General Microbiology, Concise Notes

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GENERAL MICROBIOLOGY

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Microbiology

The word microbiology is derived from the Greek word, MICRO means small, BIOS means life and LOGIA means study. It is the study of microscopic organisms either unicellular, multicellular or acellular.

Microbiology include disciplines of Virology, mycology and bacteriology etc.

- Eukaryotic microorganisms exhibit cell organelles and include fungi, protists and algae.
- Prokaryotic microorganisms are conventionally classified as lacking organelles and include Eubacteria, Archaebacteria.
- Microbiologist traditionally relayed on culture, staining and microscopy. However, if only 1 percent of microbes present in the environment are culture able. Microbiologist often relay on extraction or detection of nucleic acid either DNA or RNA.
- Viruses are not always classified as organisms as they have been identified either as very simple microorganisms or very complex molecules.
- Prions are never considered microorganisms and they have been investigated by virologist as the clinical effects traced to them were originally presumed due to chronic viral infection and virologist identified them as infectious proteins, e.g. mad cow disease in cattle and Scrapie disease in sheep.

Branches of Microbiology:

Two main branches

1. Pure microbiology
2. Applied microbiology

Pure microbiology:

- **Bacteriology**: study of bacteria
- **Mycology**: study of fungi
- **Phycology**: study of algae
- **Immunology**: study of immune system
- **Virology**: study of viruses
- **Microbial physiology**: study of microbial cell function. It includes the study of microbial growth, metabolism and cell function.
- **Microbial cytology**: study of microscopic and sub-microscopic details of organisms.
- **Microbial genetics**: study of organization and regulation of genes in microbes in relation to their cellular function.

Applied microbiology:

- **Veterinary microbiology**: study of microbes of veterinary importance such as probiotics and pathogens which are related to veterinary medicine.
- **Pharmaceutical microbiology**: study of microorganisms which are related to the production of antibiotics, enzymes, vitamins, biologics and other pharmaceutical products and those microbes which cause pharmaceutical contamination and spoilage.
- **Microbial biotechnology**: manipulation of microorganisms at genetics and molecular level to generate useful products e.g. generation of insulin from E. coli.
- **Food microbiology**: study of microorganism causing food spoilage and food borne illness. It is all the use of microbes to produce food.

History of Microbiology:

Recent discovery of Mycobacterium Tuberculosis, DNA in the three thousand years old Egyptian mummies reminds us that microorganisms have been around us for a much longer period of time. Infect Bacterium ancestors were the firsts living cells to appear on the earth.

Golden age of microbiology:

From 1857-1940 has been named Golden age of microbiology. During this period, rapid advancement spread hold mainly by Pasture & Robert Koch lead to the establishment of microbiology as a science. During this era

- Discovery of immunity
- Discovery of disease causing agent

Robert Koch a Germen physician discovered the cause of Anthrax (Bacillus anthracis) in 1870's.

Koch postulates:

1. Same pathogen must be present in every case of the disease.

2. Pathogen must be isolated from the diseased host & grown in a pure culture

3. Pathogen from the pure culture must cause the disease when it is inoculated into susceptible lab. Animal

4. The same pathogen must be isolated from the inoculated animal and it must be same as original organism

Vaccination:

Edward Jenner a young British Physician used scraping of cowpox blister to vaccinate against sheep pox.

Fermentation:

Microorganism like yeast converts sugar to alcohol in the absence of air this process is called fermentation. It is used to make wine & beer. In the presence of air bacteria convert alcohol into acetic acid or vinegar.

Pasteurization:

It is the heat treatment of beverage & milk at 72°C for 30 min to kill microorganism (which causes spoilage of liquid) in food without compromising on its quality. Now a day it is only used for milk product.

Germ theory of diseases:

Microorganisms are the cause of disease. In 1860's English surgeon Joseph Lister use phenol, Carbolic acid as a disinfectant & antiseptic solution. This practice reduced the incidence of infection & death others surgeon readily adopted it. Lister technique was the earliest medical attempt to control infection caused by microorganism.

Morphology of Bacteria

Size:

Bacteria come in a great many sizes and several shapes. Most bacteria range from 0.2 to 2.0 µm in diameter and from 2 to 8 µm in length. (an overall average size of 1 to 10 µm.)

Shape:

They have a few basic shapes:

1. Spherical coccus (plural: cocci, meaning round),
2. Rod-shaped bacillus (plural: bacilli)

3. Spiral.

Cocci:

Cocci are usually round but can be oval or elongated. When they divide to reproduce, they remain attached to each other.

Classification According to Plane of Division:

Diplococci:

Those Cocci that remain in pairs after dividing are called diplococci.

Streptococci:

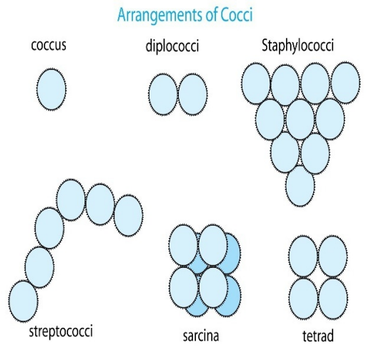
Those cocci that divide and remain attached in chainlike patterns are called streptococci.

Tetrad:

Those cocci that divide into two planes and remain in groups of four are known as tetrads.

Sarcinae:

Those cocci that divide in three planes and remain attached in cube like groups of eight are called sarcina.



Staphylococci:

Those cocci that divide in multiple planes and form grapelike clusters or broad sheets are called staphylococci.

Bacilli:

Bacillus are the rod like bacteria e.g. Bacillus anthracis

Single bacillus:

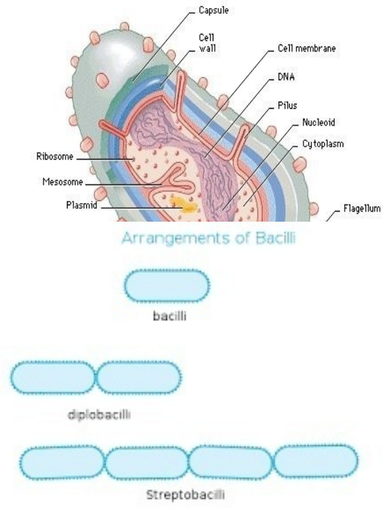
Most bacilli appear as single rods, called single bacilli.

Diplobacilli:

Those bacilli which are appear in pairs after division called Diplobacilli

Streptobacilli:

Those bacilli that found in chains in single plane are called Streptobacilli.



Monomorphic:

Bacteria that maintain single shape called monomorphic or the bacteria having same shape.

Pleomorphic:

Some bacteria can have many shapes known as pleomorphic. e.g: Corynebacterium, Pyogenesis.

- Some bacteria occur in the shape of star e.g. *Stella.*
- Some bacteria occur in the shape of rectangle e.g. *Haloarcula.*
- Some bacteria also appear triangular in shape.

Functional Anatomy of Bacteria:

Brief functions:

- **Cell Wall**: acts as an antigen, provide protection and rigidity
- **Cell membrane**: serve as a barrier through which materials enter and exit the cell

- **Capsule**: acts as an antigen, has feeding importance, sticking features and cause disease
- **Mesosome**: role in metabolism
- **Fimbriae**: helps in motility, jerky movement, has sticking feature and acts as an antigen
- **Pilus**: helps in reproduction(conjugation)
- **Ribosomes**: protein formation
- **Nucleoid**: transcription and translation
- **Chromosomes**: hereditary material
- **Droplets**: helpful in storage
- **Flagella**: act as an antigen and helps in motility
- **Plasmid**: has special features of resistance and infection

Appendages external to bacteria

Fimbriae

- Made up of fimbrine protein and range hundreds in number.
- Helps in motility and jerky movement
- Acts as a poor antigen thus helps in recognition
- Used for attachments and formation of biofilms e.g. fimbriae of bacteria Neisseria gonorrhoeae helps to colonize in mucous membrane and fimbriae of E.coli helps to adhere in small intestine
- Acts as a disease causing agents after attachment
- Cause ascending infection e.g. in excretory system

Pilli

- They are longer then fimbriae and range in number from 1 to 10.
- They are made up of pillin protein. Its function is reproduction i.e. sexual reproduction in bacteria known as conjugation. (F^+, the bacteria which give genetic material and F^-, which receive). The rest of the functions are same as fimbriae.

Glycocalyx (capsule)

Prokaryotic cell sometimes secrete a substance outside the cell wall known as glycocalax.

- If the substance is organized and is firmly attached to the cell wall, the glycocalyx is described as a capsule.
- If the substance is unorganized and only loosely attached to the cell wall, the glycocalyx is described as a slime layer.
- If a number of bacteria are present close together and the capsule of a single cell cannot identified the layer formed is known as saline layer.
- If glycocalyx is removed from cell wall or if it is not present at all then a thin layer is present known as “S” layer.
- If a colony forms LPS capsule the appearance of colony will be smooth.
- If a colony forgets to form LPS capsule the colony will b rough.
- If protein capsule is present in colony then appearance will be rough
- If protein capsule absent then the appearance will be smooth.

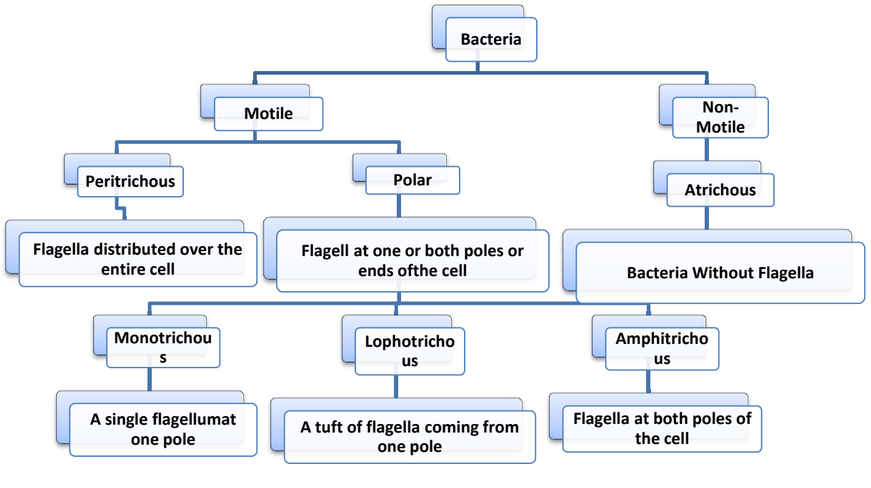
Function

- Protection because of slimy nature
- Protection against immune system so they can cause disease
- Capsule can serve as a food source e.g. Streptococcus mutants.
- Used for attachment
- Serve as a good antigen.

Flagella

Flagella are long filamentous appendages that propel bacteria.

Classification of Bacteria on the basis of Flagella



Structure of Flagella

A flagellum has three basic parts:

- The long outermost region, the filament, is constant in diameter and contains
  the globular (roughly spherical) protein flagellin.(The flagellar protein called **H antigen**)
- The filament is attached to a slightly wider hook.
- The third portion of a flagellum is the basal body, which anchors the flagellum
  to the cell wall and plasma membrane.

When a bacteria wants to grow in size it produces its protein that moves towards the tipoff flagella and increase its size.

Movement Due to Flagella

The Basal body is composed of a small central rod inserted into a series of rings.

- Gram-negative bacteria contain two pairs of rings; the outer pair of rings is anchored to various portions of the cell wall, and the inner pair of rings is anchored to the plasma membrane.
- In gram-positive bacteria, only the inner pair is present. Each prokaryotic flagellum is a semi rigid, helical structure that moves the cell by rotating from the basal body. The rotation of a flagellum is either clockwise or counter clockwise around its long axis the flagella rotate, they form a bundle that pushes against the surrounding liquid and propels the bacterium. The energy is provided by **"The electron motive force"**

Bacteria move by propelling the flagella when a bacteria move in any direction it is called **"run"**. Sometime when a bacteria come across some chemicals it start to move in random direction it is called **"Tumbles"** then it start to move move in any direction.

Axial Filament/Endofilament

Some species of bacteria i.e *Spirochete* have unique structure and motility. One of the best-known spirochetes is ***Treponema pallidum***

Spirochetes move by means of **axial filaments,** or **endoflagella,** bundles of fibrils that arise at the ends of the cell beneath an outer sheath and spiral around the cell Axial filaments, which are anchored at one end of the spirochete, have a structure similar to that of flagella.

The rotation of the filaments produces a movement of the outer sheath that propels the spirochetes in a spiral motion. This type of movement is similar to the way a corkscrew move through a cork.

Inclusions Bodies

Within the cytoplasm of prokaryotic cells are several kinds of reserve deposits, known as **inclusions.** Cells may accumulate certain nutrients when theyare plentiful and use them when the environment is deficient.

Some inclusions are common to a wide variety of bacteria,whereas others are limited to a small number of species and therefore serve as a basis for identification

Metachromatic granules

- Metachromatic granules are large inclusions Collectively they are known as volutin. Volutin represents a reserve of inorganic phosphate (polyphosphate) that can be used in the synthesis of ATP. It is generally formed by cells that grow in phosphate-rich environments. Metachromatic granules are found in algae, fungi, and protozoa, as well as in bacteria.

- **Polysaccharide Granules**

Inclusions known as polysaccharide granules typically consist of glycogen and starch, and their presence can be demonstrated when iodine is applied to the cells

- **Lipid Inclusions**

Lipid inclusions is a common lipid-storage material appear in various species of Mycobacterium, Bacillus,Azotobacter, Spirillum and other genera.They are revealed by staining cells with fat-soluble dyes, such as Sudan dyes.

- **Sulfur Granules**

Certain bacteria derive energy by oxidizing sulfur and sulfur-containing compounds. These bacteria may deposit sulfur granules in the cell, where they serve as an energy reserve.

- **Carboxysomes**

Carboxysomes are inclusions that contain the enzyme ribulose 1,5-diphosphate carboxylase. Photosynthetic bacteria use carbon dioxide as their sole source of carbon and require this enzyme for carbon dioxide fixation. i.e cyanobacteria, and thiobacilli.

- **Gas Vacuoles**

Hollow cavities found in bacteria are called gas vacuoles.Each vacuole consists of rows of several individual gas vesicles. maintain buoyancy so that the cells can remain at the depth in the water appropriate for them to receive sufficient amounts of oxygen, light, and nutrients.These gas vacules are found in cyanobacteria, anoxygenic photosynthetic bacteria etc

Magnetosomes

- Magnetosomes are inclusions of iron oxide (Fe_3O_4) surrounded by invaginations of the plasma membrane. Magnetosomes are formed by several gram-negative bacteria such as Magnetospirillum magnetotacticum and act like magnets. Magnetosomes may protect the cellagainst hydrogen peroxide accumulation

Ribosomes

Ribosomes are used for protein synthesis. In bacteria the ribosomes present are 70s and made up of to smaller and larger subunits one is 20s and other is 50s. these subunits consist of protein and ribosomal RNA.

Endospore

Endospores are the specialized resting cells produced by gram positive bacteria when the essential nutrients are not available any more. Examples of pathogenic bacteria forming endospore are *Bacillus* and *Colstridium.*

- The process of formation of endospore within a vegetative cell takes several hours and the process is known as **sporulation** or **sporogenesis.**
- Endospore can remain dormant for thousands of years and when an endospore return to its vegetative state the process is given the name of **germination**.
- Endospores are highly durable dehydrated cells with thick walls and additional layers. They are formed internal to the bacterial cell membrane. When released into the environment, they can survive extreme heat, lack of water, and exposure to many toxic chemicals and radiation.

Stages in Endospore Formation

1. **I**n first observable stage, a newly replicated bacterial chromosome and a small portion of cytoplasm are separated by an inward growth of cell membrane **spore septum**.
2. The spore septum becomes a double-layered membrane that surrounds the cytoplasm and chromosome and a thick layer of peptidoglycan is laid down between the two membrane layers. this structure is entirely enclosed within the original cell and is called a **forespore**.
3. A thick spore coat of protein forms around the outside membrane, responsible for resistance of endospore to many harsh chemicals. The original cell is degraded or ruptures and endospore is released.

- The diameter of the endospore may be smaller or larger than the size of vegetative cell.
- The endospore may be located *terminally,subterminally* or *centrally* inside the vegetative cell.
- Endospore contain a large amount of organic acid called dipicolinicacid(DPA) which is accompanied by a large number of calcium ions.
- One vegetative cell produces only one endospore and one endospore converts to one vegetative cell only. Hence endospore cannot serve as a source of reproduction because the number of cells does not increase.
- Endospores are resistant to the process of heating, freezing , desiccation, chemicals or radiations which causes death of vegetative cells hence endospore are a problem in food industry.

Cell Wall

The cell wall of the bacterial cell is a complex, semi rigid structure responsible for the shape of the cell.

- The cell wall surrounds the underlying, fragile plasma membrane and protects it and the interior of the cell from adverse changes in the outside environment.
- The major function of the cell wall is to prevent bacterial cells from rupturing when the water pressure inside the cell is greater than that outside the cell.
- Also, act as an antigen.

Composition

The bacterial cell wall is composed of a macromolecular network called peptidoglycan (also known as murein). Peptidoglycan consists

of a repeating disaccharide attached by polypeptides to form a lattice that surrounds and protects the entire cell. The disaccharide portion is made up of monosaccharides called N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), which are related to glucose. Alternating NAM and NAG molecules are linked in rows of 10 to 65 sugars to form a carbohydrate "backbone". Adjacent rows are linked by polypeptides. Although the structure of the polypeptide link varies, it always includes tetrapeptide side chains, which consist of four amino acids attached to NAMs in the backbone. The amino acids occur in an alternating pattern. Parallel tetrapeptide side chains may be directly bonded to each other or linked by a peptide cross-bridge, consisting of a short chain of amino acids.

Gram positive bacteria

- In most gram-positive bacteria, the cell wall consists of many layers of peptidoglycan, forming a thick, rigid structure. By contrast, gram-negative cell walls contain only a thin layer of peptidoglycan.
- The cell walls of gram-positive bacteria contain teichoic acids, which consist primarily of an alcohol and phosphate.
- There are two classes of teichoic to the plasma membrane, and wall teichoic acid, which is linked to the peptidoglycan layer.
    1. Lipoteichoic acid
    2. Wall teichoic acid
- The wall of gram negative bacteria cannot be broken down mechanically. On the other hand the wall of gram negative can be broken down.

Gram negative bacteria

- The cell walls of gram-negative bacteria consist of one or a very few layers of peptidoglycan and an outer membrane.
- The outer membrane of the gram-negative cell consists of lipopolysaccharides (LPS), lipoproteins, and phospholipids. The outer membrane has several specialized functions. Its strong negative charge is an important factor in evading phagocytosis. The outer membrane also provides a barrier to certain antibiotics, digestive enzymes.
- The outer membrane does not provide a barrier to all substances in the environment because nutrients must pass through to sustain the metabolism of the cell.
- The lipopolysaccharide (LPS) of the outer membrane is a large complex molecule that contains lipids and carbohydrates and consists of three components: (1) lipid A, (2) a core polysaccharide, and (3) an O polysaccharide. Lipid A is responsible for the symptoms associated with infections by gram-negative bacteria such as fever, dilation of blood vessels, shock, and blood clotting. The core polysaccharide is attached to lipid A and contains unusual sugars. Its role is structural—to provide stability. The O polysaccharide extends outward from the core polysaccharide and is composed of sugar molecules. The O polysaccharide functions as an antigenand is useful for distinguishing species of gram-negative bacteria.

Protoplast

If cell wall is removed from the gram positive bacteria then they are called as protoplast

Spheroplast

When the cell wall is removed from the gram negative bacteria then they are known as spheroplast.

- *Mycoplasma,Acheloplasm* and *Ureaplasm* are bacteria which have forgotten to produce cell wall.

Requirements for Microbial Growth

Microorganisms need both physical and chemical's requirements for their growth

1) Physical Requirements

➢ **Surface**

Microorganisms does not grow in air they need some medium for their growth. These medium may be liquid or solid

In liquid medium bacteria can grow at a very rapid rate up to $10^{12\text{-}14}$, and if the water is mixed regularly or agitated they can reach up to $10^{16\text{-}20}$. This is because in liquid medium nutrients are easily diffusible and easily available.

In solid medium bacterial growth rate is slow as compared to liquid medium however if the nutrients are provided is solid medium bacteria can grow up to $10^{6\text{-}8}$. Most commonly used solid media is **"Agar Agar".**

➢ **Temperature**

On the basis of temperature bacteria are divided into three classes

- **Psychrophyles: (Cold loving)**

These bacteria live in temperature range of-10-25^{o}C.Some Psychrophytes grow at -10^{o}C, while other bacteria grow at 4-25^{o}C. These include ***Staphylococcus, Streptococcus, Bacillus ceveus etc*.** These bacteria mostly cause the spoilage of food.

- **Mesophiles: (Moderate tem. loving)**

These bacteria range from 25-40^{o}C most of these bacteria live at 37^{o} C. About 99% of disease causing bacteria is included in this group. These bacteria do not cause as much spoilage of food.

- **Thermophiles: (Heat loving)**

These bacteria live at 100^{o}C or above 100^{o}C. The bacteria living above 100^{o}C are called **Extreme thermophiles.** These are not pathogenic bacteria .These bacteria found in volcanic, thermal springs, geyser etc. These bacteria are used in **PCR (Thermal Aquaticus).**

➢ **Humidity**

Bacteria grow at humid environment of humidity 40-60%. If bacteria are grown in dry environment they will start to die.

➢ **Light**

UV light is bad for the growth of bacteria. UV light will change the structure of DNA and cause mutation. UV light will make loops in DNA.

Simple light is necessary for some bacteria i.e. photosynthetic bacteria which need light to prepare their food. Some bacteria are not affected by light but they behave different in light and dark medium.

➢ **Osmolarity**

Microorganisms obtain almost all their nutrients in solution from the surrounding water. Thus, they require water for growth, and their composition is 80–90% water. If the bacteria are grown in **Hypertonic** environment the water inside the cell start to move outside the cell and bacterial cell shrink and will die, If the bacteria is grown in **Hypotonic** environment the water will start to move inside the cell and bacterial cell will swell and burst.

Different bacteria live in different osmotic pressure.

Some organisms, called **extreme halophiles**, have adapted so well to high salt concentrations that they actually require them for growth.

Obligate halophiles. Organisms from such saline waters as the Dead Sea often requirenearly 30% salt.

Facultative halophytes, which do not require high salt concentrations but areable to grow at salt concentrations up to 2%, a concentration thatinhibits the growth of many other organisms. A few species offacultative halophiles can tolerate even 15% salt.

2) Chemical Requirements:

- **Carbon**

Besides water, one of the most important requirements for microbial growth is carbon. it is needed for all the organic compounds that make up a living cell. Half the dry weight of a typical bacterial cell is carbon. **Chemoheterotrophs** get most of their carbon from the source of their energy—organic materials such as proteins, carbohydrates, and lipids. **Chemoautotrophs** and **photoautotrophs** derive their carbon from carbon dioxide.

- **Nitrogen, Sulfur, and Phosphorus**

In addition to carbon, microorganisms need other elements to synthesize cellular material. For example, protein synthesisrequires considerable amounts of nitrogen as well as some sulfur.The syntheses of DNA and RNA also require nitrogen andsome phosphorus, as does the synthesis of ATP, the moleculeso important for the storage and transfer of chemical energywithin the cell.Organisms use nitrogen primarily to form the aminogroup of the amino acids of proteins. Many bacteria meet thisrequirement by decomposing protein-containing material. Some bacteria usenitrogen from ammonium ions ($NH4+$),nitrate ion, $NO3-$ and some bacteria use direct nitrogen from environment This process is called **nitrogenfixation.**

Sulfur is used to synthesize sulfur-containing amino acidsand vitamins such as thiamine and biotin. Important naturalsources of sulfur include the sulfate ion ($SO4^{2-}$), hydrogen sulfide,and the sulfur-containing amino acids.

Phosphorus is essential for the synthesis of nucleic acids andthe phospholipids of cell membranes. A source of phosphorusis the phosphate ion ($PO4^{3-}$).

Potassium, magnesium, and calcium are also elements that microorganisms require, often as cofactors for enzymes

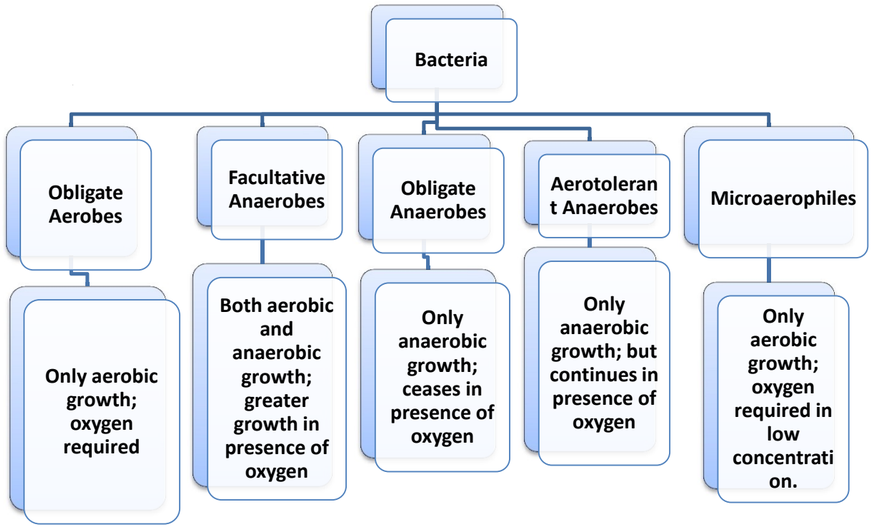
- **Trace Elements**

Microbes require very small amounts of other mineral elements, such as iron, copper, molybdenum, and zinc; these are referred to as **trace elements.** Most are essential for the functions of certain enzymes, usually as cofactors.

- **Oxygen**

Oxygen is needed for the breakdown of food but some bacteria can live without oxygen. However the energy extract is greater if the bacteria use oxygen. There are some bacteria that live in oxygen environment and without oxygen.

Classification of Microbes on basis of Oxygen Demand



Bacterial Growth & Multiplication

Bacterial growth refers to the increase in the no. of individual cell. Normally bacteria reproduce by **Binary Fission.** Asexual reproduction is a separation of a body into two new bodies in the process of a binary fission. Organisms duplicates its genetic material & then divides into two parts with each new organism receiving one copy of DNA A few bacterial species also reproduce by **Budding**. Budding is formation of small initial outgrowth which is called **Bud** then it enlarges until its size approaches to the parent cell & then it separate.

Generation Time

The time required for a cell to divide (and its population to double) is called the **generation time.** *E. coli* under favorable conditions double in 20 minutes & *Mycobacterium* in 24hours.

Logarithmic Representation of Bacterial Populations

Phases of Growth

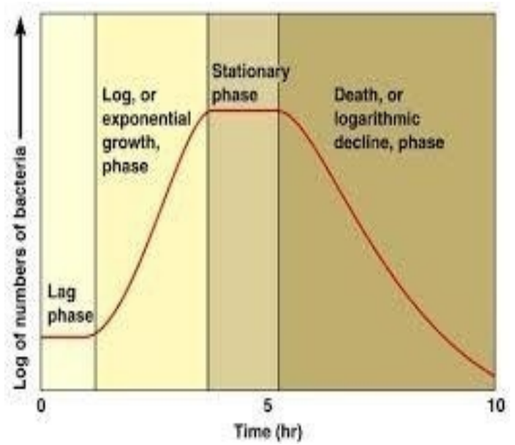
When a few bacteria are inoculated into a liquid growth medium and the population is counted at intervals, it is possible to plot a **bacterial growth curve** that shows the growth of cells over time. There are four basic phases of growth: the **lag, log**, **stationary**, and **death phases.**

- **The Lag Phase**

This period of little or no cell division is called the **lag phase,** and it can last for 1 hour or several days depending upon the species of bacteria. The microbial population is undergoing a period of intense metabolic activity involving, in particular, synthesis of enzymes and various molecules.

- **The Log Phase**

In this phase the cells begin to divide and enter a period of growth, or logarithmic increase, called the **log phase,** or **exponential growth phase.** Cellular reproduction is most active during this period, and generation time reaches a constant minimum. Because the generation time is constant, a logarithmic plot of growth during the log phase is a straight line. The log phase is the time when cells are most active metabolically and is preferred for industrial purposes where, for example, a product needs to be produced efficiently.



- **The Stationary Phase**

If exponential growth continued unchecked, startlingly large numbers of cells could arise but in reality this does not happen. In this phase the growth rate slow down the number of microbial deaths balances the number of new cells, and the population stabilizes. The metabolic activity of individual cell serving also slows down at this stage.

- **The Death Phase**

The number of deaths eventually exceeds the number of new cells formed, and the population enters the **death phase,** or **logarithmic decline phase.** This phase continues until the population is diminished to a tiny fraction of the number of cells as compared to the previous phase or until the population dies out entirely. Many bacterial cells often undergoes involution during this phase meaning that their morphology changes dramatically & make them difficult to identify.

Types of Culture Medium

Culture Medium

A nutrient material prepared for the growth of microorganisms in a laboratory is called a culture medium.

1. Solid media known as Nutrient Agar
2. Liquid media known as Nutrient Broth

Inoculum

Microbes that are introduced into a culture medium to initiate growth are called an inoculum.

Properties of Culture Media

1.It must contain the right nutrients for the specific microorganism we want to grow.i.e. MacConkey Agar contain Bile salt which is Required for the growth of E. coli.

2.It should also contain sufficient moisture.

3.The pH of media must be properly, adjusted as per requirement of microorganism to be grown e.g. pH 7.0-7.2 for bacteria and 5.5-5.6 for fungi.

4.Suitable level of oxygen or no oxygen at all.

5.The medium must initially be sterile that mean it must initially contain no living microorganism. Most of these media, which are available from commercial sources, have premixed components and require only the addition of water and then sterilization. When it is desirable to grow bacteria on a solid medium, a solidifying agent such as agar is added in a medium. Agar is a complex polysaccharide derived from marine algae.

Properties of Agar

1. Only a few microbes can degrade agar, so it remains solid.
2. Agar liquefies at about 100°C (the boiling point of water) and at sea level remains liquid until the temperature drops to about 40-45C.
3. Agar media are usually contained in test tubes or Petri dishes. The test tubes are called slants. when they are allowed to grow microairofiles with the tube held at an angle so that a large surface area for growth is available. It has two portions a Slant and a Butt.

Chemically Defined Media

A chemically defined medium is one whose exact chemical composition is known.

Organisms that require many growth factors are described as fastidious. Organisms of this type, such as Lactobacillus are sometimes used in tests that determine the concentration of a particular component in a substance.

Complex Media

Complex media made up of nutrients including extracts from yeasts, meat, plants , or digests of proteins from these and other sources.

Composition of Nutrient agar

Peptons : 5 grams

Beef extract: 3 grams

NaCl: 8 grams

Agar: 15 grams

Distal water : 1000 ml(q.s)

Reducing Media

These media contain ingredients such as sodium thioglycolate which chemically combine with dissolved O_2 or deplete O_2 in culture media.

Selective media

Such type of media which suppresses the growth of unwanted bacteria and encourages the growth of desired microbes e.g. MacConkey agar , which contain bile salts which make it selective for intestinal bacteria.

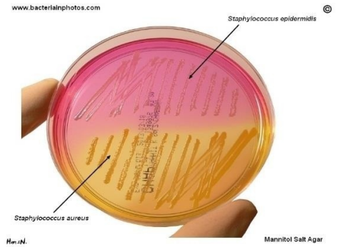
Another example is of Bismuth sulphite agar which is used to isolate gram negative bacteria Salmonella typhi

And Sabouraud's dextrose agar have a pH 5.6 and used to isolate fungi.

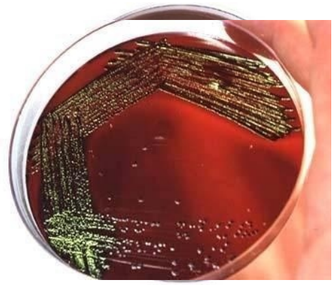
Differential media

Such type of media which makes it easier to distinguish colonies of desired microbes from other colonies growing on the same media. Examples

1. MacConkey agar contain lactose and microbes which ferment lactose appear pink e.g. E.coli. And the non-fermenters appear off-white e.g. Salmonella typhi.
2. On blood agar some bacteria show alpha hemolysis(incomplete hemolysis) while other show beta hemolysis (complete hemolysis) and appear transparent.

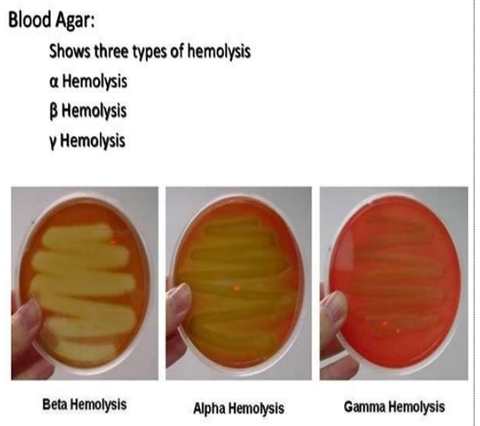


3. On Manitol salt agar Staphylococcus aureus produces yellow colonies while Staphylococcus epidermis produce pink colonies.
4. On eosin-methionine blue agar E.coli produces black colonies with metallic sheen.



While Enterobacter aeroguns' give dark centered colonies without any metallic sheen.

MacConkey agar is selective as well as differential media. It is selective as it only allows enteric bacteria to grow and is differential as E.coli give pink color colonies on it being a lactose fermenter.



Enrichment media

It is usually liquid and provide nutrient and environmental condition which favors the growth

of particular microbes but not others. It is a selective media but designated to increase very small number of desired type organism to detectable level. Example, Rappaport or Selenite F Broth used for enrichment of Salmonella typhi.

General Characteristics of Viruses

The word *virus,* the Latin word for poison,

Viral Size

Viruses range from 20 to 1000 nm in length. Different viruses vary considerably in size. Although most are quite a bit smaller than bacteria, some of the larger viruses.For Example: Adenovirus 90 nm , Poliovirus 30 nm ,

General properties

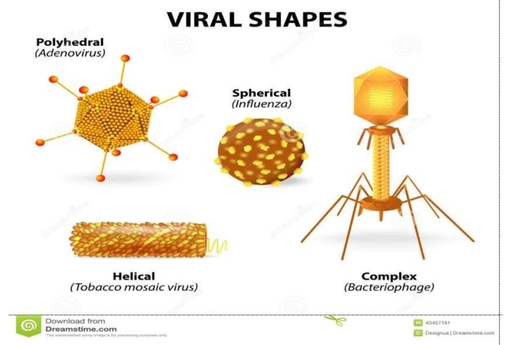
Viruses are inert/crystaline outside the body of host as soon as they come into the host they become living. they are **obligatory intracellular parasites** that is, they absolutely require livinghost cells in order to multiply.

The truly distinctive features of viruses are.

- Contain a protein coat (sometimes itself enclosed by an envelope of lipids, proteins, and carbohydrates) that surrounds the nucleic acid.
- Multiply inside living cells by using the synthesizing machinery of the cell.
- Cause the synthesis of specialized structures that can transfer the viral Contain a single type of nucleic acid, either DNA or RNA.
- nucleic acid to other cells.

Viruses have few or no enzymes of their own for metabolism; for example, they lack enzymes for protein synthesis and ATP generation. To multiply, viruses must take over the metabolic machinery of the host cell.

Viruses are Host Specific



Viruses are host specific There are viruses that infect invertebrates, vertebrates, plants, protists, fungi, and bacteria. However, most viruses are able to infect specific types of cells of only one host species. In rare cases, viruses cross the host-range barrier, thus expanding their host range. The particular host range of a virus is determined by the virus's requirements for its specific attachment to the host cell and the availability within the potential host of cellular factors required for viral multiplication. For the virus to infect the host cell, the outer surface of the virus must chemically interact with specific receptor sites on the surface of the cell.

Viral Structure

Viruses may be Hexagonal, Octagonal or have any other shape and have a 3D structure a **virion** is a complete, fully developed, infectious viral particle composed of nucleic acid and surrounded by a protein coat outside of a host cell, and is a vehicle of transmission from one host cell to another.

Nucleic Acid

A virus can have either DNA or RNA—but never both. The nucleic acid of a virus can be single-stranded or double-stranded.

There are viruses with the familiar:

- double-stranded DNA
- single-stranded DNA
- double-stranded RNA
- single-stranded RNA

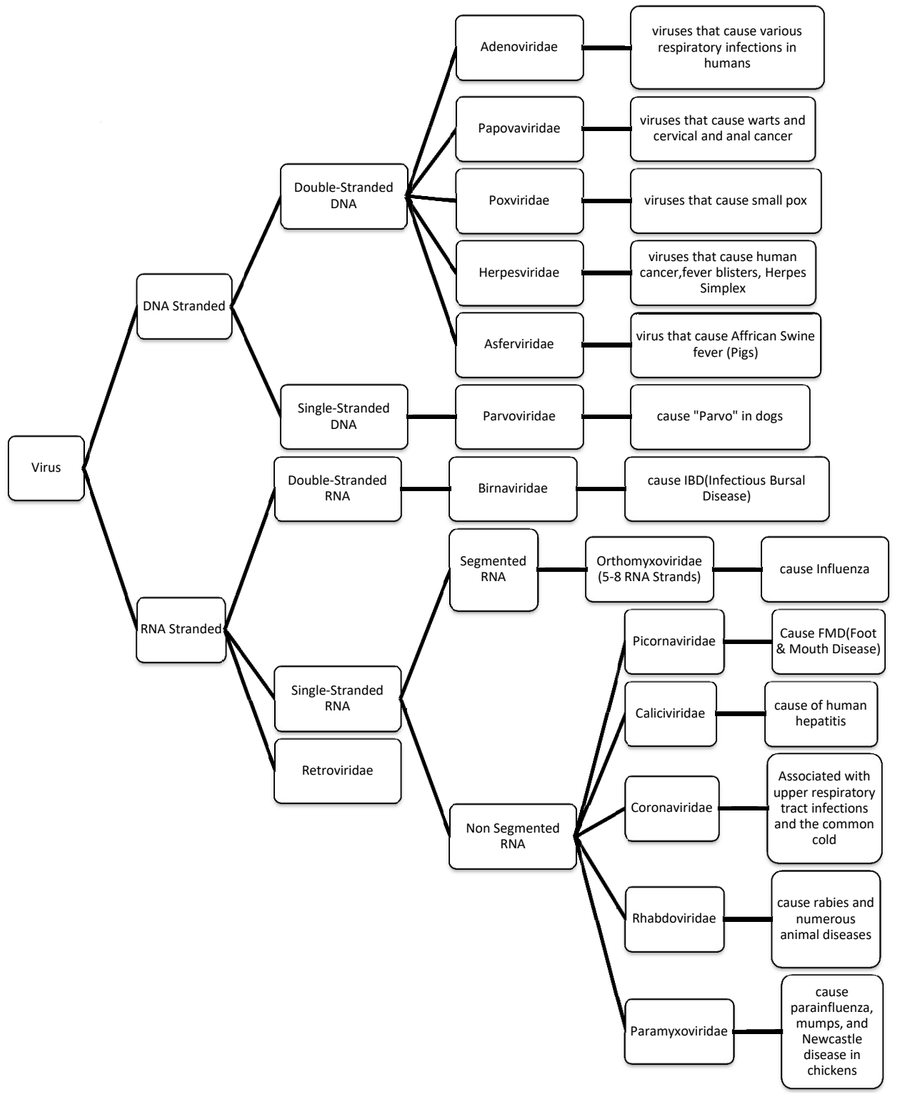
Depending on the virus, the nucleic acid can be linear or circular. In some viruses (such as the influenza virus) the nucleic acid is in several separate segments.

Capsid and Envelope

The nucleic acid of a virus is protected by a protein coat called the **capsid.**Each capsid is composed of protein subunits called **capsomeres.** In some viruses, the proteins composing the capsomeres are of a single type; in other viruses, several types of protein may be present. a particular type of virus. In some viruses, the capsid is covered by an **envelope** which usually consists of some combination of lipids, proteins, and carbohydrates., that is derived from the host cell membrane. Some viruses are not covered by an envelope are known as **Naked** Virus/**non-enveloped viruses.** The capsid of a non-enveloped virus protects the nucleic acid from nuclease enzymes in biological fluids and promotes the virus's attachment to susceptible host cells.

Depending on the virus, envelopes may or may not be covered by **spikes,** which are carbohydrate-protein complexes that project from the surface of the envelope. Some viruses attach to host cells by means of spikes. Spikes are such a reliable characteristic of some viruses that they can be used as a means of identification. The ability of certain viruses, such as the influenza virus to clump red blood cells is associated with spikes. Such viruses bind to red blood cells and form bridges between them. The resulting clumping is called ***hemagglutination***.

Classification of Viruses



Viral Multiplication

Viruses can multiply by two alternative mechanisms: the lytic cycle or the lysogenic cycle. The **lytic cycle** ends with the lysis and death of the host cell, whereas the host cell remains alive in the **lysogenic cycle.**

The lytic cycle

The multiplication cycle of these phages, like that of all viruses, occurs in five distinct stages: **attachment, penetration, biosynthesis, maturation, and release**.

1. **Attachment**

After a chance collision between phage particles and bacteria, *attachment,* or *adsorption,* occurs. During this process, an attachment site on the virus attaches to a complementary receptor site on the bacterial cell. The complementary receptor sites are on the bacterial cell wall.

2. **Penetration**

After attachment virus injects its DNA (nucleic acid) into the cell. To do this, the bacteriophage's tail releases an enzyme, **phage lysozyme,** which breaks down a portion of the bacterial cell wall. During the process of *penetration,* the tail sheath of the phage contracts, and the tail core is driven through the cell wall. The capsid remains outside the bacterial cell.

3. **Biosynthesis**

Once **6**the**7** bacteriophage DNA has reached the cytoplasm of the host cell, the biosynthesis of viral nucleic acid and protein occurs. Host protein synthesis is stopped by virus induced degradation of the host DNA.Viral RNA/DNA is translated to form 3 types of protein.

1. Early Protein
2. Intermediate Protein
3. Late Protein

Early protein shutdown the protein synthesis of host cell it is **Immune Depressor Protein.**

Intermediate Protein starts replication of DNA/RNA & start protein synthesis of virus.

Late Protein starts producing the structural protein i.e. **Capsid of Virus**.

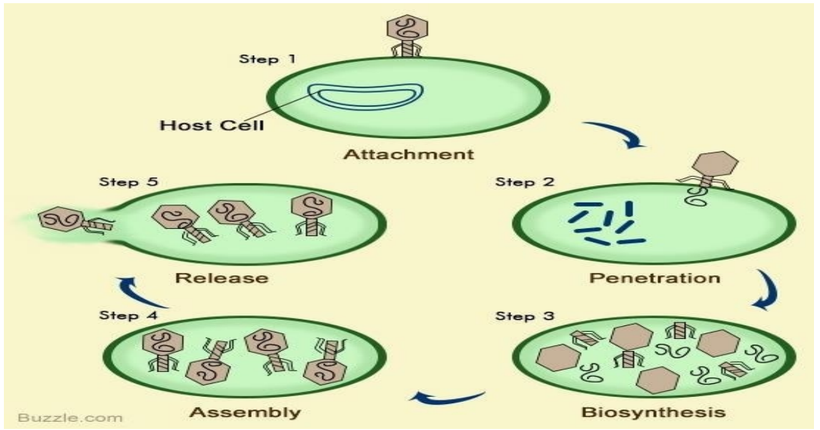
The host cell's ribosomes, enzymes, and amino acids are used for translation. For several minutes following infection, complete phages cannot be found in the host cell. Only separate components— DNA and protein—can be detected. The period during viral multiplication when complete, infective virions are not yet present is called the **eclipse period**

4. **Maturation**

In this process, bacteriophage DNA and capsids are assembled into complete virions. The phage heads and tails are separately assembled from protein subunits, and the head is filled with phage DNA and attached to the tail.

5. **Release**

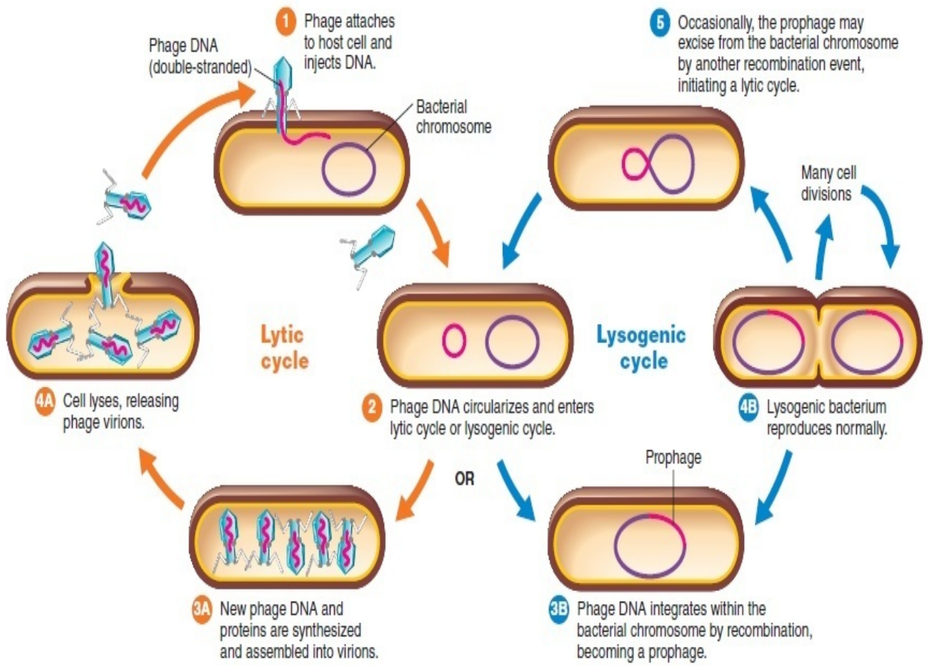
The final stage of viral multiplication is the *release* of virions from the host cell. Lysozyme, enzyme causes the bacterial cell wall to break down, and the newly produced bacteriophages are released from the host cell. The released bacteriophages infect other susceptible cells in the vicinity, and the viral multiplication cycle is repeated within those cells



The Lysogenic Cycle

In contrast some viruses do not cause lysis and death of the host cell when they multiply. These *lysogenic phages* (also called *temperate phages*) may indeed proceed through a lytic cycle, but they are also capable of incorporating their DNA into the host cell's DNA to begin a lysogenic cycle. In **lysogeny,** the phage remains latent (inactive). Every time the host cell's machinery replicates it also replicates the viral DNA. The viral DNA remains latent within the progeny cells.

However, a rare spontaneous event, or the action of UV light or certain chemicals, can lead to the excision (popping-out) of the phage DNA, and to initiation of the lytic cycle



Viral Cultivation

How can we grow Viruses

We must keep following points in mind

1) Host specific, age specific, sex specific
2) Strict(obligate) parasite i.e it need a living organism.
3) E.g to grow polio-virus we need human child(we can not afford)
   a) To grow Avian influenza we need poultry bird of any age.
   b) Equine influenza _ __ __ __ __ we need horses.
   c) Canine distemper we need a dog(puppy).
   d) Faline Leukopenia we need a cat(kitten).

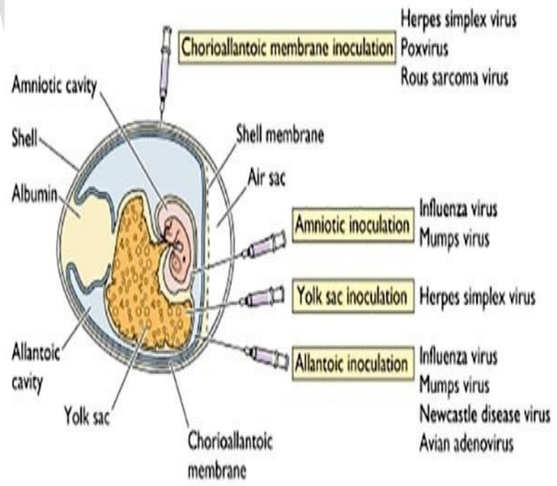
Viruses can spread and can affect other organisms.

The animal to be infected must be specific pathogen free (S.P.E)

We have to build a specific kind of room for them in which every thing which goes in and goes out get filtered.

- Lab animals include
  a) Guinea pig
  b) Albino mice
  c) Rabbits
  d) Poultry

We can grow viruses in eggs which fertilized at the age of 9-11 days.



Eggs do not move and donot

excrete.

Layers eggs can't be used.

Breeder eggs are used because they are incubated for 9 days and following condition appears

After inoculating virus ,incubate the egg at 37^{o}C and 60% humidity for 2-3 days.

- Allentoid inoculation
- CAS/CAM inoculation
- Yolk inoculation

We can infect 0.1-0.2ml of viruses.

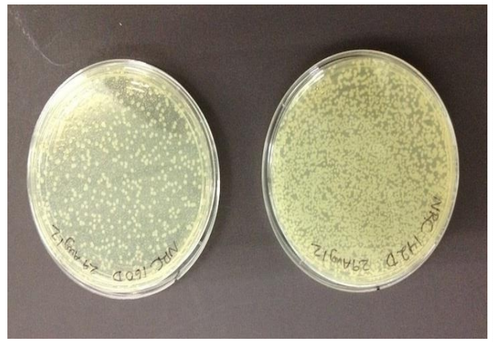
Viruses which we can grow are

Aves virus,NDV,IBN,IBV,IBH and ILH.

- Short egg passage (SEP) and long egg passage (LEP)
- In LEP the virus infect ability is lost and it becomes a vaccine.

Growing viruses in bacteria

For example we have 100 phage viruses. We take 100 petri dishes and make media and grow bacteria in it which forms lawn and place each kind of phage on each dish. When viruses grow then the lawn formed by the bacteria will disappear. Or we can grow bacteria in a large dish and make map on it.



Growing viruses on tissue

Take a tissue ,ground it, then tryspinize it and put it in a solution which contain H_2O , phosphate buffer solution , normal saline solution and nutrients.
We supply air (O_2, CO_2) and then insert viruses.
The bottle in which tissue is placed is called Roux bottle.

- Baby Hamster kidney – FMD virus
- Vero cell line - Rabies virus.
- These cells have no specific receptors for viruses.

Hemagglutination and Elution

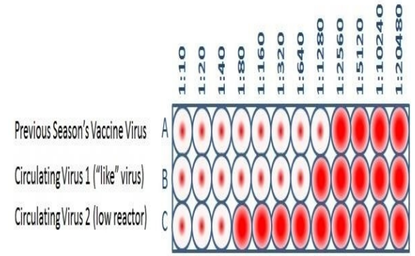
Hemagglutination is a process in which silica acid receptors on the surface of RBCs binds to hemagglutinin glycoprotein found on the surface of influenza virus and other various viruses.

It creates a network or lattice structure of interconnected RBCs and virus particles. The agglutinated lattice maintain the RBCs in a suspended distribution typically viewn as diffused reddish solution.

The formation of lattice depend on the concentration of virus and RBCs when the relative virus concentration is too low the RBCs are not constrained by the lattice and settle to the bottom of well.

The bottom of the well can be

i) Flat
ii) V-shaped (pointed)
iii) U-shaped (rounded)
(1)First of all we put 50 microliter of normal saline solution in A-row.



(2)Then we add 50 microliter of virus/bacteria/HA agent in 1A then mix and take 50 microliter from it and add it to 2(A) and repeat the process.

(3)In this way two fold scrial dilution is produced in each well and hence we can see that to which extent HOA antigen or virus can cause agglutination of RBCs

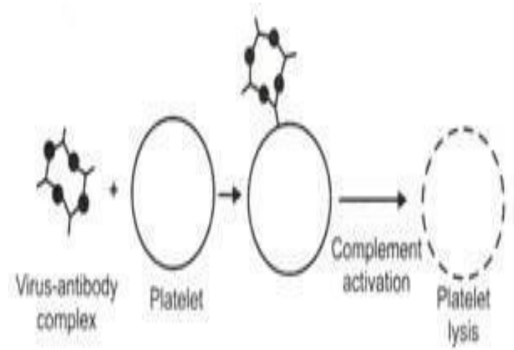
Hemagglutination is observed in the presence of streptococci and other specis similar to the mechanism which virus uses to cause agglutination of RBCs.

The RBCs used in hemagglutination(HA) and hemagglutination inhibition(H.I) are typically from chicken, turkey, horses, guinoe pig or human being depending upon the selectivity of targeted virus. It has been observed that adsorption of virus particles to red blood cells is transitory.

The dissociation of virus particles from RBCs is called Elution.

Hemagglutination and elution depends on environmental temperature , sensitivity of cells, balance of electrolytes, pH of medium and the concentration of components
which is antigen and RBCs.

(in elution the RBCs separate)



Fungus

1. **Yeast:**

- Scientific name:

Sachromyces Cervisae

- Yeasts are unicellular and round in shape.
- 8-10times longer than long bacteria.
- Used to make alcohol,vinegar and lactic acid etc.
- It replicate through Budding having a central Nucleus

2. **Fungus:**

- Fungi are both unicellular and multicellular. The body of fungi consist mostly of three parts i.e. spore, areal hyphae, ground hyphae.
- Size range from micrometer to millimeter.\

Ground Based Hyphae

- A horizontal hyphae.It spread in ground.Its size range few cm to many Km.
- Every hyphae have multiple spores,Ground hyphae are also called **Mycelium.**
- They are whole bed of ground.
- They secrete an exoenzymes that breakdown the food.They are proteolytic and hydrolytic.
- They grow in dead matter and humid environment at 25C temperature
- Present in tropic and subtropic forests.

Every hyphae has a cell wall made up of **Chitin** and have a nucleus that moves upward through diffusion.Cell wall have pores due to which it move and other vacuoles also move through it.

They are false multinucleated i.e. pores are so large that cell wall is not seen.it is also called **Coenocytes.**

Algae

Algae are unicellular, round shaped or multicellular.

- We get agar agar and vetigel from algae.

	Acellular		Prokaryotes			Eukaryotes		Items	Sr.NO.
	Prions	Virus	Bacteria		Fungus	Algae	Protozoa		
	Protein(Chromosomes absent)	DNA or RNA or never both	Single chromosome,some have 2	Chromosome(Multiple)		Chromosome(Multiple)	Chromosome(Multiple)	Genome	1
	Absent	Absent	Present(Important)	Absent		Absent	Absent	Plasmid	2
	Absent	Absent	Tetramer Protein called HU-Proteins	Present		Present	Octomers	Histone Bodies	3
	Absent	Nucleocapsid present	Nucleoid present	Multiple nucleus		one nucleus	one nucleus	Nucleus	4
	Absent	Host dependent transcription and translation	Trascription and translation at same time	Present		Present	Nucleus>Transcription,Cytoplasm>Translation	Transcription/Translation/Central Dogma	5
	Absent	Host dependent	70s	Present		Present	80s	Ribosomes	6
	absent	Absent	absent	Present		Present	Present	Membrane bound organelles	7
	absent	Absent	present	Present		Present	Present	Inclusion bodies	8
	Absent	Non-metabolic	Various	Saprotrophes		Autotrophes	Heterotrophes	Eating habits	9
	Absent	Absent	present	Present		present	present	Metabolic enzymes	10
	Absent	present	present	Present		Present	Present	Replicating enzymes	11
	Photocopy	Host dependent	Binary fission	Single state mitosis		present	mitosis, meosis	Cell division	12
	Non-motile	Non-motile	Varied	Present		Present	Present	Motility	13
	Absent	Absent	absent	present		Present	Present	Cytoskeleton, fibrils	14
	Absent	Absent, capsid present	peptidoglycins	chitin		cellulose	Absent	Cell wall	15
	Absent	absent	False,varied with metabolic enzymes	Present		Present	Present, true	Cell membrane	16
	absent	absent	endospore(one bacteri one spore)	multiple spores		Absent	Absent	Spores	17
)	absent	absent,peptomers present	single fiber	present		present	present	flagella, cilia	18
	Proteins	acellular	unicellular	uni and multicellar		multicellular	single	Organization	19

DNA Replication

In DNA replication one parental DNA molecule is converted into two identical daughter molecules. As DNA is a double stranded molecule. the complementary structure of nitrogenous bases in the DNA molecule is key to understand DNA replication. As the bases along the two strands of double helical DNA are complementary, one strand acts as a template for the production of the other strand. Enzymes required in DNA replication are:

DNA Gyrase: it relaxes the super coiling of ahead of replication fork.

DNA Ligase: it joins the oka-zaki fragments and makes covalent bond to join DNA strands.

DNA polymerase: it synthesizes DNA and also it proof reads and repair DNA molecule.

Helicase: it unwinds double stranded DNA molecule.

Primase: makes RNA primase from DNA template.

Topo isomerase: it relaxes super coiling a head of replication fork and also separate DNA circles at the end of DNA replication.

When replication begin the super coiling is relaxed by topo isomerase or Gyrase and two strands of parent DNA are unwound by helicase and separated from each other by one small DNA segment. Free nucleotides present in the cytoplasm of the cell are matched up to exposed bases of single stranded parental DNA. Where thymine is present on original strand only Adenine can fit into place on new strand and where guanine is present on the original strand only cytosine can fit into place and so on.

Any bases that are improperly paired are removed and replaced by replication enzyme called DNA polymerase. Once aligned the newly added nucleotide is joined to the growing strand of DNA. By an enzyme called DNA polymerase. The parental DNA unwound a bit further to allow the addition of next nucleotide. The point at which replication occurs is called replication fork. As the replication fork moves along the parental DNA. Each of the unwound single strands combines with new nucleotide. The original strand and this newly synthesized daughter strand then rewinds because each new double stranded molecule contain one original conserved strand and one new strand. The process is referred as **semi-conservative replication.**

Leading strand is synthesized continuously as the DNA polymerase moves toward the replication fork making DNA in 5’ to 3’ direction.

Lagging strand is synthesized in pieces consisting of about 1000 nucleotides, called as oka-zaki fragments, as the DNA polymerase moves away from the replication fork.

- DNA polymerase converts the RNA primer into DNA strand.

Transcription

Transcription is the synthesis of complementary strand of RNA from DNA. Three types of RNA are rRNA , mRNA and tRNA . Ribosomal RNA forms an integral part of ribosomes, which are cellular machinery. Transfer RNA contains anticodon to one end & specific amino acid to the other end it is involved in translation. mRNA carries the coded information for making specific protein from DNA. During translation a strand of mRNA is synthesized using a specific gene or DNA as template. In other words genetic information stored in sequence of nitrogenous babes of DNA is rewritten so that the same information

appears in the base sequence of mRNA. The base sequence will be as follow. If **Guanine** is in base template then in mRNA it will be replaced by **Cytosine**. If **Cytosine** is DNA template it will be replaced by **Guanine**. If there is **Thymine** in DNA template it will be replaced by **Adenine**. If there is **Adenine** in DNA template it is replaced by **Uracil.**

The process of transcription requires an enzyme called RNA Polymerases & a supply of RNA nucleotide. Transcription begins when RNA polymerase bind to the DNA a specific site called promoter region. Only one of the two DNA strands serves as a template strand for RNA synthesis for a given gene. Like DNA, RNA is synthesized in 5'-3' direction. RNA polymerase assembles free nucleotide into a new chain using complementary base paring as a guide. As the new RNA chain grows RNA polymerase moves along the DNA. RNA synthesis stops when RNA polymerase reaches a site on the DNA called Terminator region. After this RNA polymerase & newly formed single stranded mRNA are released from the DNA. The process of transcription allows cell to produce short term copy of gene that can be used as direct source of information for protein synthesis mRNA act as an intermediate between the permanent storage from DNA & the process which uses this information for protein synthesis called Translation.

Translation

Protein synthesis is called **translation.**It involves decoding the "language" of nucleic acids and converting that information into the "language" of proteins.

Codon

Codon is defined as the group of three nucleotides, such as AUG, GGC, or AAA. The language of mRNA is in the form of codons. The sequence of codons on an mRNA molecule determines the sequence of amino acids that will be in the protein being synthesized. Each codon "codes" for a particular amino acid. Codons are written in terms of their base sequence in mRNA. There are 64 possible codons but only20 amino acids.

Degeneracy.

Most amino acids are signaled by several alternative codons, a situation referred to as the **degeneracy** of the code. For example, **leucine** has six codons, and **alanine** has four codons. Degeneracy allows for a certain amount of change, or mutation, in the DNA without affecting the protein ultimately produced

Sense codons

Those codons which code for amino acids known as **Sense codons.** Of the 64 codons, 61 are sense codons,

Nonsense codons

Nonsense codons not code for any amino acid 3 are nonsense codons. (also called *stop codons*) The nonsense codons UAG—UAA, UGA—signal the end of the protein molecules synthesis.The start codon that initiates the synthesis of the protein molecule is **AUG,** which is also the codon for **Methionine.** In bacteria, the start AUG codes for **Formylmethionine.** The site of translation is the **ribosome**, and **transfer RNA (tRNA)** molecules both recognize the specific codons and transport the required amino acids. Each tRNA molecule has an **anticodon,** a sequence of three bases that is complementary to a codon. A tRNA molecule can base-pair with its associated codon. The ribosome moves along the mRNA in the 5′ : 3′**direction.** As a ribosome moves along the mRNA, it will soon allow the start codon to be exposed Additional ribosomes can then assemble and begin synthesizing protein. In this way, there are usually a number of ribosomes attached to a single mRNA, all at various stages of protein

synthesis. In prokaryotic cells the translation of mRNA into protein can begin even before transcription is complete

Genetic Engineering

Biotechnology

It is the use of microorganism, cell or cell components to make products. Microbes have been used in commercial production of food, vaccine, antibiotics and vitamins for years.

Recombinant DNA technology

Microorganisms as well as entire plants are used to produce chemicals which that specific organism can produce naturally. This was made possible by inserting gene into cell by recombinant DNA technology, sometimes called as genetic engineering. The development of recombinant DNA technology is expanding the practical application of biotechnology beyond imagination, As recombination of DNA occurs naturally in microbes, in 1970s and 1980s scientist developed artificial technology for making recombinant DNA. A gene from vertebrate animal including humans can be inserted into the DNA of bacterium or a gene from virus can be inserted into yeast. Then the gene is expressed in recipient which code for a commercially useful product. A bacteria with a gene for human insulin are now being used to produce insulin for treating diabetes. Vaccine for hepatitis B is being made by yeast carrying a gene for parts of hepatitis virus. Viral protein coats are produced by recombinant DNA technology from yeast. Scientist hope that such approach may prove useful in producing vaccine against other infectious agents thus eliminating the need to use whole organism as in conventional vaccines.

Procedure of Recombinant DNA Technology

1. A vector such as plasmid is isolated from the bacterium.
2. DNA containing the gene of interest is cleaved into fragments by enzyme called restriction endonuclease.
3. Gene is inserted into the plasmid.
4. Plasmid is taken up by the cell to make component cell.
5. Cell with gene of interest can be cloned.
6. Now there are two possibilities.
    - Goal is to make copies of gene, the copies of gene will be harvested e.g. a gene of interest can be inserted into a bacteria which can clean up different ponds or lakes or they can be introduced in plants to develop resistance against pests.
    - Goal is to make protein product of gene. the produced protein are harvested from bacterial biomass e.g. amylase, cellulose and other such enzymes prepare fabrics for manufacturing clothes and human growth hormone which treats shunted growth.

Mutation

It is a change in the base sequence of DNA. Such change in the base sequence of a gene will some time cause a change in the product encoded by that gene e.g. When a gene for an enzyme mutates the enzyme encoded by the gene may become inactive or less active because its amino acid sequence has changed.

Types of mutation

Base substitution or point mutation

At one point a single base is replaced in DNA sequence by a different base. When this DNA replicates it results in substituted base pairing. It has two types.

Missense mutation

if a base substitution results in amino acid substitution in the synthesized protein, this change is known as missense mutation**.** The effect of such kind of mutation can be dramatic e.g. sickle cell disease is caused by a single change in the gene of globin which is a protein component of hemoglobin. A change from A to T at a specific site results in change from glutamic acid to valine in the protein. The effect is that the shape of the hemoglobin molecule changes under low O_2 altering the shape of the RBCs such that the movement of cell though the small capillaries is greatly impeded.

Non sense mutation

if base substitution results in the creation of nonsense or stop codon in the middle of mRNA molecules thus it stops protein synthesis. This type of base substitution is known as nonsense mutation.

Frame shift mutation

If one or few nucleotides are deleted or inserted in the DNA then it can shift the translation reading frame e.g. deleting one nucleotide pair in the middle of a gene cause change in many amino acids downstream from the site of mutation. Frame shift mutation always result in a long stretch of altered protein or amino acid and the production of an inactive protein from a mutated gene.

Mutagens

Agents in the environment such as certain chemicals and radiations which directly bring about mutation are called mutagens.

Chemical Mutagens

One of the many chemicals known to be mutagen is Nitrous acid. Exposure of DNA to nitrous acid can convert the base adenine to a form that no longer pairs with instead it pairs with cytosine. When DNA containing such modified adenine replicates one daughter DNA molecule will have base pair sequence different from that of parent DNA eventually some AT base pairs of parent will have been changed to GC base pair in the grand daughter cells. Nitrous acid makes a specific base pair change in DNA like all mutagens it alter DNA at random locations. Another type of chemical mutagen is nucleoside analogue. These molecules are structurally similar to normal nitrogenous base but they have slightly altered base paring property. For example 2-amino protein and 5-bromouracil are analogue to adenine and thymine nucleotide respectively. Aflatoxins produced by Aspergillus flavous is a frame shift mutagen

Radiations

x-rays and gamma rays are form of potent mutagens because of their ability to ionize atoms and molecules the penetrating rays of ionizing radiations cause electrons to pop out of their usual cells. These electrons bombard other molecules and cause more damage. Many of the resulting ions and free radicals are very reactive. Another form of mutagenic radiations is UV light a non-ionizing component of ordinary sunlight. The most important effect of direct UV light on DNA is the formation of harmful covalent bond between certain bases adjacent T in DNA strand can cross link to form T dimers unless repaired may cause serious damage or death to the cell because it can not properly transcribe or replicate such DNA.

Vaccination

A **vaccine** is a suspension of organisms or fractions of organisms that is used to induce immunity.

In 1798 Edward Jenner inoculated people with cowpox in an attempt to prevent smallpox. Cowpox is a mild disease that causes lesions on cows' udders; dairymaids' hands often became infected during milking and after this they become immune to Small pox. To honor Jenner's work, the term *vaccination* (from the Latin ***vacca*****,** meaning cow) was coined. Two centuries later, the disease of smallpox has been eliminated worldwide by vaccination, and two other viral diseases, measles and polio, are also targeted for elimination.

Principles and Effects of Vaccination

In a vaccination against small pox The injection/by skin scratches, provoked a primary immune response in the recipients, leading to the formation of antibodies and long-term memory cells. Later, when the recipient encountered the smallpox virus, the memory cells were stimulated, producing a rapid, intense secondary immune response. Many communicable diseases can be controlled by behavioral and environmental methods. For example, proper sanitation can prevent the spread of cholera, and the use of condoms can slow the spread of sexually transmitted infections. Viral diseases, however, often cannot be effectively treated once contracted. Therefore, vaccination is frequently the only feasible method of controlling viral disease.

Types of Vaccines

There are now several basic types of vaccine. Some of the newer vaccines take full advantage of knowledge and technology developed in recent years.

- **Live Attenuated Vaccines**

In this type of vaccine Live Attenuated viruses are used to develop immunity against a specific disease. In the autumn of 1881 Louis Pasteur used the culture that had been left on the bench during the summer to inoculate some chickens to cause cases of chicken cholera, the birds remained healthy. A fresh culture of the pathogen was then used to inoculate these birds, but, surprisingly, they remained healthy. Pasteur concluded that the culture left on the bench that summer had been weakened and was now unable to cause disease—but had rendered the chickens immune.

Live vaccines more closely mimic an actual infection. Lifelong immunity, especially in the case of viruses, is often achieved without booster immunizations, this long-term effectiveness probably occurs because the attenuated viruses replicate in the body, increasing the original dose and acting as a series of secondary (booster) immunizations.

- **Inactivated Killed Vaccines**

Inactivated killed vaccines use microbes that have been killed, usually by formalin or phenol. Inactivated virus vaccines used in humans include those against rabies, influenza and polio. Generally speaking, these vaccines are considered safer than live vaccines. Compared to live attenuated vaccines, these inactivated vaccines often require repeated booster doses. They also induce a mostly humoral antibody immunity, which makes them less effective than attenuated vaccines in inducing cellular immunity.

- **Conjugated Vaccines**

Conjugated vaccines have been developed in recent years to deal with the poor immune response of children to vaccines based on capsular polysaccharides. polysaccharides are T-independent antigens; children's immune systems do not respond well to these antigens until the age of 15 to 24 months. Therefore, the polysaccharides are combined with proteins such as diphtheria or tetanus toxoid; this approach has led to the very successful vaccine for ***Haemophilus influenzae*** type b (Hib), which gives significant protection even at 2 months.

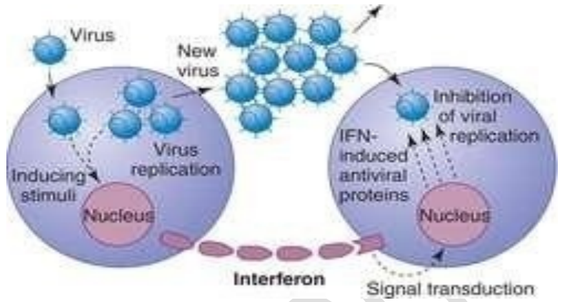
Interferon

As viruses depend on their host cell to produce many function of viral multiplication. So, it is difficult to inhibit viral multiplication without affecting host cell itself. One way by which infected host cell counter viral infection is with a family of cytokines called interferon.

Interferons are a class of similar antiviral protein produces by certain animal cells such as lymphocytes & macrophages after viral stimulation. One of principal function of interferon is to interfere with viral multiplication. All interferon are small protein with weight between 15000-30000 Daltons. They are quiet stable at low pH & fairly resistant to heat. There are three types **α, β, ϒ.**

ϒ-interferon is produced by lymphocytes. It includes neutrophils & macrophages to kill bacteria. ϒ Interferon causes macrophages to produce nitric oxide which kills bacteria as well as tumor cell by inhibiting ATP production.

Antiviral Action of Interferon (α &β)



Viral genome enters into host cell infecting virus replicates into new viruses. Mean while infecting virus also induces host cell to produce interferon mRNA which is translated into interferon α and interferon β. Interferon released by virus infect host cell , bind to plasma membrane receptors on uninfected neighboring host cell inducing cell to synthesize antiviral protein.

New viruses released by viruses infect host cell infect the neighboring cell .Antiviral protein degrade viral genome & inhibits protein synthesis and interfere with viral replication.

An interesting feature of interferon is that, they are host cell specific, not virus specific. Interferon produced by human cell protect human cell but produces little antiviral activity for cells of other species i.e. mice or chicks. However interferon of a species is active against a number of different viruses.

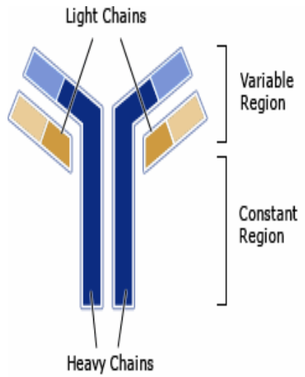
Mode of action of interferons

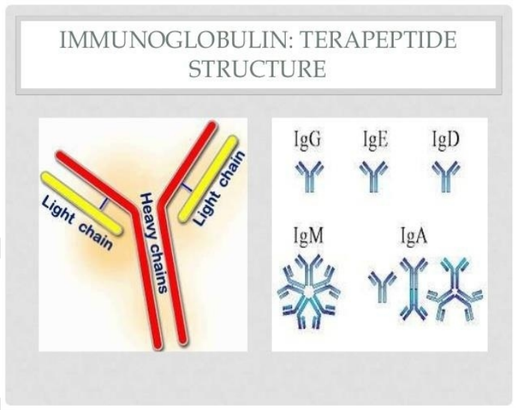
Interferons are produced in the nucleus and transported through cytoplasm out of the cell and enter the surrounding cells. There are three types of interferons ***Translation blockers***, ***Transcription blockers*** and ***replication blockers***. So when they enter the surrounding cells they block the above three cell processes and hence do not allow the viruses to replicate in the surrounding nearby cells.

Interferons can also be used for long term interferon therapy for viral diseases.

Antibodies

Antibodies are produced by the plasma proteins and travel through blood to the target and attaches to the antigen and stops its activity. Antibodies are composed of variable and constant fragments. Variable fragments of antibodies are different for each kind of antigen.





Interferons VS Antibodies

8. Interferons block genetic codes.	• Blocks the antigen synthesis.

Interferons:	**Antibodies:**
1. They are generalized in their action e.g. interferons for NDV virus also block other viruses.	• They are highly specific in their action. E.g. NDV antibody controls NDV virus only.
2. Interferon is a part of the infected cell.	• Antibodies are formed by plasma cells.
3. They are produced in small amounts.	• They are produced in large numbers.
4. They are chemical mediators.	• They are Igs and are "Y" shaped.
5. Present in the intercellular spaces.	• Present in the whole body, blood, tissue, mucous membrane.
6. They are classified as α, β and γ.	• They are classified as IgG, IgD, IgM, IgE, and IgA.
7. They do not bind to the antigen but enter the surrounding cells.	• They do not enter the cell but bind the antigen.

Practical portion

Experiment NO.1

Biosafety Measures in Microbiology Laboratory

1. Only authorized persons are allowed n Laboratory.
2. Students without Lab coat are not allowed to enter in the Lab.
3. Stay in laboratory only during practical work.
4. Before operating equipments,carefully read its manual.
5. Always follow teacher's instructions during practical work in lab.
6. Before and after working in laboratory must wash your hands.
7. Don't try to taste and smell any chemical or media in laboratory.
8. Use personal protective equipment (PPE) during lab work to avoid contamination.
9. Don't wash or reuse disposable gloves.
10. Mouth pipetting is prohibited,instead use mechanical devices for pipetting.
11. Eating,drinking,smoking,handling contact lenses are prohibited durin lab work.
12. Use of cosmetics and high heels is prohibited in lab.
13. In case of any injury/accidents/splashes immediately inform supervisor.
14. Carefully handleinstruments especially sharp objects,needles,broken glasswares to reduce chances of injury.Don't handle it directly,remove them with brush.
15. Decontaminate work surfaces with proper disinfectant fter completion of work or in case in case of any splashes of infectious material.
16. Decontaminate(Auto clave) all cultures, stocks & other potentionally infectious material before disposal.

Experiment NO.02

List of Micro Lab Instruments:

1. Autoclave

An Electric or Automatic Machine used to sterilize Equipment by killing microbes at 121^{o}C ,15psi and for 15min.

About 99% microbes die at this temperature. Although this temperature is adjustable.

2. **Hot Air Oven**

This is also used to sterilize Equipments at High temperature Ranging from 140-180^{0}C.

This is also used as Desiccator.

3. **Incubators (temp.range 20-40^{o}C)**

i. **BOD Incubator**

- This is used to Grow Microbes(Bacteria,Fungi etc) at Required Oxygen demand.

ii. **CO_2 Incubator**

- Used to Grow Microbes in the absence of oxygen ,so in place of oxygen ,nitrogen&CO_2 is provided

4. **Refrigerator fridge**

- Used to store things at cold temperature

5. Microwave Oven

- Used to heat substances via using radiations.

6. **Water Bath**
   - Used to heat some chemicals
   - We kept agar in water bath so that it don't solidify.Temp. range 20-100^{o}C.
7. **Safety Cabinet/Laminar air flow cabinet.**
   - It provide sterilized area for culturing.
   - In cabinet the air don't contaminate culture or performer with germs because air is filtered by High efficiency particulate filters. The size of pore in filter is 2 microns.
8. **Microscope**
   - Used to observe microbes at 4x,10x,40x and 100x of objective lenses.
9. **Cedar Wood Oil**
   - This is a liquid that is used to aid in seeing objects at 100x in microcope.

8. PH Meter

- Use to observe the pH and temperature of liquid

10. **Weight Balance**
    - Used to weigh of sample in milli,micro grams.
11. **Petri Plate**
    - Used to grow Microbes on it.
12. **Colony Counter**
    - Used to count microbes grown on petriplate.
13. **Bunsen Burner**
    - Used to provide a hot environment for culturing,
14. **Vertical Gel Apparatus**
    - Used to check protein/DNA type via Gel Electrophoresis.
15. **Sonicator**
    - Used to kill bacteria using sonicator waves.
16. **Stains**
    - Used to dye/color slide sample
17. **Micropettes**
    - Used to pick samples in very small quantity in microns.
18. **Vortex Mixer**
    - Used to mix different samples in test tube.
19. **Desiccator**
    - Used to dry sample or evaporate water from sample
20. **Lab Animals**
    - Are used to test the affect of different microbes and chemicals on them.

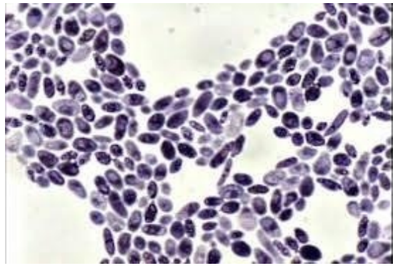
Experiment NO.02

Preparation of slides:

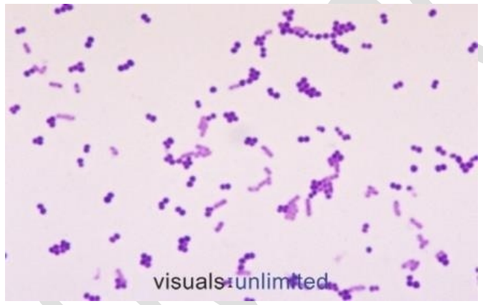
Steps involved

1. Drop the sample on slide
2. Spread the drop with the help of platinum loop
3. Heat fix the slide
4. Primary staining
5. Wait for 30 sec
6. Wash the slide
7. Dry on flame
8. Microscopy

Slide of yeast:



Slide of cocci and bacillus:



Experiment NO.04

Agar Making

Instruments Required

Flask,petridish,autoclave machine,stirrer etc.

Procedure

1. Agar is basically a solid substance that is ground to a powder and then mixed with water to make usually a Nutrient Agar
2. Usually 28g/liter of solution is required but to make 100ml of solution we require 2.8g agar.
3. This 100ml solution is then autoclaved in autoclave machine.
4. After autoclavation solution is cool down to 45-55C temperature.
5. Then this solution is poured on a petriplate.

Sterilization process

1. Auto-claving
2. Hot Air Oven

Autoclave

If instruments are to be reused then autoclave is used.

1. Lubricate all instruments which have any "metal to metal" action such as scissors, hemostats, needle holders, self-retaining retractors, petriplates etc.
2. Recommend surgical lubricants such as instrument milk are best. Do not use oil or other industrial lubricants.
3. Then put instrument in a autoclave machine at 15psi, 121C for 15min.
4. All microbes (about 99%) died in autoclave machine.

Caution

At the end of the autoclave cycle – before the drying cycle – unlock autoclave door and open it more than a crack (about 3/4").

Then run dry cycle for the period recommended by the autoclave manufacturer.

If the autoclave door is opened fully before the drying cycle, cold room air will rush into the chamber, causing condensation on the instruments. This will result in water stains on instruments and also cause wet packs. If you have any unusual staining on your instruments during sterilization, contact your local instrument representative.

Hot Air Oven

This is also used for sterilization

1. The instrument is firstly washed with water.
2. Then it is wrapped in covering of paper.
3. Then it is put into hot air oven at 180C for 3-4hours.
4. All microbes (about 99%) died in hot air oven.

Experiment No. 5

Simple Staining

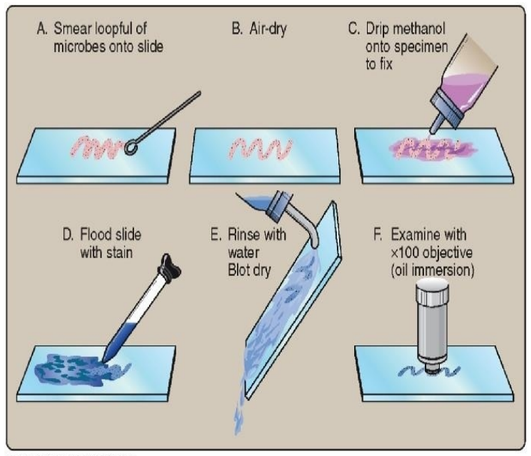
Objective:

The refrective index of sample layer is nearly equal to that of glass slide and the sample vision is not clear when seen in microscope so staining is used to increase refrective index of sample layers.

Only one dye is used called principle dye or primary dye.

Procedure

1) Take slide and put a sample.
2) Heat fix it(protein will coagulate and sample stick to the slide).
3) Add dye or stain and wait 30sec-1min.
4) Wash with water.
5) Air dry,heat dry,bloat dry.
6) Observe in microcope.



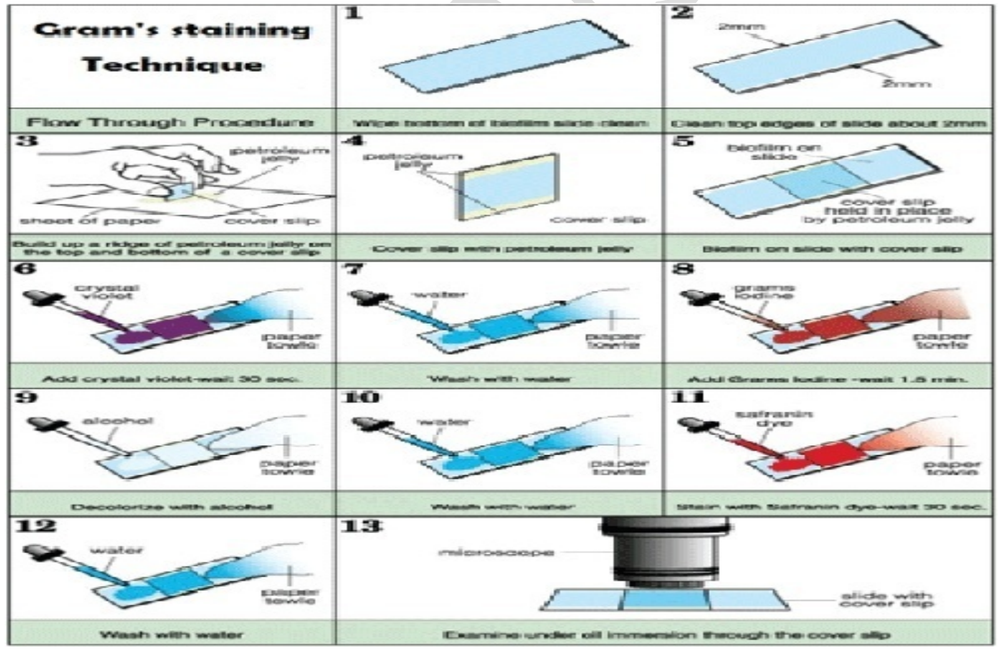
Experiment No.6

Gram staining

I. Take a slide.
II. Put a drop of water.
III. Put bacterial colony.
IV. Heat fix it.
V. Add crystal violet stain(principle stain) and wait for 1 min and wash.
VI. Add ethyl iodine(mordant) and wash.
VII. Add ethyl alcohol(decolorizer) ,wait for 10-15sec and wash.
VIII. Add carbol fucksin or saphranin(secondary dye),wait for 30sec and wash.
IX. Then air dry, bloat dry,heat dry.
X. Observe in microscope

Experiment No.7

Sample of Morbid Bacteria



1) Blood is used as sample

i) Is septicemic form
ii) or As it contain obligatory pathogens.

Blood collection sites

Mice	tail cut
Dog/cat	saphanous vein or cephalic vein
Cow/buffalo/sheep/goat	juggler vein or coxygeal vein
Horse	juggler vein
Chicken	wing vein

Blood is collected in heparanized vaccutainers.

We transport blood in cold form(4C)

We should use sterilized equipment's.

Affected organ

These affected organs are transported at 4c.
We add formalin ,water ,normal saline/phosphate buffer saline to preserve affected organ.

If we have to observe organ then we section it.

If we have to observe bacteria then we add pbs,saline etc.

If we have to observe virus then we add Antibiotic and Antifungal because majority of bacteria suppress virus activity.

3) excretion/urine/feaces.
4) Secretions/eye/lacrimal/saliva/nasal/tonsilar mucous
5) Cavities/pleural cavity/peritonial cavity/cerebrospinal cavity.

Mostly we collect sample when diseased case or postmortem case occur.

Sterilization

Sterilization is the removal or destruction of life of any kind.

Methods of Sterilization

- Flame sterilization
- Incarnation
- Heat sterilization
- Boiling sterilization
- Pasteurization
- Sonicated sterilization
- Use of U.V
- Filtration
- Freeze and throwing

(1) Flame sterilization;

Flame has three parts

I. Lower part contains unburn gases
II. Upper part is hottest in which platinum loop is placed
III. Middle part is not so hot

(2) Incarnation

There are two type of inceration

I. Open
II. Close

In open inceration all things are burned in open aera. In this way environment is polluted.
In close inceration closed incerator are used.These incerator may be..

I. Electrical
II. Gaseous

Principle of Incarnation

- Burning
- Dehydration
- Coagulation of protien
- Redox reaction

(3) Heat

One of the most common methods of food preservation

- Heat is usually used to sterilize laboratory media and glassware and hospital instruments.
- Heat appears to kill microorganisms by denaturing their enzymes

Moist Heat Sterilization

Moist heat kills microorganisms primarily by coagulating proteins (denaturation), which is caused by breakage of the hydrogen bonds that hold the proteins in their three-dimensional structure. This coagulation process is familiar to anyone watched an egg white frying.

Autoclaving is the preferred method of sterilization,

In autoclave steam at a pressure of about 15 psi (121°C) will kill *all* organisms and their endospores in about 15 minutes.

Autoclaving is used to sterilize culture media, instruments, dressings, intravenous equipment, applicators, solutions, syringes, transfusion equipment etc

Dry Heat Sterilization

- For dry heat Hot air oven is used.
- Time and temperature of autoclave and Hot air oven depend upon Surface area Amount of organic and inorganic mater

Depth of sterilization

Principle

- Dehydration
- Coagulation of proteins

(4) Boiling sterilization

One type of moist heat "sterilization" is boiling, which kills vegetative forms of bacterial pathogens, almost all viruses, and their Endospores. Some viruses, however, are not destroyed for example hepatitis and some bacterial endospores Boiling is therefore not always a reliable sterilization procedure. However, brief boiling, even at high altitudes, will kill most pathogens. The use of boiling to sanitize glass baby bottles is a familiar example.

(5) Pasteurization

Louis Pasteur found a practical method of preventing the spoilage of beer and wine. Pasteur used mild heating, which was sufficient to kill the organisms that caused the particular spoilage problem without seriously damaging the taste of the product. The same principle was later applied to milk that we now call pasteurization Products other than milk, such as ice cream, yogurt, and beer, all have their own pasteurization times and temperatures

High Temperature Short-Time (HTST) Pasteurization

Most milk pasteurization today uses temperatures of at least72°C, but for only 15 seconds. This treatment, known as **high temperature short-time (HTST) pasteurization**.Milk can also be sterilized—something quite different from pasteurization—by **ultra-high-temperature (UHT) treatments.**It can then be stored for several months without refrigeration

(6) Use of U.V

- UV light damages the DNA of exposed cells by causing bonds to form between adjacent pyrimidine bases, usually thymine's, in DNA chains.These *thymine dimers* inhibit correct replication of the DNA during reproduction of the cell. The UV wavelengths most effective for killing microorganisms are about 260 nm; these wavelengths are specifically absorbed by cellular DNA.
- UV radiation is also used to control microbes in the air.
- UV is commonly found in hospital rooms, nurseries, operating rooms, and cafeterias.

- UV light is also used to disinfect vaccines and other medical products.
- A major disadvantage of UV light as a disinfectant is that the radiation is not very penetrating,so the organisms to be killed must be directly exposed to the rays

(7) Filtration

F*iltration* is the passage of a liquid or gas through a screen like material with pores small enough to retain microorganisms

High-efficiency particulate air (HEPA) filters remove almost all microorganisms larger than about 0.3 µm in diameter

(8) Freezing and Thawing

Freezing and Thawing also kill the microbes.

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