insects, and vertebrates.

It used to be thought that the changes that allow microorganisms to adapt to new environments or alter their virulence capabilities was a relatively slow process occurring within an organism primarily through mutations, chromosomal rearrangements, gene deletions and gene duplications. Those changes would then be passed on to that microbe's progeny and natural selection would occur. This gene transfer from a parent organism to its offspring is called vertical gene transmission.

It is now known that microbial genes are transferred not only vertically from a parent organism to its progeny, but also horizontally to relatives that are only distantly related, e.g., other species and other genera. This latter process is known as horizontal gene transfer. Through mechanisms such as transformation, transduction, and conjugation, genetic elements such as plasmids, transposons, integrons, and even chromosomal DNA can readily be

spread from one microorganism to another. As a result, the old three-branched "tree of life" in regard to microorganisms (Figure 1.3.11.3.1) now appears to be more of a "net of life."

Microbes are known to live in remarkably diverse environments, many of which are extremely harsh. This amazing and rapid adaptability is a result of their ability to quickly modify their repertoire of protein functions by modifying, gaining, or losing their genes. This gene expansion predominantly takes place by horizontal transfer.

Summary

1. Phylogeny refers to the evolutionary relationships between organisms.
2. Organisms can be classified into one of three domains based on differences in the sequences of nucleotides in the cell's ribosomal RNAs (rRNA), the cell's membrane lipid structure, and its sensitivity to antibiotics.
3. The three domains are the Archaea, the Bacteria, and the Eukarya.
4. Prokaryotic organisms belong either to the domain Archaea or the domain Bacteria; organisms with eukaryotic cells belong to the domain Eukarya.
5. Microorganism transfer genes to other microorganisms through horizontal gene transfer - the transfer of DNA to an organism that is not its offspring.

HACKEL'S THREE KINGDOM CLASSIFICATION:

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. Then in the 1860s, the German investigator Ernst Haeckel proposed a

three-kingdom system of classification. Haeckel's three kingdoms were Animalia, Plantae, and Protista. Members of the kingdom Protista included the protozoa, fungi, bacteria, and other microorganisms. 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).

- 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.

FEATURES OF THREE KINGDOM 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.
- Organisms lacking morphological complexities, tissue system, division of labour, and enjoying diversified type of modes of nutrition were segregated and put under the kingdom Protista (algae, fungi, protozoa and bacteria).
- 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.

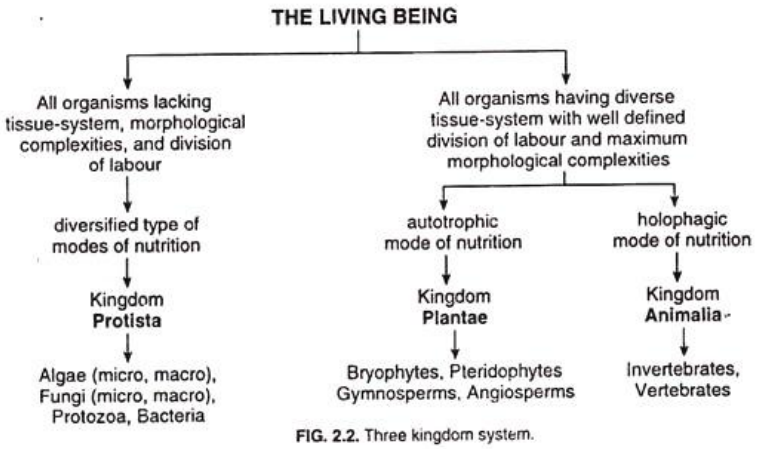


FIG. 2.2. Three kingdom system.

LIMITATIONS:

- 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.

WHITAKKER'S FIVE KINGDOM CLASSIFICATION:

Five Kingdom Classification

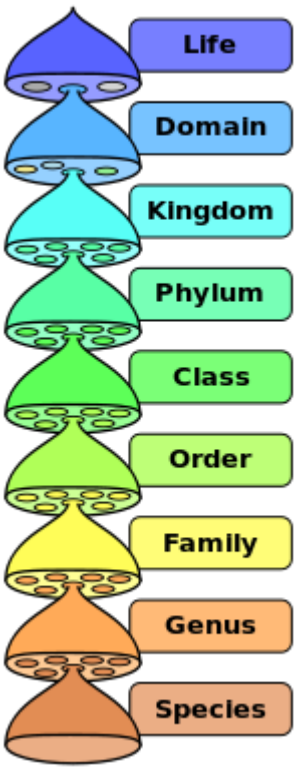
Very early on, scientists began grouping the living organisms under different categories. Some biologists classified organisms into plants and animals. Ernst Haeckel, Robert Whittaker, and Carl Woese are some biologists who attempted a broader system of classification. Amongst these, the Five Kingdom Classification proposed by Robert Whittaker stood out and is widely used.

Whitaker proposed that organisms should be broadly divided into kingdoms, based on certain characters like the structure of the cell, mode of nutrition, the source of nutrition, interrelationship, body organization, and reproduction. According to this system, there are five main kingdoms. They are:

- Kingdom Monera
- Kingdom Protista
- Kingdom Fungi
- Kingdom Animalia
- Kingdom Plantae

Kingdoms are divided into subgroups at various levels. The following flowchart shows the hierarchy of classification.

Kingdom → Phylum → Class → Order → Family → Genus → Species



Distinguishing Features of the Five Kingdoms

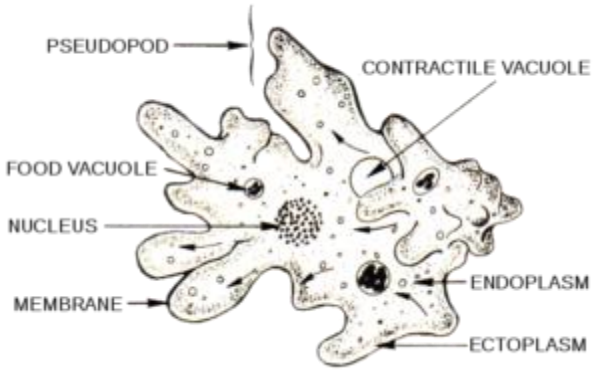
Kingdom Monera

These organisms are prokaryotic and unicellular. They do not have a well-defined nucleus and also lack cell organelles. Some organisms show the presence of cell wall while there are others without a cell wall. Consequently, some organisms are autotrophic and others are heterotrophic. Examples include *Bacteria, Cyanobacteria, and Mycoplasma.*

Kingdom Protista

Organisms grouped under Kingdom Protista are all unicellular, but eukaryotic organisms. These are the simplest forms of eukaryotes that exhibit either autotrophic or heterotrophic mode of nutrition. Some organisms have

appendages such as cilia or flagella or pseudopodia to move around. Some examples are *Diatoms, Protozoan like Amoeba, Paramoecium*



Kingdom Fungi

Heterotrophic, Multicellular and Eukaryotic organisms are grouped under Kingdom Fungi. Their mode of nutrition is saprophytic as they use decaying organic matter as food. They have cell walls, which are made up of a substance called Chitin. Fungi also form a symbiotic association with some blue-green algae. *Yeast, Mushroom, Aspergillus* are examples of Fungi.

Kingdom Plantae

These are Eukaryotic, Multicellular organisms with a cell wall that is made up of cellulose. They are autotrophs and synthesize their own food through the process of photosynthesis. This kingdom includes all plants.

Based on the body differentiation and presence or absence of specialized vascular tissue, Kingdom Plantae is divided into different divisions, namely Thallophyta, Bryophyta, Pteridophyta, Gymnosperms, and Angiosperms. Examples are *Spirogyra, Ferns, Pines, and Mango Plant* etc.

Kingdom Animalia

This Kingdom includes organisms that are Multicellular, Eukaryotic, without the presence of cell wall. They have a heterotrophic mode of nutrition. They also exhibit great diversity. Some organisms are simple while others have a complex body with specialized tissue differentiation and body organs.

The Animal Kingdom is divided into many phyla and classes. Some of the phyla are Porifera, Coelenterata, Arthropoda, Echinodermata, Chordata etc. Examples – *Hydra, Starfish, Earthworms, Monkeys, Birds* etc.

GENERAL CHARACTERISTICS OF ALGAE, FUNGI,VIRUS

ALGAE:

Algaeare *Eukaryotic, autotrophic* and usually aquatic *thallophytes*. As algae possess chlorophyll, they are photosynthetic in nature. The organization of their thallus is either unicellular or multicellular.

General characters' of Algae

1. They are usually aquatic. They occur both in marine and fresh water habitats.
2. Some of the algae are also found as terrestrial, subterranean or epiphytic forms.
3. The thallus of algae shows great degree of variation in size and form.
4. The algal cell wall is made up of cellulose.
5. They are unicellular or colonial or multicellular unbranched or branched filamentous forms or siphonous forms or heterotrichous forms.
6. Algal cells have chloroplasts or chromatophores with photosynthetic pigments.

7. These members show eukaryotic organization but one of the classes Cyanophyaceae has prokaryotic organization.
8. Algae are autotrophic and synthesize their own food. Their main reserve food material is starch. However, different algae store different food material.

- Phaeophyceae stores laminarin or mannitol.
- Rhodophyceae stores floridean starch.
- Bacillariophyceae and Xanthophyceae stores leucosin.

9. Algae reproduce by vegetative, sexual and asexual methods.
10. Sexual reproduction may be isogamous, physiologically anisogamous or oogamous type.
11. Asexual reproduction takes place by zoospores, aplanospores, endospores, harmagonia and auxospores etc.
12. Algae show progressive evolution in sexuality.
13. The motile vegetative cells, zoospores and gametes of algae (except the class Rhodophyceae and Cyanophyacae) show a pair of flagella. Each flagellum shows 9+2 arrangement of fibrils. The flagella of algae are of two types namely acronematic and pantonematic.
14. Algae exhibit haplontic life cycle or diplontic life cycle, haplodiplontic life cycle, haplobiontic life cycle and diplobiontic life cycle.
15. The photosynthetic pigments of algae are of three types:

Photosynthetic pigments of algae:

Chlorophylls: They may be of five types. Namely, chlorophyll a, b, c, d, e

- Chlorophyll a is present in all algal groups
- Chlorophyll b is present in Chloropyceae
- Chlorophyll c is present in Phaeophyceae
- Chlorophyll d is present in Rhodophyceae
- Chlorophyll e is present in zoospores of *Vaucheria*

Carotenoids: They are of two types. Namely **Carotenes and Xanthophylls**

- β-Carotenes is present in all algal groups
- Xanthophyll, Leutin is present in Chlorophyceae and
- Another xanthophyll, Fucoxanthin is present in Bacillariophyceae and Phaeophyceae

Phycobilins: These are water soluble pigments. They are two types namely, Phycocyanin and Phycoerythrin.

- Phycocyanin is predominant in Cyanophyceae
- Phycoerythrin is predominant in Rhodophyceae

FUNGI:

General Characteristics of Fungi:

- Eukaryotic
- Decomposers – the best recyclers around
- No chlorophyll – non photosynthetic
- Most multicellular (hyphae) – some unicellular (yeast)
- Non-motile
- Cell walls made of **chitin** *(kite-in)* instead of cellulose like that of a plant
- Are **more related to animals** than plant kingdom
- Lack true roots, leaves and stems
- Absorptive heterotrophs -Digest food externally and then absorb it
- *Lack of chlorophyll affects the lifestyle of fungi…*
- Not dependant on light
- Can occupy dark habitats
- Can grow in any direction
- Can invade the interior of
- a substrate with
- absorptive filaments

Structures

- Body of fungus made of tiny filaments or tubes called **hyphae** .
- Contain cytoplasm and nuclei (more than 1)
- Each hyphae is one continuous cell
- Cell wall made of chitin
- A tangled mess of hyphae is called **mycelium**
- **Rhizoids** are root-like parts of fungi that anchor them to the substrate *(whatever they are bonding to)*
- **Mycelium increases the surface area of the fungi to absorb more nutrients.**

CLASSIFICATION OF FUNGI:

- Fungi can be classified into 5 groups
- Fungi evolved from an aquatic, flagellated ancestor
- Chytrids
- Glomeromycetes (Mycorrhizae fungi)
- Mycorrhizae are mutually beneficial associations of plant roots and fungi.
- Ascomycetes **(Sac fungi)**
- Truffles and yeast
- Basidiomycetes (club fungi)
- Puff ball mushroom
- Zygomycetes (zygote fungi)

Fungus Reproduction:

- Fungi produce spores in <u>both</u> asexual and sexual life cycles.
- Mushrooms let out spores from their pores that are carried by the wind to meet other spores and become a new fungi
- Yeast are unicellular and divide into new fungal cells **(mitosis)**

In some fungi, fusion of haploid hypha produces a heterokaryotic stage containing nuclei from two parents *(fusion of cytoplasm)* o After the nuclei fuse, meiosis produces **haploid spores** *(can grow in fungi and are the asexual part of the life cycle).*

General Fungi Reproduction Cycles:

- But fungal groups do ***differ*** in their life cycles and reproductive structures

Reproduction in Basidiomycetes:

- Basidiomycota *(typical mushroom)*
- Ascomycota
- Fungi Nutrition:
- Fungi absorb food after digesting it outside their bodies o Fungi are heterotrophic eukaryotes

FUNGI NUTRITION:

- Fungi use digestive enzymes to break down their food then absorb the liquid. ***(acquire nutrients such as nitrogen)***

<u>Examples:</u>

- Trap nematodes *(little worms who feed on fungi)* and paralyze them with special juices then absorbs and digests the nitrogen out of them.

Modes of Nutrition in Fungi:

- Saprophytes
- Parasites
- Mutualists (symbionts)

Saprophytes

- Use **non-living organic material**
- Important scavengers in ecosystems
- Important in recycling carbon, nitrogen and essential mineral nutrients

Parasites

- Use organic **material from living organisms,** harming them in some way
- Range of hosts from single-celled diatoms to fungi, to plants to animals to humans
- **Mutualists (symbionts)**
- Fungi that have a **mutually beneficial relationship with other living organisms**
- **Mycorrhizae** – beneficial relationship with fungi with plant rooto More than 90% of plants in nature have a mycorrhizal in roots (***example: Truffles- expensive delicacy!)***
- **Lichens** – associations of fungi with algae or cyanobacteria

Food source for animals, breaking down rocks into soil

- Parasitic fungi harm plants and animals. Parasitic fungi cause 80% of plant diseases. Can kill plants and affect crops.

- Many fungi are harmful to humans
- Can cause human diseases – *allergies, athletes foot, ringworm, yeast infection*

Ringworm

- A contagious fungal infection having characteristic red ring that can appear on an infected person's skin
- Can affect the scalp, the body (particularly the groin), the feet, and the nails
- Also called Tinea.

ALGAE

General Characteristics of Algae:

Viruses are ultra-microscopic, non-cellular living particles, composed solely of a nucleic acid (DNA or RNA) core, surrounded by a protein envelope called capsid.

Characteristics of Viruses:

The major distinguishing characteristics of viruses are given below.

1. They are non-cellular and very simple in structure, consisting mainly of a nucleic acid surrounded by a protein envelope called capsid. Therefore, a unit of virus is referred to as 'a virus particle' rather than 'a virus cell'.

2. They are devoid of the sophisticated enzymatic and biosynthetic machinery essential for independent activities of cellular life. Therefore, they can grow only inside suitable living cells. That is why; they are cultivated in the laboratory only inside living cells, unlike bacteria and fungi, which can be cultivated in the laboratory on non-living matter like nutrient agar.

3. They are ultra-microscopic and can only be visualized under electron microscope.

4. They do not increase in size.

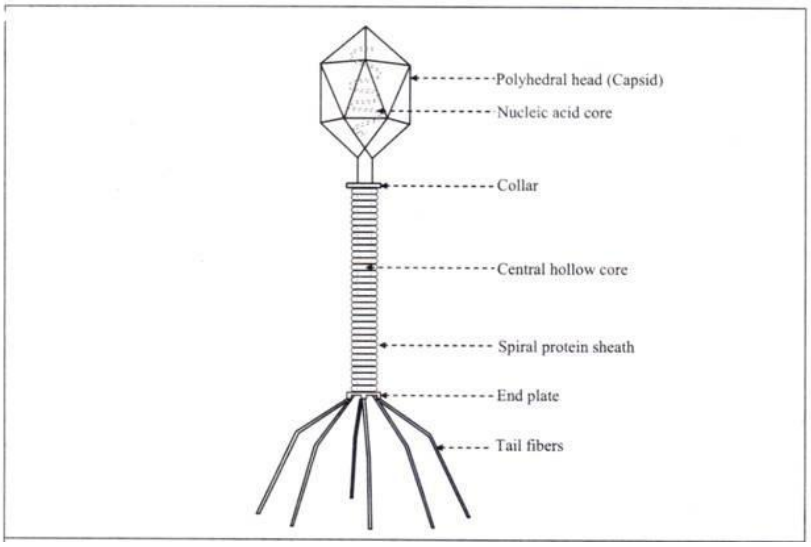
5. They can pass through filters, through which bacteria cannot pass.

6. A virus is called either 'DNA virus' or 'RNA virus' depending on whether it contains the nucleic acid DNA or RNA. A virus cannot have both DNA and RNA

Structure of Viruses:

Much of our knowledge about viruses has been gathered from the study of few viruses, which infect bacteria. These viruses, which infect bacteria, are called 'bacteriophages' or 'phages'. The bacteriophages were first described in 1915 almost simultaneously by Twort and d'Herelle.

The name bacteriophage, which in Greek means to eat bacteria, was coined by d'Herelle because of the ability of these viruses to destroy the infected bacteria cells through lysis. Bacteriophages exhibit notable variability in their size, shape and complexity of structure. The T-even (T2, T4 and T6) phages demonstrate the greatest morphological complexity



Structure of T-even Bacteriophage

The functions of its structural components are as follows:

1. Capsid (Protein Coat): Protection of nucleic acid from destruction by DNase.

2. Nucleic Acid Core: Phage genome carrying genetic information necessary for replication of new phage.

3. Spiral Protein Sheath: Retracts, so that nucleic acid can pass from capsid into host cell's cytoplasm.

4. End Plate and Tail Fibers: Attachment of phage to specific receptor sites on a susceptible host's cell wall.

Viral Infection:

Phage replication depends on the ability of the phage particle to infect a suitable bacterial host cell.

Infection consists of the following sequential events:

1**. Adsorption:**

Tail portion of the phage particle binds to receptor sites on host's cell wall.

2. **Penetration (Infection):**

Spiral protein sheath retracts and an enzyme, early muramidase, perforates the bacterial cell wall enabling the phage nucleic acid to pass through the hollow core into the host cell's cytoplasm. The empty protein shell remains attached to the cell wall and is called the protein ghost.

3. **Replication:**

The phage genome subverts the synthetic machinery, which is then used for the production of new phage components.

4. **Maturation:**

The new phage components are assembled and form complete, mature virulent phage particles.

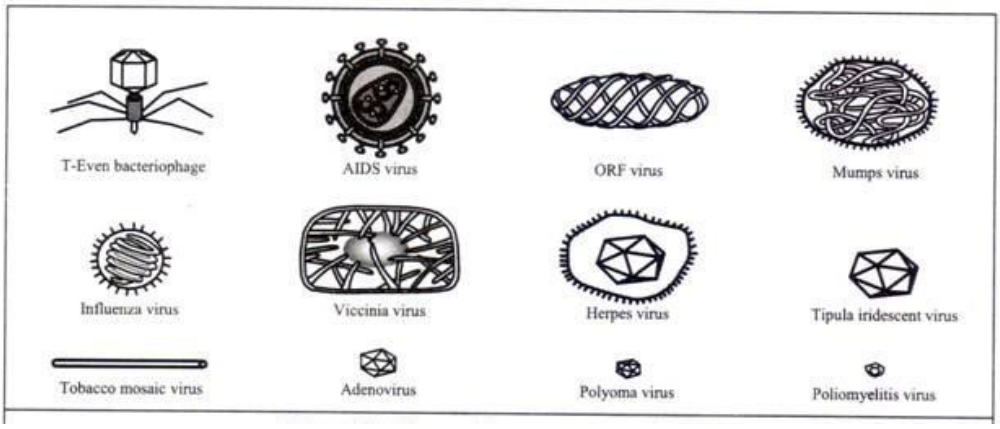
5**. Release:**

Late muramidase lyses the cell wall, liberating infectious phage particles that are now capable of infecting new susceptible host cells, thereby starting the cycle over again.

Shape of Viruses:

The shape of some of the important viruses has been revealed by electron microscope (Figure 8.2). All of them cause different diseases in plants and animals. The bacteriophages infect bacteria cells.

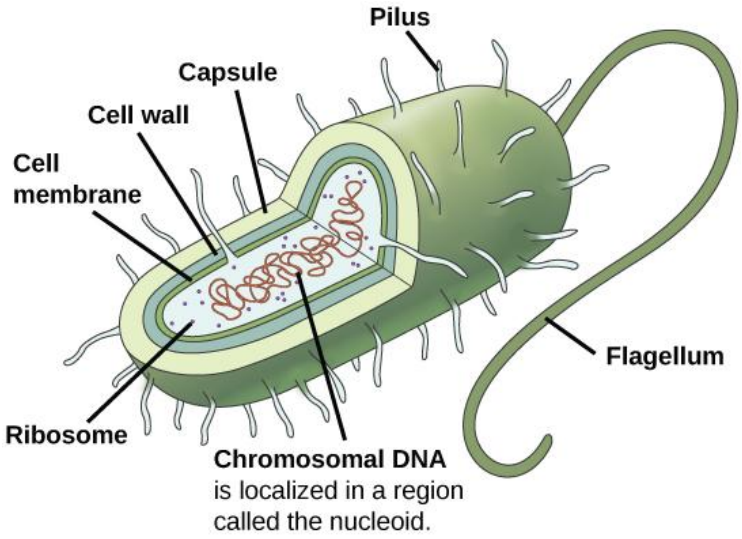
Shape of Some Common Viruses



ANATOMY OF PROKARYOTES

The Prokaryotic Cell

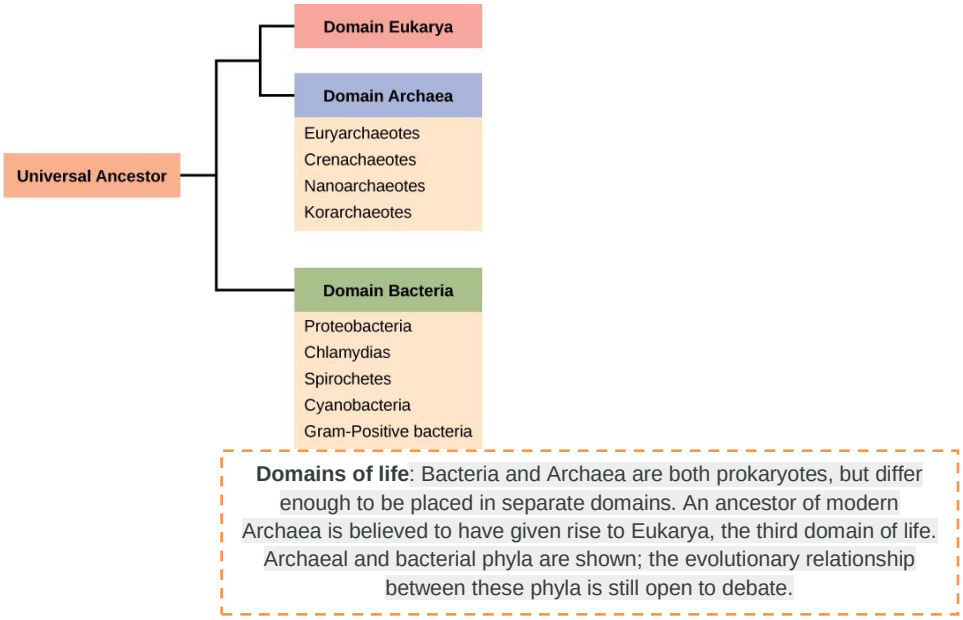
Prokaryotes are unicellular organisms that lack organelles or other internal membrane-bound structures. Therefore, they do not have a nucleus, but, instead, generally have a single chromosome: a piece of circular, double-stranded DNA located in an area of the cell called the nucleoid. Most prokaryotes have a cell wall outside the plasma membrane.



The composition of the cell wall differs significantly between the domains **Bacteria and Archaea**, the two domains of life into which prokaryotes are divided. The composition of their cell walls also differs from the eukaryotic cell walls found **in plants (cellulose) or fungi and insects (chitin).**

The cell wall functions as a protective layer and is responsible for the organism's shape. Some bacteria have a capsule outside the cell wall. Other structures are present in some prokaryotic species, but not in others. For example, the capsule found in some species enables the organism to attach to surfaces, protects it from dehydration and attack by phagocytic cells, and increases its resistance to our immune responses.

Some species also have flagella used for locomotion and pili used for attachment to surfaces. Plasmids, which consist of extra-chromosomal DNA, are also present in many species of bacteria and archaea.



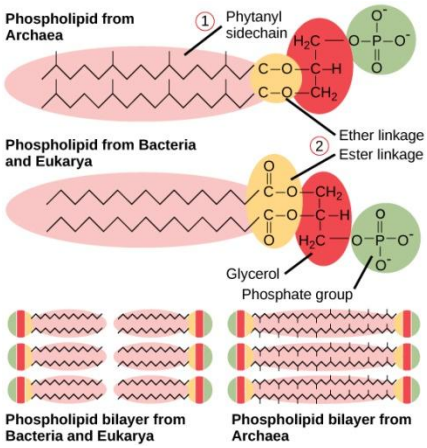
Domains of life: Bacteria and Archaea are both prokaryotes, but differ enough to be placed in separate domains. An ancestor of modern Archaea is believed to have given rise to Eukarya, the third domain of life. Archaeal and bacterial phyla are shown; the evolutionary relationship between these phyla is still open to debate.

The Plasma Membrane

The plasma membrane is a **thin lipid bilayer** (6 to 8 nanometers) that completely surrounds the cell and separates the inside from the outside.

Its selectively-permeable nature keeps ions, proteins, and other molecules within the cell, preventing them from diffusing into the extracellular environment, while other molecules may move through the membrane.

The general structure of a cell membrane is a **phospholipids bilayer** composed **of two layers** of lipid molecules. In archaeal cell membranes, isoprene (phytanyl) chains linked to glycerol replace the fatty acids linked to glycerol in bacterial membranes. Some archaeal membranes are **lipid monolayer instead of bilayers.**



Plasma membrane structure: Archaeal phospholipids differ from those found in Bacteria and Eukarya in two ways. First, they have branched phytanyl sidechains instead of linear ones. Second, an ether bond instead of an ester bond connects the lipid to the glycerol.

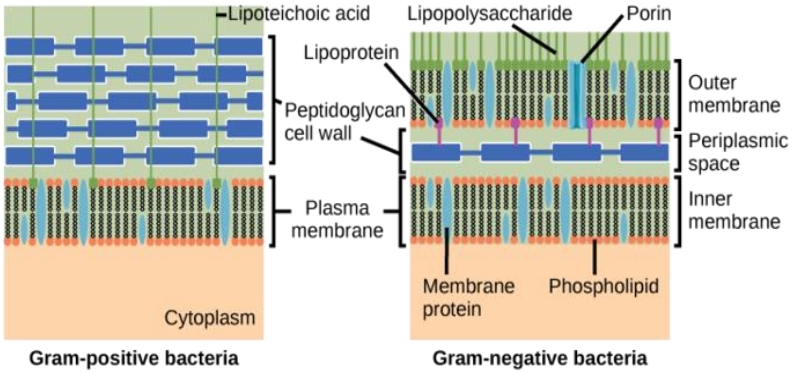
The Cell Wall

The cytoplasm of prokaryotic cells has a high concentration of dissolved solutes. Therefore, the osmotic pressure within the cell is relatively high. The cell wall is a protective layer that surrounds some cells and gives them shape and rigidity. It is located outside the cell membrane and prevents osmotic lysis (bursting due to increasing volume). The chemical composition of the cell walls varies between **archaea and bacteria.** It also varies between bacterial species.

Bacterial cell walls contain **peptidoglycan** composed of polysaccharide chains that are cross-linked by unusual peptides containing both L- and D-amino acids, including **D-glutamic acid and D-alanine**. Proteins normally have only L-amino acids; as a consequence, many of our antibiotics work by mimicking D-amino acids and, therefore, have specific effects on bacterial cell wall development. There are more than 100 different forms of peptidoglycan. S-layer (surface layer) proteins are also present on the outside of cell walls of both archaea and bacteria.

Bacteria are divided into two major groups:

gram-positive and gram-negative, based on their reaction to gram staining. Note that all gram-positive bacteria belong to one phylum; bacteria in the other phyla (Proteobacteria, Chlamydias, Spirochetes, Cyanobacteria, and others) are gram-negative. The gram-staining method is named after its inventor, Danish scientist Hans Christian Gram (1853–1938). The different bacterial responses to the staining procedure are ultimately due to cell wall structure. Gram-positive organisms typically lack the outer membrane found in gram-negative organisms. Up to 90 percent of the cell wall in gram-positive bacteria is composed of **peptidoglycan**, with most of the rest composed of acidic substances called teichoic acids. **Teichoic acids** may be covalently linked to lipids in the plasma membrane to form lipoteichoic acids**.** **Lipoteichoic acids** anchor the cell wall to the cell membrane. Gram-negative bacteria have a relatively thin cell wall composed of a few layers of peptidoglycan (only 10 percent of the total cell wall), surrounded by an outer envelope **containing lipopolysaccharides (LPS)** and lipoproteins. This outer envelope is sometimes referred to as a second lipid bilayer. The chemistry of this outer envelope is very different, however, from that of the typical lipid bilayer that forms plasma membrane.



Flagellum:

Flagellum Definition:

A flagellum is a microscopic hair-like organelle used by cells and microorganisms for movement. The word *flagellum* in Latin means whip, just like the whipping motion flagella (plural) often use for locomotion. Specialized flagella in some organisms are also used as sensory organelles that can detect changes in temperature and pH.

Function of Flagellum:

Flagella are filamentous protein structures found in bacteria, archaea, and eukaryotes, though they are most commonly found in bacteria. They are typically used to propel a cell through liquid (i.e. bacteria and sperm). However, flagella have many other specialized functions. Some eukaryotic cells use flagellum to increase reproduction rates. Other eukaryotic and bacterial flagella are used to sense changes in the environment, such as temperature or pH disturbances. Recent work with the green alga *Chlamydomonas reinhardtii* has shown that flagellum may also be used as a secretory organelle, but this discovery needs more time to be fully understood.

Examples of Flagellum:

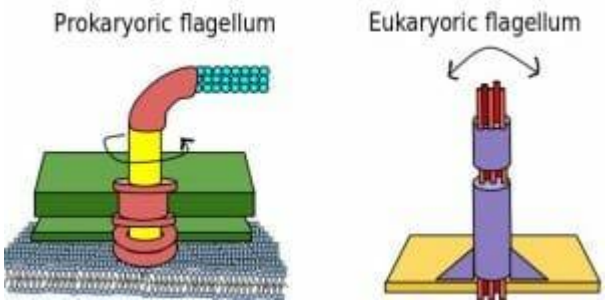
A flagellum can be comprised of different structures depending on the organism, especially when flagellum from eukaryotes and bacteria are compared. Since eukaryotes are usually complex organisms, the attached flagellum is more complex as well. The flagellum is made up of microtubules composed from a protein called tubulin. Nine microtubule pairs surround another two pairs of microtubules in the center to form the core of the flagellum; this is known as the nine-plus-two arrangement. The whole nine-plus-two structure is anchored in a basal body within the organism. These

bundled microtubules use ATP to bend back and forth in a whip-like motion together.

Although few multicellular eukaryotes have true flagellum, almost half the human population produces cells with them in the form of sperm. This is the only cell in the human body with flagellum, and for good reason. In order to move through the vaginal tract to meet the egg, sperm must be able to swim, or move, very long distances (in comparison of cell to body size). Without the flagellum, there would be very little chance of fertilization or population stability.

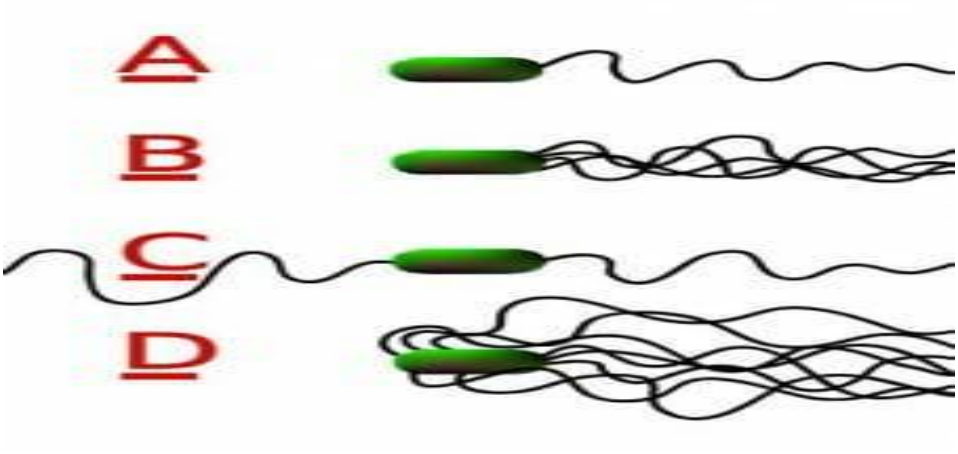
On the other hand, bacterial flagella are structured and function completely differently than the eukaryotic counterparts. These flagella are made of a protein called flagellin. ATP isn't needed because bacterial flagellum can use the energy of the proton-motive force. This means the energy is derived from ion gradients – usually hydrogen or sodium – which lie across cell membranes. These flagella are helix shaped and rotate quickly like a windmill to move the organism instead of whipping back and forth. The bacteria *Escherichia coli* uses this windmill-like locomotion to propel up the urethra to cause urinary tract infections. *Salmonella enterica*, a harmful pathogen, uses several windmill-like flagella to infect human hosts.

Comparison of flagellum motion in bacterial (prokaryotic) and eukaryotic organisms:



Types of Flagellum:

The flagellar structure consists of three different parts: rings embedded in the basal body, a hook near the surface of the organism to keep it in place, and the flagellar protein filaments. Every flagellum has these three things in common, regardless of organism. However, there are four distinct types of bacterial flagellum based on location:



A. Monotrichous: A single flagellum at one end of the organism or the other.

B. Lophotrichous: Several flagellum on one end of the organism or the other.

C. Amphitrichous: A single flagellum on both ends of the organism.

D. Peritrichous: Several flagellum attached all over the organism.

Monotrichous, amphitrichous, and lophotrichous flagellum are considered polar flagellum because the flagellum is strictly located on the ends of the organism. These flagella can rotate both clockwise and counterclockwise. A clockwise movement propels the organism (or cell) forward, while a counterclockwise movement pulls the organism backwards.

Peritrichous flagella are not considered polar because they are located all over the organism. When these flagella rotate in a counterclockwise movement, they form a bundle that propels the organism in one direction. If a few of the flagellum break away and begin rotating clockwise, the organism then begins a tumbling motion. During this time, the organism cannot move in any real direction.

Slime layers:

Diffuse and easily removed and capsules (organized and hard to remove)

Usually network of polysaccharides extending from the surface (called glycocalyx) but can be made of other materials too.

Functions:

a) Protection against phagocytosis, dehydration, viruses, detergents

b) Attachment

c) Nutrient reserve

S-layer–crystalline surface layer composed of outer layer of protein subunits arranged in a regular pattern

Functions:

a) Protection

b) Adherence

a) Selectively permeable "membrane"

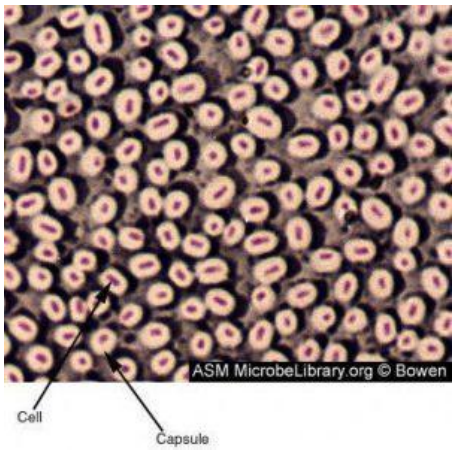
Structure:

1) Frequently functions as the cell wall in Archae bacteria

CAPSULE:

Capsule is a

- Gelatinous layer covering the entire bacterium
- Composed of polysaccharide (i.e. poly: many, saccharide: sugar). These polymers are composed of repeating oligosaccharide units of two to four monosaccharides.



Bacterial Capsule

Capsule is located immediately exterior to the murein (peptidoglycan) layer of gram-positive bacteria and the outer membrane (Lipopolysaccharide layer) of gram-negative bacteria**.**

- The sugar components of polysaccharide varies within the species of bacteria, which determines their serologic types. Example: *Streptococcus pneumoniae* has 84 different serologic types discovered so far.

Importance of Bacterial Capsule:

1. **Virulence determinants:**

   Capsules are anti-phagocytic. They limit the ability of phagocytes to engulf the bacteria. The smooth nature and negative charge of the capsule prevents the phagocyte from adhering to and engulfing the bacterial cell. If

a pathogenic bacteria lose capsule (by mutation), they wont be able to cause disease (i.e. loses disease causing capacity).

2. **Saving engulfed bacteria from the action of neutrophil:**
   Bacterial capsule prevents the direct access of lysosome contents with the bacterial cell, preventing their killing.
3. Prevention of complement-mediated bacterial cell lysis.
4. Protection of anaerobes from oxygen toxicity.
5. **Identification of bacteria**:
   1. Using specific antiserum against capsular polysaccharide. E.g. **Quellung reaction**
   2. **Colony characteristics in culture media**: Bacteria with capsules form smooth (S) colonies while those without capsules form rough (R) colonies. A given bacterial species may undergo a phenomenon called S-R variation whereby the cell loses the ability to form a capsule. Some capsules are very large and absorb water; bacteria with this type of capsule (e.g., *Klebsiella pneumoniae*) form mucoid (M) colonies.
6. **Development of Vaccines**: Capsular polysaccharides are used as the antigens in certain vaccines. For examples:
   - Polyvalent (23 serotypes) polysaccharide vaccine of *Streptococcus pneumoniae* capsule.
   - Polyvalent (4 serotypes) vaccine of *Neisseria meningitidis* capsule.

   A monovalent vaccine made up of capsular material from *Haemophilus influenzae*.

7. Initiation of infection: Capsules helps the organism to adhere to host cells.The capsule also facilitates and maintains bacterial colonization of biologic (e.g. teeth) and inanimate (e.g. prosthetic heart valves) surfaces through formation of **biofilms**.

8. Receptors for Bacteriophages.

Examples of Capsulated bacteria/yeasts:

Mneomonics to remember capsulated bacteria–
Some **K**illers **H**ave **P**retty **N**ice **C**apsule

1. ***S****treptococcus pneumoniae*
2. ***K****lebsiella pneumonia*

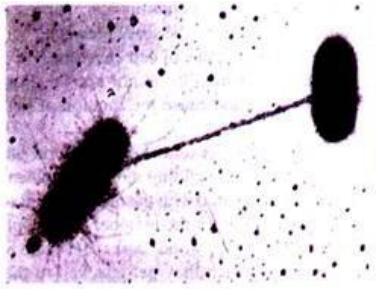
PILI:

Structure of Pili and Fimbriae:

Both fimbriae and pili are like flagella as both are the appendages on bacterial cell wall. They originate from cytoplasm that protrudes outside after penetrating the peptidoglycan layer of cell wall. Fimbriae are made up of 100% protein called fimbrilin or pilin which consists of about 163 amino acids Molecular structure of bacterial flagellum

The number of fimbriae is around 1,000. However, a similar structure has also been observed only in Corynebacterium renale, a Gram-positive bacterium. Pili differ from flagella in being shorter and thinner, straight and less ligid. But they are in large number. They occur either at the poles of bacterial cell or evenly distributed over the entric surface of the cell. The pili are 0.2-20 μm long with a diameter of about 250 Å.

Pili are genetically governed by plasmids, the number of which varies from 3 to 5. Fimbrilin has a molecular weight of about 16,000 Daltons. In addition, the sex pili are helical tubules consisting of a hollow core (25-30Å).



Structure of pili during conjugation.

The sex pili are the cylinder of repeating protein units. Its filamentous structure is governed by the sex factor (plasmid) of the bacterium for example F factor. Col I factor and R factor.

As compared to fimbriae the sex pili have a greater diameter (65-135 Å diam and length upto 20 μm), and a terminal knob of 150-800 Å diam-eter. There are two types of pili in E. coli for example F-pili (determined by F factor) and i-pili (determined by Col I factor. They have several receptor sites on which bacteriophages (e.g. f2, Ms2, M13, etc.) get adsorbed.

Structure of pili during conjugation Functions of Pili and Fimbriae:

There are several functions of fimbriae and pili as given below:

(a) Bacteria containing fimbriae are called fimbriate bacteria. Fimbriae have the adhesive properties which attach the organism to the natural substrate or to the other organism. Fimbriae agglutinate the blood cells such as erythrocytes, leucocytes, eplithelial cells, etc.

(b) Fimbriae are equipped with antigenic properties as they act as thermo labile nonspecific agglutinogen.

(c) Fimbriae affect the metabolic activity. The Fim+ cells (cells containing fimbriae) possess higher rate of metabolic activity than the Fim– cells (cells devoid of fimbriae). Moreover, they function as aggregation organelles i.e. they can form stellate aggregation on a static liquid medium.

(d) The sex pili make contact between two cells. Since they posses hollow core, they act as conjugation tube. The tip of pilus recognises the female (F–) cell through which the genetic material of donor (F+) cell passes to the recipient (female) cell. Only F-pili (not I-pili) contain axial hole.

Nutrient storage structures:

Most bacteria do not live in environments that contain large amounts of nutrients at all times. To accommodate these transient levels of nutrients bacteria contain several different methods of nutrient storage in times of plenty for use in times of want. For example, many bacteria store excess carbon in the form of polyhydroxyalkanoates or glycogen. Some microbes store soluble nutrients such as nitrate in vacuoles.

Sulfur is most often stored as elemental (S^0) granules which can be deposited either intra- or extra cellularly. Sulfur granules are especially common in bacteria that use hydrogen sulfide as an electron source. Most of the above-mentioned examples can be viewed using a microscope and are surrounded by a thin non unit membrane to separate them from the cytoplasm.

***Inclusions**:*

Inclusions are considered to be nonliving components of the cell that do not possess metabolic activity and are not bounded by membranes. The most common inclusions are glycogen, lipid droplets, crystals, and pigments. Volutin granules are cytoplasmic inclusions of complexed inorganic polyphosphate. These granules are called metachromatic granules due to their displaying the metachromatic effect; they appear red or blue when stained with the blue dyes methylene blue or toluidine blue.

Gas vacuole:

Gas vacuoles are membrane-bound, spindle-shaped vesicles, found in some planktonic bacteria and *Cyanobacteria*, that provides buoyancy to these

cells by decreasing their overall cell density. Positive buoyancy is needed to keep the cells in the upper reaches of the water column, so that they can continue to perform photosynthesis.

They are made up of a shell of protein that has a highly hydrophobic inner surface, making it impermeable to water (and stopping water vapour from condensing inside) but permeable to most gases. Because the gas vesicle is a hollow cylinder, it is liable to collapse when the surrounding pressure increases.

Natural selection has fine tuned the structure of the gas vesicle to maximise its resistance to buckling, including an external strengthening protein, GvpC, rather like the green thread in a braided hosepipe. There is a simple relationship between the diameter of the gas vesicle and pressure at which it will collapse – the wider the gas vesicle the weaker it becomes. However, wider gas vesicles are more efficient, providing more buoyancy per unit of protein than narrow gas vesicles. Different species produce gas vesicle of different diameter, allowing them to colonise different depths of the water column (fast growing, highly competitive species with wide gas vesicles in the top most layers; slow growing, dark-adapted, species with strong narrow gas vesicles in the deeper layers). The diameter of the gas vesicle will also help determine which species survive in different bodies of water. Deep lakes that experience winter mixing expose the cells to the hydrostatic pressure generated by the full water column. This will select for species with narrower, stronger gas vesicles.

The cell achieves its height in the water column by synthesising gas vesicles. As the cell rises up, it is able to increase its carbohydrate load through increased photosynthesis. Too high and the cell will suffer photobleaching and possible death, however, the carbohydrate produced during photosynthesis increases the cell's density, causing it to sink. The daily cycle of carbohydrate build-up from photosynthesis and carbohydrate catabolism during dark hours is enough to fine-tune the cell's position in the water column, bring it up

toward the surface when its carbohydrate levels are low and it needs to photosynthesis, and allowing it to sink away from the harmful UV radiation when the cell's carbohydrate levels have been replenished. An extreme excess of carbohydrate causes a significant change in the internal pressure of the cell, which causes the gas vesicles to buckle and collapse and the cell to sink out.

***Microcompartments**:*

Bacterial micro compartments are widespread, membrane-bound organelles that are made of a protein shell that surrounds and encloses various enzymes. Provide a further level of organization; they are compartments within bacteria that are surrounded by polyhedral protein shells, rather than by lipid membranes. These "polyhedral organelles" localize and compartmentalize bacterial metabolism, a function performed by the membrane-bound organelles in eukaryotes.

Carboxysomes:

Carboxysomes are bacterial micro compartments found in many autotrophic bacteria such as Cyan bacteria, Knallgasbacteria, Nitroso- and Nitrobacteria. They are proteinaceous structures resembling phage heads in their morphology and contain the enzymes of carbon dioxide fixation in these organisms (especially ribulose bisphosphate carboxylase/oxygenase, RuBisCO, and carbonic anhydrase). It is thought that the high local concentration of the enzymes along with the fast conversion of bicarbonate to carbon dioxide by carbonic anhydrase allows faster and more efficient carbon dioxide fixation than possible inside the cytoplasm.[11] Similar structures are known to harbor the coenzyme B12-containing glycerol dehydratase, the key enzyme of glycerol fermentation to 1,3-propanediol, in some Enterobacteriaceae (e. g. Salmonella).

Magnetosomes:

Magnetosomes are bacterial microcompartments found in magnetotactic bacteria that allow them to sense and align themselves along a magnetic field (magnetotaxis). The ecological role of magnetotaxis is unknown but is thought to be involved in the determination of optimal oxygen concentrations. Magnetosomes are composed of the mineral magnetite or greigite and are surrounded by a lipid bilayer membrane. The morphology of magnetosomes is species-specific.

The bacterial endospore:

A. Special resistant, dormant structure formed by vegetative cells of several gram-positive organisms

B. Resistant to heat, desiccation, radiation, chemicals

A. Functions: Survival

A. Structure

1. Core (contains plasma membrane and cytoplasm of cell)

a) Contains dipicolinic acid complexed with calcium ions

b) Contains SASPs–small acid- soluble pore proteins that protect DNAc) dehydrated–only10-30%water

2. Spore cell wall (core wall)

3. Cortex–loosely cross-linked peptide glycan.

1.Spore coat–several layers of protein which are impermeable to many chemicals.

2.Exosporium–thin, protein layer.

E.Sporogenesis or sporulation=Spore formation

1. Induced by lack of nutrients.

2. Complex process that requires 200 genes and takes 8-10hours

a) Stage 1: DNA becomes denser and formsan "axial filament" of nuclear material

b) Stage 2: invagination of plasma membrane engulfs the DNA and produce the forespore septum

a) Stage 3: forespore is engulfed by second membrane

a) Stage 4: cortex formation between the two membranes; calcium and DPA accumulate

a) Stage 5: sporecoat synthesis around cortex

b) Stage 6: maturation of spore: development of resistance to heat and chemicals

c) Stage 7: lysis of cell and release of spore.

F.Breaking of the endospore dormant state

1. Activation–reversible process usually from heat treatment that conditions cells so that they will germinate when placed in nutrient rich medium

2. Germination–breaking of spore's resistant state

a) Spores welling

b) Rupture of the coat

c) Loss of DPA and calcium

a) Degradation of SASPs

3. Out growth–synthesis of new components (RNA,proteins,etc).

UNIT III
STERILIZATION

DEFINITION:

A process in which complete elimination microorganisms from an object is done is known as sterilization. Sterilization is done by several methods includes physical methods & chemical methods.

PHYSICAL METHODS: Physical methods includes

1. Sunlight'
2. Drying
3. Heat
4. Filtration &
5. Radiation

CHEMICAL METHODS: Chemical methods include 1. Variety of Chemicals as antiseptics & disinfectants

PHYSICAL METHODS:

SUN LIGHT:

Sunlight possess appreciable bactericidal activity. It plays an important role in the spontaneous sterilization of materials under natural conditions. The bactericidal activity of sunlight is mainly due to the combination of UV rays along with Heat rays. When typhoid bacilli exposed to sunlight, the bacilli will be killed in two hours. But, exposure of the bacilli in dark, the bacilli will be surviving for six days. Bacteria suspended in water are readily destroyed by sunlight.

DRYING:

Moisture is essential for the growth of microorganisms. About 80% of the body weight of MO made of water. So, drying in air has deleterious effect on MO. But, spores are unaffected by drying. So, drying is an unreliable method of sterilization for sensitive lab works.

HEAT:

Heat is the reliable method of sterilization. Materials can be damaged by heat can be sterilized at low temperatures for longer periods or by repeated cycles.

FACTORS WHICH INFLUENCES STERILIZATION BY HEAT INCLUDES,

- Nature of heat,
- Temperature,
- Holding period,
- Number of MOs present on the material,
- Types of MOs on the material, &
- Type of material from which the organism will be eliminated.

Sterilization by heat is done by two methods. Known as, DRY Heat & MOIST Heat

DRY HEAT:

The killing effect of dry heat is due to Protein denaturation. Oxidative damage & toxic effect of elevated levels of electrolytes. High amount of heat is required to carry out all these effects.

Dry heat includes, Flaming, Incineration & Hot air oven.

Flaming: showing off an article through the Bunsen burner's flame till the article becomes red hot. This process is referred as flaming.

Example: Sterilization of an inoculation loop, and
Sterilization of the tip of a foreceps.

Incineration: burning of an infected material or waste materials in open place is said to be incineration.
Example: burning of hospital wastes,
Burning of animal carcasses, Burning of contaminated materials.

Hot air oven:

- An instrument which works on dry heat principle is known as hot air oven.
- This is the most widely used method of sterilization by dry heat.
- The sterilization temperature of hot air oven is 160^0C carried out for one hour(160^0C/1Hr).
- All types of dried glass wares, foreceps, scalpels, syringes, some pharmaceutical powders, fats & grease are sterilized by hot air oven.
- Hot air is the bad conducter of heat & its penetrating power is low. The oven is heated by electricity. The heating elements present in the walls of the instrument will get heated. The oven also contains a fan, to ensure the circulation the hot air among the materials. The oven should not be over loaded. Glass wares should be dried thoroughly before keeping it in hot air oven for sterilization.
- Certain materials will be sterilized at 150^0C for two hours.
- After sterilization over, the oven must be cooled for atleast 2 hours before opening. to avoid the cracking of glass wares.

Disadvantages:

- Cotton swabs are not sterilized by hot air oven, because the cotton will be charred (powdered).
- Rubber materials are not sterilized.
- Heat labile (or) heat sensitive materials cannot be sterilized.

Sterilization control:

The spores of non-toxigenic strains of Clostridium tetani are used as sterilization control agents. Sterile filter paper strips soaked in Clostridium's spore suspension and kept in suitable packs and then sterilized by in hot air oven. After sterilization over, the strips are removed from the pack & inoculated into suitable medium (Thioglycollate broth or Robertson's cooled meat medium). Then, incubated anaerobically at 37^0C for 5 days or till the growth appears.

In another method, a special device called Browne's tube is used. After proper sterilization (160^0C/1Hr), a GREEN COLOUR will be produced on the tube.

MOIST HEAT

The killing effect or lethal effect of moist heat is due to the protein denaturation & protein coagulation in MOs. Moist heat can be carried out in diferent methods. Which includes,

1. Temperature below 100^0C (Pasteurization),
2. Temperature at 100^0C (boiling),
3. Steam at atmospheric pressure (100^0C) &
4. Steam under pressure.

1. Temperature below 100^0C: Sterilization is carried out at temperature below 100^0C is said to be Pasteurization. A process in which milk is sterilized at 62.8^0C for 30 minutes and then subjected to immediate cooling at 13^0C is known as Pasteurization. This technique was introduced by Louis Pasteur. All the non-sporing vegetative bacteria are killed by this method.
2. Temperature at 100^0C:

Sterilization is carried out at temperature of 100^0C is said to be boiling. Vegetative bacteria are killed almost by boiling. But, spores are not killed, they require longer time period to kill. In this method, hard water should not be used. Sterilization can be promoted by the addition of 2% Sodium bicarbonate to the water. However, boiling is not recommended for the sterilization of all the glass wares. It is not suitable for most of the microbial works.

3. Steam at atmospheric pressure (100^0C):

An atmosphere of free steam is used to sterilize culture media.

A Koch or Arnold steamer is used. This is an inexpensive method. The container and the medium are simultaneously sterilized. The apparatus requires little or no attention.

A steamer consists of tinned copper cabinet. It contains a conical shaped lid which ensures the drainage of condensed steam. A perforated tray is present at the bottom water level to ensure the steam to surround the material. A single exposure of 90minutes gives successful sterilization for certain media.

TYNDALLIZATION:

A method of sterilization in which a medium containing sugar or gelatin will be sterilized at 100^0Cfor 20 minutes on three successive days is referred as Tyndallization or intermittent sterilization. The principle behind this method is, on the first day, the medium will be sterilized. Cooled & incubated. The first exposure kills all the vegetative bacteria present in the medium but not the spores. During incubation the spores will be germinated & give raise to vegetative cells. On second day, again the medium will be sterilized at 100^0C/20 minutes, cooled & incubated. Vegetative cells will be killed, spores germinated at incubation. The same thing will be continued for the 3rd day. But, this method is not widely used in the laboratories.

4. Steam under pressure (Autoclaving or steam sterilizer):

The principle of an Autoclave or a steam sterilizer:

Water boils when its vapour pressure equals to that of the surrounding atmosphere. Hence, when pressure inside a closed vessel increases, the temperature at which water boils also increases. The saturated steam has high penetrative power. When steam comes into contact with a cooler surface it condenses into water and gives up its LATENT HEAT (hidden heat) to that surface. This process happening continuously till the heat of that surface raised to that of the steam. The condensed water ensures moist heat to kill the pathogens present in the object.

Sterilization by steam under pressure is carried out at temperatures between 1080C to 147^0C, mostly at 121^0C/ 15 minutes. Variety of materials such as dressings, instruments, media, colon swabs & pharmaceutical things can be sterilized by autoclaving.

The autoclave is made of a gunmetal or stainless steel. The instrument is either vertical or horizontal. It contains a conical shaped lid with some screws. The autoclave have a discharge tap (for air & steam) on the upper region. A pressure gauge & a safety valve are also present. The autoclave is heated by either gas or electricity.

Sterilization control:

The spores of non-pathogenic strains of Bacillus stearothermophilus are used as sterilization control agents. Sterile filter paper strips soaked in Bacillus spore suspension and kept in suitable packs and then sterilized by autoclaving. After sterilization over, the strips are removed from the pack & inoculated into suitable medium (Nutrient agar medium). Then, incubated at 37^0C for 5 days or till the growth appears.

FILTRATION

A method of sterilization in which HEAT SENSITIVE (or) HEAT LABILE materials will be filtered through sterile filters. It is known as filtration.Through filtration bacteria are removed from the liquid matter.Bacterial toxin can be obtained by passing bacterial cultures through the filters. Different types of filters are used in microbiology. These filters are made with different porosity(holes – usually in microns- μ).

TYPES OF FILTERS:

1. Candle filters,
2. Asbestos filters,
3. Sintered glass filters and
4. Membrane filters.

1. Candle filters:

- These filters are manufactured with different porosity.
- Widely used in water purification process in industries.
- It is of two types.

A). Unglaced ceramic filters (Chamberland & Doulton filters),

B). Diatomaceous earth filter (Berkefeld & mandler filters)

2. Asbestos filters:

- These are disposable, single use filter discs.
- They have high adsorbing capacity.
- These make the solutions alkaline while filtering.
- These filters are carcinogenic in nature. So, their use will be very much limited.
- Example: Seitz filters & Sterimat filters.

3. Sintered glass filters:

- These filters are prepared by heat-fusing of finely powdered glass particles.
- They possess different porosity.
- They have low adsorptive capacity.
- Theas filters can be cleaned easily.
- These are very expensive.

4. Membrane filters:

- These filters are made up of either Cellulose or other polymers.
- These filters are widely used in all the industries.
- Membrane filters are widely used in water purification, analysis, sterilization & sterility testing.
- They have the porosity of 0.22 μm.

RADIATION

By using certain rays, the microorganisms will be eliminated from the objects. This is known as radiation. Radiation is of two types. Ionizing radiation & Non-ionizing radiation.

IONIZING RADIATION:

- Alpha, beta, gamma, cosmic, X-rays & high energy electrons are used in ionizing radiation.
- These rays are highly lethal (dangerous) to the DNA & other vital (important) components of the MOs.
- These rays possess high penetration power on MOs. During radiation process, minimum amount of heat is liberated. Hence, this method is also known as COLD STERILIZATION.
- Gamma rays are used to sterilize plastics, syringes, swabs, catheters, animal feeds, oils & metal foils.
- High energy electrons are not used in the field of medicine.

NON-IONIZING RADIATION:

- Ultraviolet (UV) & Infrared (IR) rays are commonly used in non-ionizing radiation.
- During sterilization more amount of heat is liberated. Hence, this method is also known as hot air sterilization.
- IR rays are used to sterilize prepacked items like syringes & catheters.
- UV rays are used to disinfect the areas such as operation theatres, laboratories & entryways of hospitals & industries.

ULTRASONIC & SONIC VIBRATIONS:

- Ultrasonic & sonic waves have enough amount of bactericidal activity. But, MOs showed considerable variation in the sensitivity to these waves. Surviving MOs were found after the treatment. Hence, this method is have no practical value.

CHEMICAL METHODS OF STERILIZATION

Several chemical agents are used as antiseptics & disinfectants. The properties of an ideal disinfectant or an antiseptic agent: An ideal antiseptic or disinfectant should

1. Have a wide spectrum of activity,
2. Be effective against all microorganisms,
3. Be active in the presence of an organic matter,
4. Have speedy action,
5. Have high penetrating power,
6. Be stable,
7. Not corrode metals, not cause any irritation,
8. Not be toxic if absorbed into circulation,
9. Be easily available.
10. Be inexpensive,
11. Be safe,
12. Be very easy to use,
13. Be effective in acid & alkaline media &
14. Be compatible with other antiseptics & disinfectants.

Antiseptic agent: A chemical agent, which is applied on living things (or) animate objects is said to be an antiseptic.

Example: Savlon, Dettol

Antisepsis: A process in which an antiseptic agent is used is known as antisepsis.

Example: Savlon, Dettol

Disinfectant: A chemical agent, which is applied on non-living things (or) inanimate objects is said to be a disinfectant.

Example: Phenol, Lizol

Disinfection: A process in which a disinfectant is used is known as disinfection.

Example: Phenol, Lizol

All the chemical agents act in various ways, like,

- Protein coagulation,
- Disruption of cell membrane,
- Removal of free sulphydryl groups from the co-enzyme of the organism,
- Substrate competition

The chemical agents includes – Alcohols,
Aldehydes,
Dyes,
Halogens,
Phenols,
Gases &
Surface active agents.

ALCOHOLS: Example: ethyl alcohol, Iso propyl alcohol &methyl alcohol.

- These are mainly used as skin antiseptic agents.
- They denature bacterial proteins.
- They have no action on spores.
- To make so effective, these agents must be used with water.
- Methyl alcohol shows its effectiveness especially on fungal spores.
- The laminar chambers, incubator's inside can be wiped with methanol for the removal of MOs.

ALDEHYDES: Example: Formaldehyde.

- It is bactericidal & sporicidal.
- It affects the action of proteins of MOs.
- It is lethal for viruses also.
- This aldehyde destroys anthrax spores in hair & wool.
- 10% formalin containing 0.5% sodium tetraborate is used to sterilize clean metal instruments.

- Formaldehyde gas is widely used in the sterilization of surgical instruments & catheters, fumigating wards & sick rooms.
- This gas is irritable in nature. So, after disinfection over, ammonia gas will be exposed on that area to nullify the effect of formaldehyde gas.
- Gluteraldehyde is similar to formaldehyde in action. This gas is specifically effective against tubercle bacilli, fungi & viruses.
- It is less irritant than formaldehyde gas.
- Gluteraldehyde gas is used in the treatment of anesthetic tubes, face masks & endotracheal tubes, etc.,

DYES:

- Two groups of dyes are used. Aniline dyes & acridine dyes.
- These are used as skin & wound antiseptics.
- Both are bacteriostatic as well as bactericidal.
- Aniline dyes includes – brilliant green, malachite green & crystal violet..
- These aniline dyes very effective against Gram positive organisms.
- Acridine dyes are effective against Gram positive organisms.
- These dyes includes proflavine, acriflavine & euflavine.
- They impair the DNA complexes of organisms and kill them.

HALOGENS: Example: Iodine, Chlorine.

- Iodine is widely used as a skin antiseptic agent.
- It is bactericidal & shows moderate effect on spores.
- It is active against tubercle bacilli & viruses.
- Compounds of iodine with non-ionic wetting or surface active agents known as iodophores. These are more active against the organisms.
- Chlorine & its compounds are widely used as disinfectants.

- Chlorines are used as hypochlorites. These are bactericidal in nature.
- Hypochlorites are widely effective against viruses.
- Water supplies, swimming pools, food & dairy industries are disinfected by chlorine compounds.

PHENOLS: Example: Lysol, cresol

- Phenol is obtained from coal through distillation process.
- Phenol is also known as Carbolic acid. It is a powerful microbicidal agent. It is toxic for humans.
- Joseph Lister, the father of antiseptic surgery, first introduced them in surgery.
- From this event a wide range of phenolic compounds have been developed & used as disinfectants.
- Treatment with phenol makes lethal effects on the cell membrane of MOs.
- Phenolic compounds disrupts cell membranes thus leads the release of cell contents & causing cell lysis.
- Low concentration of phenol precipitate proteins.
- Lysol & cresol are active against wide range of microorganisms.
- Various combinations of the phenolic agents are used in the control of pathogens in surgical & neonatal units of hospitals.

GASES: Example: Ethylene oxide, formaldehyde gas & Betapropiolactone.

- Ethylene oxide (EO) has high penetrative power.
- It is highly inflammable. If the concentration of ethylene oxide in air is greater than 3%, it is highly explosive.
- EO reacts with DNA, RNA protein molecules & modify them.
- EO exhibits a potential toxicity to humans.

- EO is especially used in the sterilization of heart-lung machines, dental equipments, plastics, metal & paper surfaces, soil & some foods.
- Betapropiolactone (BPL): it is a condensation product of ketane & formaldehyde.
- It is used in fumigation process.

SURFACE ACTIVE AGENTS: Example: Detergents

- Substances that alter the energy relationship at interfaces, producing a reduction in, the surface tension are called as surface active agents (SAA).
- These SAA are either anionic/cationic/ nonionic/amphoteric in nature.
- SAA are active in alkaline pH.
- These agents act on the phosphate group of the cell membranes. The semipermeability of the membrane will be lost thus leads the release of intracellular contents.
- Quaternary ammonium compounds (QAC) are cationic surface active agents possess microbicidal activity. Ex: Benzalkonium chloride.
- The amphoteric or ampholytic compounds are known as Tego compounds. These are effective against both Gram positive & Gram negative bacteria.

METALLIC SALTS: Heavy metal salts

- Salts of heavy metal have a greater action against MOs.
- Metallic salts are also used as disinfectants.
- They denature & coagulate the proteins & enzymes of MOs.
- These are also used as fungicides.

ANTIMICROBIAL CHEMOTHERAPY

Definition:

A Biological component produced by one group of microorganism, which is inhibitory for other group of microorganism is known as "Antibiotic".

- Penicillin
- Streptomycin
- Tetracyclin
- Vancomycin

In 1929 Alexander fleming discovered penicillin.

The discover was "Serendipity".

SERENDIPITY- Accidental discovery.

SALIENT FEATURES OF AN ANTIBIOTIC:

1. The antibiotic may be a broad spectrum antibiotic.
2. It should prevent the ready development of the resistant forms of organisms.
3. It should not cause any side effects like allergic reactions.
4. It should not cause any nerve damage or irritation to the kidneys.
5. It should not eliminate the normal microbial flora from the host cells.

Broad Spectrum:

The ability of the substances (antibiotic) to destroy or inhibit different kinds of pathogens is known as "Broad Spectrum".

TYPES OF ANTIBIOTICS:

Antibiotics are classified in different ways, i.e.,

1. Based on the effect bactericidal and bacteriostatic
2. Based on the chemical nature
3. Based on the mode of action.

1. Based on the effect antibiotics are two types they are

Bactericidal.

Bacterostatic

Bactericidal: (Killing)

Agents which kill the microbial cell are called bactericidal agents.

Bacteriostatic: (Inhibition)

Agents which inhibit the growth of microbial cell are called bactericidal agents. Based on the chemical structure;

The antibiotics are of different types known as

1. β- Lactams ----- Penicilin. It is classified into two types such as, natural and semi synthetic penicillin.
2. Polypeptidies ---- Bacitracin
3. Polyenes ----- Nystatin
4. Amino glycosides ----Streptomycin.
5. Tetracyclines----- Tetracyclin
6. Macrolides ------ Erythromycin.
7. Sulfonamides -----Sulphanilamide, Sulfathiazole.
8. Nitrobenzene compounds ----- Chloromphenicol

- ✓ These antibiotics may have broad spectral activity.
- ✓ They may be bactericidal \ bacteriostatics
- ✓ They may be active against both Gram positive and Gram negative bacteria.
- ✓ They may cause detrimental effect on cell wall synthesis, membrane permeability , protein synthesis etc…

3. Based on the mode of action :

Based on the mode of action the antibiotics are of four types namely,

1. Cell wall synthesis inhibitors
2. Antibiotics cause damage to cytoplasmic membrane

3. Nucleic acid and protein synthesis inhibitors.
4. Specific enzyme system inhibitor.

1. Cell wall synthesis inhibitors:

Antibiotics which inhibit the biosynthesis of peptidoglycan and prevents the formation of cell wall are called as Cell wall synthesis inhibitors.

Eg; Penicillin
Cephalosporin.
Ampicillin
Cycloserine.

Peptidoglycan:

- A substances that gives rigidity to the bacterial cell wall, made up of monomers of N-Acetyl glucosamine and N-Acetyl muramic acid which have linked by means of peptide bonds.
- The PG is the protective covering of the bacterial cell.
- Various numerous steps occurs in the biosynthesis of PG and Cell wall.
- Any interruption in any of the steps leads to inhibition of PG biosynthesis and leads to no proper formation of cell wall.

Eg: Inhibition of trans peptidase reaction between the two polymers.

- Certain antibiotics, which inhibits the transpeptidase reactions and damage the cross linking between the two polymers.
- This leads the inability of the bacterium to survive due to no protective covering.

Eg: β- lactams (penicillin) – inhibits the Transpeptidation reaction in peptidoglycan synthesis.

2 . Damage to cytoplasmic membrane:

Certain antibiotic have the ability to damage cell membrane structure. They adversely affect the “Normal permeability” of the membrane. The antibiotics which cause damage to cytoplasmic membrane are “Bactericidal” in nature [polymyxin antibiotics].

Eg: 1 These **polymyxins** cause damage in CM thus leads "leakage" of the contents from cell inside to outside.

Eg: 2 Polyenes- These antibiotics act upon the "sterols" in the cytoplasmic membrane. They act upon fungi and animal cells but do not affect bacteria.

NOTE: [Bacteria does not contain sterol on their cell wall].

3. Inhibition of nucleic acid and protein synthesis:

Nucleic acids and proteins are synthesized by a number of intricate biochemical reactions. Interruption in any of these steps inhibits the synthesis of nucleic acid or proteins.

Eg: Streptomycin, Tetracycline, Chloramphenicol, and Erythromycin.

These antibiotics have adverse effects on the synthesis of nucleic acid / proteins.

Streptomycins:

It Combines irreversibly with the 30S subunit rRNA and inhibits protein synthesis.

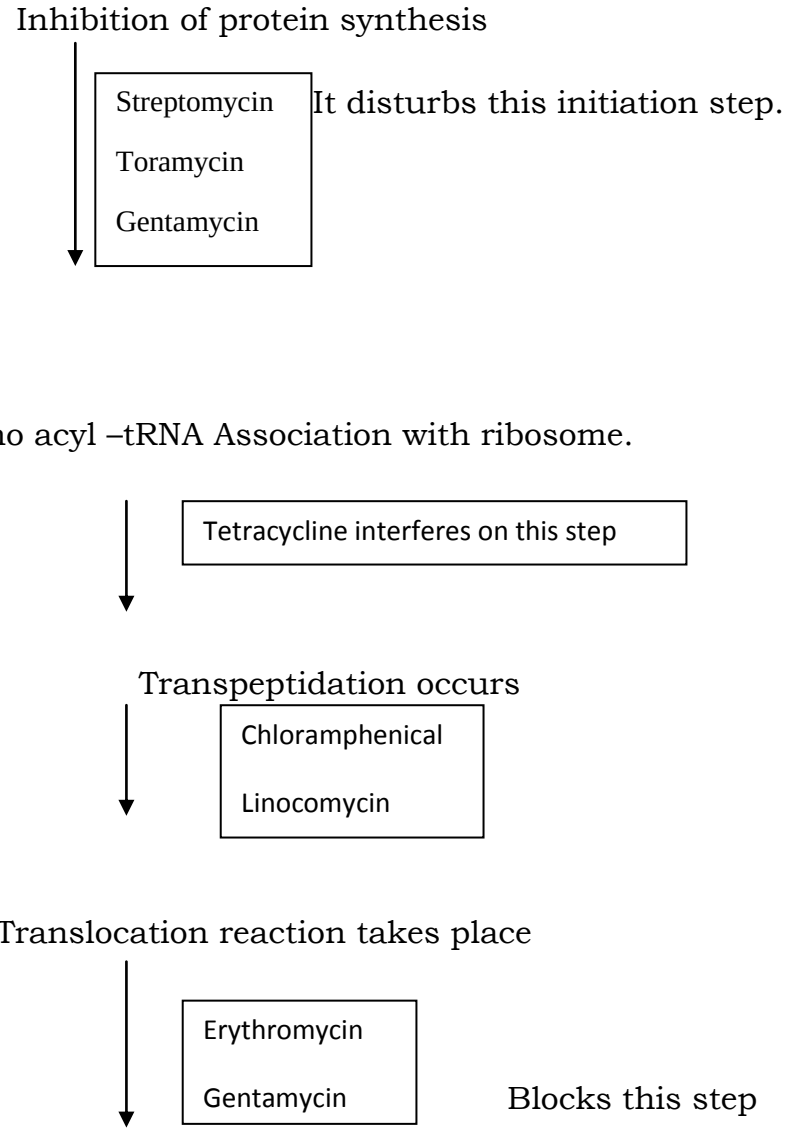
Tetracyclines:

- These antibiotics are bacteriostatic.
- They inhibits protein synthesis by blocking the binding of " amino acyl – Trna to the 30 S subunit of Rrna.

Chloramphenicol & Erythromycin:

- These antibiotics inhibits protein synthesis by combining with 50S subunit of ribosome.
- The transpeptidase and translocation functions associated with this sites are blocked.

THE ACTION OF VARIOUS ANTIBIOTICS ON THE INHIBITION OF PROTEIN SYNTHESIS:



4). **INHIBITION OF SPECIFIC ENZYME SYSTEMS :**

- Many bacteria require **P-amino benzoic acid (PABA)** as a precursor for the synthesis of an essential Co-enzyme **Tetrahydrofolic acid (THFA). PABA** is the structural part of THFA molecule.
- Hence "THFA" molecule (Co-enzyme) is very essential for the synthesis of amino acids, thymidine synthesis etc..
- **SULFONAMIDES –** A group of compound which are very similar to the structure of PABA molecule. So, in the synthesis of THFA molecule instead of PABA, sulphonamide will bind with THFA and blocks its synthesis.

ANTIBIOTIC SENSITIVITY TEST

An **antibiotic sensitivity** (or **susceptibility**) **test** is done to help choose the **antibiotic** that will be most effective against the specific types of bacteria or fungus infecting an individual person. ... Infections caused by resistant bacteria or fungi are not cured by treatment with those **antibiotics**.

Principle:

- The introduction of various antimicrobials for treating variety of infections showed the necessity of performing antimicrobial susceptibility testing as a routine procedure in all microbiology laboratories. In laboratories it can be made available by using antibiotic disk which will diffuse slowly into the medium where the suspected organism is grown. The basic principle of the antibiotic susceptibility testing has been used in microbiology laboratories over 80 years. Various chemical agents such as antiseptics, disinfectants, and antibiotics are employed to combat with the microbial growth.

- Among these, antibiotics are generally defined as the substances produced by the microorganism such as Penicillium, which has the ability to kill or inhibit the growth of other microorganisms, mainly

bacteria. Antimicrobial susceptibility tests (ASTs) basically measures the ability of an antibiotic or other antimicrobial agent to inhibit the invitro microbial growth.

- There are many different procedures that microbiologists use to study the effects of various antimicrobial agents in treating an infection caused by different microorganisms. Mueller Hinton Agar is considered as best for the routine susceptibility testing since it is has batch-to-batch reproducibility, low concentration of inhibitors of sulphonamide, trimethoprim and tetracyclines and produce satisfactory results for most of the non-fastidious pathogens. Fastidious organisms which require specific growth supplements need different media to grow for studying the susceptibility patterns.

- The Kirby Bauer test is a qualitative assay whereby disks of filter paper are impregnated with a single concentration of different antibiotics or any chemicals that will diffuse from the disk into the agar. The selected antibiotic disks are placed on the surface of an agar plate which has already been inoculated with test bacteria. During the incubation period, the antibiotics/chemicals diffuse outward from the disks into the agar.

- This will create a concentration gradient in the agar which depends on the solubility of the chemical and its molecular size. The absence of growth of the organism around the antibiotic disks indicates that, the respected organism is susceptible to that antibiotic and the presence of growth around the antibiotic disk indicates the organism is resistant to that particular antibiotic. This area of no growth around the disk is known as a zone of inhibition, which is uniformly circular with a confluent lawn of growth in the media.

- The diameters of the zone of inhibition are measured (including disk) using a metric scale or a sliding caliper. The measured zone diameter

can be compared with a standard chart for obtaining the susceptible and resistant values. There are zone of intermediate resistance which means that the antibiotic may not be sufficient enough to eradicate the organism from the body.

Factors affecting Antibiotic Susceptibility Testing

Many conditions can affect the accuracy of the AST results, which is described in detail below.

1. pH

pH of the medium is an important factor which influences the accuracy of the antibiotic susceptibility testing. If the pH of the medium is too low than the desired pH, certain drugs such as amino glycosides, quinolones and macrolides lose their potency, on the other hand, antibiotic classes such as tetracyclines appear to have excess activity a lower pH and the vice versa happens in the case of the higher pH.

2. Moisture

The presence of moisture content on the medium can counter act with accuracy of the susceptibility testing. It is important to remove the excess moisture present in the agar surface, by keeping it in the laminar flow hood for few minutes.

3. Effects of medium components

If the media selected for the antibiotic susceptibility contains excessive amounts of thymine or thymidine compounds, they will reversibly inhibit the action of certain antimicrobial agents such as trimethoprim groups. This reversible inhibition yields smaller or less distinct or even no zones and will be misinterpreted as resistant antibiotics. MHA is low in thymine and

thymidine content and it can be used successfully to study the susceptibility of antibiotics. Also the medium containing excessive cation reduces the zone size, while low cation content results in unacceptably large inhibition zones.

4. Amount of organism

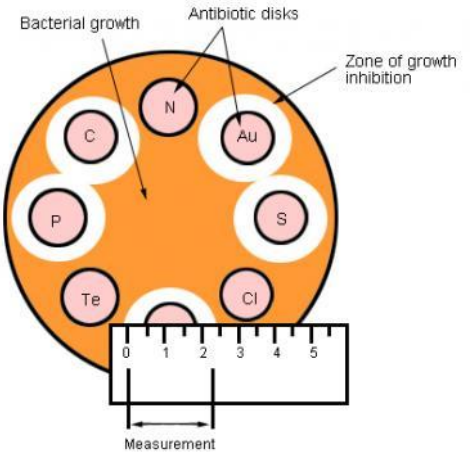
The amount of the organism used for the susceptibility testing is standardized using a turbidity standard. This is obtained by a visual approximation using McFarland standard of 0.5 or else it can be determined by using a spectrophotometer with Optical density of 1 at 600 nm wave length. In addition to this, the antibiotic concentration for the susceptibility testing is pre-determined and is commercially available.

Procedure:

Both disk diffusion and MIC methods employ the phenotypic identification of susceptibility, and therefore, requires the following process:

- Preparation of a standardized inoculum from a bacterial culture:
    - Choosing well-isolated colonies
    - Creating a bacterial suspension (inoculum)
    - Standardizing the bacterial suspension using McFarland standards
- Dilution of bacterial suspension (only for MIC method)
- Inoculation of bacterial suspension to one of the following:
    - A particular growth medium (e.g., Mueller Hinton Agar, MHA for disk diffusion)
    - An MIC panel
- Addition of antimicrobial disks (only for disk diffusion)
- Incubation of plates (disk diffusion) or panels (MIC)
- Measuring the zone of inhibition or reading MIC panel

- Interpretation of AST results



UNIT IV

CULTURE MEDIA AND ITS TYPES

What is culture media ?

- A **culture media** is a special **medium** used in microbiological laboratories to grow different kinds of microorganisms. A **growth** or a **culture medium** is composed of different nutrients that are essential for microbial **growth**.
- Culture media contain nutrients and physical growth parameters necessary for microbial growth. All microorganisms cannot grow in a single culture medium and in fact many can't grow in any known culture medium.
- Organisms that cannot grow in artificial culture medium are known as **obligate parasites**. *Mycobacterium leprae*, *rickettsias, Chlamydias,* and *Treponema pallidum* are obligate parasites.

Classification of bacterial culture media on the basis of consistency

1. **Solid medium**

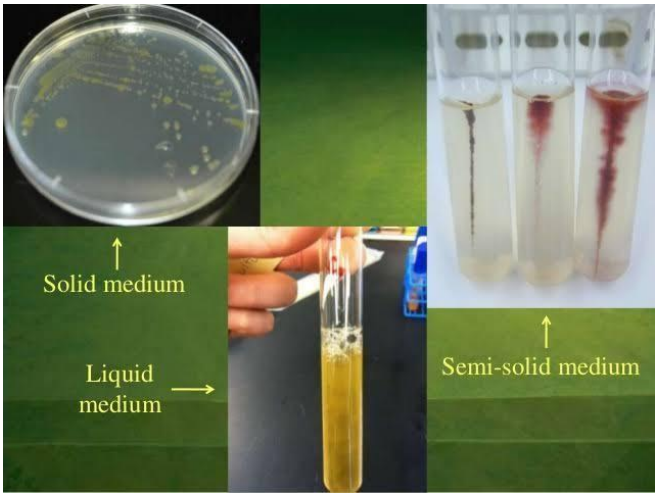
Solid medium contains agar at a concentration of 1.5-2.0% or some other, mostly inert solidifying agent. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as colonies or in streaks). Solid medium is useful for **isolating bacteria** or for determining the colony characteristics of the isolate.

2. **Semisolid media**

Semisolid media are prepared with agar at concentrations of 0.5% or less. They have soft custard like consistency and are useful for the cultivation of **microaerophilic bacteria** or for **determination of bacterial motility.**

3. **Liquid (Broth) medium**

These media contains specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of large number of organisms, fermentation studies, and various other tests. e.g. **sugar fermentation tests, MR-VR broth**.



Classification of culture media on the basis of composition

1. **Synthetic or chemically defined medium**

A chemically defined medium is one prepared from purified ingredients and therefore its exact composition is known.

2. **Non synthetic or chemically undefined medium**

Non-synthetic medium contains at least one component that is neither purified nor completely characterized nor even completely consistent from batch to batch. Often these are partially digested proteins from various organism sources. Nutrient broth, for example, is derived from cultures of yeasts.

Synthetic medium may be simple or complex depending up on the supplement incorporated in it. A simple non-synthetic medium is capable of meeting the nutrient requirements of organisms requiring relatively few growth factors where as complex non-synthetic medium support the growth of more fastidious microorganisms.

Classification of Bacterial Culture media on the basis of purpose/ functional use/ application

Many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs, numerous media are available.

1.General purpose media/ Basic media

Basal media are basically simple media that supports most non-fastidious bacteria. **Peptone water,** nutrient broth and **nutrient agar (NA)** are considered as basal medium. These media are generally used for the primary isolation of microorganisms.

Nutrient agar

2.Enriched medium (Added growth factors):

Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium

makes enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria **blood agar**, chocolate

agar, Loeffler's serum slope etc are few of the enriched media. Blood agar is prepared by adding 5-10% (by volume) blood to a blood agar base. **Chocolate agar** is also known as heated blood agar or lysed **blood agar**.

3.**Selective and enrichment media** are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest. Various approaches to make a medium selective include **addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.**

a. Selective medium

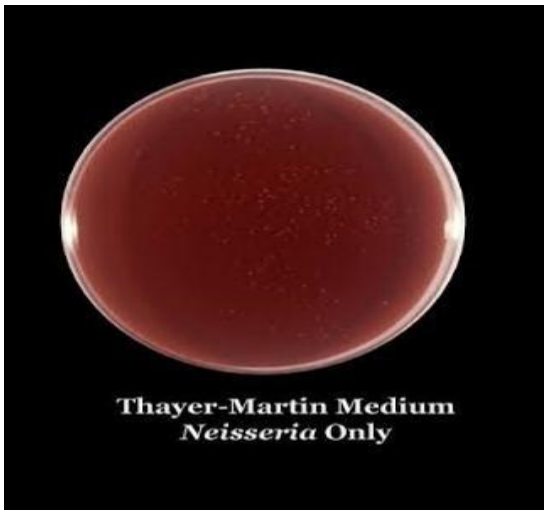
Principle:

Selective medium is designed to suppress the growth of some

microorganisms while allowing the growth of others. Selective medium are agar based (solid) medium so that individual colonies may be isolated.

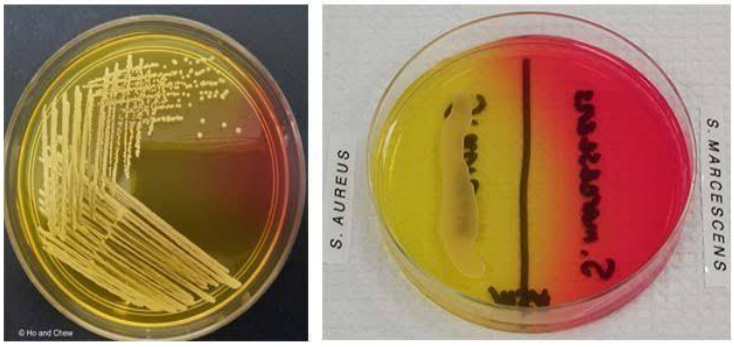
Examples of selective media include:

1. Thayer Martin Agar used to recover *Neisseria gonorrhoeae* contains antibiotics; vancomycin, colistin and nystatin.



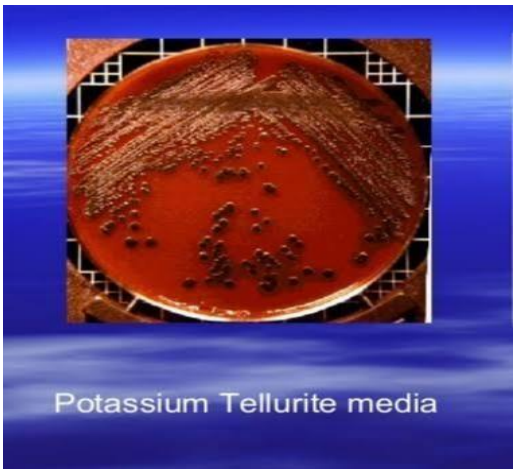
2. **Mannitol salt agar** and Salt Milk Agar used to recover *S.aureus* contains 10% NaCl.

Mannitol Salt Agar (MSA) for the isolation of *Staphylococcus aureus*

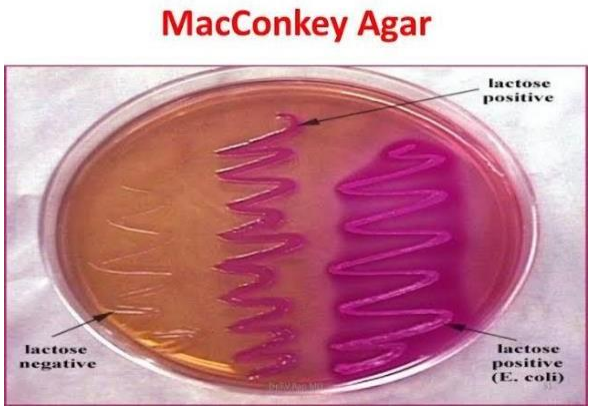


Yellow colonies of *Staphylococcus aureus* — *Staphylococcus aureus* and *Serratia marcescens* on MSA

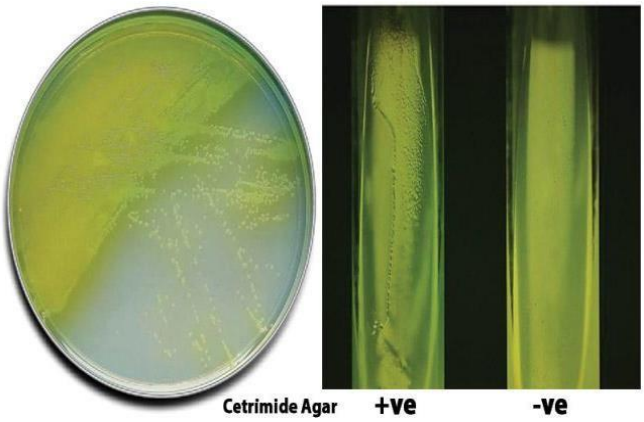
3. **Potassium tellurite medium** used to recover *C.diphtheriae* contains 0.04% potassium tellurite.



4. **MacConkey's agar** used for Enterobacteriaceae members contains bile salt that inhibits most gram positive bacteria.



5. **Pseudosel Agar (Cetrimide Agar)** used to recover *P. aeruginosa* contains cetrimide (antiseptic agent).

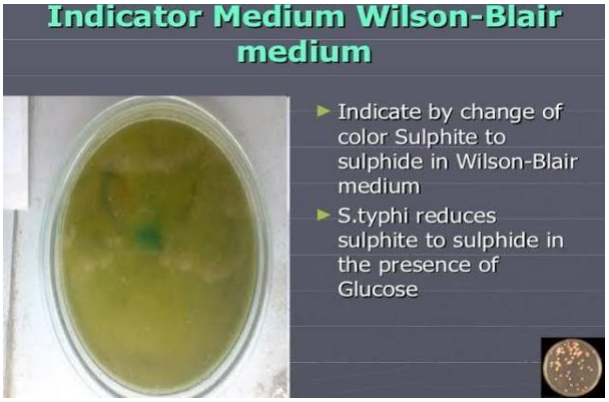


6. **Crystal Violet Blood Agar** used to recover *S. pyogenes* contains 0.0002% crystal violet.

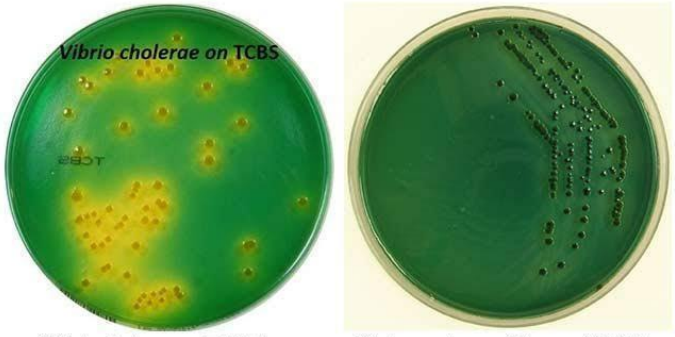
6. **Lowenstein Jensen Medium** used to recover *M.tuberculosis* is made selective by incorporating malachite green.

7. Wilson and Blair's Agar for recovering *S. typhi* is rendered

selective by the addition of dye brilliant green.



8. Selective media such as **TCBS Agar** used for isolating *V. cholerae* from fecal specimens have elevated pH (8.5-8.6), which inhibits most other bacteria.



Vibrio cholerae on TCBS Agar *Vibrio parahaemolyticus* on TCBS Agar

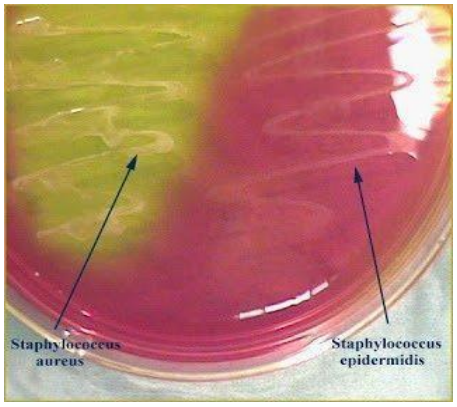
3.Enrichment culture medium

Enrichment medium is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium. Unlike selective media, enrichment culture is typically used as **broth medium**. Enrichment media are liquid media that also serves to inhibit commensals in the clinical specimen. **Selenite F broth, tetrathionate broth** and **alkaline peptone water (APW)** are used to recover pathogens from fecal specimens.

4.Differential/ indicator medium: differential appearance:

Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.

Examples of differential media include:



1. MANNITOL SALTS AGAR (Mannitol fermentation = Yellow)
2. BLOOD AGAR (various kinds of hemolysis i.e. α, β and γ hemolysis)
3. MAC CONKEY AGAR (lactose fermenters, pink colonies whereas non- lactose fermenter produces pale or colorless colonies.)
4. TCBS (*Vibrio cholerae* produces yellow colonies due to fermentation of sucrose)

5. Transport media:

Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of

contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors.

Examples of transport media

- **Cary blair transport medium** and **Venkatraman Ramakrishnan (VR) medium** are used to transport feces from suspected cholera patients.
- **Sach's buffered glycerol saline** is used to transport feces from patients suspected to be suffering from bacillary dysentery.
- **Pike's medium** is used to transport streptococci from throat specimens.

6. Anaerobic media:

Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients. Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Such media may also have to be reduced by physical or chemical means. Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings can render a medium reduced. Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

Examples of anaerobic media

1. **Robertson cooked meat (RCM)**

   Medium that is commonly used to grow *Clostridium*spps contains a 2.5 cm column of bullock heart meat and 15 ml of nutrient broth.

2. **Thioglycollate broth**

It contains sodium thioglycollate, glucose, cystine, yeast extract and casein hydrolysate.

Methylene blue or resazurin is an oxidation-reduction potential indicator that is incorporated in the medium. Under reduced condition, methylene blue is colorless.

7. Assay media

These media are used for the assay of vitamins, amino acids and antibiotics. E.g. antibiotic assay media are used for

determining antibiotic potency by the microbiological assay technique.

LYOPHILISATION

WHAT IS LYOPHILISATION ?

- **Lyophilisation** is the process of preserving something by freezing it very quickly and then subjecting it to a vacuum which removes ice.
- **Lyophilisation** *is one of the most effective methods for the long-term preservation of cells.*

PRINCIPLE

The term "**lyophilisation**" describes a process to produce a product that "loves the dry state". The main **principle** involved in **freeze drying** is a phenomenon called sublimation, where water passes directly from solid state (ice) to the vapour state without passing through the liquid state.

LYOPHILIZER and FREEZE DRYING

Lyophilizer and **freeze dryer** are synonymous names for the same equipment. A lyophilizer executes a water removal process typically used to preserve perishable materials, to extend shelf life or make the material more convenient for transport. Lyophilizers work by freezing the material, then reducing the pressure and adding heat to allow the frozen water in the material to sublimate.

ESSENTIAL COMPONENTS OF FREEZE DRYER

CHAMBER:

- This is the vaccum tight box, sometimes called as Lyophilization chamber or cabinet.
- This chamber contains shelves or shelf for processing product.
- This chamber can also be fitted with the stoppering system.
- It is typically made with a stainless steel and usually highly polished on the inside and insulated and clad on the outside.
- The door locking arrangement by a hydraulic or electric motor.

SHELVES:

- The shelf act as a heat exchanger, supplying energy to the product during the primary and secondary drying segments of the freeze drying cycle.
- The shelves will be connected to the silicone oil system through either fixed or flexible hoses.

REFRIGERATION SYSTEM:

- The product to be freeze dried is frozen whilst on the shelves.
- Compressors or sometimes liquid nitrogen supplies the cooling energy.
- Most often multiply compressors are needed and the compressor may perform two duties, one to cool the shelves and second to cool the process condenser.

SHELF FLUID SYSTEM:

- The freeze-drying process requires that the product is first frozen and then energy in the form of heat is applied throughout the drying phases of the cycle.

- This energy exchange is traditionally done by circulating a silicone oil through the shelves at a desired temperature.
- The temperature is set in an external heat exchange system consisting of cooling heat exchangers and an electrical heater.

PROCESS CONDENSER:

- The process condenser is sometimes called as just the condenser or the cold trap.
- It is designed to trap the solvent, which is usually water during the dry process.
- The process condenser will consist of coils or sometimes plates which are refrigerated to allow the temperature.
- These refrigerated coils or plates may be in a vessel separate to the chamber, or they could be located within the same chamber as the shelves.
- Hence there is designation, “external condenser” and “internal condenser". Physically the external condenser is traditionally placed behind the chamber.
- For an internal condenser the refrigerated coils or plates are placed beneath the shelves. The position of the condenser does not affect the trapping performance.

STEPS INVOLVED IN LYOPHILIZATION

- ✓ Pre-treatment
- ✓ Freezing stage
- ✓ Primary drying stage
- ✓ Secondary drying stage
- ✓ Packing

1. PRE-TREATMENT

Pre-treatment includes and method of treating the product prior to freezing. This may include,

- Concentrating the product
- Formulation revision(i.e., addition of components to increase stability and/or improve processing)
- Decreasing a high vapour pressure solvent
- Increasing the surface area.

2. FREEZING

- The product is frozen to low enough temperature to be completely solidify.
- The method of prefreezing and the final temperature of the frozen product can affect the ability to successfully freeze dry the material.
- Rapid cooling results in small ice crystals, useful in preserving structures to be examined microscopically, but resulting in a product that is more difficult to freeze dry.
- Slower cooling results in large ice crystals.
- Most samples that has to be freeze dried are eutectics, which are mixtures of substances that freeze at lower temperature than the surrounding water.
- It is very important in freeze-drying to prefreeze the product to below the eutectic temperature before beginning the freeze-drying process.
- The second type of frozen product is the suspension that undergoes glass formation during the freezing process.

3. PRIMARY DRYING

- After prefreezing the product, conditions must be established in which ice can be removed from the frozen

product via sublimation, resulting in a dry, structurally intact product.

- This requires very carefully control of the two parameters.
    1. Temperature (-10deg C to 30deg C) and
    2. Pressure involved in freeze-drying system (50mm Hg)
- The rate of sublimation of ice from a frozen product depends upon the difference in vapour pressure of the product compared to the vapour pressure of the ice collector.
- Heat enters the product by one of several mechanisms:
    1. By direct contact between the container base and the shelf, so here the shape of the container is important.
    2. By conduction across the container base and then through the frozen mass to the drying front (also called the sublimation interface)
    3. By gaseous convection between the product and the residual gas molecules in the chamber.
    4. By radiation.

Convection is certainly the most important of these mechanisms.

4. SECONDARY DRYING

- After primary freeze-drying about 7-8% residual moisture content is present, so continuous drying at warmer temperature is necessary to reduce the residual moisture content to optimum values.
- This process is called “Isothermal desorption" as the bound water is desorbed from the product.
- Secondary drying is normally continued at a product temperature higher than ambient but compatible with the sensitivity with the product.
- In contrast to processing conditions for primary drying which use low shelf temperature and a moderate vaccum,

desorption drying is facilitated by raising shelf temperature (50-60deg C) and reducing chamber pressure to a minimum.

- Care should be exercised in raising shelf temperature too highly; since, protein polymerization and biodegradation may result from using high processing temperature during secondary drying.
- Secondary drying is usually carried out for about 10-20 hours, whereas primary drying is carried out for 2 hours.

5. *PACKING*

- After drying the vaccum is replaced by filtered dry air or nitrogen to establish atmospheric preasure.
- Ampoules are sealed by either tip sealing or pull sealing method.
- Vials and bottles are sealed with rubber closures and aluminium caps.

SOME LYOPHILIZED FORMULATIONS

Drug	Category	Route Of Administration	Marketed Name
Amphotericin B & Cholestryl sulphate	Anti-fungal	IV Infusion at 2-4 mg/kg/hr	Amphotec® (Sequus pharmaceuticals)
Chlorthiazide sodium	Diuretic & anti-hypertensive	IV Infusion , IV bolus	Diuril® (Merck)
Cisplastin	Anti-neoplastic	IV Infusion,	Platinol® (Bristol Myers Oncolgy)
Gemcitabine	Anti-neoplastic	IV Infusion over 30 min	Genzer® (Lilly)
Thiopental sodium	Short acting anesthetic	IV Infusion	Pentothal sodium® (Baxter)

ADVANTAGES

- Thermo labile products can be dried.
- Loss of volatile materials is less.
- Moisture level can be kept as low as possible.
- Sterility can be maintained.
- Final product can be stored in ambient temperature, if well sealed with inert atmosphere.

DISADVANTAGES

- Equipment and running costs are high.
- Increased handling and processing time.
- Need for sterile diluents upon reconstitution.
- It is difficult to adopt the method for solutions containing non aqueous solvents.
- Product is prone to oxidation so it should be packed in vaccum.

APPLICATIONS:

In the aseptic pharmaceutical and biotechnology production, **freeze drying/Lyophilization** are often applied to the following products:

- antibiotics,
- bacteria,
- vaccines,
- hormones,
- vitamins, enzymes, and peptides,
- liposome and collagen,
- plants and liver extracts,
- radio-immuno essays,
- Blood plasma, plasma fractions and antibodies.

CULTIVATION OF BACTERIA

CULTIVATION OF AEROBIC BACTERIA

Important steps in Isolation of Bacteria in pure culture:

1. First – It is required to isolate and grow the bacteria from natural environment to laboratory medium. It requires the knowledge of growth requirements of bacteria of interest.

2. Second – Bacteria of interest should be separated from other bacteria to obtain a pure culture of one type of bacteria.

3. Third – Once a pure culture is achieved, maintenance of pure culture without contamination is a task again.

Pure Culture : Population of cells arising from a single bacterial cell, to study characteristics in detail.

Pure Culture Technique – Developed by Robert Koch.

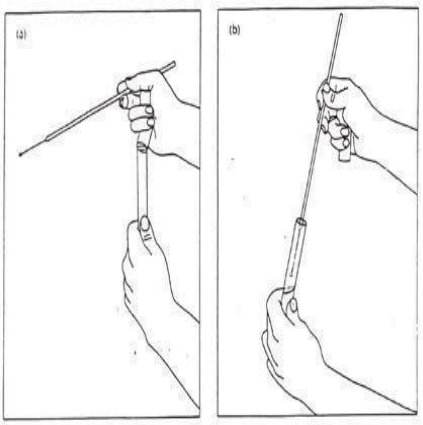
In natural habitat i.e., clinical sample, bacteria of interest usually grow in complex mixed population. It is required to separate the bacteria of interest from the mixed population, as pure culture to study the characteristics of the bacteria in detail.

Generally, initial incubation of clinical sample is done using liquid broth i.e., Nutrient Broth for growing the bacteria or increasing the number of bacteria in the given clinical sample, in laboratory using artificial culture media.

Inoculation of Nutrient Broth : The clinical samples should be added to the nutrient broth aseptically to avoid contamination and incubate the nutrient broth for 24 Hrs at 37OC.

In nutrient broth after incubation:

Turbidity : Indicates the growth of bacteria Transparency : Indicates no growth of bacteria.



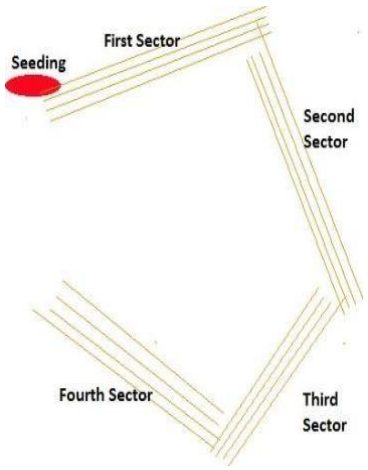
2. Streak Plate Method:

To obtain a pure culture, it is required to obtain separate, single bacterial colony. To attain this, well known 'Quadrant streaking method' can be used. The microbial mixture is seeded on the edge of an agar plate with inoculating loop and then streaked out over the surface.

After seeding the culture, first sector is streaked originating from seeding area. After first sector is streaked, inoculum loop is sterilized and second sector is streaked using inoculum from the first sector. Similarly, Third sector is streaked .

Inoculation loop will develop into separate colonies.

Separate single colony is picked up , streaked on fresh plate to get a pure culture.

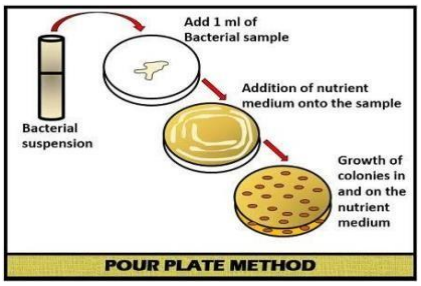


3. Pour Plate Method

The sample is diluted several times to reduce the microbial count sufficiently to obtain separate colonies. When plating, small volumes of several diluted samples are mixed with molten agar that has been cooled to 45OC before pouring into sterile plates.

Bacteria and fungi are not killed at 45OC for a short exposure. After solidification of agar each bacterial cell is fixed in a place and form an individual colony.

Like the spread plate, pour plate can also be used to determine the number of cells in a population. Plates containing 30-300 clonies are counted. The total number of



4. Spread Plate/Lawn Culture

In this method, small volume of dilute microbial mixture containing around 30-300 bacterial cells is transferred to the centre of agar plate and with the help of spreader, spread evenly over the surface of agar medium. The dispersed cells develop into a single colony.

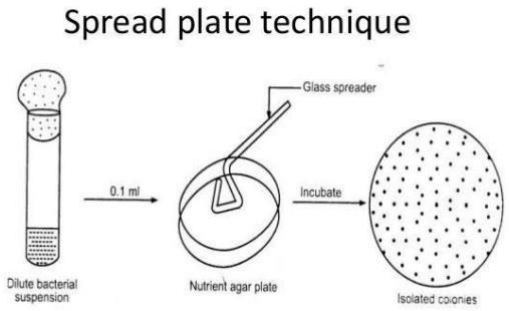
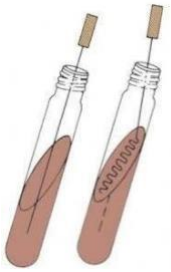


Fig. 6.3 : Spread plate technique

5. Slant and stab culture

Slants of agar in a test tube are generally used for maintaining the bacteria in pure culture. The bacterial colonies are streaked on the surface of slants.



An anaerobic bacteria culture is a method used to grow anaerobes from a clinical specimen. Obligate anaerobes are bacteria that can live only in the absence of oxygen. Obligate anaerobes are destroyed when exposed to the atmosphere for as briefly as 10 minutes. Some anaerobes are tolerant to small amounts of oxygen. Facultative anaerobes are those organisms that will grow with or without oxygen. The methods of obtaining specimens for anaerobic culture and the culturing procedure are performed to ensure that the organisms are protected from oxygen.

Description

Anaerobes are normally found within certain areas of the body but result in serious infection when they have access to a normally sterile body fluid or deep tissue that is poorly oxygenated. Some anaerobes normally live in the crevices of the skin, in the nose, mouth, throat, intestine, and vagina. Injury to these tissues (i.e., cuts, puncture wounds, or trauma) especially at or adjacent to the mucous membranes allows anaerobes entry into otherwise sterile areas of the body and is the primary cause of anaerobic infection. A second source of anaerobic infection occurs from the introduction of spores into a normally sterile site. Spore-producing anaerobes live in the soil and water, and spores may be introduced via wounds, especially punctures. Anaerobic infections are most likely to be found in persons who are immunosuppressed, those treated recently with broad-Spectrum antibiotics**,** and persons who have a decaying tissue injury on or near a mucous membrane, especially if the site is foul-smelling.

Specimens

Some specimens from which anaerobes are likely to be isolated are:

- blood
- bile
- bone marrow
- cerebrospinal fluid
- direct lung aspirate

- tissue biopsy from a normally sterile site
- fluid from a normally sterile site (like a joint)
- dental abscess
- abdominal or pelvic abscess
- knife, gunshot, or surgical wound
- severe burn

Specimen collection

The keys to effective anaerobic bacteria cultures include collecting a contamination-free specimen and protecting it from oxygen exposure. Anaerobic bacteria cultures should be obtained from an appropriate site without the health care professional contaminating the sample with bacteria from the adjacent skin, mucus membrane, or tissue. Swabs should be avoided when collecting specimens for anaerobic culture because cotton fibers may be detrimental to anaerobes. Abscesses or fluids can be aspirated using a sterile syringe that is then tightly capped to prevent entry of air. Tissue samples should be placed into a degassed bag and sealed, or into a gassed out screw top vial that may contain oxygen-free prereduced culture medium and tightly capped. The specimens should be plated as rapidly as possible onto culture media that has been prepared.

Culture

Cultures should be placed in an environment that is free of oxygen, at 95°F (35°C) for at least 48 hours before the plates are examined for growth.

Gram staining is performed on the specimen at the time of culture. While infections can be caused by aerobic or anaerobic bacteria or a mixture of both, some infections have a high probability of being caused by anaerobic bacteria. These infections include brain abscesses, lung abscesses, aspiration pneumonia, and dental infections.

Anaerobic organisms can often be suspected because many anaerobes

have characteristic microscopic morphology (appearance). For example, *Bacteroides* spp. are gram-negative rods that are pleomorphic (variable in size and shape) and exhibit irregular bipolar staining. *Fusobacterium* spp. are often pale gram-negative spindle-shaped rods having pointed ends. *Clostridium* spp. are large gram-positive rods that form spores. The location of the spore (central, subterminal, terminal, or absent) is a useful differential characteristic.

The presence of growth, oxygen tolerance, and Gram stain results are sufficient to establish a diagnosis of an anaerobic infection and begin antibiotic treatment with a drug appropriate for most anaerobes such as clindamycin, metronidazole, or vancomycin.

Methods of cultivation

1. Special anaerobic culture media (prereduced media)

Of all the methods available for the cultivation of anaerobic bacteria, exclusion of oxygen from the medium is the simplest method. During preparation, the liquid culture medium is boiled by holding in a boiling water both for 10 minutes to drive off most of the dissolved oxygen.
Liquid media soon become aerobic thus a reducing agent (e.g., cysteine 0.1%, ascorbic acid 0.1%, sodium thioglycollate 0.1%), is added to further lower the oxygen content.
Oxygen-free N_2 is bubbled through the medium to maintain anaerobic condition. The medium is then dispensed into tubes, which are stoppered tightly and sterilized by autoclaving. Such tubes can be stored for many months before being used. During inoculation, the tubes are continuously flushed with oxygen free CO_2 by means of gas cannula, re-stoppered, and incubated

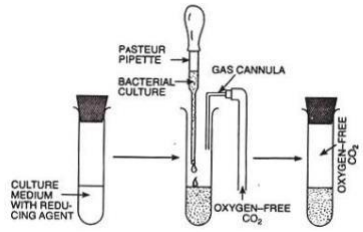


FIG. 16.18. Preparation of special anaerobic culture medium (prereduced medium) for cultivation of obligate anaerobic bacteria.

Cooked meat broth Robertson's bullock-heart medium

This medium has a special place in anaerobic bacteriology, and thioglycollate broth and its modifications are also very useful. CMB is suitable for growing anaerobic bacteria in air and also for the preservation of their stock cultures.

The inoculum of the bacterium is introduced deep in the medium in contact with the meat. Meat particles are placed in 30 ml bottles to a depth of about 2.5 cm and covered with about 15 ml broth. However, some other media which can be used for recovering anaerobes are Brucella blood agar, Bacteroides bile aesculin agar, phenylethyl alcohol agar, kanamycin blood agar, etc. Anaerobic bacteria have special nutritional requirements for vitamin K, haemin and yeast extract, and all primary isolation media for anaerobes should contain these three ingredients.

2. Anaerobic chamber

- Anaerobic chamber is an ideal anaerobic incubation system, which provides oxygen- free environment for inoculating media and incubating cultures. It refers to a plastic anaerobic glove box that contains an atmosphere of H_2, CO_2, and N_2. Glove ports and rubber gloves are used by the operator to perform manipulations within the chamber. There is an air-lock with inner and outer doors.

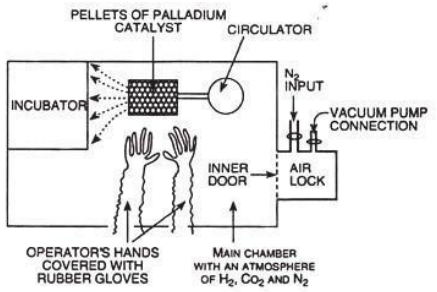


FIG. 16.19. Schematic representation of an anaerobic chamber showing its various parts.

- Culture media are placed within the air-lock with the inner door. Air of the chamber is removed by a vacuum pump connection and replaced with N_2 through outer doors.
- The culture media are now transferred from air-lock to the main chamber, which contains an atmosphere of H_2, CO_2, and N_2. A circulator fitted in the main chamber circulates the gas atmosphere through pellets of palladium catalyst causing any residual O_2 present in the culture media to be used up by reaction with H_2.
- When the culture media become completely anaerobic they are inoculated with bacterial culture and placed in an incubator fitted within the chamber. The function of CO_2present in the chamber is that it is required by many anaerobic bacteria for their best growth.

3. Anaerobic Bags or Pouches:

- Anaerobic bags or pouches make convenient containers when only a few samples are to be incubated anaerobically. They are available commercially. Bags or pouches have an oxygen removal system consisting of a catalyst and calcium carbonate to produce an anaerobic, CO2- rich atmosphere.
- One or two inoculated plates are placed into the bag and the oxygen removal system is activated and the bag is sealed and incubated. Plates can be examined for growth without removing the plates from bag, thus without exposing the colonies to oxygen.
- But as with anaerobic jar, plates must be removed from the bags in order to work with the colonies at the bench. These bags are also useful in transport of biopsy specimen for anaerobic cultures.

4.Anaerobic Jars (or GasPak Anaerobic System):

- When an oxygen-free or anaerobic atmosphere is required for obtaining surface growth of anaerobic bacteria, anaerobic jars are the best suited. The most reliable and widely used anaerobic jar is the Melntosh-Fildes' anaerobic jar. It is a cylindrical vessel made of glass or metal with a metal lid, which is held firmly in place by a clamp.

- The lid possesses two tubes with taps, one acting as gas inlet and the other as the outlet. On its under surface it carries a gauze sachet carrying palladium pellets, which act as a room temperature catalyst for the conversion of hydrogen and oxygen into water. Palladium pellets act as catalyst, as long as the sachet is kept dry.

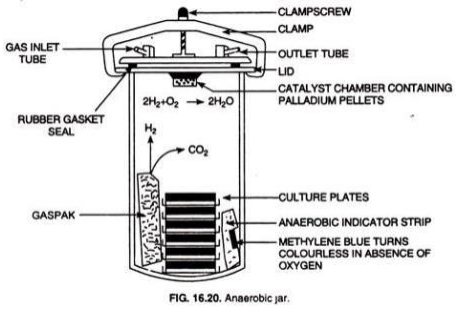


FIG. 16.20. Anaerobic jar.

- Inoculated culture plates are placed inside the jar and the lid clamped tight. The outlet tube is connected to a vacuum pump and the air inside is evacuated. The outlet tap is then closed and the gas inlet tube connected to a hydrogen supply. Hydrogen is drawn in rapidly. As soon as this inrush of hydrogen gas has ceased the inlet tube is also closed.
- After about 5 minutes inlet tube is further opened. There occurs again an immediate inrush of hydrogen since the catalyst creates a reduced pressure within the jar due to the conversion of hydrogen and leftover oxygen into water.

- If there is no inrush of hydrogen, it means the catalyst is inactive and must be replaced. The jar is left connected to the hydrogen supply for about 5 minutes, then the inlet tube is closed and the jar is placed in the incubator. Catalysis will continue until all the oxygen in the jar has been used up.

- The gasPak is now the method of choice for preparing anaerobic jar. The gasPak is commercially available as a disposable envelope containing chemicals, which generate hydrogen and carbon dioxide when water is added. After the inoculated plates are kept in the jar, the gasPak envelope with water added, is placed inside and the lid screwed tight.

- Hydrogen and carbon dioxide are liberated and the presence of a cold catalyst in the envelope permits the combination of hydrogen and oxygen to produce an anaerobic environment.

- The outstanding feature of the gasPak system is the disposable gas generator envelope, which does away with the need for a vacuum pump and cylinders of compressed gas; the operation of the jar is consequently very quick and simple. As the standard gasPak jar is not evacuated before use a relatively large volume of water is formed during catalysis.

- An indicator should be used for verifying the anaerobic condition in the jar and for this purpose methylene blue is generally used. When it is placed in an anaerobic environment, it is reduced from its coloured oxidized form to a colourless reduced leuco-compound.

- Removal of the culture plates from the jar for microscopic examination is the major disadvantage of any anaerobic jar system. This, of course, results in the exposure of the colonics to oxygen, which is especially hazardous to the anaerobes during their first 48 hours of growth. For

this reason, a suitable oxygen-free holding system always should be used in conjunction with anaerobic jars.

- The culture plates should be removed from the jar and placed in the oxygen-free holding system. From there they should be removed one by one for rapid microscopic examination of colonies, and then quickly returned to the holding system. Plates never should remain in room air on the open bench.

Anaerobes and infections they produce

Gram-negative anaerobes and some of the infections they produce include the following genera:

- *Bacteroides* (the most commonly found anaerobes in cultures; intra-abdominal infections, rectal abscesses, soft tissue infections, liver infection)
- *Fusobacterium* (abscesses, wound infections, pulmonary and intracranial infections)
- *Porphyromonas* (aspiration pneumonia, periodontitis)
- *Prevotella* (intra-abdominal infections, soft tissue infections)

Gram-positive anaerobes include the following:

- *Actinomyces* (head, neck, pelvic infections; aspiration pneumonia)
- *Bifidobacterium* (ear infections, abdominal infections) *Clostridium* (gas, gangrene, food poisoning, tetanus, pseudomembranous colitis)
- *Peptostreptococcus* (oral, respiratory, and intra-abdominal infections)
- *Propionibacterium* (shunt infections)

Identification of anaerobes

- The identification of anaerobes is highly complex, and laboratories may use different identification systems. Partial identification is often the goal. For example, there are six species of the

Bacteroides genus that may be identified as the *Bacteroides fragilis* group rather than identified individually.

- Organisms are identified by their colonial and microscopic morphology, growth on selective media, oxygen tolerance, and biochemical characteristics. These include sugar fermentation, bile solubility, esculin, starch, and gelatin hydrolysis, casein and gelatin digestion, catalase, lipase, lecithinase, and indole production, nitrate reduction, volatile fatty acids as determined by gas chromatography, and susceptibility to antibiotics.
- The antibiotic susceptibility profile is determined by the microtube broth dilution method. Many species of anaerobes are resistant to penicillin, and some are resistant to clindamycin and other commonly used antibiotics.

Results

Negative results will show no pathogenic growth in the sample. Positive results will show growth, the identification of each specific bacterium, and its antibiotic susceptibility profile.

STAINS AND STAINING TECHNIQUE

Introduction:-

- Microbes are colourless in nature, to visualize and to study their structure, shape and other structural characteristics, the organism should be contrasted from their environment.

Stain:-

- Stain is a dye used to colour the living or dead organism.
- Example: Methylene blue and Safranin.

Composition of Stain:-

- Stain or dye is the synthetic chemical which is derived from nitrobenzene or aniline. Stains are use commonly in microbiology to

increase the contrast between microorganism or parts of organism and the background, so that it can be easily visible. The process of giving colour to particular organism or part of organism is called staining.

- Each stain or dye is composed of three components. They are Benzene ring, Chromophore, and Auxochrome.
    - **Benzene ring: It is a colourless part of the dye and it is basic structural component of dye.**
    - **Chromophore: It is the functional group of a dye that gives colour of the stain.**
    - **Auxochrome: It is the group that gives ionic property to the stain.**

Types of Stain:-

Based on the nature of **chromogen**, there are three types of stains. They are Acidic stain, Basic stain and Neutral stain.

Acidic stain or Anionic stain:-

- Chromogen of aicidic stain is negatively charged. So, it is called as Anionic stain.
- Acidic stain is used to stain the positively charged components such as background staining.
- Histone protein is positively charged so it can be stained by acidic stain.
- Acidic stain cannot stain bacterial cell due to repulsion of same charge.
- **Example: Eosin, Nigrosin, Indian ink.**

Basic stain or Cationic stain:-

- Chromogen or coloured part of basic stain is positively charged. So, it is also known as cationic stain.
- Basic stain is used to stain negatively charged components such as bacterial cell.
- **Example: Methylene blue, Safranin, Malachite green, Basic fuchsin, Crystal violet.**

Neutral stain:-

- In neutral stain, both cation and anion are coloured, such that net charge is zero.

- **Example: Giemsa stain.**

Types of staining:-

A. **Positive staining**
B. **Negative staining**
C. **Simple staining**
D. **Differential staining**
E. **Special staining**

a) Positive staining :-

- In this process, the actual cells are themselves coloured and appear in a clear background.
- **Example: Acid fast staining, Gram staining.**

b) Negative staining:-

- In this process, the cells remain clear and the background is coloured to create a contrast to aid better visualization of the image.
- **Example: Capsule staining.**

c) Simple staining:-

- In this process, only one type of stain is used to colour the cells, where all the cells appear in same colour.
- **Example: Malachite green is only used.**

d) Differential staining:-

- In this process, different stains are used to colour different bacteria.
- **Example: Gram staining and Acid fast staining.**

e) Special staining:-

- In this process, a stain highlights the features of cell, or organism that cannot be identified by routine staining techniques.

- Example: Capsule staining and Endospore staining.

GRAM STANING

Introduction:-Gram staining method was named after its inventor the Danish scientist Gram in 1884.

- Gram stain or Gram staining or Gram's Method of staining is used to distinguish and classify bacterial species into two large groups gram positive and gram negative.

Aim:-

- To perform gram staining of the given sample and to differentiate the bacteria into gram positive and gram negative.

Principle:-

- The structure of organism's cell wall determines whether the organism is gram positive or gram negative. When stained with primary stain and fixed by mordant, bacteria which retain the primary stain are called gram positive.
- Gram positive cell wall contains a thick layer of peptidoglycan with numerous teichoic acid cross linking which resist the decolourization. In aqueous solution crystal violet dissociates into cv+ and cv- ions that penetrates through the wall and membrane of both gram positive and gram negative cells. The cv+ interacts with negatively charged particles of bacteria and stained purple.
- When iodine is added, it interacts with cv+ to form large crystal violet iodine complex (cv-I) within the cytoplasm and outer layer of cell wall.
- Then decolorizing agent (ethanol or acetone) is added, it interacts with lipid membrane of both gram positive and gram negative bacteria.
- In gram negative cells, when decolourizer is added, the thick layer of lipopolysacchide dissolves and the cells become leaky and allow cv-I complexes to wash out from the cell.
- In gram positive cells, when decolourizer is added, the highly cross linked and thick layer of peptidoglycan becomes dehydrated and traps

cv-I complex inside the cell.

- After decolourization gram positive cell remain purple in colour, whereas the gram negative cell wall loses purple colour and counter stained with safranin and appears as pink in colour.

Procedure:-

- Flood the slide with Crystal violet, after 1 minute wash with water.
- Flood with Gram's iodine and wash after 1 minute with water.
- Decolorize with 95% ethanol until thinnest parts of the smear are colourless. (Wash with water).
- Flood with Safranin and after 1 minute wash with water.
- Air dry, or blot with absorbent paper.

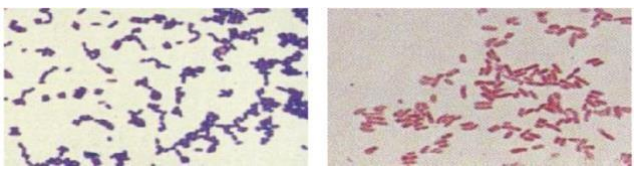
Result:-

Gram positive:-

- Organisms that retain the primary stain after washing with ethanol, appears purple and are termed as Gram positive.
- **Example: *Streptococcus, Staphylococcus, Enterococcus, Corynebacterium, Clostridium.***

Gram negative:-

- Organisms which looses primary stain and appears pink in colour are termed as Gram negative.
- Example: ***Escherichia coli, Pseudomonas, Salmonella, Shigella, klebsiella , Proteus.***



Gram positive **Gram negative**

ACID FAST STANING

Introduction :

- It was first discovered by Ehrlich in 1881 and modified by Zeihl & Neelsen also called Zeihl-Neelsen staining techniques.
- It is a differential stain used mainly to detect ***Mycobacteria***.

Aim:-

- To differentiate bacteria into acid fast group and non-acid fast groups. This method is used for those microorganisms which are not stained by simple or gram staining method, particularly the member of genus *Mycobacterium*, are resistant and can only be visualized by acid fast staining.

Principle:-

- ***Mycobacteria***, which do not stain well by Gram stain, are stained with **carbol fuchsin combined with** phenol.

- In this technique, the phenol-carbol fuchsin stain is heated to enable the dye to penetrate the waxy mycobacterial cell wall.

- The stain binds to the mycolic acid in the mycobacterial cell wall. After staining, an acid decolorizing solution is applied. This removes the red dye from the background cells, tissue fibres, and any organism in the smear except mycobacteria which retain (hold fast to) the dye and are referred to as acid fast bacilli (AFB).

- Following decolorization, smear is counterstained with malachite green, or methylene blue which stains the background material, providing a contrast colour against which the red acid fast bacilli can be seen.

- Among the ***Mycobacterium*** *species,* ***M. tuberculosis and M. ulcerans*** are strongly acid fast.

Materials required:-

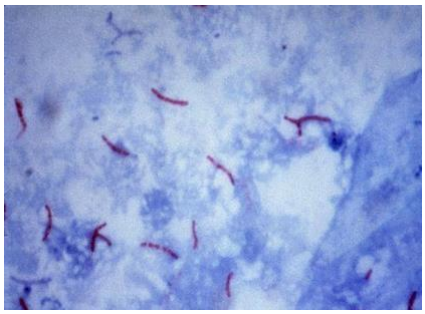
- **Primary stain**: Strong carbol fuchsin (consists of basic fuchsin and carbolic acid phenol)
- **Decolourizer**: Acid alcohol 3% or 20% sulphuric acid
- **Counter stain**: Methylene blue or Malachite green

Procedure:-

- Smear prepared directly from a patient specimen prior to processing or smear prepared from a processed specimen after centrifugation (it is used to concentrate the material).
- Heat fix dried smear.
- Cover smear with carbol fuchsin stain, heat the smear until vapour just begin to rise (about 60 degree Celsius)
- Allow the heated stain to remain on slide for 5 minutes then wash.
- Cover the smear with decolourizer for 2-5 minutes. Stain with methylene blue for 1-2minutes.
- Examine the smear microscopically using (100X) oil immersion objective lens.

Result:-

- Pink or red coloured rod-shaped bacilli were observed. Slightly curved bacilli in singles or in small clumps seen against a blue background of epithelial cells and pus cells.



Acid fast bacilli

CAPSULE STAINING

- Capsule is a type of special staining where capsule of the organism is visualized by negative staining.
- Capsule is clearly visible in the light microscope when negative stains or special capsule stains are employed.

Aim:-

- To demonstrate the presence of capsule by negative staining.

Principle:-

- Capsules are gelatinous layer accumulates the outer surface of the cell wall. An acidic stain such as Indian ink or Nigrosin is used to reveal the presence of capsule by negative staining methods.
- Acidic stain Nigrosin is negatively charged so it will stain the positively charged background and basic stain is positively charged so it will stain the negatively charged bacterial cell. Bacterial capsule is non-ionic, so neither acidic nor basic stains will stain the capsule.
- Negative stain will stain the back ground into dark colour and the counter stain will stain the bacterial cells. Capsule appears colourless with stained cells against dark background.

Procedure:-

- Place a drop of negative stain on the slide. Add a loopful of culture to slide, smearing it in the dye.
- With an another slide, drag the smear along the first slide and let for 5-7 minutes. Allow to dry. Flood the smear with crystal violet stain for about 1 minute.

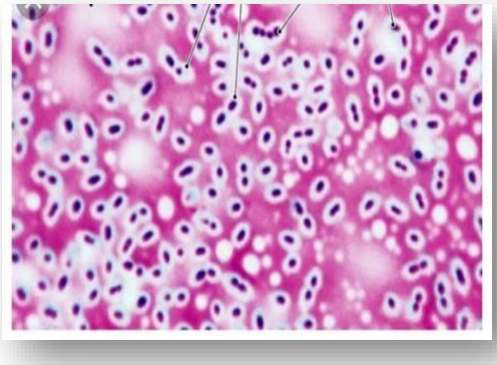
- Drain the crystal violet by tilting the silde at a 45 degree angle and let stain run off until it dries.
- Examine the smear microscopically under100x oil immersion lens for the presence of encapsulated cells as indicated by clear zones surrounding the cells.

Observation:-

- Clear or hollow area was observed surrounding the cells against black background.

Result:-

- Capsulated organism is demonstrated.



Capsulated organism

Clear zone surrounding the bacteria

ENDOSPORE STAINING

Introduction

- In 1922, Dorner published a method for staining endospres. Schaeffer and fulton
  modified the Dorner's method to make the process faster.

- Endospore stain is differential stain which selectively stains bacterial endospores. The main purpose of endospore staining is to differentiate bacterial spores from other vegetative cells and to differentiate spore formers from non- spore formers.

Principle:-

- Bacterial endospore is highly resistant and inactive form of especially bacteria. Spore consists of 2,4 dipicolinic acid and calcium ions which contributes resistance. Spores are covered with thick walls thus make them difficult for staining and decolourization.
- The special staining technique developed by Schaeffer fulton in which heat is employed to reveal the presence of bacterial spores.
- In this method, malachite green is forced into the spore by steaming the bacterial emulsion. Malachite green is water soluble and has low afffinity for cellular material, so vegetative cells may be decolourized with water. Safranin is then applied to counter stain any cells which have been decolourized. At the end of the staining process, vegetative cells will be pink, and endospores will be dark green.
- Spores may be located in the middle of the cell, or in the end of the cell. Spores may be spherical or elliptical.

Reagents required:-

- Primary stain: Malachite green
- Decolourizing agent: Distilled water or tap water Counter stain: Safranin

Procedure:-

- Take a clean glass slide and make a smear using sterile technique. Air dry and heat fix the organism on a glass slide.
- The smear was flooded with primary stain malachite green. Slide

is then kept on a warm hot pate and the preparation allowed for 2-3 minutes. The smear should not get dried up. So excess stain should be added.

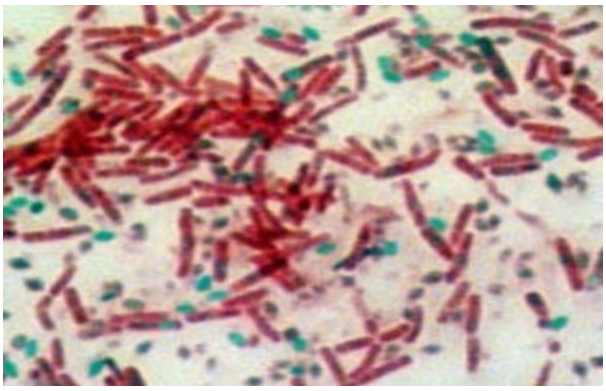
- The smear was removed from hot plate and cooled. The smear is decolorized with water. Then it is counter stained with safranin for 30 seconds.
- Then the slide was washed with water, dried and observed under low power and high power objective lens of microscope.

Observation:-

- Bright coloured bacterial endospores are observed inside pink coloured vegetative cells.

Result:-

- Bacterial endospores were demonstrated.



Green coloured endospores

UNIT -V

- ✓ Growth factors
- ✓ Nutritional Requirements
- ✓ Uptake of Nutrients
- ✓ Growth curve

GROWTH FACTOR

- All organisms require and share certain nutrition's for growth and normal functioning. The major nutritional requirement for microorganisms include,

1.Carbon 6.Sulfur and Phosphorus

2. Electron 7.Metal ions

3.Energy 8.Vitamins

4.Nitrogen 9.Water

5.Oxygen

NUTRITIONAL TYPES OF ORGANISMS

Based on the different nutritional requirements, the organisms are of different types.

1. SOURCE OF ENERGY;

Based on the energy source the organisms are of two types, known as,

a. **Chemotroph**

b. Phototroph

a. Chemotroph: Organisms require chemical compounds for their energy are called chemotrophs. Eg. *Escherichia coli*

b. **Phototroph** : organisms require radiant energy (light) are called phototrophs. Eg. *Pseudomonas sp*

2. ELECTRON SOURCE

- All organisms require a source of electrons for their metabolisms. Based on electron source, the organisms are of two types known as ,

a. Lithotrophs

b. Organotrophs

a. Lithotrophs : organisms are inorganic compounds as electron donors are called lithotrophs.

Eg.*Rhodospirillum*

Lithotrophs is further classified into 2 types, called

1. Chemolithotroph

2. Photolithotroph

b. Organotroph: organisms require organic compounds as electron donors are called organotrophs. It is of two types.

1. Chemoorganotroph

2. Photoorganotroph

Eg. *Escherichia coli.*

3. SOURCE OF CARBON: ("C" SOURCE)

- All organisms require a source of carbon for synthesizing their cell components. Based on "C"source, the organisms are of two types, named as,

a. Autotrophs

b. Heterotrophs

a. Autotroph: Organisms those require "CO2" as their major or sole source of carbon to synthesize their cellular components are termed "autotrophs".

Eg.*Nitrosomonas, Pseudomonas*

b. Heterotroph: Organisms those require organic compounds as their "C" source are termed as "heterotrophs"

Eg. *Escherichia coli*

4. SOURCE OF NITROGEN

All organisms require nitrogen source for synthesizing their cellular components.

- Some organisms use-atmospheric nitrogen.
- Some organisms use-organic compounds as their "N" source {amino acids}
- Certain organisms use-Inorganic compounds as their "N" source like { ammonium salts, Nitrates and Nitrites}.

5. SOURCE OF OXYGEN

- Organisms obtain their O_2 from water, or from various nutrients or use "molecular O_2.

6. SOURCE OF SULFUR

Organisms require sulphur for synthesizing certain amino acids for their growth. The sulphur source obtained may be from,

- Organic sulphur compounds
- Inorganic sulphur compounds
- Elemental sulphur.

7. SOURCE OF PHOSPHORUS

All organisms require a source of phosphorus – in the form of

phosphate – to synthesize their essential compounds like nucleotides, nucleic acids, phospholipids, teichoic acids and other compounds.

8. METAL IONS

- All organisms require certain metal ions for their normal growth.

Eg. K^+,Ca^{2+},Mg^{2+} and Fe^{2+}=> These are required in major quantities, so called major elements.

- Certain metal ions required in small quantities so called minor elements or trace elements. Eg. Mo^{6+},Co^{2+},Zn^{3+}, Mn^{2+}.
- These metal ions may act as "co factors" for various enzymes.

9. VITAMIN SOURCE

All living organisms contain vitamin and vitamin like compounds. These vitamins function either as co enzymes for several enzymes or as the building blocks for coenzymes.

- Certain bacteria are capable of synthesizing their vitamin requirement.
- For others we should supply the vitamins along with production medium.

Eg. Thiamine (B1) → *Bacillus anthracis*

Riboflavin → *Clostridium tetani*

Biotin → *Leuconostoc*

Folic acid → *Leuconostoc*

Vitamin B12 → *Lactobacillus*

Vitamin K → *Bacteroides*

10. WATER

All organisms required water, to synthesize their protoplasm. Water act as a vehicle for entry and exit of substance in and out of the cells. Most of the chemical reaction are mediated by the water.

UPTAKE OF NUTRIENTS

- The first step in nutrients use is uptake of the required nutrient by the cell.
- The uptake mechanism must be specific.

Nutrients from outer environment → through plasma membrane → To inner environment of the cell

- This process known as nutrient uptake.
- Micro-organisms use different transport mechanism for nutrient uptake, includes,

1. Passive diffusion

2. Facilitated diffusion

3. Active transport

4. Group translocation.

1. PASSIVE DIFFUSION

- Certain compounds from outside can pass through the cytoplasmic or plasma membrane and enters inside the cell. This process known as simple or passive diffusion.

- The solute from higher concentration (outside) to lower concentration (inside) through a membrane called simple diffusion.

Eg. Very small molecules such as H2O ,O2 and CO2 move across membranes by passive diffusion.

2. FACILITATED DIFFUSION

- A type of diffusion process by which certain substances enters inside the cell through a carrier molecule which are embedded in the plasma membrane. The carrier called " permeases" aids the diffusion process so called facilitated diffusion.
- The carrier molecule combines reversibly with the solute and forms carrier - solute complex.
- This complex moves towards the interior of cell.
- The membrane carrier can change their confirmation after binding with an external molecule.
- Subsequently release the molecule on the cell interior.
- The carrier molecule returns to its original shape and is ready to bind with another solute molecule.
- Molecules will continue to enter the cell inside as long as their concentration is greater on the outside.
- This method requires no energy input by the organism.

Eg. Transportation of glycerol and water.

3. ACTIVE TRANSPORT

- The transport of solute molecules to higher concentrations, or against a concentration gradient, with the use of metabolic energy input is known as Active transport. This method needs a carrier protein molecule to transport the solutes.
- Active transport occurs in microorganisms, when they have a high concentration gradient (inner nutrient content will be high).

- The carrier molecule have greater specificity to the solutes to be transported.

Symport: Transport of two substances in the same direction is called "symport".

Antiport: Transport of two substances in opposite directions is called "antiport".

ACTIVE TRANSPORT USING PROTON AND SODIUM GRADIENTS

- Bacteria also use proton gradients generated during electron transport chain to drive the active transport mechanism.

PROTON PUMP

- A higher concentration of protons is maintained outside the membrane by ETC activity.
- The proton pump is active in cells where the carrier molecules lacks the binding proteins for solutes to be transported.

Eg. Lactose permease of *E.coli* → A carrier molecule which lacks the binding proteins for a solute Molecule.

- This Lactose permease transport a lactose molecule inward as a proton simultaneously enters the cell from ETC activity. The proton and the lactose are transported in same directions so called "symport".
- Here, the energy stored as a proton gradient drives the solute transport. The proton may change the shape and affinity of the carrier molecule (Lactose permease) for the solute to be transported.

Eg. *E.coli* → uses proton symport to takeup aminoacids and organic acids.

4. GROUP TRANSLOCATION

- A process in which the solute is altered during transport. It's a metabolic energy dependent process known as group translocation.

Eg. Phosphoenolpyruvate : sugar -phosphotransferase system [PTS].

- This system transports a variety of sugar and sugar derivatives into bacteria while phosphorylating them using phosphoenol pyruvate [PEP]. This system uses several enzymes for phosphorylation process. These solutes enters and accumulated inside the cells as phosphates. PTS in *E.coli*.
- This system consists of two enzymes and a low molecular weight heat stable protein [HPr].
- Two enzymes are

E 1

E II →made up of three subunits.

E II enzyme →E II A -- soluble portion.

E II B-- hydrophilic portion

E II C -- hydrophobic portion -- embedded in the membrane.

PHOSPHORYLATION

- A high energy phosphate is transferred from PEP to enzyme II with the aid of enzyme I and HPr. Then, a sugar molecule is phosphorylated and it is carried across the membrane by enzyme II. This enzyme II transports only specific sugars and varies with PTSs, where as enzyme I and HPr are common to all PTSs.

CONTINOUS CULTURE SYSTEM

DEFINITION

- A method in which microorganisms are cultivated in an open system is known as “continous culture system”. By this method , constant environmental conditions maintained through continual addition of nutrients and removal of wastes.
- By this method, a constant biomass concentration can be maintained for longer periods.
- Continuous culture system is widely used as two methods, known as ,
  - Chemostat
  - Turbidostat

Chemostat:

It is system constructed, so that a sterile medium is fed into the culture vessel and at the same time, same rate of media containing microorganism is removed.

Turbidostat:

It is a system contain a “photocell” that measures the “absorbance” or “turbidity” of the culture in the vessel.

BATCH CULTURE

DEFINITION

- A method in which microorganisms are cultivated in a closed environment is known as “batch culture”.
- In this method, a single medium will be used for particular period. During incubation no fresh medium or no nutrients will be added.

- On batch culture method, nutrient concentration will be declined and the cell wastes are increased.
- The growth of microorganisms obtained has 4 curves collectively called “Growth curve”.

BACTERIAL GROWTH CURVE

DEFINITION

- When a single bacterium is inoculated into a suitable medium and incubated subsequently, it's growth follows a definite course. The measurement of bacterial growth at regular interval (times) will give a curve like appearance called bacterial growth curve.

The bacterial growth curve consists of 4 phases, known as,

1. Lag phase
2. Log phase
3. Stationary phase
4. Death phase

1. LAG PHASE :

The time required for adaptation to the new environment medium is known lag phase. In this phase, the bacterial cell is not multiplied, instead its increased in size. During this stage, the organism make ready of their enzymes to carryout metabolic reactions during log phase.

- The lag phase may be vary with
- The speices
- The size of the inoculums
- The nature of the bacterium
- The environmental factors like temperature and pressure.

2. LOG PHASE:

- A stage at which the bacterial cell is multiplied vigorously and their number increased exponentially so called log/exponential/logarithmic phase. The logarithm of viable count is plotted against time, a straight line will be obtained.
- During this phase, the organism synthesizes certain metabolites need for their growth. Those metabolites are called primary metabolites. Eg. Vitamins, aminiacids.

3. STATIONARY PHASE:

After the log period, cell division stops due to depletion of nutrients. Accumulation of toxic metabolites may also occurs, during this phase the cells are maintained steadily so called stationary phase.

- i.e., no increase or decrease in cell numbers.

In this phase, the bacteria synthesize certain substances which may or maynot influence their growth. Those substances are called secondary metabolites.

Eg. Exotoxins

Antibiotics

Enzymes

4. DEATH PHASE:

After the stationary phase, the bacterial population gradually decreases due to cell death. Besides nutrient depletion and toxic substance accumulation, the cell death may also be caused by autolytic enzymes. Sporulation occurs in death phase due to nutrient depletion.

BACTERIAL METABOLISM

METABOLISM

A group of chemical activities performed by a cell in which much if free energy will be produced and utilized by the cell. This process known as metabolism. The energy is utilised for

1. The construction of cell wall, membrane,

2. Enzyme synthesis

3. Nucleic acid synthesis

4. Polysaccharide synthesis

5. Other chemical component synthesis

6. Repair of damage

7. Growth and multiplication

8. Motility

9. Accumulation of certain components in high concentration.

Micro-organisms obtain their energy through

1. Respiration

2. Fermentation

3. Photosynthesis

1. Respiration:

Two types

- Aerobic respiration ETC and TCA cycle
- Anaerobic respiration Glycolysis and HMP shunt

Aerobic respiration:

In this process, the electron donor is an oxidizable substrate the electron acceptor is oxygen. Eg. ETC, TCA cycle.

THE RESPIRATION CHAIN:

The respiratory chain is an electron transport chain (ETC). It is a sequence of oxidation - reduction reactions mediated by a number of electron carriers and electron- carrier enzymes. As the electrons flow through the chains, much of their free energy is liberated in the form of ATP. This process is called oxidative phosphorylation.

OXIDATION : Loss of electrons (hydrogen ions) [dehydrogenation process]

REDUCTION: Gain of electrons [hydrogenation process].

A respiratory chain consists of many enzymes, includes, dehydrogenases and oxidative enzymes.

1. NAD Nicotinamide adenine dinucleotide.

2.NADP Nicotinamide adenine dinucleotide phosphate.

3. FAD Flavin adenine dinucleotide

4. FMN Flavin mononucleotide

5. Co enzyme Q also called Ubiquinone

6. CYTOCHROMES (Oxidative enzyme)

NAD & NADP

certain enzymes which remove electrons and hydrogen ions from substrates so called dehydrogenases have NAD + or NADP + as their co enzyme.

FAD & FMN

A type of dehydrogenases enzyme called Flavoprotein contains either FAD or FMN as their co enzymes.

- The reduced forms of FAD & FMN are FADH2 & FMNH2.

CO enzyme Q

- This enzyme also called as Ubiquinone.
- It is present in all cells.
- It is a fat - soluble co enzyme.
- It accepts the electrons from the Flavoprotein.

NAD +

NADP+ These co enzymes carry both 2H+ and 2e-

Flavoprotein & Ubiquinone

CYTOCHROMES :

These enzymes transfer only electrons not protons (hydrogens).

- Oxidative enzymes.
- It is of 3 types (based on absorption)
- Cytochromes a → cyte. a
  cyte. a3
- Cytochromes b
- Cytochromes c → cyte.c
  cyte. c1

- These enzymes have different function in ETC.
- Each type may exist in either an oxidized or reduced form.
- These cytochromes act sequentially to transport electrons from Ubiquinone (co Q) to oxygen (O2).

TRICARBOXYLIC ACID CYCLE

- ✓ Krebs cycle
- ✓ TCA cycle
- ✓ Citric acid cycle

TCA cycle is one of the aerobic process by which the organisms obtain their energy.

- From glycolysis –"1 glucose" molecule is brokendown to "2 pyruvate"molecules. This pyruvate molecules are further degraded aerobically to co2 and acetyl co A so called TCA is an aerobic process.
- Acetyl – co A arises from the catabolism of many carbohydrates, lipids and aminoacids. This acytyl co A can be further degraded in the TCA cycle. (degraded aerobically).
- The TCA cycle produces 2 co2s

3 NADH2S ======➔ for each acetyl co A oxidized.

1 FADH2

1 GTP

NET Production:

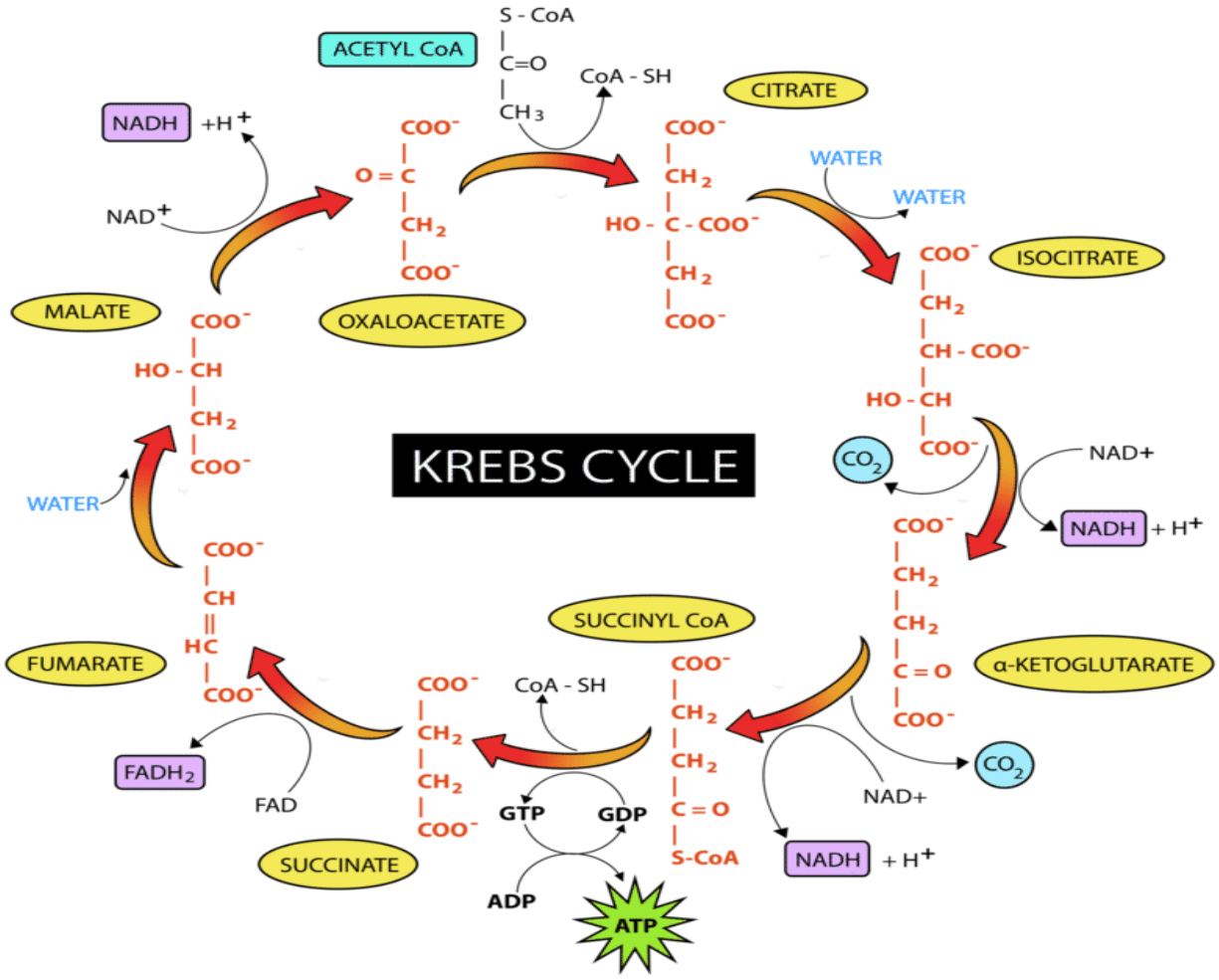
For 2 Acetyl co A molecules → 4 co2

6NADH2S

2FADH2S

2GTP

- TCA cycle is an "amphibolic cycle" which means both catabolic and anabolic reactions will takes place.



GLYCOLYSIS -→ Anabolic as well as catabolic processes.

- Glycolysis "splitting of sugar"
  - The most common pathway of glucose catabolism is known as glycolysis.
  - Glycolysis doesnot require the presence of oxygen. It can occur in both aerobic and anaerobic cells.
- Aerobic cells degrade glucose by oxidation (glycolysis)

Glucose ----------→ pyruvate----------→co2 + H2o

- In anaerobic cells, the glucose is converted to fermented products.

Glucose----------→ fermentation products.

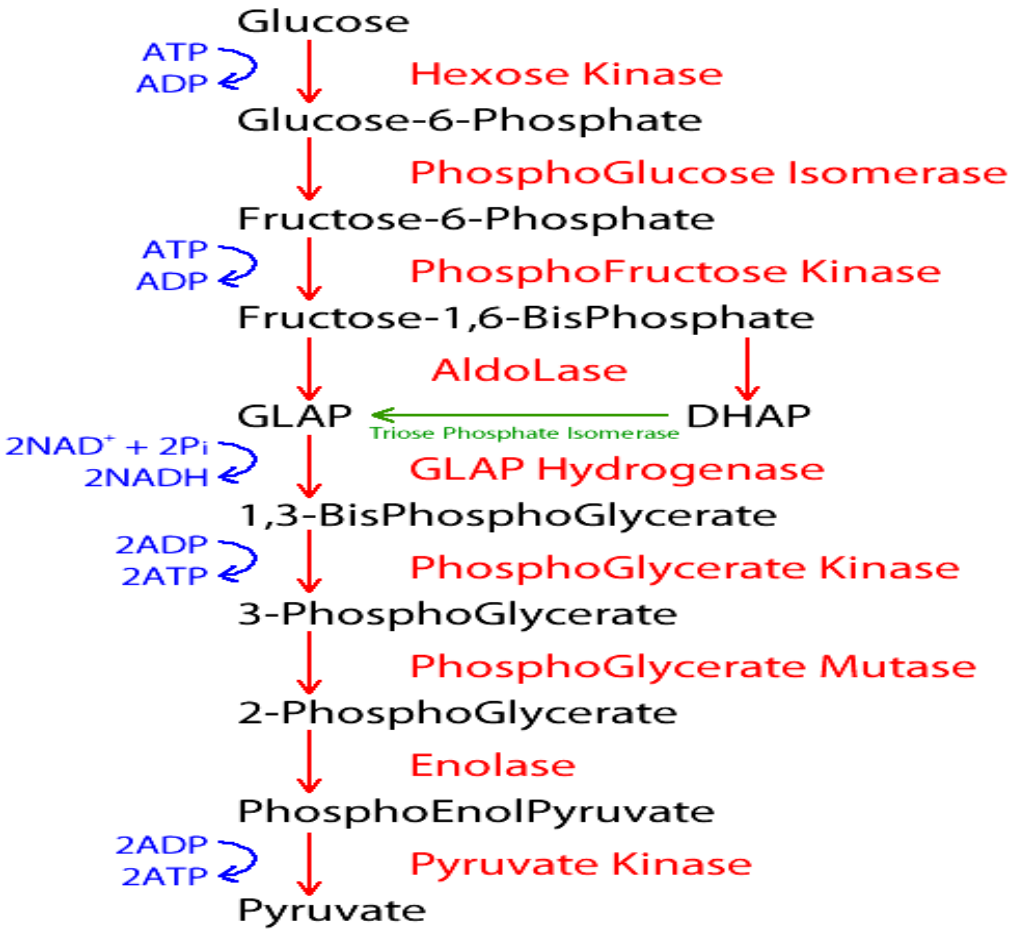
- In glycolysis, the glucose is converted to pyruvate through oxidation reduction reaction. In this process, the oxidation of Glyceraldehyde -3-phosphate releases a pair of electrons.
- In the presence of oxygen, these electrons may enter the respiratory chain.

Glucose --→pyruvate-→co_2 +H_2O

- In the absence of oxygen, these electrons may be used to reduce the pyruvic acid to lactic acid or ethanol.

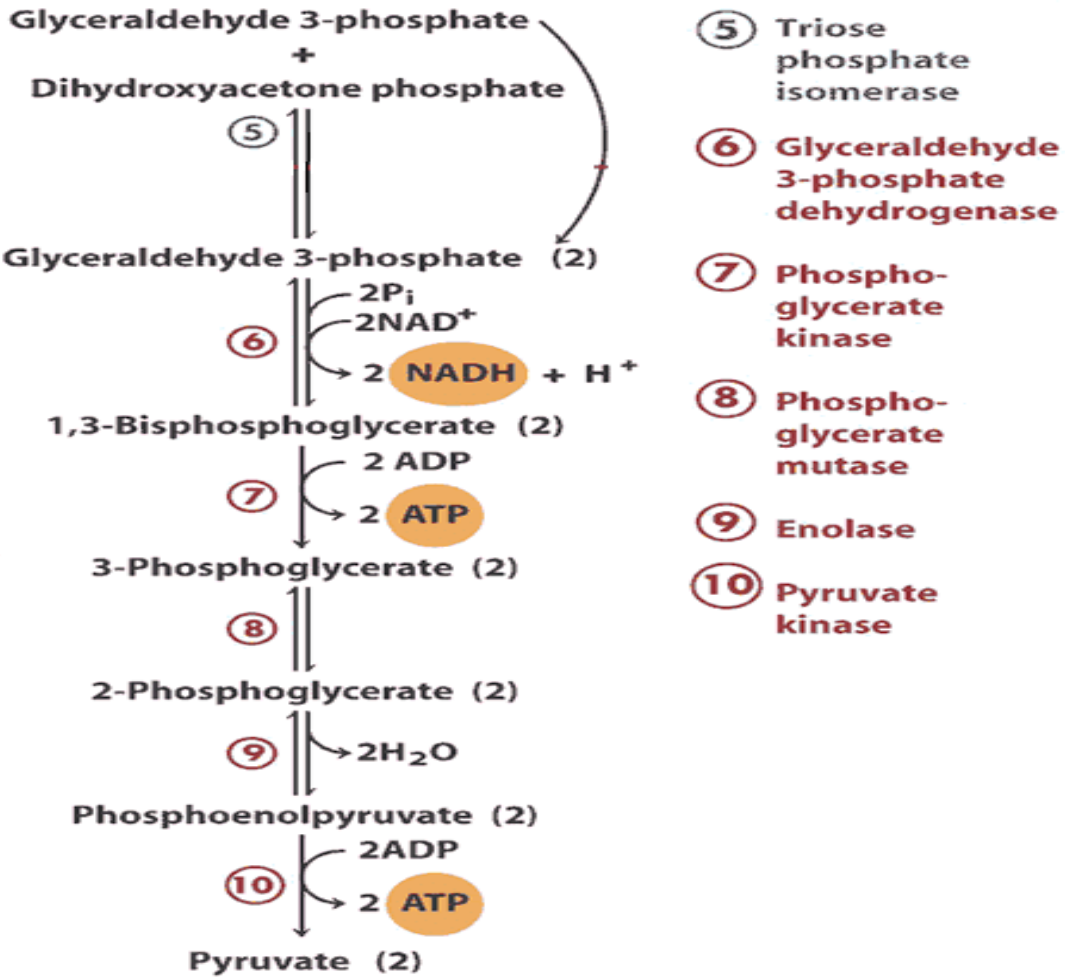
Glucose----------→ fermentation products.

- In glycolysis process, synthesis as well as breakdown of glucose occurs.
- This glycolysis process gives 2ATPS /1 Glucose molecules.



Simplified Glycolysis diagram. Molecule names contain extra capitals to illustrate components. 21/02/2010 followchemistry.wordpress.com

➢

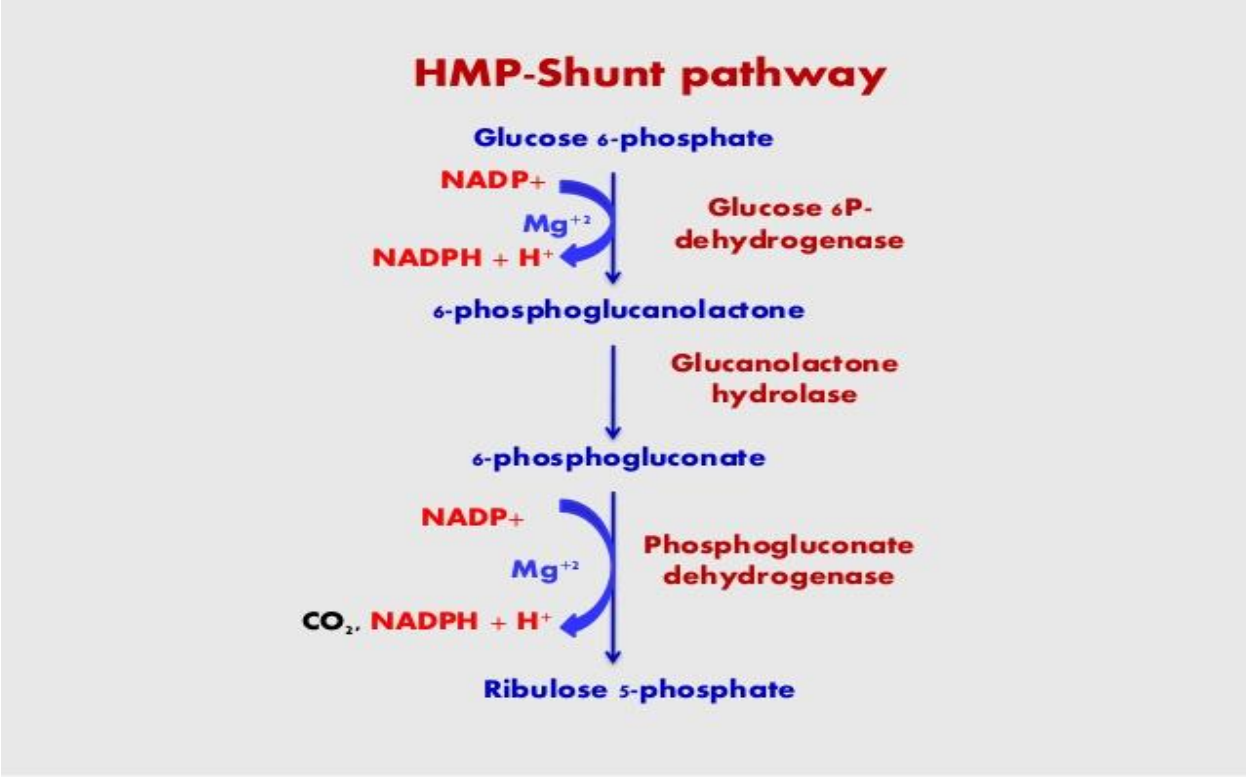

Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Glyceraldehyde 3-phosphate (2)
2Pi
2NAD+
6
2 NADH + H+
1,3-Bisphosphoglycerate (2)
2 ADP
7
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H2O
Phosphoenolpyruvate (2)
2ADP
10
2 ATP
Pyruvate (2)
5 Triose phosphate isomerase
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phospho-glycerate kinase
8 Phospho-glycerate mutase
9 Enolase
10 Pyruvate kinase

HMP SHUNT (Hexose mono phosphate pathway)

PENTOSE PHOSPHATE PATHWAY= PPP

PHOSPHOGLUCONATE PATHWAY

- It is catabolic pathway.
- In this pathway, the glucose is oxidized with liberation of an electron pair-which may enter the respiratory chain (ETC).
- This pathway yield energy in the form of NADPH+H+. This energy used up by the organisms to carry out their biosynthesis process.
- This "PPP"feeds into glycolytic pathway by means of fructose -6-phosphate and glyceraldehydes-3- phosphate so called, HMP shunt.
- Through this pathway, the cell can carry out the complete oxidation of glucose to co2. These reactions are mediated by means of certain enzymes called transketolases and transaldolases.



PHOTOSYNTHESIS

Microorganisms may also derive their energy from light sources. Those are called “photoautotrophs”.

A process in which the light energy is converted to chemical to energy is called “photosynthesis”.

$2H_2A+Co_2$ ---→$(CH_2O)+2A+H_2O$

(In the presence of light and bacterio chlorophyll, the compound is converted to carbohydrate)

- Anoxygenic photosynthetic bacteria possess chlorophyll called “bacteriochlorophyll”. They are found in membrane system throughout the bacterial cell.
- These absorbs light from Infra Red region.
- Photosynthesis is of two types, known as,

1.cyclic photosynthesis

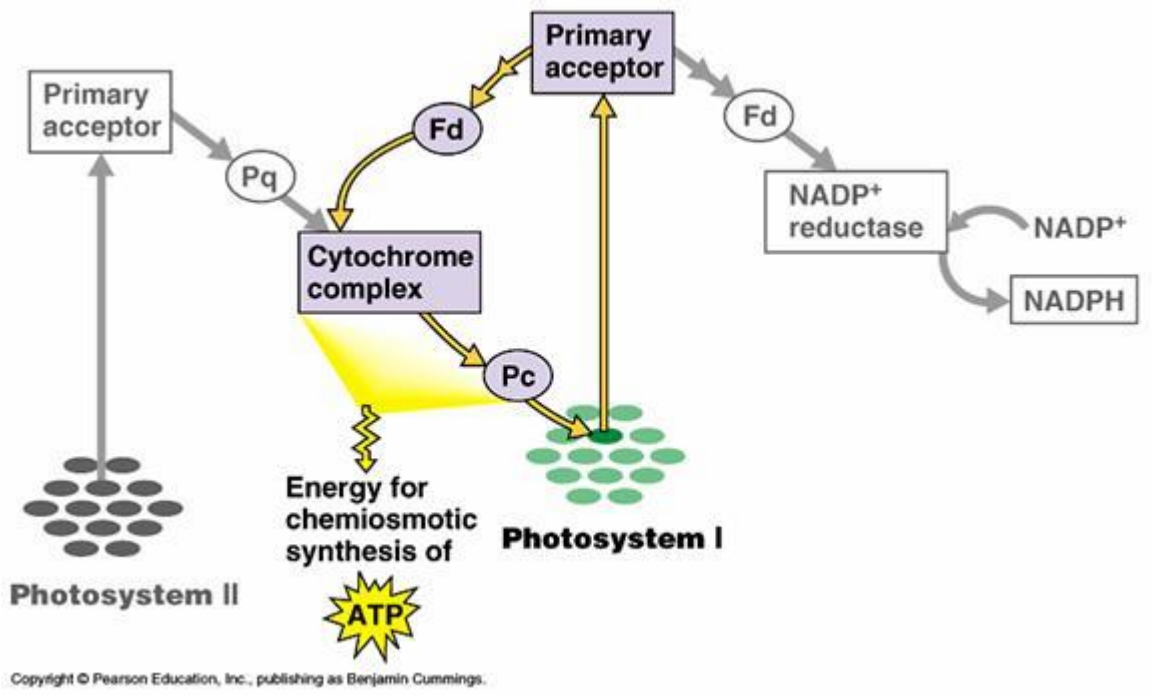
2. Non cyclic photosynthesis

1. CYCLIC PHOTOSYNTHESIS:

- Occurs in anoxygenic photosynthetic bacteria (Bacteria that donot use water as electron donor and donot produce oxygen on photosynthesis.)
- Bacteria possess chlorophyll called bacteriochlorophyll which is found in membranes of bacteris throughout the cell. Which absorbs light (Radiant energy) from Infrared region.
- When a molecule of bacteriochlorophyll absorbs a quantum of light, the chlorophyll get excited and release an electron. This electron carrying some energy is transferred to an iron containing heme protein known as Ferredoxin. Then it is passed to Ubiquinone, to cytochrome b and to

cytochrome f and finally back to the bacteriochlorophyll, where it has been released. The electron has gone around in a cycle {beginning with and returning to=same chlorophyll} so called "cyclic photosynthesis".

- Only "pigment system –I" involves in cyclic photosynthesis process.



2. NON CYCLIC PHOTOSYNTHESIS:

- Non cyclic photosynthesis occurs in plants, algae and cyanobacteria (oxygenic photosynthesis bacteria).
- In this process two pigment systems are involved, known as, Pigment system I and Pigment system II
- This process also called as "oxygenic photosynthesis".

PROCESS:

- When a molecule, from Pigment system II absorbs light, the chlorophyll get excited and release an electron. This electron to "plasquinone", to cytochrome b, to cytochrome f and finally reaches the Pigment system I.
- When : Pigment system I" absorbs light, it get excited and release an electron. This electron transferred to ferredoxin, to flavoprotein and to NADP+.
- The electron lost by Pigment system II is not cycled back to it. Instead, electrons are replaced in Pigment system II by the light mediated breakdown of water, called "photolysis". So, this process called as Non-cyclic photosynthesis.

FERMENTATION

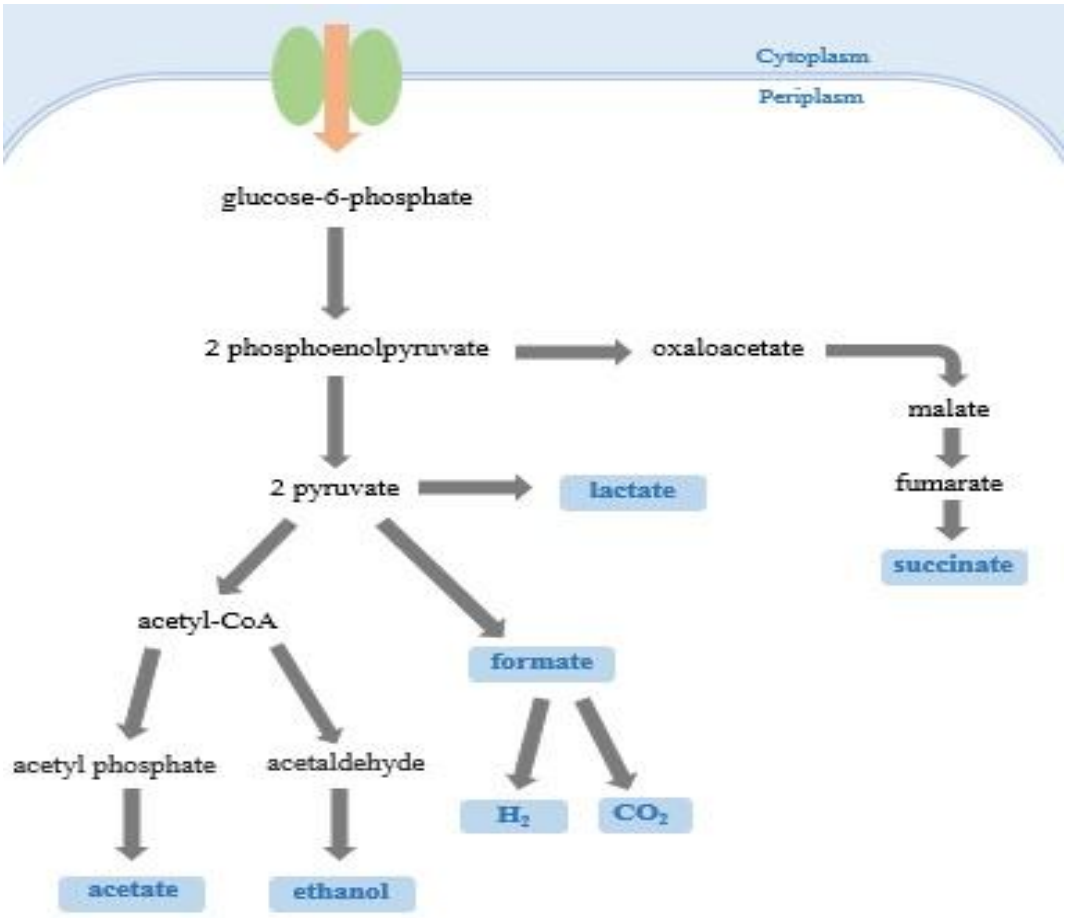
- Anaerobes also produce energy by a process called "fermentation".
- The anaerobes and facultative anaerobes produce energy through fermentation process.
- These organisms use organic compounds as electron donors and electron acceptors.

Eg. 1. Lactic acid production by *Streptococcus lactis* from glucose.

Glucose → pyruvic acid → lactic acid.

- First the glucose is converted to pyruvate. Then, the pyruvate is converted to lactic acid.

Eg. 2. *E.coli* produces a mixture of products from glucose through fermentation.



TEXT BOOKS:

Sl no:	**Book Name**	**Author**	**Publisher**	**Year of Publication**
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