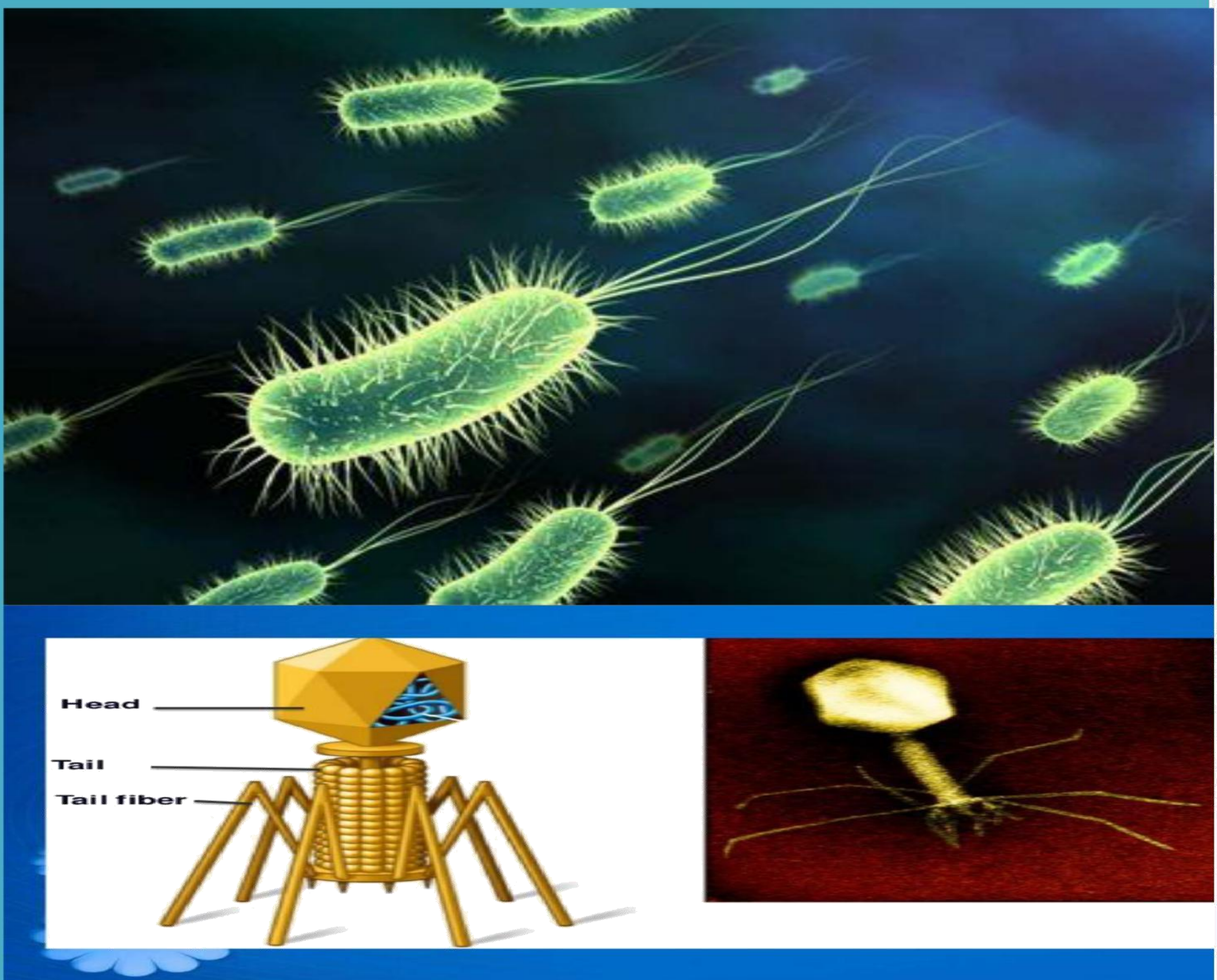
EASY & CONCISE APPROACH TO

MICROBIOLOGY

FIRST-EDITION 2013



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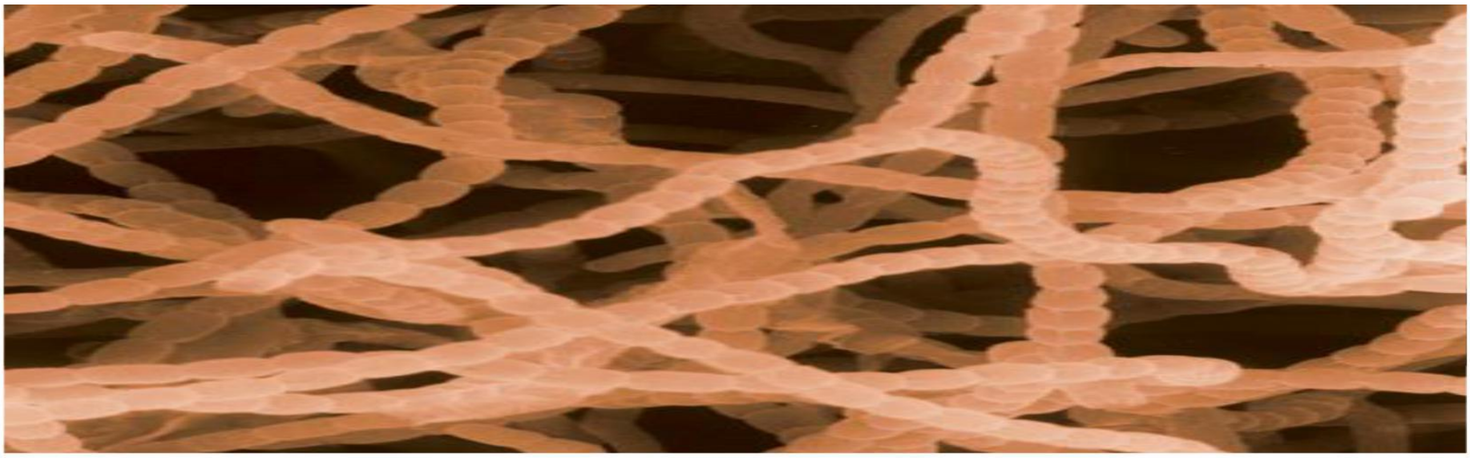
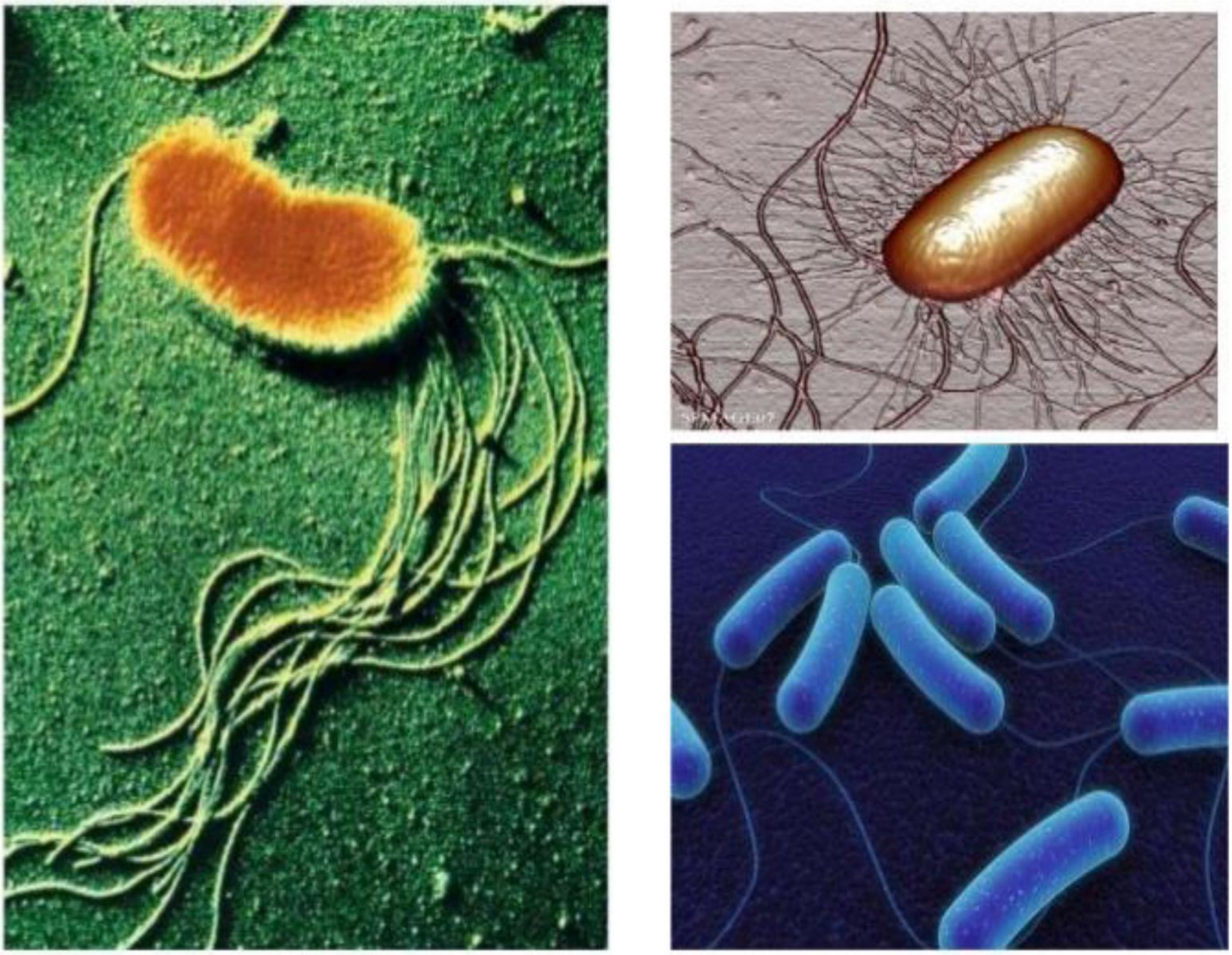
PUNJAB PHARMACY COUNCIL, LAHORE



E. Coli – what is the long thing at the end of each bacterium called?



PREFACE

MICROBIOLOGY IS EMERGING AS KEY BIOLOGICAL SCIENCES.THE FIRST EDITION OF EASY&CONCISE MICROBIOLOGY HAS OBJECTIVE TO PROVIDE THE READER WITH A BASIC UNDERSTANDING OF THE FUNDUNDAMENTALS OF MICROBIOLOGY THAT CAN BE APPLIED IN FURTHER RESEARCH .MICROORGANISMS AND THEIR ACTIVITIES ARE INCREASINGLY CENTRAL TO MANY OF THE CONCERNS OF SOCIETY BOTH NATIONALY AND INTERNATIONALLY.THE RECOGNITION OF THE NEED TO RECYCLE THE NATURAL RESOURCES,THE DISCOVERY OF RECOOMBINANT DNA,ROLE OF MICROBIOLOGY IN DIAGNOSIS OF MANY HUMAN AILMENTS AND RESULTING HIGH TECHNOLOGY OF GENETIC-ENGINEERING,AND MANY OTHER DEVELOPMENTS HAVE PLACED MICROBIOLOGY IN THE LIMELIGHT.

Dedication

This book is dedicated to specially to mine
***Parents** for their dedicated parternership for success in my life and **Sir.Javed Iqbal** ,who guide and cooperated with me to have time for the compilation of the book.*

Dr.Rabia Tabassum

To succed....You need to find something to hold on to, something to motivate you, something to inspire you.

CONTENT

Chapter 1 The Introduction of Microbiology

MICROBIOLOGY The word microbiology is derived from the two Greek words

- Micron means small
- Biologia means studying life.

Hence microbiology is the study of micro-organisms and their activities.

It is concerned with form, structure, reproduction, physiology, metabolism and identification of microbes. It includes the study of their distribution in nature, their relationship to each other and to other living things, the beneficial and detrimental effects on man, and the physical and chemical changes, they make in their environment. It may also be defined as.

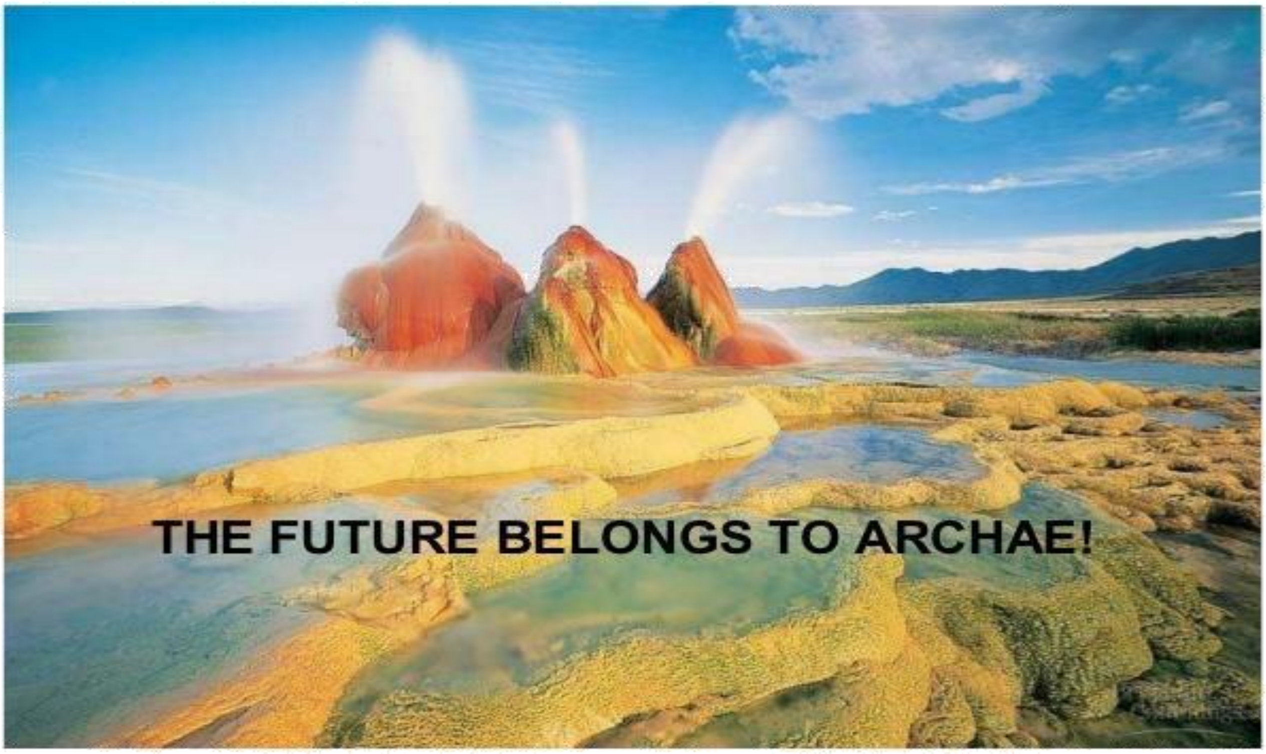
“The branch of biology that deals with microorganisms and their effects on other living organisms.”

Microbe Microbe is a term for tiny creature that individually is too small to be seen with unaided eye.

Microbes include:

- Bacteria
- Virus
- Archaea
- Fungi
- Protozoa,
- Mycoplasma,
- Rickettsia, etc.

THERMOACIDOPHILE – thrives in super-hot, super-acidic conditions
the red stuff on the rocks
This is an archaebacteria.



History of Microbiology

In 1665 Robert hook an English scientist gave deception of microscope and its uses, but he did not invent it. He described a slice of cork and suggested that cork was composed of compartment, which he called cells.

The discoveries of Hook and other scientist showed that microscope is an important tool for discover the secrets of life.

Leven hooke revealed his descriptions of microorganism in 1670 by the invent of his simple and single lens microscope.

In 1674 he called tiny microorganism as animalcules which was a term used for microorganisms by him. He outlined the structural details of the familiar protozoa and paramecium and ameba. He died in 1723.

Scope of Microbiology

1. The science of microbiology includes the study of micro-organism algae bacteria, rickettciae, protozoa, fungi, yeasts and viruses.
2. Without the **harmless organisms** which convert complex material into simple substances, life would be uncertain.
3. Interest in how micro-organism affects human existence has been the foundation of microbiology.
4. The **fermentation of fruit juice and the souring of milk** are natural processes long known to man. Yet why these changes occurred were unknown until Pasteur, in the last part of the 19^{th} century, showed a skeptical world that yeasts and bacteria converted sugars to alcohols and acids.
5. In fact, micro-organism has had a decisive role in shaping world history.
6. The growth of **the pharmaceutical industry** in recent decades has been paralleled by rising standards for product quality and more rigorous regulation of manufacturing procedure
7. For **a sterile product** criterion of quality is simple there should be no living detective microorganism what so ever.
8. The product should, therefore, be able to pass a test for sterility, and knowledge of the procedure and interpretation of results of such tests is an important aspect of pharmaceutical microbiology.
9. It is obvious that medicines contaminated with potentially pathogenic (disease causing) microorganisms are a safety hazard, so medicines administered by vulnerable routes (e.g. injections) or to vulnerable areas of the body (e.g. eyes) are manufactured as sterile products.
10. **Disinfection** and the properties of chemicals (biocides) used as antiseptics, disinfectants and preservatives are subjects of which pharmacists and other persons responsible for the manufacturing of medicines should have a knowledge, both from the perspective of biocide use in product formulation and manufacture, and because antiseptics and disinfectants are pharmaceutical products in their own right.
11. **Injections** are also subjected to a test for pyrogens these are substances that cause a rise in body temperature when introduced into the body.
12. **Sterile medicines** may be manufactured by two different strategies.

    1. The most straight forward and preferred opinion is to make the product, pack it in its final container and sterilize it by heat, radiation or other means.
    2. The alternative is to manufacture the product from sterile ingredients under conditions that do not permit the entry of

contaminating microorganisms. Spoilage of medicines as a result of microbial contamination, although obviously undesirable, has as its main consequence financial loss rather than ill health on the part of the patient.

Microbiology in Your Future

- ✓ Science dominates the twentieth century, and microbiology is one of the newer sciences. Microbiology is dynamic, exploding and revolutionary .

- ✓ The microbiologist has **discovered vaccines** for the prevention of disease such as smallpox and polio, and drugs such as penicillin, for the treatment of disease.
- ✓ The **discovery of some of the vitamins** and other basic food materials has resulted from the study of microorganisms.
- ✓ Knowledge of the **chemistry of the cell** and the importance of certain cell parts in heredity is a contribution of the microbiologist.

- ✓ The biologist may use microorganisms as possible sources of food and oxygen for the **space travelers**.

Vegetables grown in space

The **PHYSICIAN** needs knowledge of microbiology to fight disease.

✓ Industrial scientists often utilize microbiology in the manufacturing of useful chemical products.

✓ The **GEOLOGIST** frequently uses information about microorganism in his search for oil.

✓ Thus microbiology is important from the core of the earth tothe far reaches of outer space

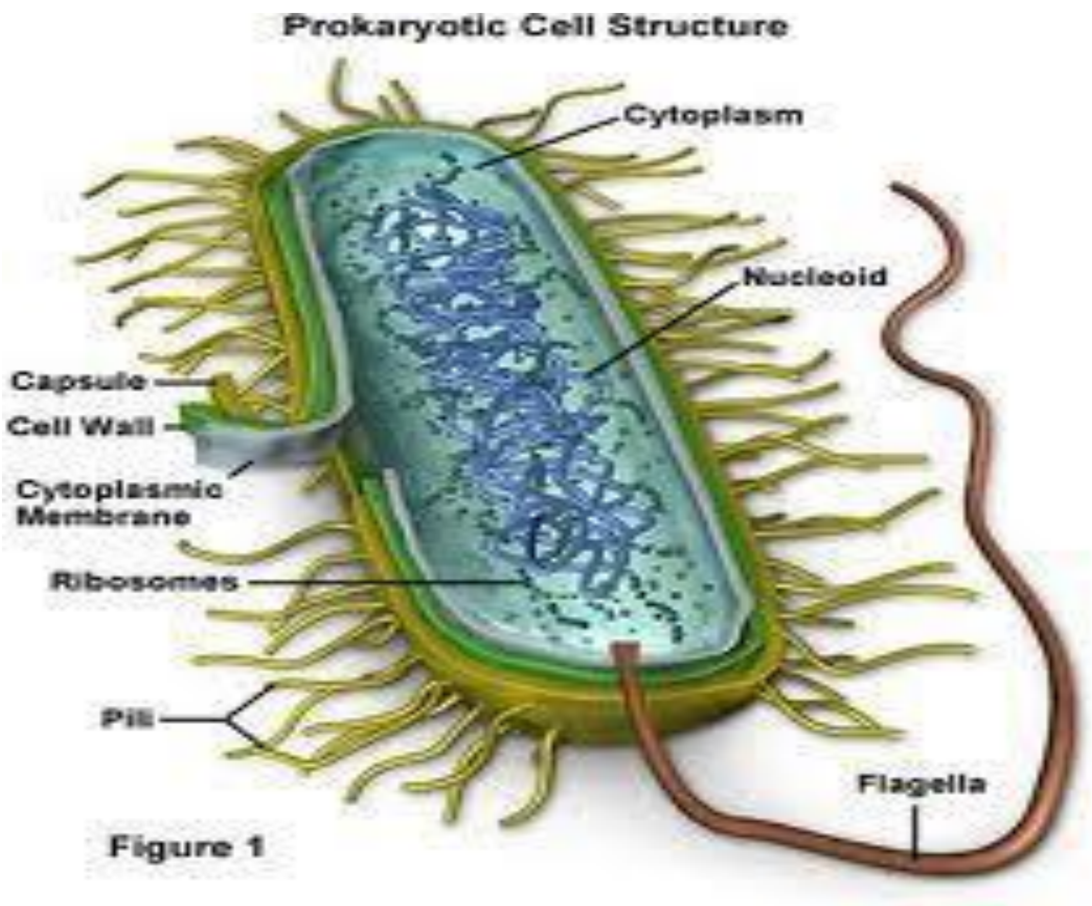
Bacteria

Discovery

The bacteria were discovered by Leeuven van Hoek in 1673. He named them “Little Animal’. In 1773 a Denish scientist Fredrick Muller naemed them Bacilli. In 1850s French biologist Casimir Bavaine named them Bacteria.

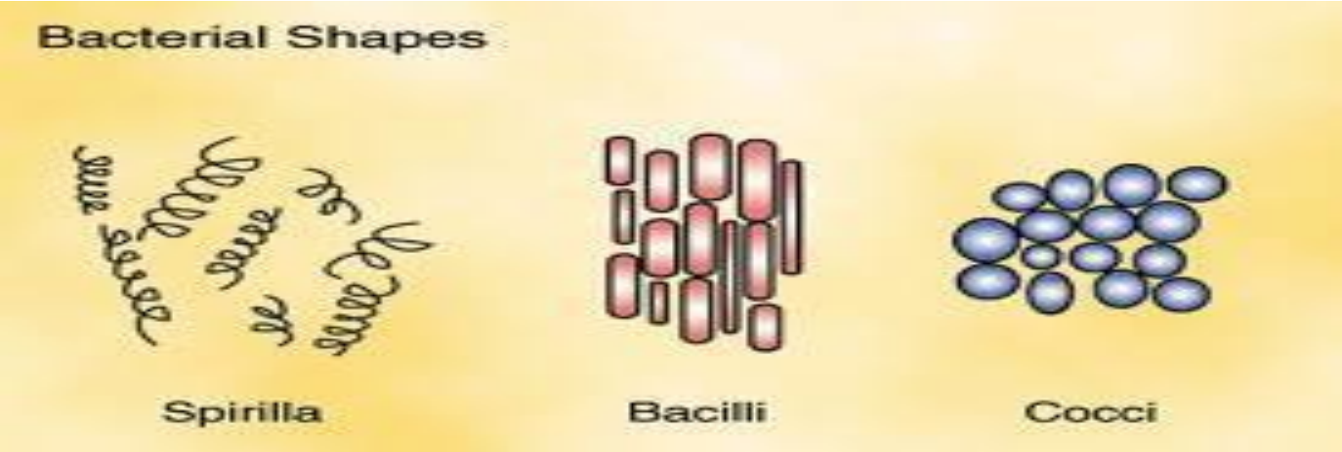
Bacteria

The microscopic, unicellular, prokaryotic organisms characterized by the lack of membrane bound nucleus and membrane bound organelles. The bacteria are the descendants of the earliest form of the life and are unicellular prokaryotes or simple association of similar cells.



Classification of Bacteria on the basis of Cellular morphology

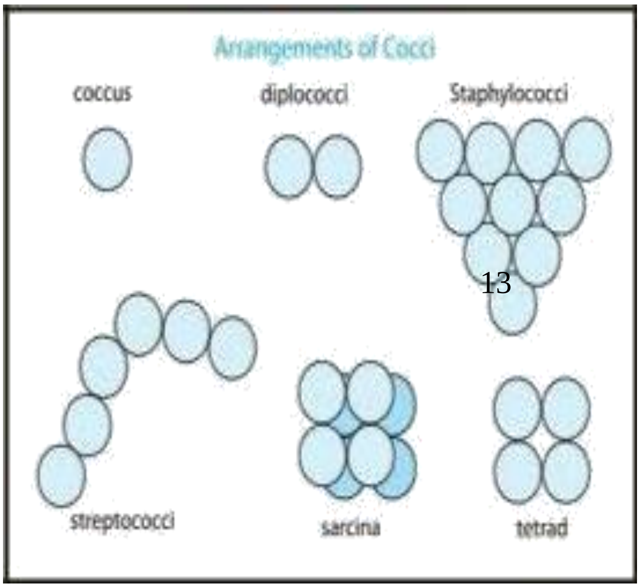
The general appearance of an individual cell as seen under bright field



compound the microscopic is known as cellular morphology. Coccus (spherical) Bacillus (rod like) Spirillum (spiral) Filamentous

Bacilli

As suggested by Muller, the rod is known as bacillus. In various species of rod shaped bacteria, the cylindrical may be as long 20µm or as short as o.5µm.



Cocci

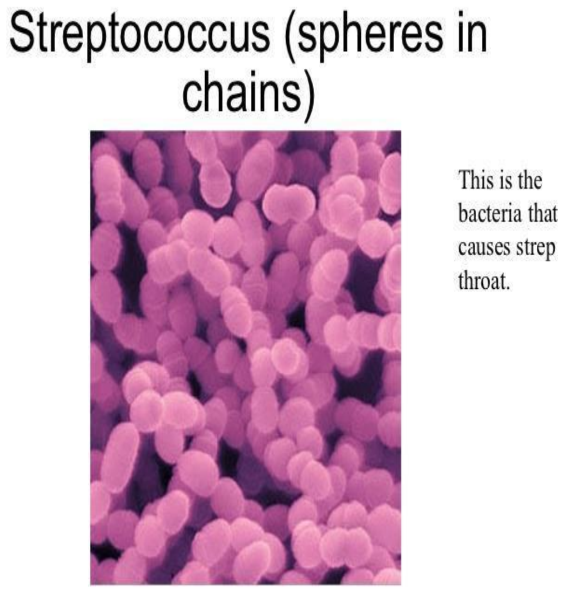
A spherically shaped bacterium is known as coccus, a term derived from greak kokkos, meaning, and berry. Cocci tend to be quite small being only 0.5µm to 1.0µm in diameter.

Diplococci

Those cocci that remain in pairs after reproduction are called diplococci.

Example.

1. Neisseria gonorrhoeae.
2. N. meningitidus.

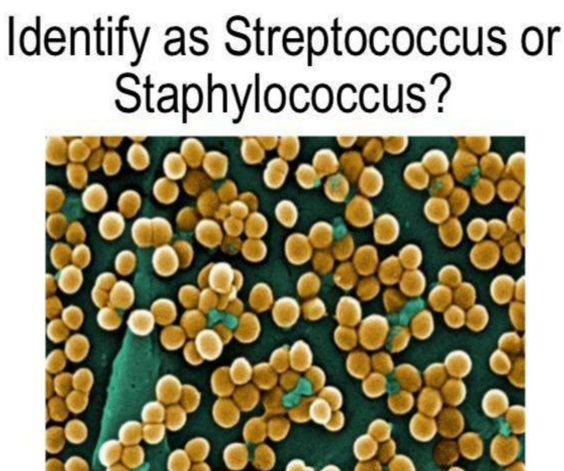


Streptococci

Cocci that remain in chains called streptococci.

Examples

1. Streptococcus pyogenes (involved) in strep throat)
2. S.mutans
3. (involved in tooth decay)
4. S.lactis (involved in producing dairy products such as yogurt)



Staphylococcus

The cocci which divide randomly and form irregular grapes like cluster of cells is called as staphylococcus.

1. Staphylococcus aureus

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Spirals

Spirals may take one of the following three forms.

Vibrios

They are the curved rods that resemble commas.

Example

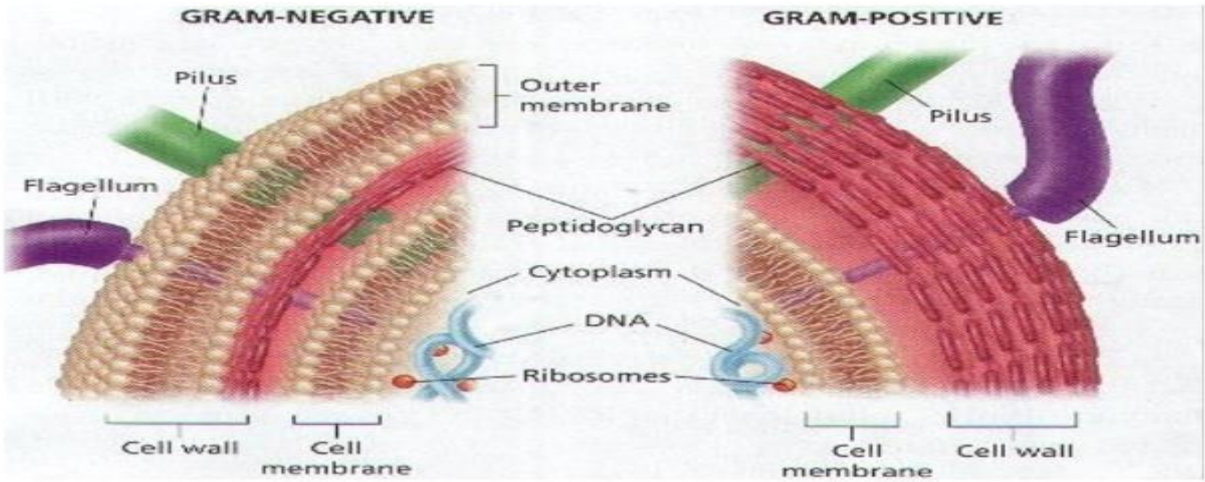
Vibrio cholerae (causing cholera)

Spirilla

They are helical shaped with a thick, rigid cell wall
and flagella that assist movement.

Example

Spirillum volutan



Cell Wall of Bacteria:

With the exception of **mycoplasmas**, all bacteria have a cell wall. Function of cell wall is to protect the cell and determine it shape.

Chemical Composition of cell Wall:

The important component of bacterial cell wall is Peptidoglyan. **Peptidoglycan** is a large molecule and it contains two amino-containing carbohydrates

i) N-acetylglucosamine
ii) N-acetylmuramic acid.

These two molecules are joined by cross bridges of amino acid.

Cell Wall of Gram-Positive:

1-In these Gram-Positive bacteria peptideglycan is about 25 nm wide and contains and additional polysaccharide called **teichoic acid**.

2-About 60-90 % of cell wall is peptidoglycan.

Cell Wall of Gram-Negative Bacteria:

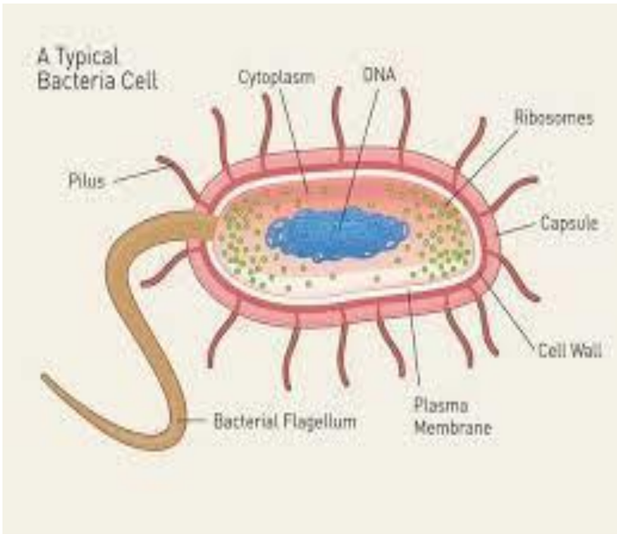
1-In Gram-Negative bacteria the cell wall is only 3 nm in thickness and contains no **teichoic acid**.

2-The cell wall in these bacteria contains various polysaccharide, proteins and lipids. This cell wall is more complex than that of Gram-positive bacteria.

The cell wall is surrounded by an outer membrane. The space between this membrane and cell wall is called **periplasmic** space. The **periplasmic space** contains a gel-like material called periplas

Capsule:

Many species of cocci and bacilli bacteria secrete a sticky, gelatinous layer of poly saccharides and proteins around the cell wall this layer is called Capsule. Spiral bacteria do not form capsule.



Glycocalyx:

The loose layer of capsule is called **Glycocalyx**. It contains a mass of tangled fibers of **dextrin**, a polysaccharide. These fibers help bacteria attach to the surface of the host.

Slime producing bacteria may render the food products unattractive and distasteful.

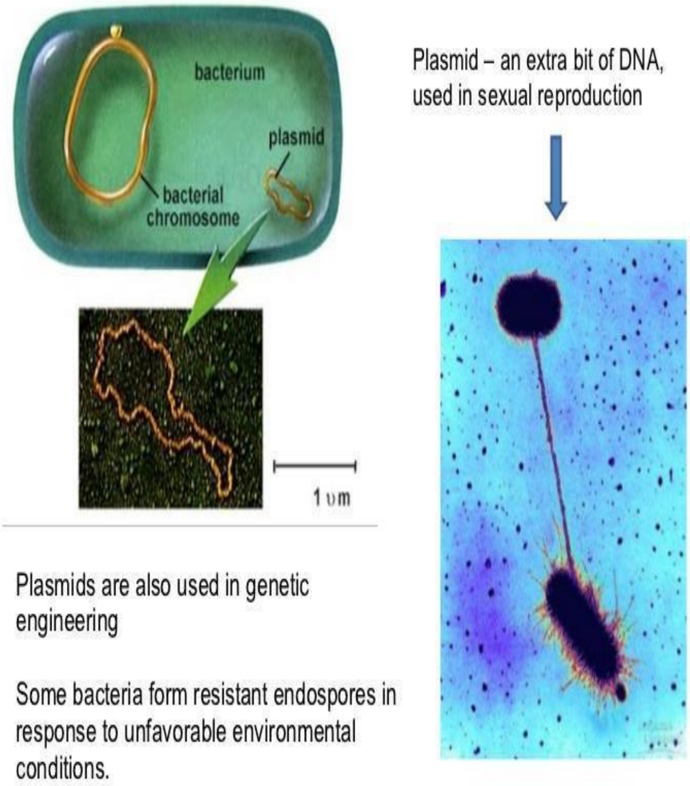
Cytoplasm

Inside the cell membrane lies the cytoplasm. It is semi-transparent and semi-fluid. It contains proteins, carbohydrates, lipids, nucleic acids, salts, and inorganic ions, all dissolved in water.

Chromosome:

The bacteria have no distinct nucleus and are hence called Prokaryotes.

Bacterial chromosome lie suspended in the cytoplasm. It also lack protein.The chromosome region is called Nucloid.



Plasmids:

They are extra-chromosomal rings of DNA. Although they contain few genes and are not essential for bacterial growth, plasmids are significant because many carry genes for drug resistance. For this reason they are often called R factors ("R" for resistance). They are very important in genetic engineering.

Ribosomes:

Ribosomes are bodies of RNA and protein. They are associated with the synthesis of protein.

Inclusion Bodies:

Globules of starch, glycogen or lipids in the cytoplasm are called Inclusion Bodies. They store nutrients for periods of starvation.

Volutin:

They are depots of phosphate. Volutins stain deeply with dyes such as methylene blue. Their presence in diphtheria bacilli assists identification procedures.

Magnetosome:

It helps certain bacteria orient themselves to the environment toward their preferred habitat.

Cell Membrane

The cell membrane (also called the plasma membrane) is the boundary layer of the bacterial cell. It exists inside the cell wall in plants and bacteria.

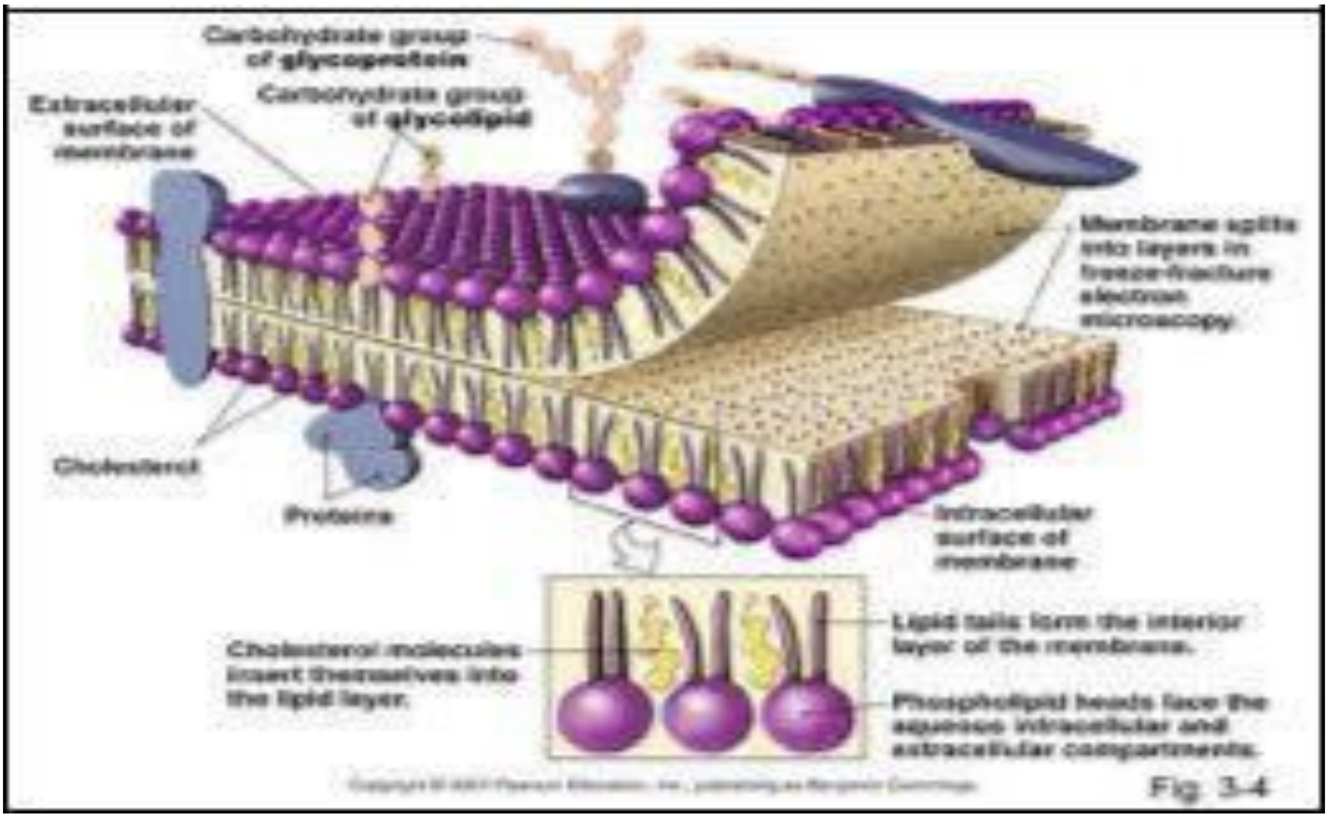
Cell Envelope

Some microbiologists combine the cell membrane, cell wall and capsule and term them Cell Envelope.

Chemical Composition:

Cell membrane contains approximately 60% proteins and 40 % lipids (mainly Phospholipids.)

Fluid Mosaic Model Of Cell Membrane:



Phospholipids Bi-layer:

The phospholipids molecules are arranged in two parallel layers called phospholipids bilayer.

Protein Globules:

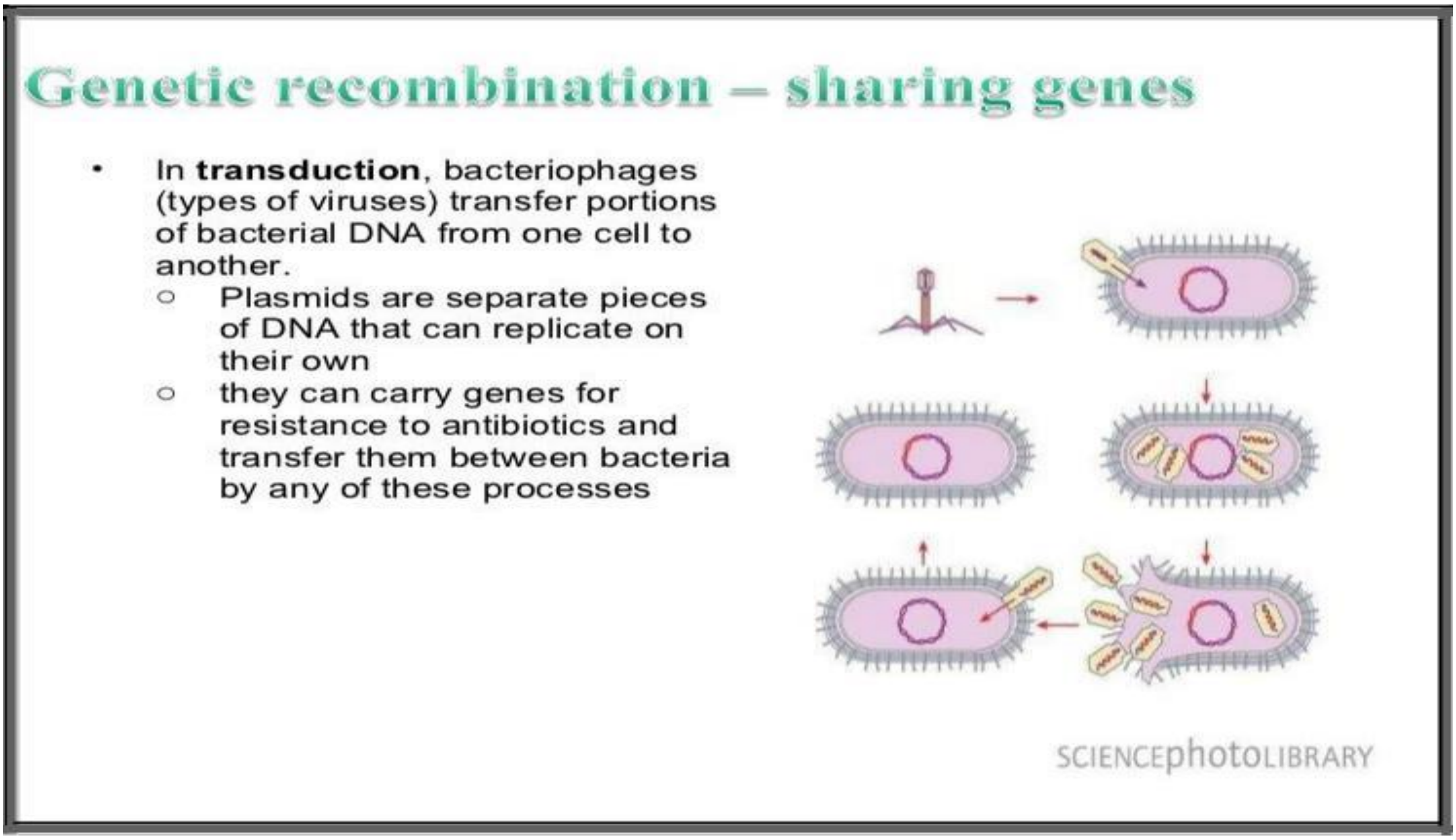
The proteins molecules are arranged as globules floating like icebergs at or near the inner and outer surfaces of the membrane. Some globules extend from one surface of the membrane to the other. This model of the membrane, called the fluid mosaic model.

This molecule accounts for the membrane's appearance under the electron microscope and helps how it allows passage of certain substances.

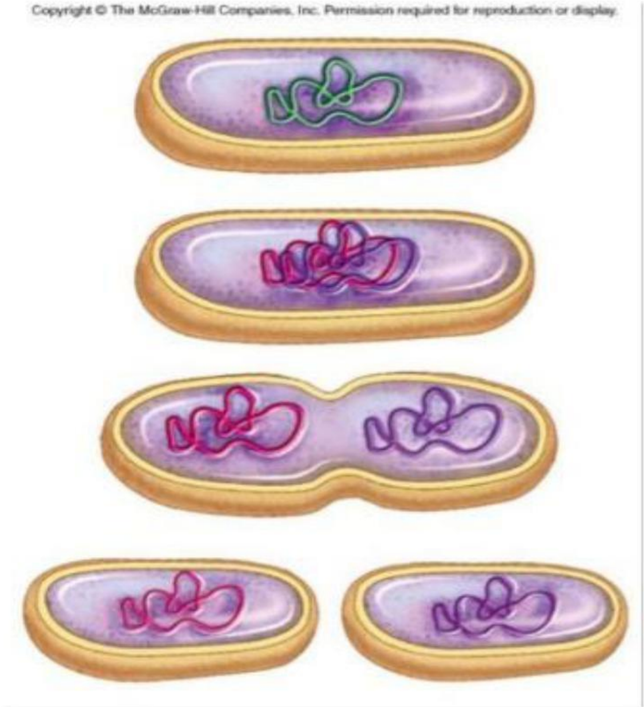
Movement of Molecules Across the Cell Membrane:

1- Lipid-soluble molecules dissolve in the phospholipids layer and pass through the membrane.

2- Acids and nitrogenous bases, which do not dissolve in lipids, move through protein passageways.

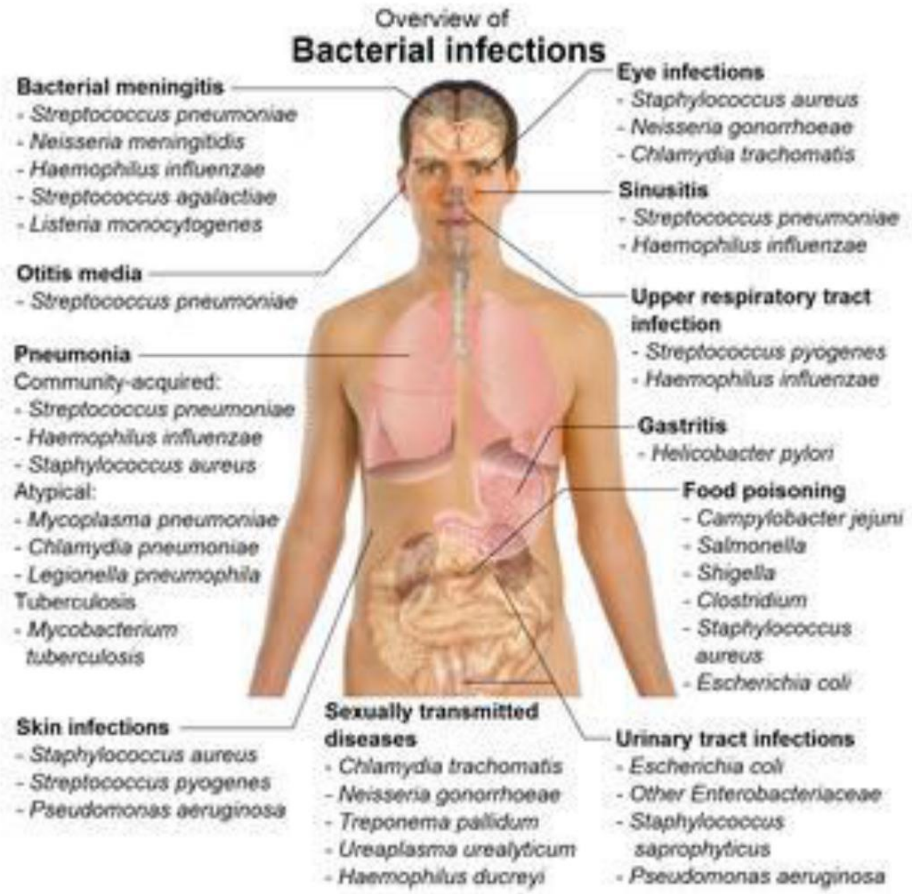


II. Reproduction in Prokaryotes



Binary fission is the splitting of a parent cell into two daughter cells; it is asexual reproduction in prokaryotes.

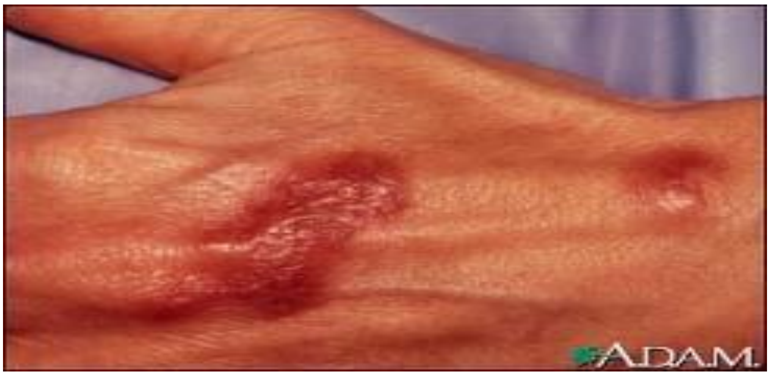
DNA makes a copy of itself, then cell splits



INFECTION AND DISEASE

The Host-Parasite Relationship

Infection



It is the relationship between two organisms, the host and the parasite, and the competition for supremacy that takes place between them.

A host whose resistance is strong remains healthy and the parasite is either driven from the host or assumes a benign relationship with the host. By contrast, if the host loses the competition, disease develops

Disease:

Disease may be conceptualized as any change from the general state of good health .

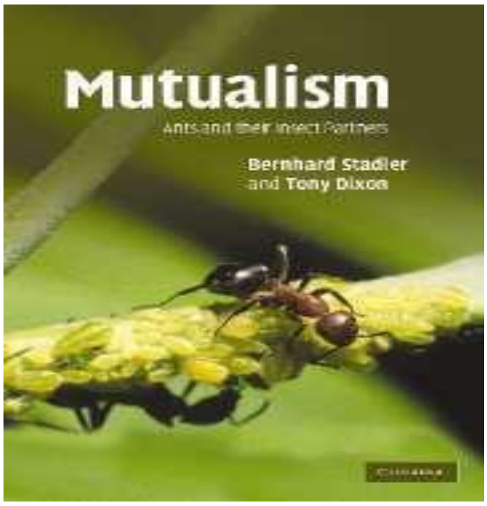
It is important to note that disease and infection are not synonymous; a person may be infected without becoming diseased.

The Normal Flora

The normal flora is a population of microorganisms that infect the body without causing disease.

The relationship between the body and its normal flora is an example of a symbiosis.

Mutualism:

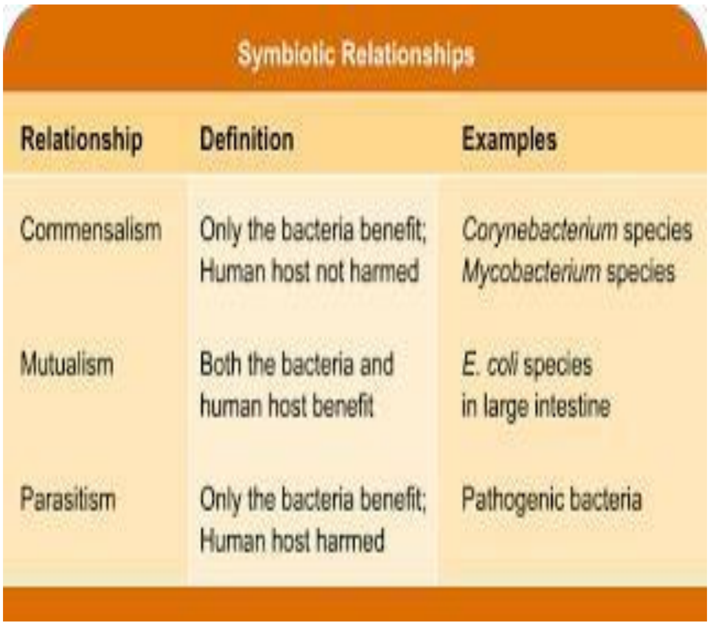


In some cases the symbiosis is beneficial to both the body and the microorganisms. This relationship is called mutualism.

Species of Lactobacillus live in the human vagina and derive nutrients from the environment while producing acid to prevent the overgrowth of other organisms.

Commensalism:

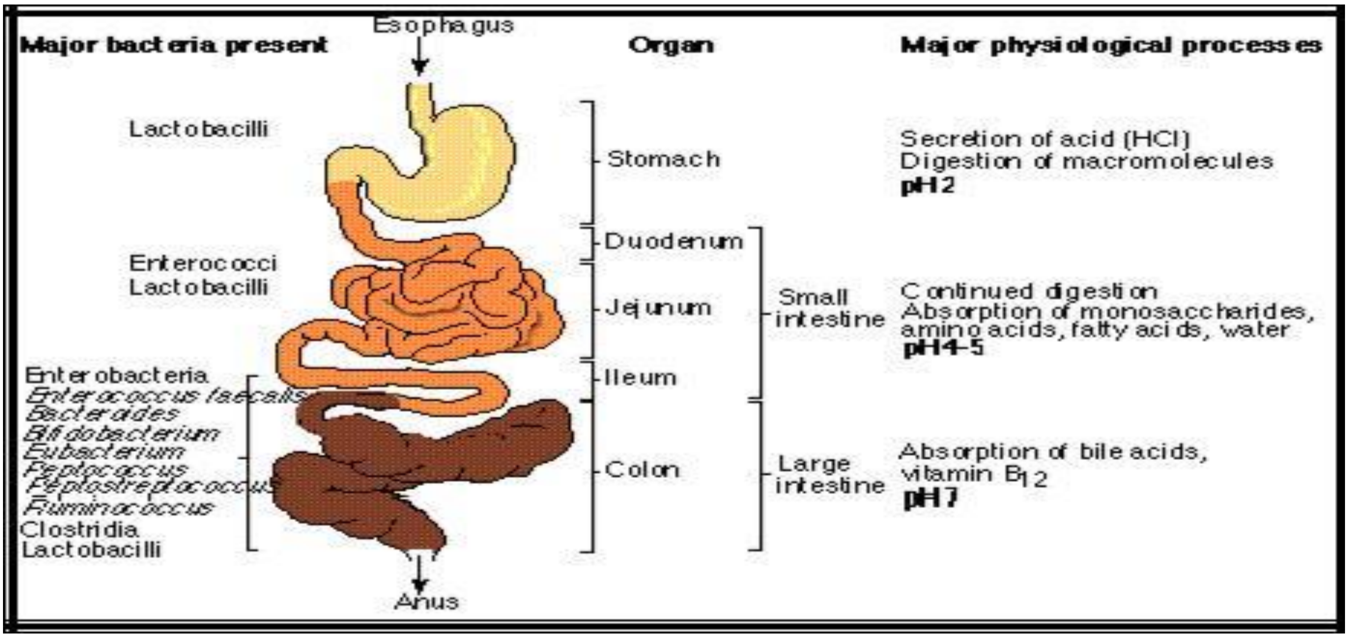
In some cases, the symbiosis is beneficial only to the microorganisms, in which case the symbiosis is called commensalism. Escherichia coli is generally pre sumed to be a commensal in the human intestine.



Symbiotic Relationships

Relationship	Definition	Examples
Commensalism	Only the bacteria benefit; Human host not harmed	*Corynebacterium* species *Mycobacterium* species
Mutualism	Both the bacteria and human host benefit	*E. coli* species in large intestine
Parasitism	Only the bacteria benefit; Human host harmed	Pathogenic bacteria

Occurrence of Normal Flora



A normal flora may be found in several body tissues.

1-Skin:

On the skin, for instance, there are various forms of viruses, fungi, and bacteria, particularly staphylococci and Propionibacterium acnes.

2-Oral Cavity:

The oral cavity commonly contains members of the genera Neisseria, Leptotrichia, and Bacteroides, as well as many diphtherialike bacilli (diphtheroids), fungal spores, and streptococci.

3-Respiratory Tract:

The upper respiratory tract is the site of all these organisms, as well as pneumococci and species of Haemophilus and Mycoplasnia. These organisms may cause respira. tory disease if the body defenses are compromised.

4-Small and Large Intestine:

Later part of the small Intestine and the large intestine abound with microorganisms. Bacteroide species are numerous, together with Clostridium spores, various streptococci, and a number of

Gram-negative rods including species of Enterobacter, Kiebsiella, Proteus, and Pseudomonas. Escherichia coli is a well-known resident of the intestine, as is Candida albicans, the yeast.

5-Vagina:

In females, Lactobacillus is a notable component of the vagina; other organisms may be located near the urogenital orifices in both males and females.

6-Blood and Urine:

The blood and urine are usually sterile unless disease is in progress.

7-Stomach:

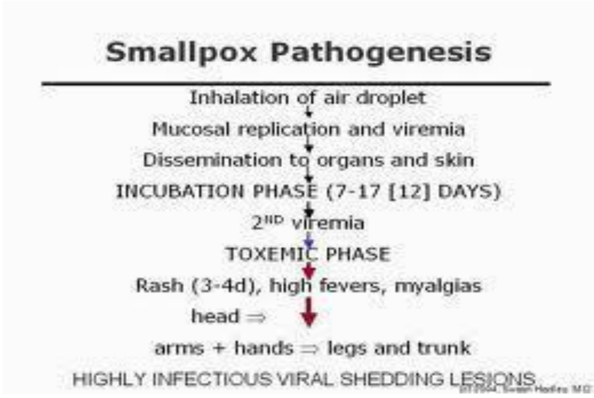
The stomach in humans is generally without a normal flora mainly due to the low pH of its contents.

Introduction of Normal Flora in the Neonates:

Organisms of the normal flora are introduced when the child passes through the birth canal. Additional organisms enter when breathing begins and upon first feeding. Within two to three days most organisms of the flora have appeared. During the next few weeks, contact with the mother and other individuals will expose the child to additional microorganisms. The normal flora remains throughout life, undergoing changes in response to the internal envi ronment of the individual.

Pathogenicity

Pathogeniclty refers to the ability of a parasite to gain entry to the host's tissues and bring about a physiological or anatomical change resulting in a change of health and thus disease.



Pathogen:

An organism having pathogenicity. The symbiotic relationship between host and parasite is called parasitism.

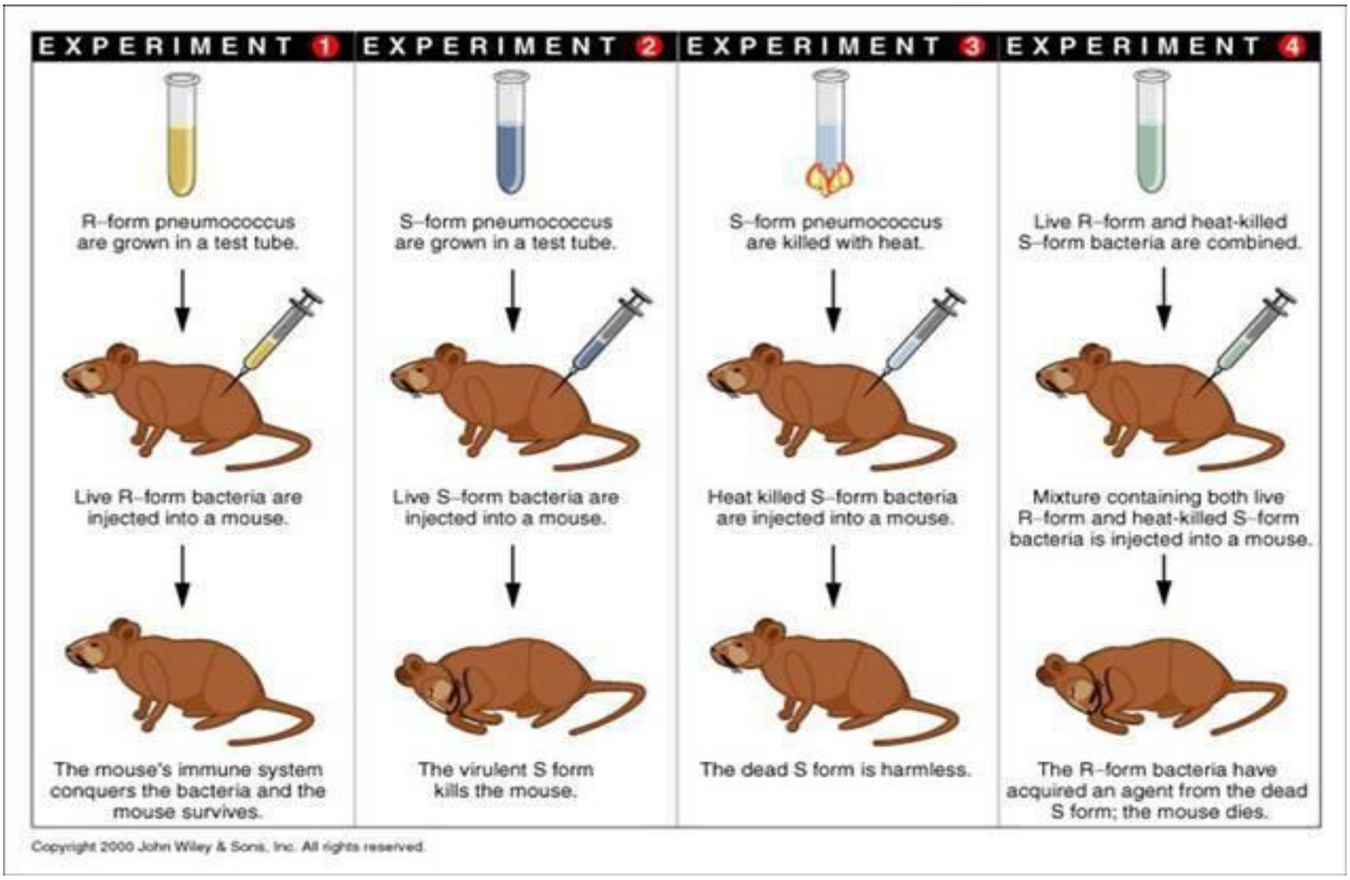
Parasites vary greatly in their pathogenicity.

Virulency:

The word virulence is used to express the degree of pathogenicity of a parasite.

Virulent:

An organism such as the typhoid bacillus that invariably causes disease is said to be highly virulent. Parasites of cholera, plague, and typhoid bacilli are well known for their ability to cause serious human diseases.



Moderately Virulent :

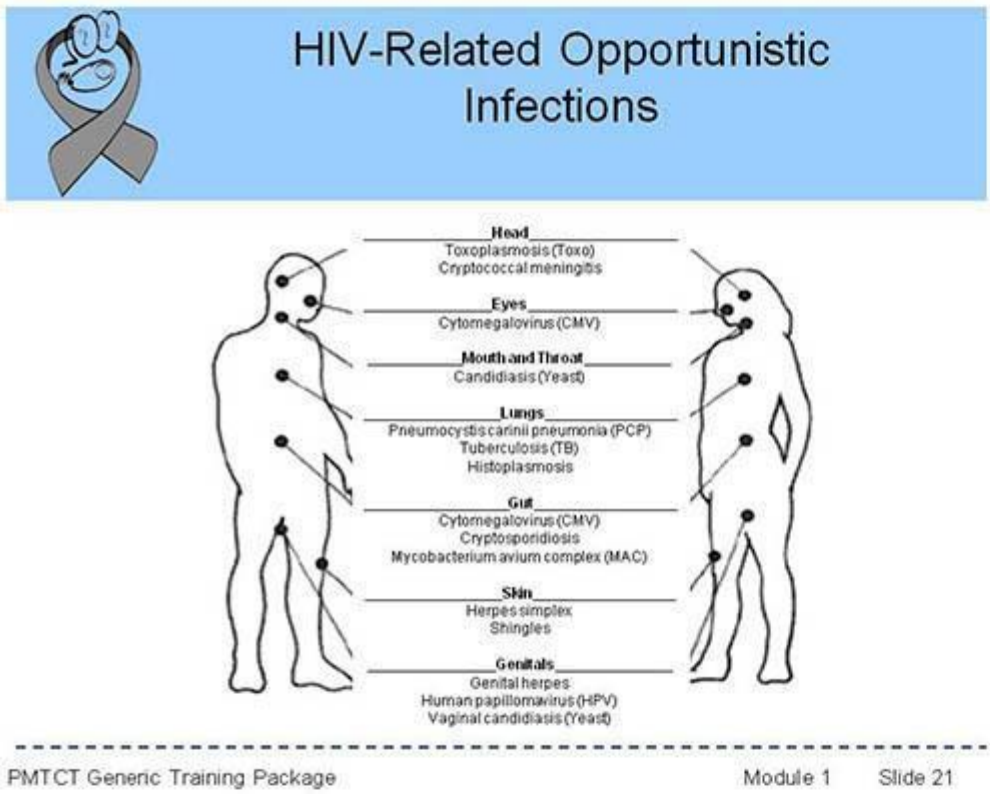
Organism such as Candida albicans that sometimes causes disease is labeled “moderately virulent.”

Avirulent:

Certain organisms described as avirulent, are not regarded as disease agents. The lactobacilli and streptococci found in yogurt are examples.

Opportunistic:

Certain commensals become parasites when the body’s normal defenses are suppressed. They invade the tissues and express their pathogenicity.



Culture Media

A- Natural Media

The media about which one cannot be certain of the exact components or their quantity are called Natural Meida for Bacteria.

(a) Beef Broth:

Since the time of Pasteur and Koch, microbiologists have used media such as beef broth for the laboratory cultivation of bacteria.

(b) Nutrient Agar

The modern form of this liquid medium, called nutrient broth, consists of:

1-Water, beef extract, and peptone. Peptone is a protein supplement from plant or animal sources.

2 -Agar. **Agar** is a polysaccharide derived from marine algae. It adds no nutrients to the medium but only serves to make it solid so that bacteria can be cultivated on the surface.

(c) <u>Enriched medium:</u>

Most common bacteria grow well in nutrient broth and nutrient agar, but certain fastidious bacteria may require specially enriched media.

Enriched media are:

(i) Blood Agar:

Some the streptococci that cause strep throat and scarlet fever grow well when whole blood is added to the nutrient medium. In this instance, the medium is called blood agar.

(ii) Chocolate Agar:

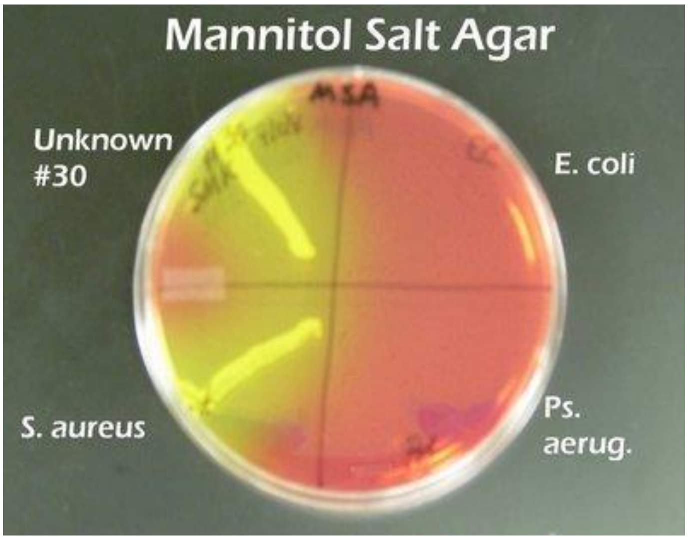
To encourage the growth of Neisseria species, blood agar is heated before solidification. Heating disrupts the red blood cells and releases the hemoglobin. The medium is now termed chocolate agar because of its charred brown appearance.

(e) <u>Slective Media</u>:

Selective media contain ingredients to inhibit the growth of certain bacteria in a mixture while permitting the growth of others. For example,

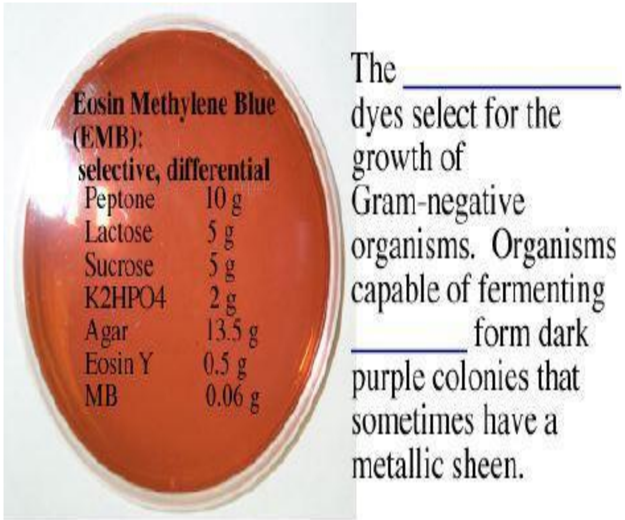
(i) <u>Mannitol Salt Agar:</u>

Staphylococci are cultivated-on **<u>mannitol salt agar.</u>** This medium contains mannitol and a high salt concentration that inhibits most other bacteria. Mannitol is an alcoholic carbohydrate fermented by staphylococci.



(ii) Eosin Methylene Blue Agar :(EMB).

This selective medium has carbohydrates fermented by E. coli and other Gram-negative bacteria. It also contains eosin and methylene blue, two dyes that inhibit Gram-positive bacteria.



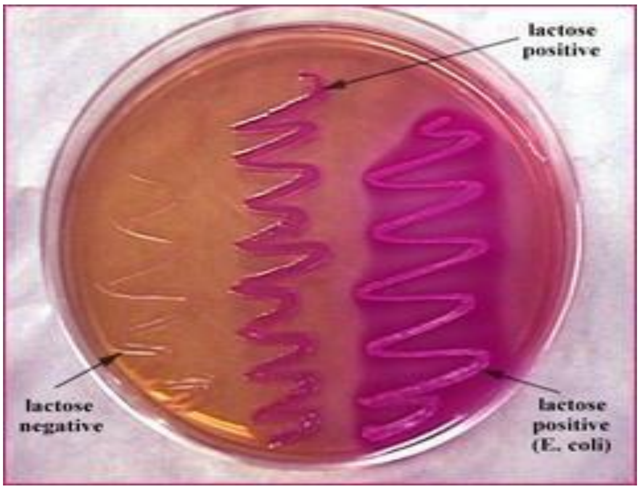
(f) Differential Medium:

Another type of medium is the **differential medium.** This medium makes it easy to distinguish colonies of one organism from colonies of other organisms on the same plate.

(i)Mac Conkey agar is typical. It contains

1-The dyes neutral red and crystal violet

2- Carbohydrate lactose. Those bacteria that ferment the lactose take up the dyes and form red colonies; other bacteria show up as colorless colonies



3-MacConkey_agar contains bile salts that inhibit the growth of Gram-positive bacteria.

This medium is thus selective as well as differential.

B-Synthetic Media

Synthetic media are chemically defined. Here the nature and amount of each component are known. Such a medium might contain glucose, ammonium phosphate, potassium phosphate, magnesium sulfate, and sodium chloride.

1) The glucose supplies energy to the cell;
2) The ammonium ions are a source of nitrogen for amino acid arid nucleic acid formation;
3) Phosphate is used in DNA and RNA synthesis;
4) Sulfur from magnesium sulfate is valuable for enzyme formation;
5) Sodium chloride maintains a stable internal environment in the cytoplasm.

BACTERIAL CULTURES

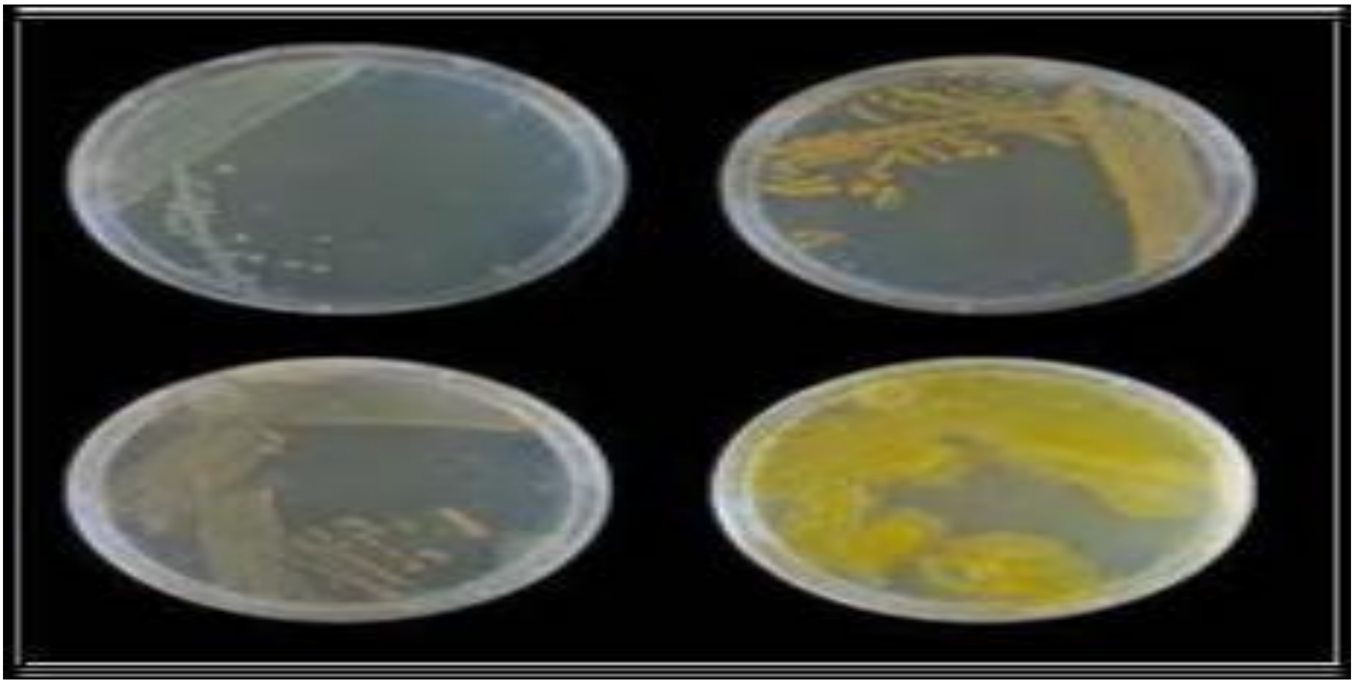
Bacteria or other microorganism grow on a laboratory they are referred to as a culture.

PURE CULTURE

A pure culture consists of a population of cells which are derived from a single cell.

METHODS OF ISOLATION OF PURE CULTURE

I. THE STREAK PLATE TECHNIQUE



The procedure of streaking a plate with an inoculating loop is used to spread millions of cells over the surface of a solid medium so that some individual cells are deposited at a distance from all others. These cells grow and reproduce, forming an isolated colony.

One or more colonies will be well Separated from all others and "represent a source of a pure culture.

The procedure is similar to the one used for streaking from the collection swab.

MATERIALS:

- Streak plates
- Nutrient agar plates (NA)
- Bunsen burner
- Bacteriological loop

STUDENT DIRECTIONS

- Examine the streak plates prepared and locate a number of well-isolated colonies.
- Now you must transfer a portion of each colony to a separate agar slant.
- To "pick" a colony you will be using an inoculating loop.
- Sterilize the loop in the burner flame, let cool 3-5 seconds then touch the end of the loop to the isolated colony, picking up the microorganisms from the colony.
- Now recover the streak plate and pick up one NA plate.
- You will now be holding the inoculating loop in your right hand and the fresh NA plate in your left hand (lefties reversed).
- Remove the lid from the plate, place the inoculating loop at one edge of the plate and with a sweeping stroke, and inoculate the agar using the

same tri-streak method as used for the initial isolation.

- Replace the lid.
- Flame the loop and proceed to inoculate another plate from different colonies.
- Try to use colonies that are visibly different in morphology.
- Incubate the plates in the 37°C incubator.

2) POUR PLATE TECHNIQUE

PURPOSE

- The pour plate technique can be used to determine the number of microbes/ml or microbes/gram in a specimen.
- It has the advantage of not requiring previously prepared plates, and is often used to assay bacterial contamination of foodstuffs.

PROCEDURE

The principle steps are to

1) Prepare/dilute the sample.

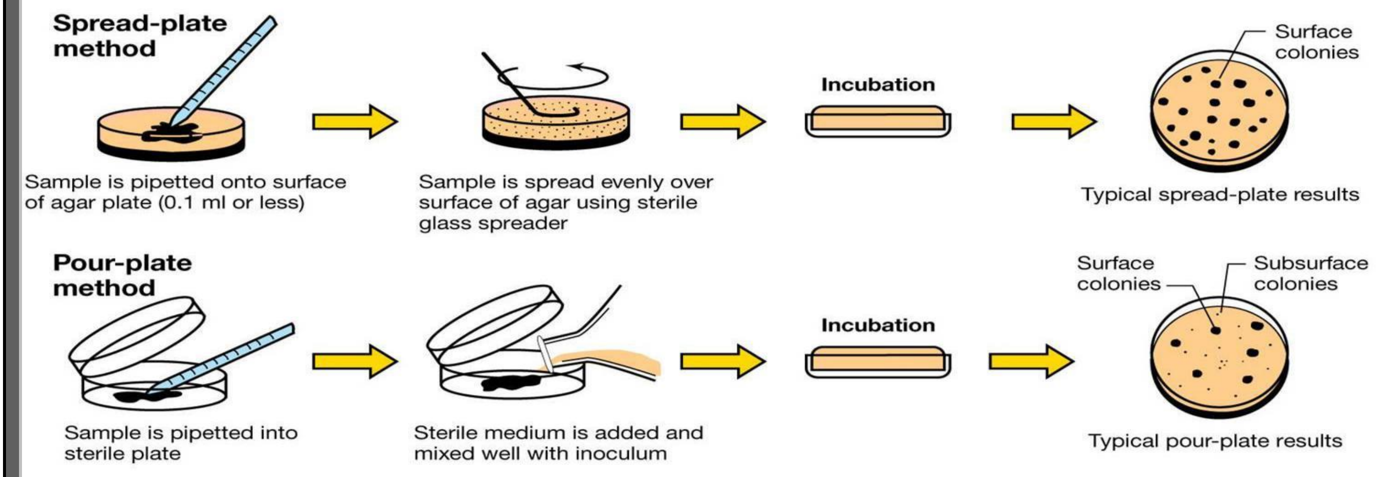
2} Place an aliquot of the diluted sample in an empty sterile plate.

3) Pour in 15 ml of melted agar which has been cooled to 45° C, swirl to mix well

4) Let cool undisturbed to solidify on a flat table top

5) Invert and incubate to develop colonies.

Each colony represents a "colony forming unit" (CFU). For optimum accuracy of a count, the preferred range for total CFU/plate is between 30 to 300 colonies/plate



DISADVANTAGE

- One disadvantage of pour plates is that embedded colonies will be much smaller than those which happen to be on the surface, and must be carefully scored so that none are overlooked.
- Also, obligate aerobes may grow poorly if deeply imbedded in the agar.

3) SPREAD PLATE METHOD

- By means of a transfer loop, a portion of bacterial specimens is spread over the surface of a solid medium.
- After appropriate incubation, growth from each culture should be checked microscopically and culturally to verify that it a pure culture.

LIMITATION:

Very small amount of specimen can be spread over the agar.

ADVANTAGE:

Minimal amount equipment s required,

4) ENRICHMENT CULTURE TECHNIQUE

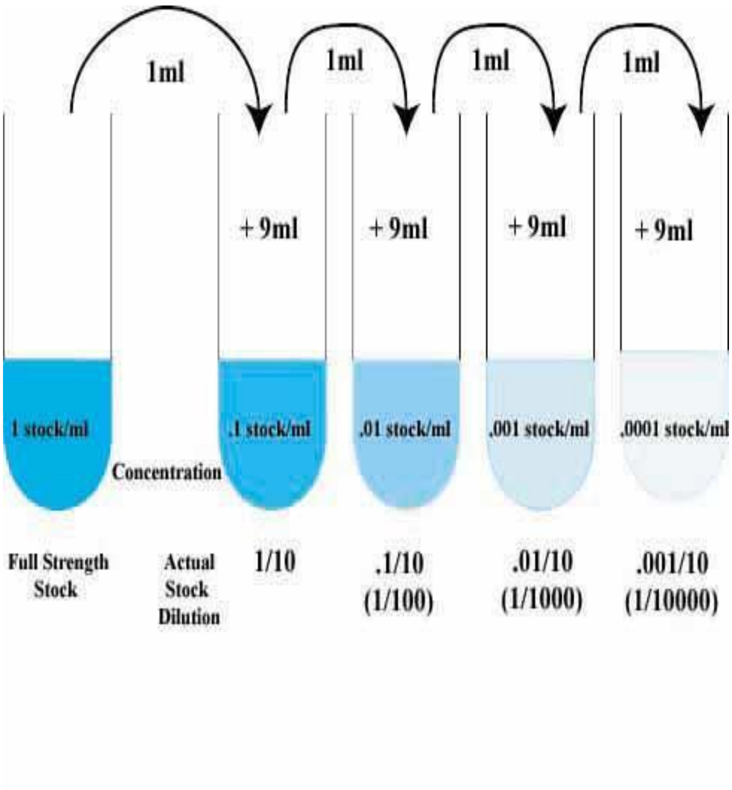
Enrichment cultures are generally used when the type of bacterium to be isolated is present in relatively small number and grows more slowly than many other species in the inoculum.

5) SERIAL DILUTION TECHNIQUE

1) If the organism in a mixed culture in present in greater number than any other organism, it may be possible to obtain it in pure culture by a series of dilution in tubes of appropriate medium,

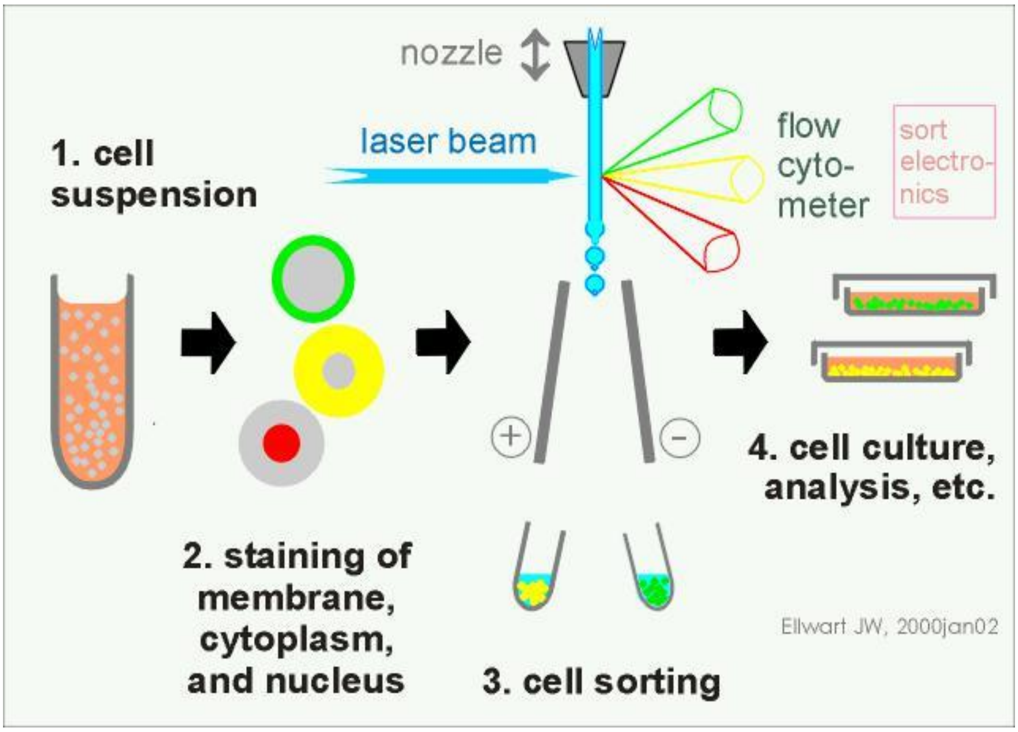
2) When greatly diluted, the specimen contains only the one specie

3) It is advisable to confirm the purity of a culture isolated in this fashion, by a plating procedure,



6) SINGLE CELL ISOLATION TECHNIQUE

- Special equipment, the micromanipulator, can be used in conjunction with a microscope to pick a single organism from a hanging drop preparation.
- The micromanipulator permits the operator to control the movement of a micropipette in the hanging drop so that a singe-cell can be taken into the tube and transferred to a suitable medium for growth.
- The technique is reserved for use in highly specialized studies as it requires skilled operator.

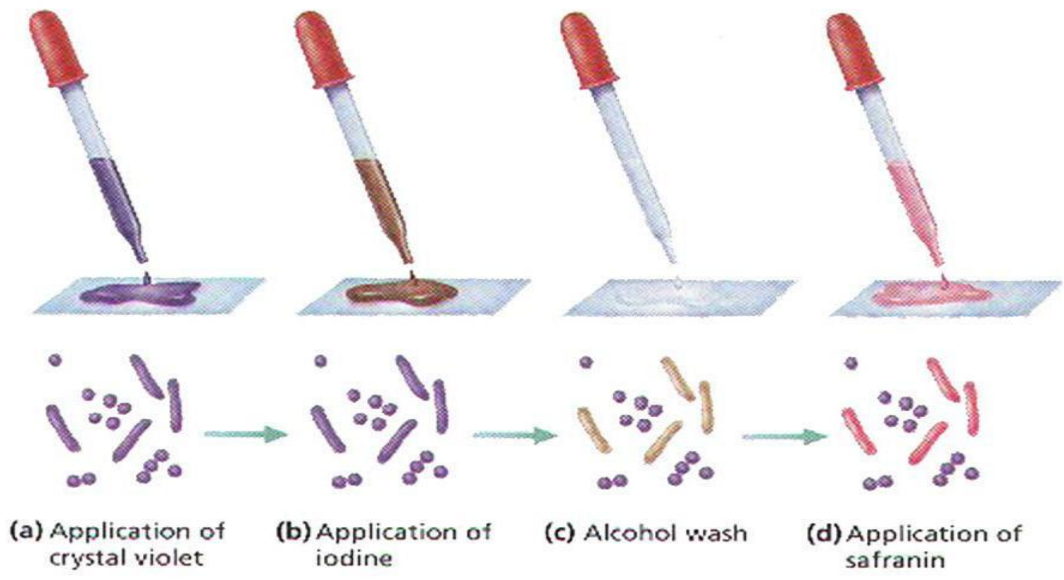


Staining Techniques

1) Gram's Stain

This is the most widely used but not a fully understood technique various theories put forward are.

- It is has been shown that gram-positive organisms contain a substance known as magnesium ribonucleate which gram positive bacteria they will react as gram negative organisms.
- When iodine is applied for staining with crystal violet or another stain of that group a compound is formed which is insoluble in water but soluble in alcohol or acetone .It is said that the more permeable the organism the more likely It is to be gram-negative since the acetone or alcohol has easier access to the compound which it will dissolve.
- It is also through that the pH of the organism has at least some influence on the reaction .Gram –positive bacteria have a more acid cytoplasm and this is increased by the addition of iodine. According to this school of through it is the acidity of the cytoplasm which helps the organism to retain the stain.



http://www.sirinet.net/~jgjohnso/monerans.html

Method

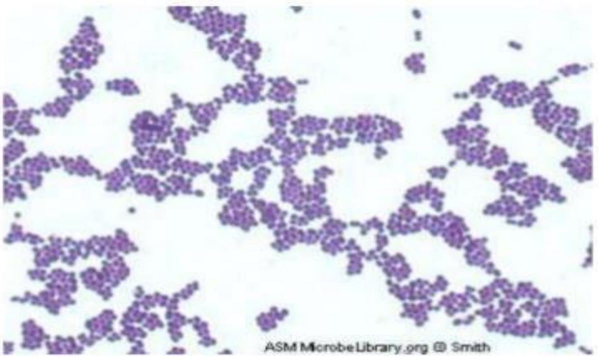
- Make a thin smear of the material or culture let it dry at room temperature .Heating should be avoided as this interferes with the staining reaction

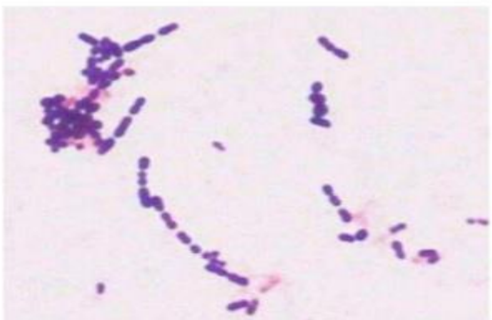
- Pass the slide through a flame once or twice or until it feels comfortable warm on the back of the hand
- Place the slide on the rack and flood with the crystal violet or gentian violet stain-stain for one minute.
- Was off the stain with gram's or Lougol's iodine and leave the slide covered with iodine for one minute.
- Rinse in water.
- Pour on acetone or alcohol till no more violet color comes from the slide
- Rinse in water again
- Stain with one of the following counter-stains; Safrainin ,Neutral red or 1:10 Carbolfuchsin
- Rinse in water and allow it to dry by standing it vertically or by blotting it with filter paper.

Results

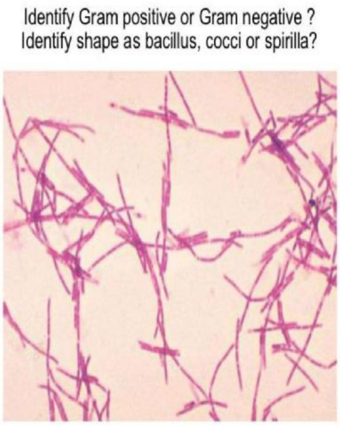
Because the gram-positive organisms retain the crystal violet after decolorized they appear violet in color

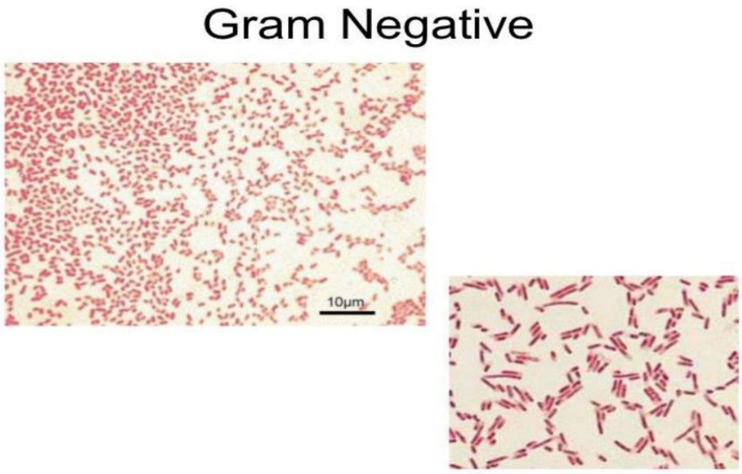
Gram Positive





.The gram negative organism are decolorized and take up the counter-stain and therefore appear pink in color .





- Crystal violet -0.5 % solution in distilled water.
- Iodine –(Lugol's)-10 gm iodine 20gm potassium iodine in 1000 ml of distilled water .Dissolve the potassium iodide in 250 ml water and then add 10 gm of iodine when dissolved make up to 1000 ml with distilled water
- Mixture of Acetone and Alcohol
- Counter stain
    - 1gm neutral red
    - 2 ml 1%Acetic acid
    - Distilled water to make 1000 ml
- Safranin
    - 1.7 gm safranin
    - 50 ml alcohol
    - Distilled water to make 500 ml
- Dilute carbolfuchsin
    - 1:10 dilution of strong carbolfunchsin

ZIEHL-NIELSEN STAIN /ACID-FAST STAINING

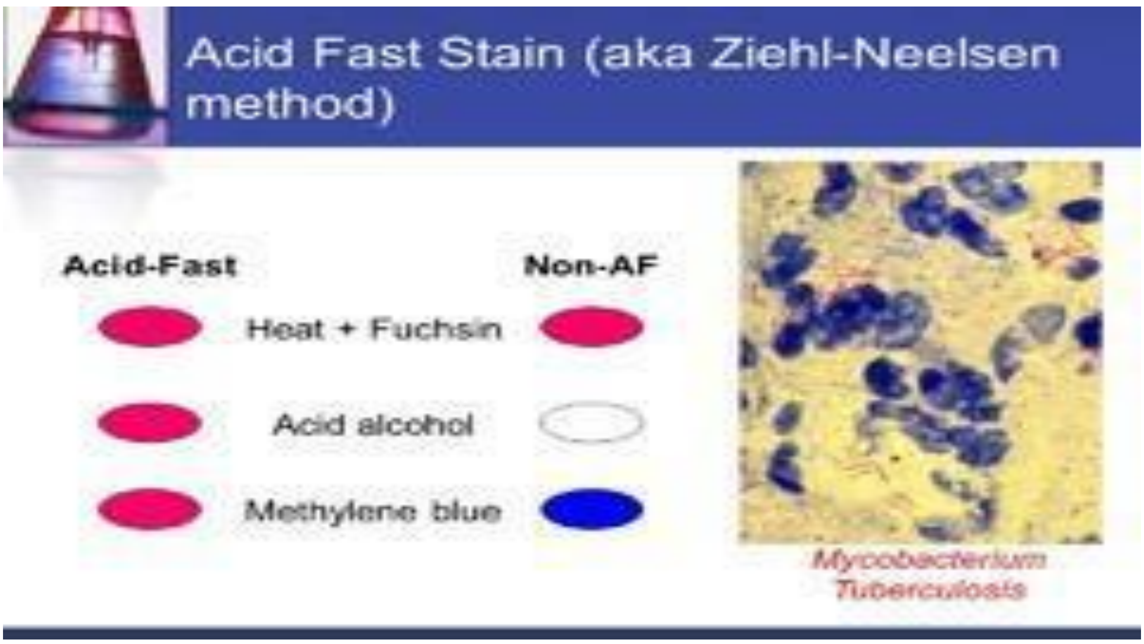
This stain is another method of categorizing certain bacteria dependind on their ability to resist decolourization by acid and alcohol a very strong stain is used basic fuchsin in a phenol solution and heat is applied in order that the stain can penetrate the waxy covering of certain bacteria.

Method

- Make a smear of the material and allow drying at room temperature.
- Flood the whole slide with strong carbolfuchsin and heat gently underneath the slide until steam is seen rising from the slide
- Rinse in water and flood the slide with 25% sulfuric acid .Leave the until the smear is pale pink in color.
- Rinse in water and pour on alcohol for a few minutes.
- Counter stain with malachite green ethylene blue or picric acid
- Dry by staining the slide vertically do not blot dry as the tubercle organism.may get attached of the paper a later may get transferred to another slide.

Result

The tubercle bacillus resists decolorizing by acid and alcohol it will remain bright red while all other organisms and material will take on the colour of the counterstain.



Viruses

Definition

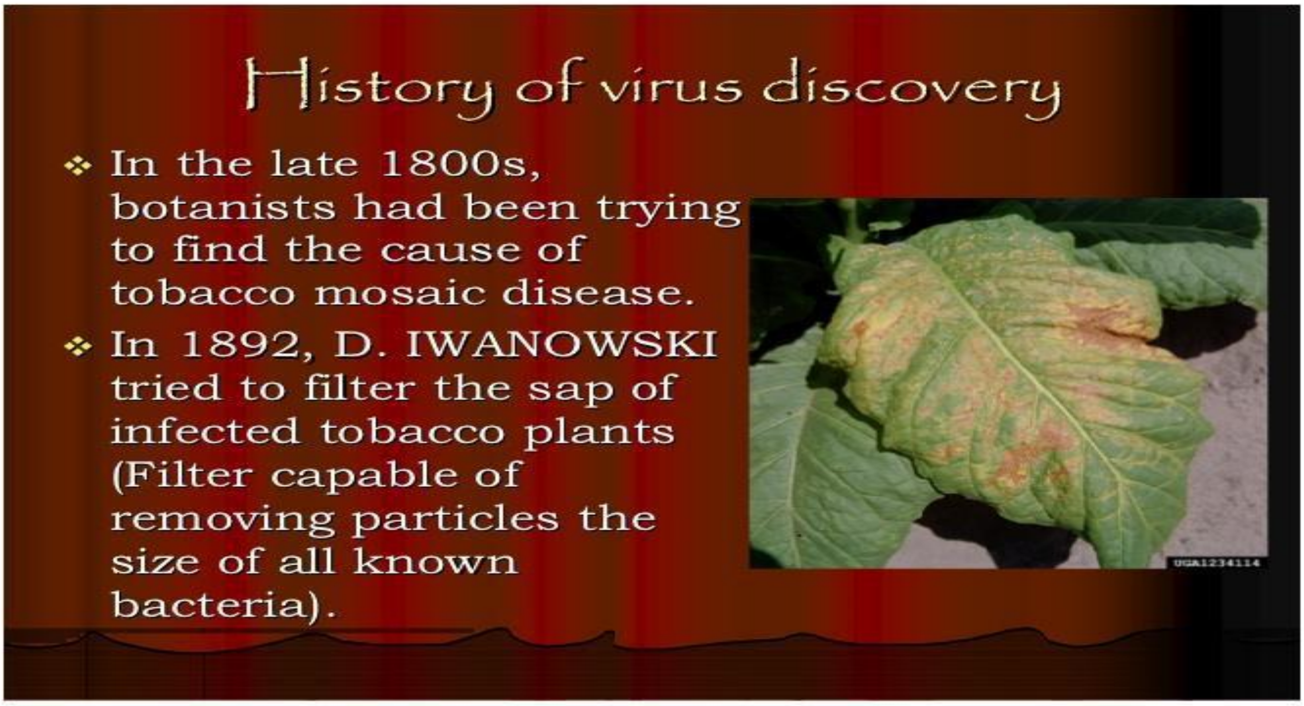
Virus is a Latin word meaning “Poison”

Viruses are obligate intracellular parasites which mean that they replicate only inside a living host cell.

Or

Viruses are non cellular infectious agents consist of either DNA or RNA, reproduce only in living cells and use the biosynthetic machinery of the host cell to direct the synthesis of virion, containing viral genome and transfer them to the other cells

Cells and Viruses		
Characteristic	Cell	Virus
Structure	Cell membrane, cytoplasm; eukaryotes also contain nucleus and organelles	
Reproduction	Independent cell division either asexually or sexually	
Genetic Code	DNA	
Growth and Development	Yes; in multicellular organisms, cells increase in number and differentiate	
Obtain and Use Energy	yes	
Response to Environment	yes	
Change Over Time	yes	

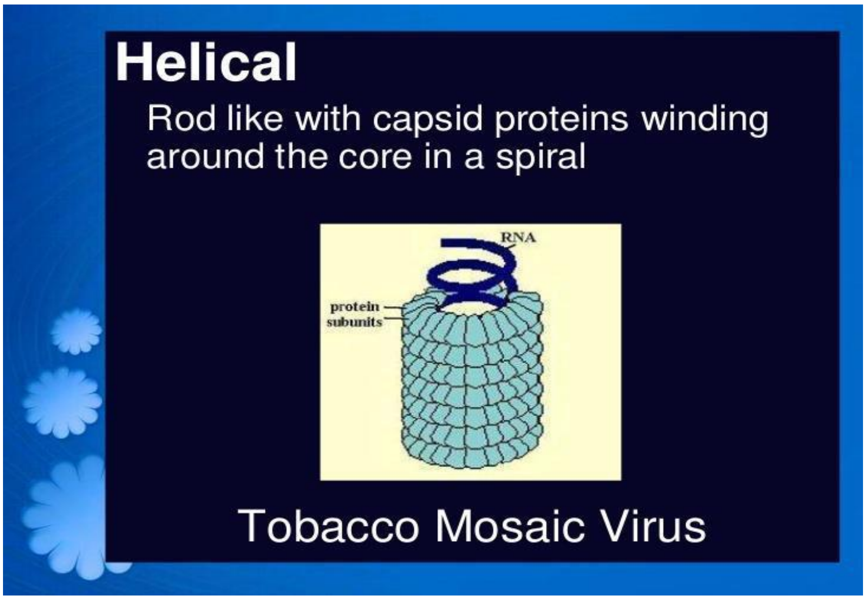


Shapes:

Viruses may appear in several shapes.

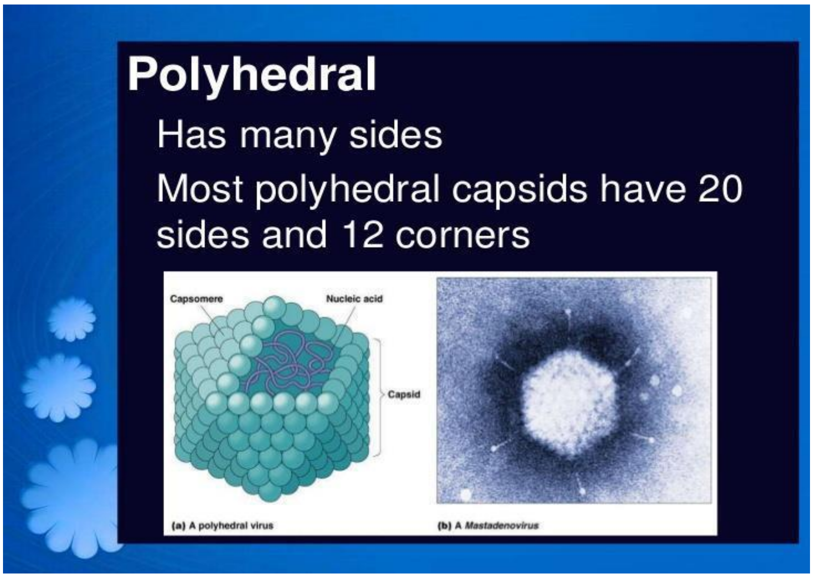
i-Helix:

The helix is a tightly wound coil resembling a corkscrew or spring. Viruses of rabies and tobacco mosaic viruses have helical symmetry.



ii- Icosahedron :

The **icosahedron** a polyhedron with20 triangular faces and 12 corners. Herpes simplex and polio viruses have icosahedral symmetry.



iii-Complex:

A combination of helical and icosahedral symmetry is described as **complex**. Some bacteriophages have complex symmetry. They have an icosahedral head and a collar and tail assembly in shape of a helical sheath.

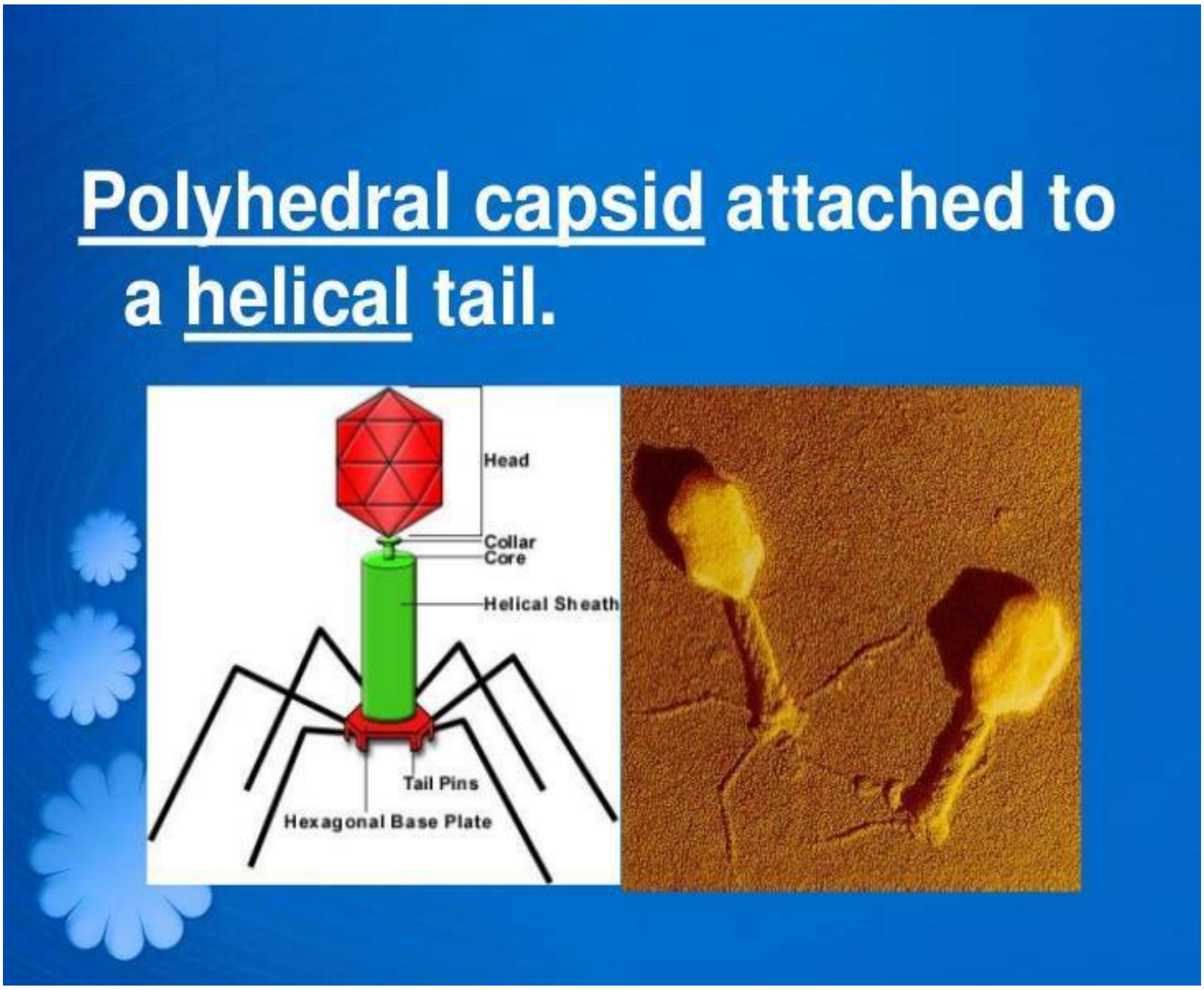


Figure 1 Enveloped viruses

Sturcutre of Virus:

All viruses consist of two basic components:

I a core of nucleic acid, the **genome**,

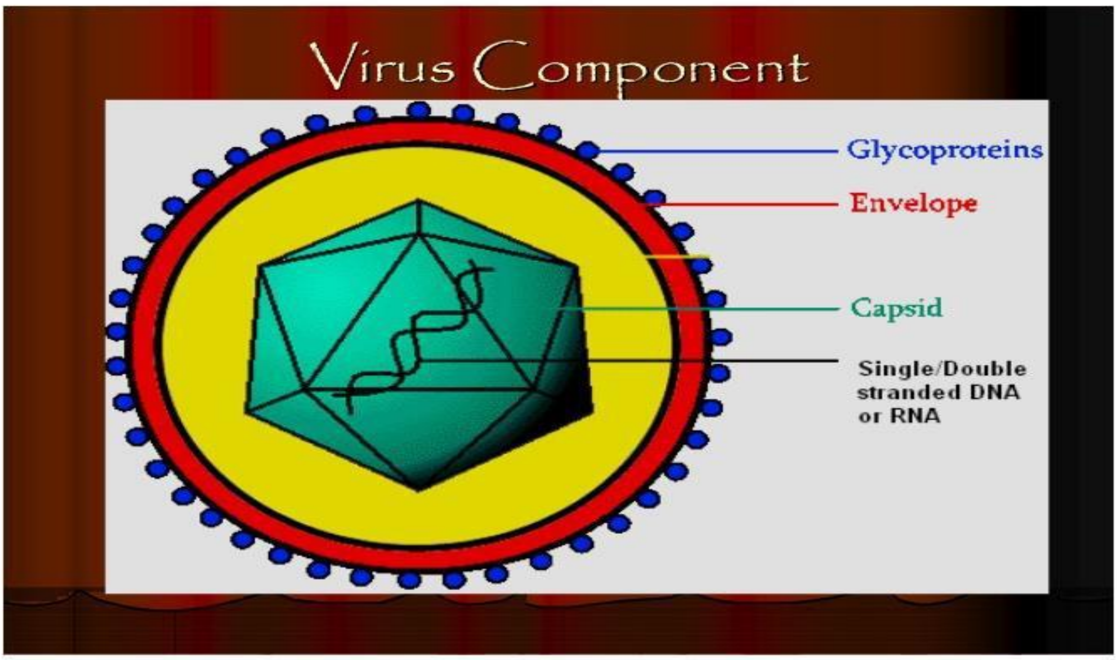
II coat of protein known as the **capsid**.

Genome:

The genome contains either DNA or RNA, but not both; and the nucleic acid occurs in double-stranded or single-stranded form. The genome may be folded, condensed, or coiled.

Capsid:

The capsid protects the genome. It also gives shape to the virus and is responsible for the helical, **icosahedral**, or complex symmetry.



Capsomers:

Generally, that capsid is subdivided into individual protein subunits called **capsomeres**
whose organization yields the symmetry. The number of capsomeres is characteristic for a particular virus.

For example,

Capsid of herpes viruses is made up of 162 capsomeres.

1-Capsid of adenoviruses which cause some common colds is made up of 252 capsomeres.

Envelope:

Many viruses are surrounded by a flexible membrane known as **envelope.**

i) It is composed of lipids and protein and is similar to the host cell membrane, except that it includes viral-specified components.

In some viruses as influenza and measles viruses, the envelope contains functional projections know as **spikes**. The spikes often contain enzymes to assist the attachment of viruses to host cells.

ii) Enveloped viruses may lose their infectivity when the envelope is destroyed.

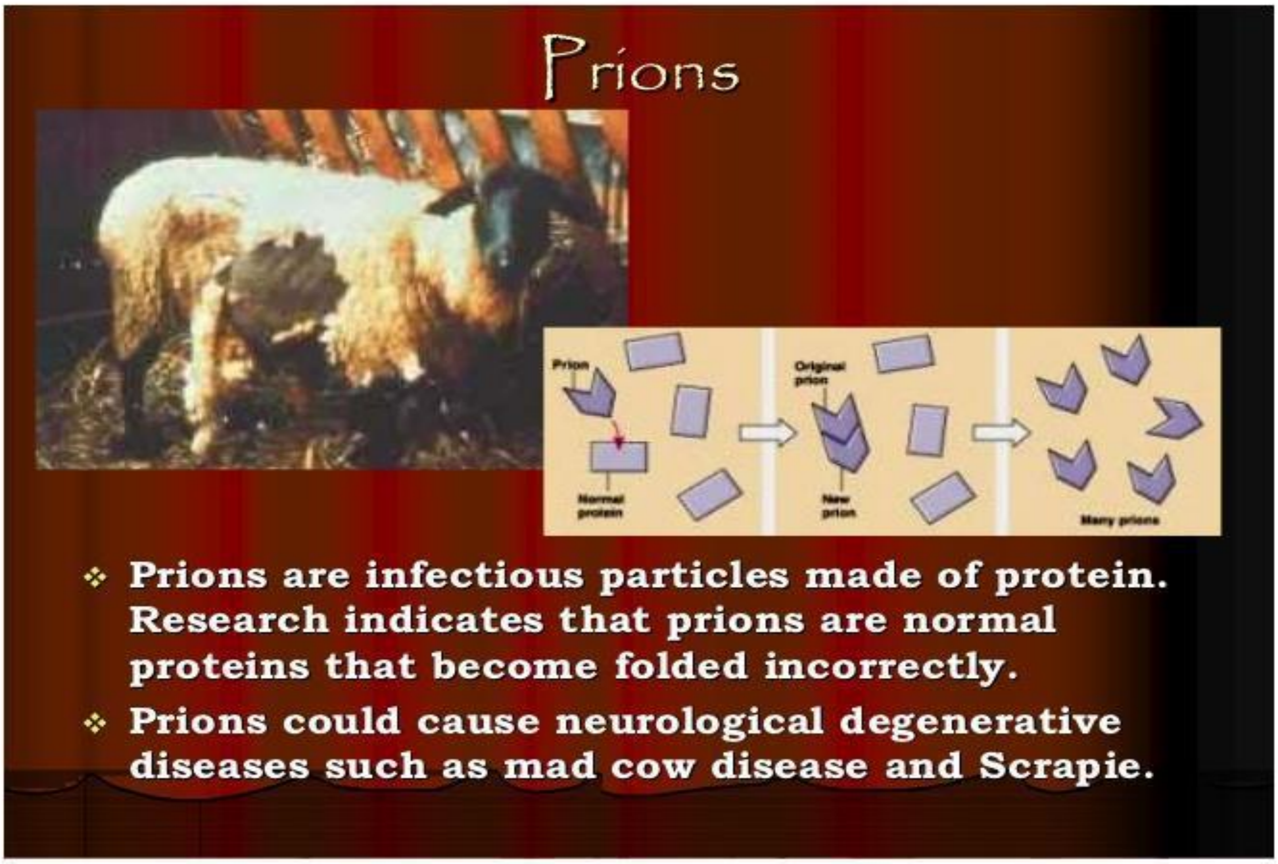
iii) Also, when the envelope is present, symmetry of the capsid may not be apparent since the envelope is generally loose-fitting structure

Virion:

A completely assembled virus outside its host cell is known as Virion. Virions lack the chemical machinery for generating energy and synthesizing large molecules. Therefore they must rely on the structures and chemical components of their host cells to replicate themselves. We shall examine how this takes place next.

Viroids

Viroids are tiny fragments of nucleic acid known to cause several diseases of plants and thought to be involved in human and animal diseases.



Viral Genome & Classification

DNA viruses

The genome replication of most DNA viruses takes place in the cell's nucleus. Most DNA viruses are entirely dependent on the host cell's DNA and RNA synthesizing machinery, and RNA processing machinery. The viral genome must cross the cell's nuclear membrane to access this machinery. The DNA may be double stranded or single stranded.

RNA viruses

These viruses are unique because their genetic information is encoded in RNA. Replication usually takes place in the cytoplasm. RNA may be single-stranded or double-stranded..

Sense:

Positive-sense viral RNA is identical to viral mRNA and thus can be immediately translated by the host cell.

Negative-sense viral RNA is complementary to mRNA and thus must be converted to positive-sense RNA by an RNA polymerase before translation.

DNA nomenclature is similar to RNA nomenclature, in that the ***coding strand*** for the viral mRNA is complementary to it (−), and the ***non-coding strand*** is a copy of it (+)

Reverse transcribing viruses

These replicate using reverse transcription, which is the formation of DNA from an RNA template. Reverse transcribing viruses containing RNA genomes use a DNA intermediate to replicate, whereas those containing DNA genomes use an RNA intermediate during genome replication. Both types use the reverse transcriptase enzyme to carry out the nucleic acid conversion

Classification

Classification seeks to describe the diversity of viruses by naming and grouping them on the basis of similarities. In 1962, André Lwoff, Robert Horne, and Paul Tournier were the first to develop a means of virus classification, based on the Linnaean hierarchical system.[95]

This system bases classification on phylum, class, order, family, genus, and species. Viruses were grouped according to their shared properties (not those of their hosts) and the type of nucleic acid forming their genomes.[96] Later the International Committee on Taxonomy of Viruses was formed.

ICTV classification

The International Committee on Taxonomy of Viruses began to advise and implement rules for the naming and classification of viruses early in the 1990s, an effort that continues to the present day.

The system shares many features with the classification system of cellular organisms, such as taxon structure. Viral classification starts at the level of order and follows as thus, with the taxon suffixes given in italics:

Order	(-*virales*)
Family	(-*viridae*)
Subfamily	(-*virinae*)
Genus	(-*virus*)
Species	

So far, **six orders** have been established by the ICTV: Names of orders and families are italicized, species names generally take the form of [Disease] virus. The establishment of an order is based on the inference that the virus families contained within a single order have most likely evolved from a common ancestor.

1-Caudovirales:

They are tailed dsDNA (group I) bacteriophages,

2-Herpesviraleso :

It contains large eukaryotic dsDNA viruses,

3-Mononegavirales :

It includes non-segmented (-) strand ssRNA (Group V) plant and animal viruses,

4-Nidovirales :

It is composed of (+) strand ssRNA (Group IV) viruses with vertebrate hosts,

5-Picornavirales

It contains small (+) strand ssRNA viruses that infect a variety of plant, insect, and animal hosts, and

6- Tymovirales

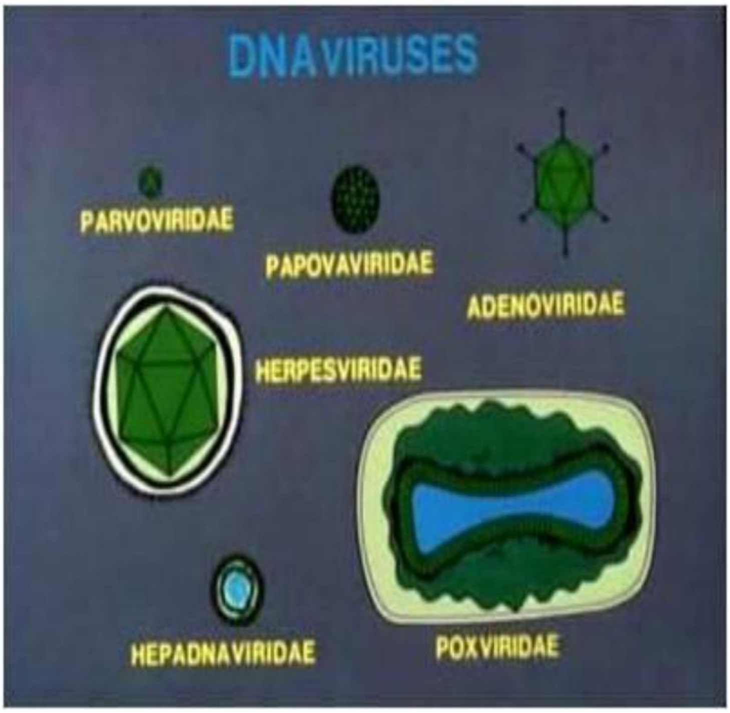
It ontains monopartite ssRNA viruses that infect plants.

Currently (2009) 6 orders, 87 families, 19 subfamilies, 348 genera, and 2,288 species of virus have been defined.

Baltimore classification

The Nobel Prize-winning biologist **David Baltimore** devised the Baltimore classification system. The ICTV classification system is used in conjunction with the Baltimore classification system in modern virus classification.

The Baltimore classification of viruse must generate mRNAs from their genomes to produce proteins and replicate s is based on the mechanism of mRNA production. Baltimore classification (first defined in 1971) is a classification system that places viruses into one of seven groups



- I: **dsDNA viruses** (e.g. Adenoviruses, Herpesviruses, Poxviruses)
- II: **ssDNA viruses** (+)sense DNA (e.g. Parvoviruses)
- III: **dsRNA viruses** (e.g. Reoviruses)
- IV: **(+)ssRNA viruses** (+)sense RNA (e.g. Picornaviruses, Togaviruses)
- V: **(−)ssRNA viruses** (−)sense RNA (e.g. Orthomyxoviruses, Rhabdoviruses)

VI:**ssRNA-RT viruses** (+)sense RNA with DNA intermediate in life-cycle (e.g.Retroviruses)

- VII: **dsDNA-RT viruses** (e.g. Hepadnaviruses
-

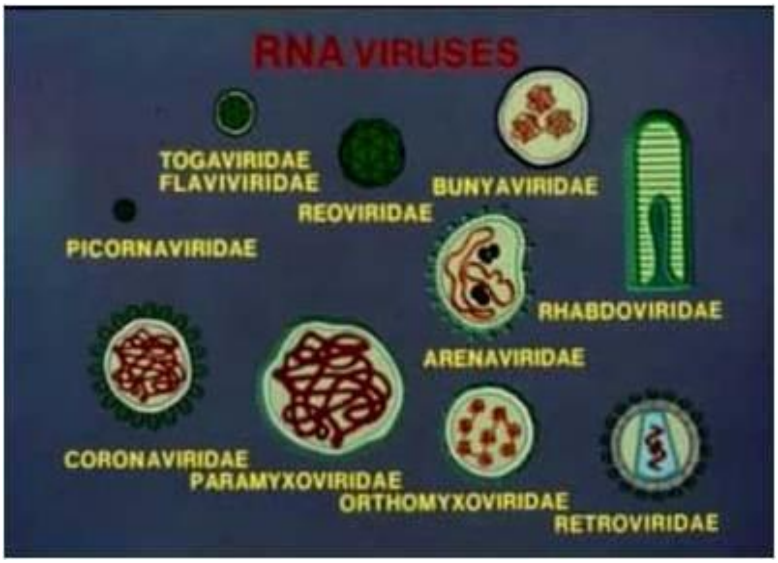
DNA viruses.

For more details on this topic, see DNA virus.

- **Group I**: viruses possess double-stranded DNA.
- **Group II**: viruses possess single-stranded DNA.

RNA viruses

For more details on this topic, see RNA virus.



- **Group III**: viruses possess double-stranded RNA genomes, e.g. rotavirus. These genomes are always segmented.
- **Group IV**: viruses possess positive-sense single-stranded RNA genomes. Many well known viruses are found in this group, including the picornaviruses (which is a family of viruses that includes well-known viruses like Hepatitis A virus, enteroviruses, rhinoviruses, poliovirus, and foot-and-mouth virus), SARS virus, hepatitis C virus, yellow fever virus, and rubella virus.
- **Group V**: viruses possess negative-sense single-stranded RNA genomes. The deadly Ebola and Marburg viruses are well known members of this group, along with influenza virus, measles, mumps and rabies.

Reverse transcribing viruses

For more details on this topic, see Reverse transcribing virus.

- **Group VI**: viruses possess single-stranded RNA genomes and replicate using reverse transcriptase. The retroviruses are included in this group, of which HIV is a member.

- **Group VII**: viruses possess double-stranded DNA genomes and replicate using reverse transcriptase. The hepatitis B virus can be found in this group.

Exampel: chicken pox virus, varicella zoster

(VZV), order Herpesvirales,

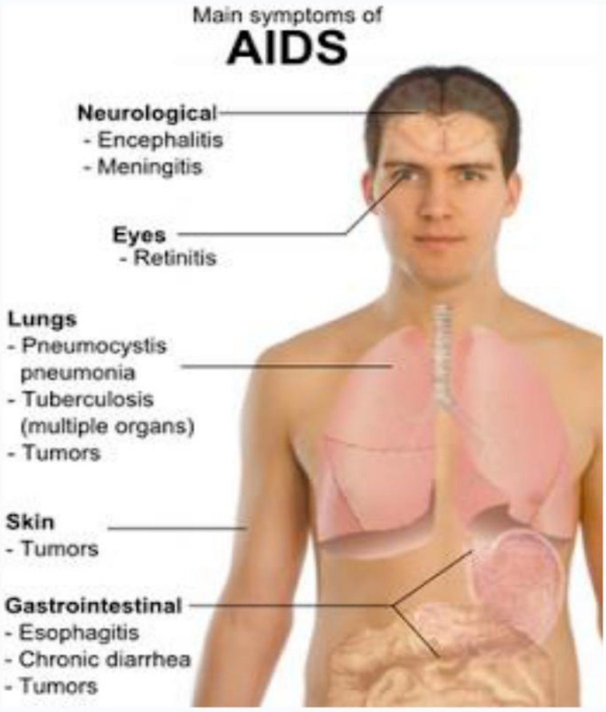
family *Herpesviridae*,

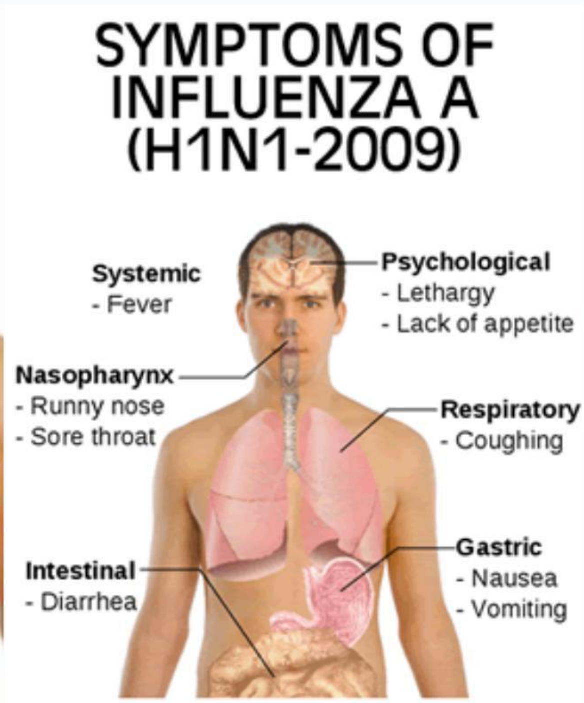
subfamily *Alphaherpesvirinae*,

genus *Varicellovirus*

VZV is in Group I of the Baltimore Classification because it is a dsDNA virus that does not use reverse transcriptase.

Reverse transcriptase, the key enzyme that retroviruses use to translate their RNA into DNA.





Fungi

Fungi(sing. Fungus) were previously were grouped as thalophytes, belonging to plant kingdom. Thallous is a plant body that has no distinct roots, shoots and stems. But now they are regarded as an independent kingdom.

About 90,000 fungal species have been described; however, some estimates of total numbers suggest that 1.5 million species may exist.

The four major Divisions of fungi are:

Zygomycetes:

The bread mold, *Rhizopus siolanifei* is a very common member of this division.

Ascomycetes:

The division *Ascomycota* contains the fungi called ascomycetcs, commonly known as the **sac fungi.** This division includes

Basidiomycetes:

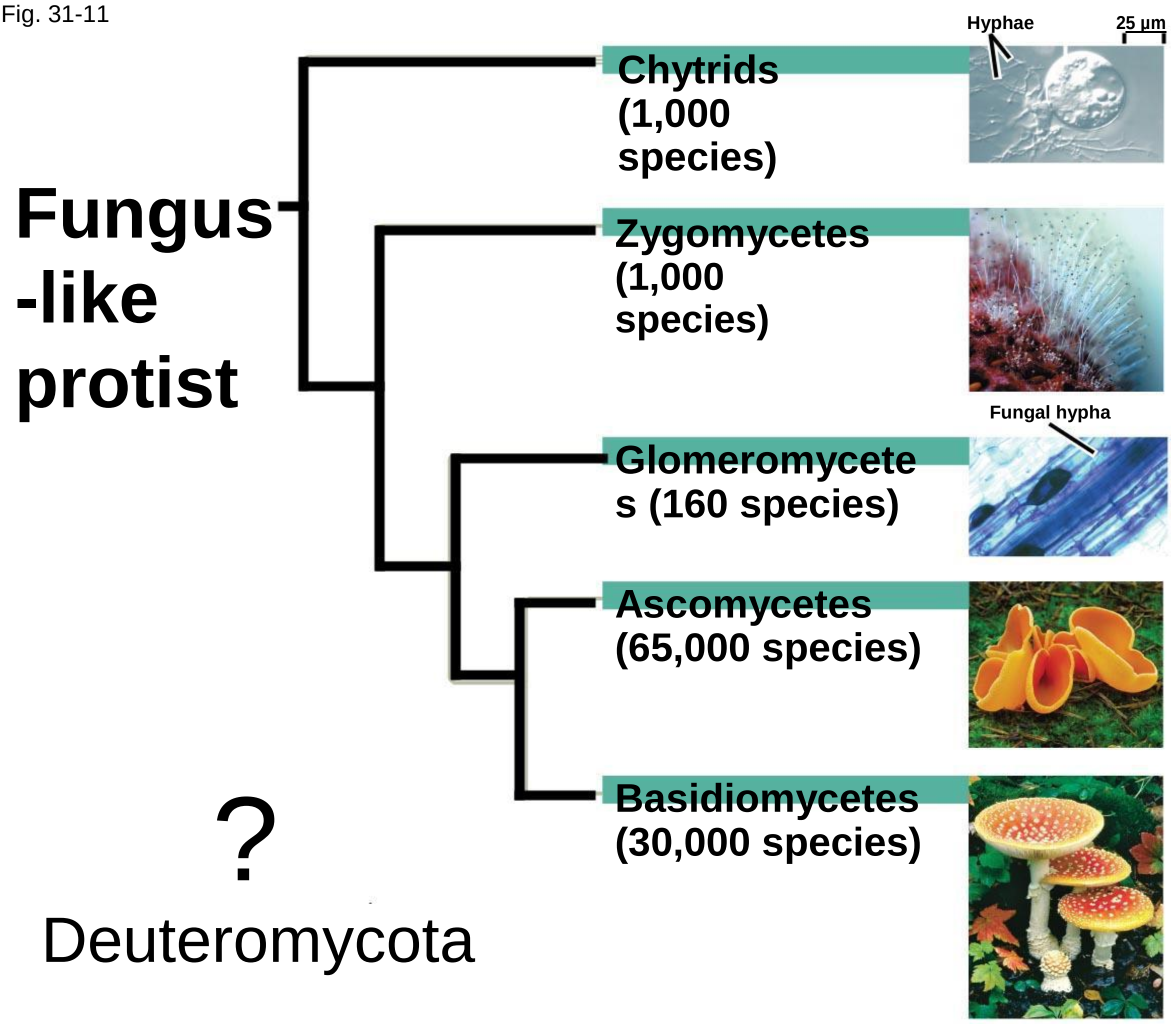
The division *Basidiomycota* contains the **basidiomycetes,** commonly known as the club fungi.

Chytridiomycetes:

The simplest of the true fungi belong to the division *Chytridiomycota.* This division contains one class, *Cliytridiomycetes,* and its members are known familiarly as the chytrids.

Chytrids are thought to have been derived from a protozoan ancestor having similar flagellation.

Fig. 31-11



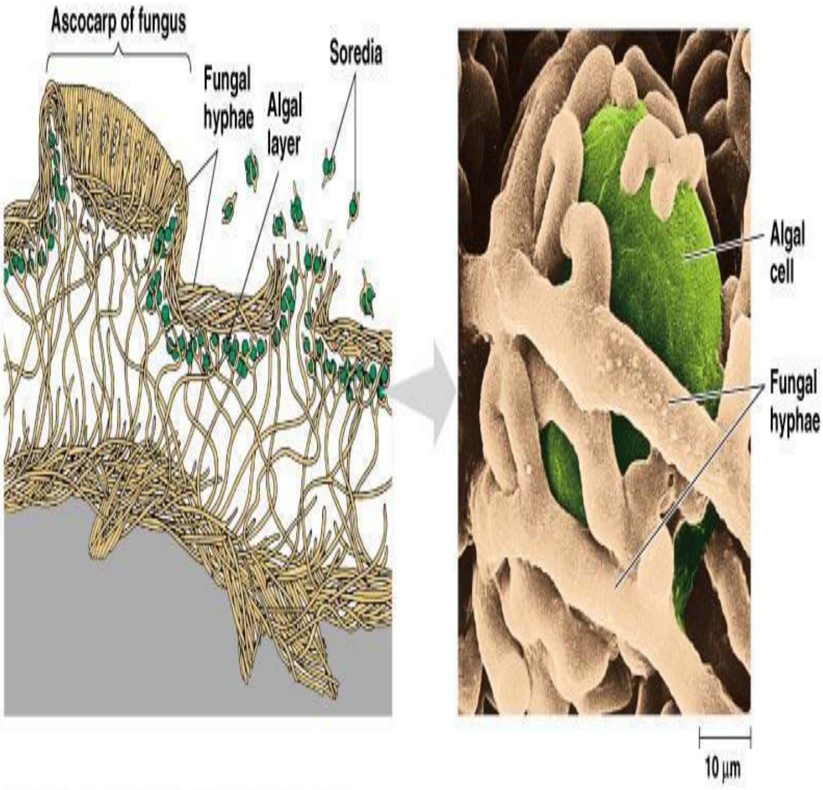
Habitat

i- Fungi are primarily terrestrial organisms, although a few are freshwater or marine. Usually they are found in the moist damp places and decomposing organic matter.

ii- Many are pathogenic and infect plants and animals.

iii- About three-fourths of all vascular plants form associations (called mycorrhizae) between their roots and fungi.

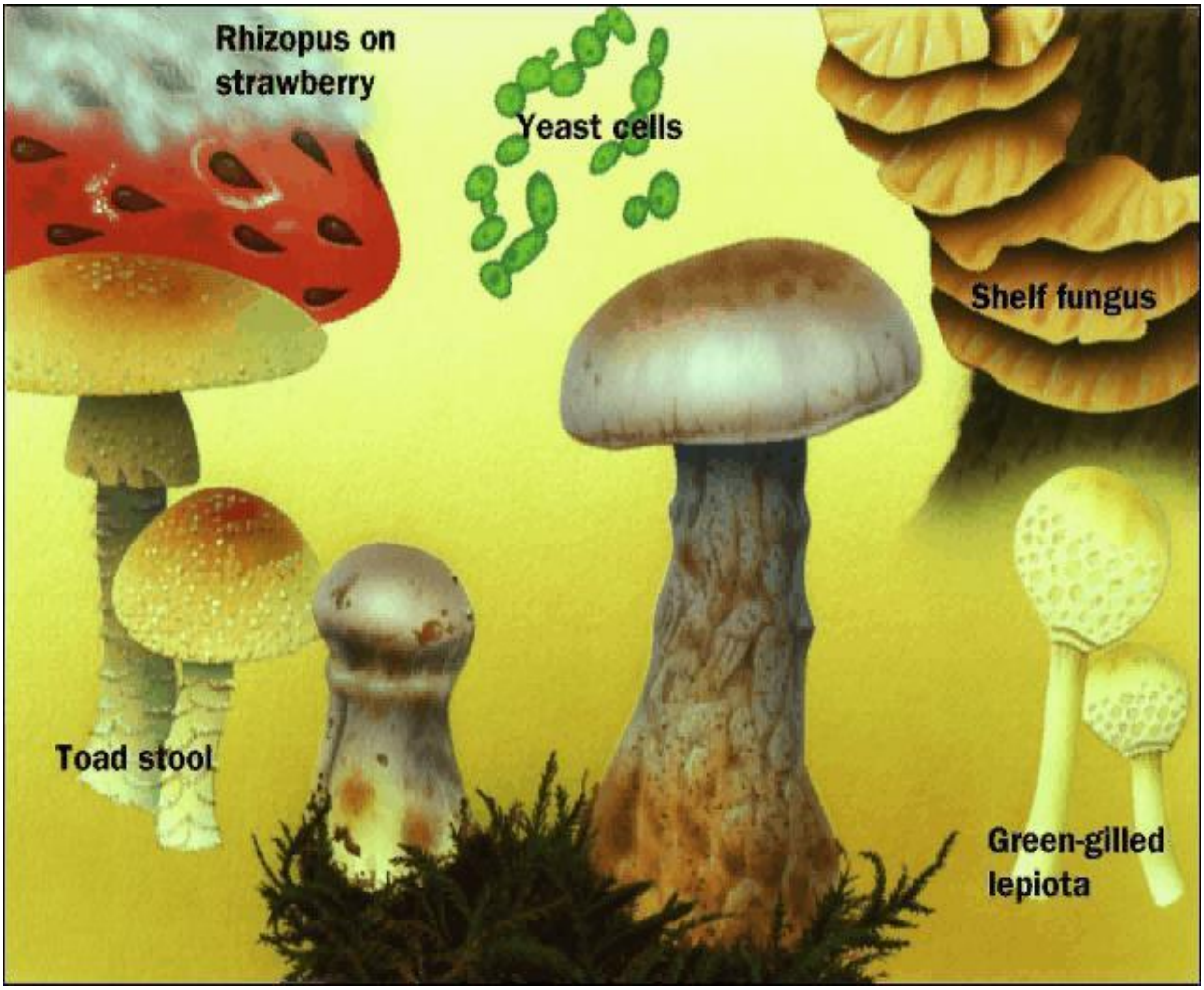
iv- Lichens are associations of fungi and either algae or cyanobacteria.



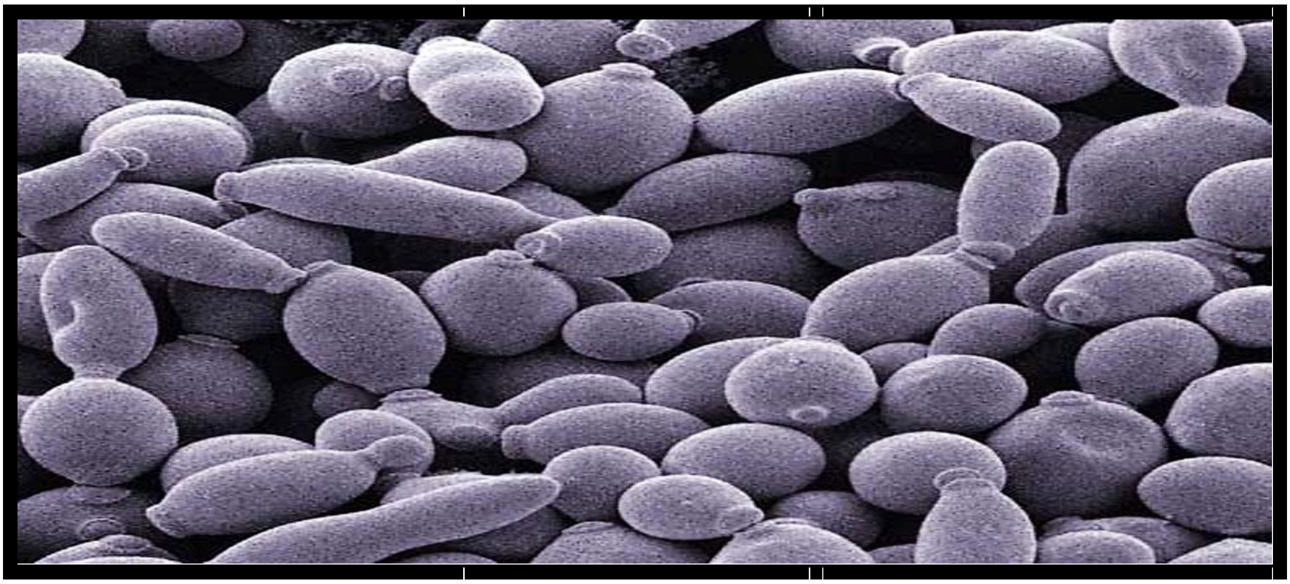
v.Fungi also are found in the upper portions of many plants. These endophytic fungi affect plant reproduction and palatability to herbivores.

Structure:

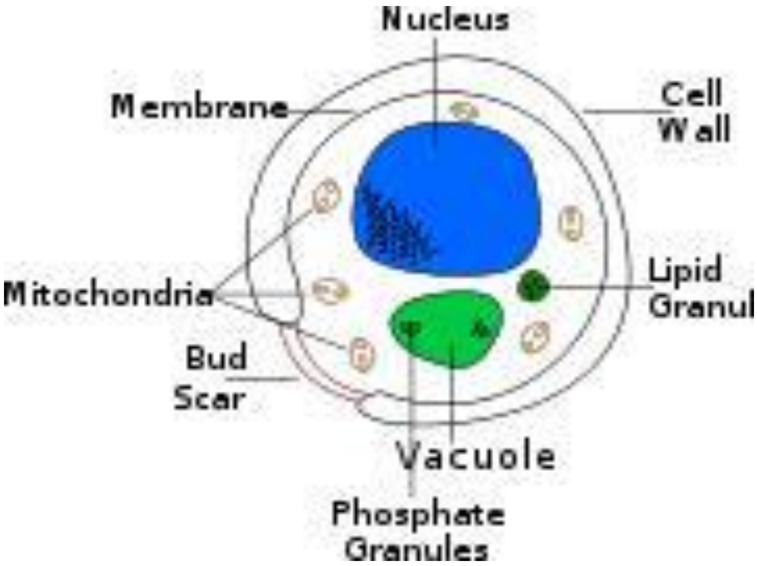
The body or vegetative structure of a fungus is called a thallus. It varies in complexity and size, ranging from the single-cell microscopic yeasts to multicellular molds, macroscopic puffballs, and mushrooms



Yeast



Yeast is a unicellular fungus that has a single nucleus. Generally yeast cells are larger than bacteria, vary considerably in size and are commonly spherical to egg shaped. They have no flagella but do possess most of the other eukaryotic organelles.



SIZE:

Yeast size can vary greatly depending on the species, typically measuring 3–4 µm in diameter, although some yeasts can reach over 40 µm.

TYPES: Almost 1500 species of yeast has been described.

Importance of Yeast

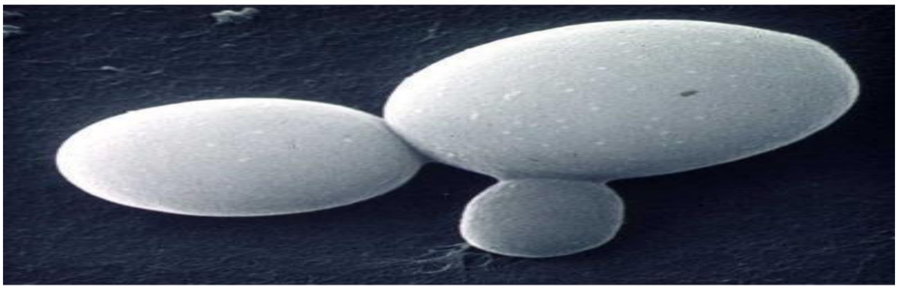
1- Fermentation: They respire by anaerobic respiration. They ferment the carbohydrates into ethanol (alcohol) and CO_2 .This process is very important in wine, beer and bread making.

Saccharomyces *cerevisiae* is used in baking and fermenting alcoholic beverages.

2-Research; It is also extremely important as a model organism in modern cell biology research, and is one of the most thoroughly researched eukaryotic microorganisms.

3-Candida albicans, are opportunistic pathogens and can cause infections in humans. *Candida* is commonly found as a commensal yeast in the mucus membranes of humans and other warm-blooded animals

Candida albicans



4- **Biofuel industry**: Yeasts have recently been used to generate electricity in microbial fuel cells, and produce ethanol for the biofuel industry.

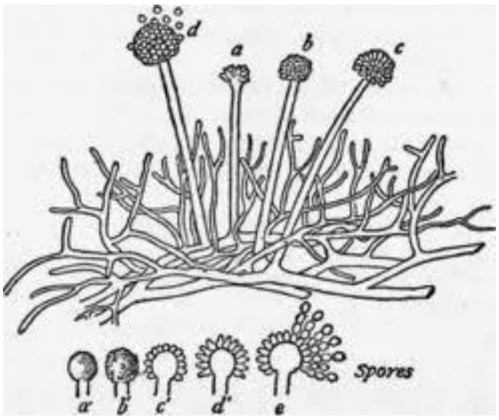
5- Spoilage of Wine: The growth of some yeast such as Zygosaccharomyces and Brettanomyces in wine can result in wine faults and subsequent spoilage.

6- **Spoilage of food:** Yeasts are able to grow in foods with a low pH, (5.0 or lower) and even in the presence of common preservatives sugars, organic acids and other easily metabolized carbon sources. In this way they spoil food.

Methylene Blue is used to test for the presence of live yeast cells.

Molds

Molds are multicellular fungi. **Hyphae: A** mold consists of long, branched, threadlike filaments of cells called **hyphae** that form a mycelium ,a tangled mass or tissue like aggregation .



Non Septate Hyphae:

They are not divide into cells by cross walls called septa. These hyphae are called **coenocytic** hyphae.

Septate Hyphae:

The hyphae of other fungi have cross walls called septa with either a single pore or multiple pores that permit cytoplasm streaming. These hyphae are termed **septate**. Hyphae are composed of an outer cell wall and an inner lumen, which contains the cytosol and organelles

<u>Dimorphic Hyphae in Animals</u>:

Many fungi, especially those that cause diseases in human and animals, are dimorphic that is, they have two forms. Dimorphic fungi can change from (1) the yeast (Y) form in the animal to (2) the mold or **mycelial** form (M) in the externernal environment in response to changes in various environmental factors (nutrients, CO2 tension, oxidation-reduction potentials, temperature). This shift is called the **YM shift**.

Yeast form --------------------> mycelial form

Animal **Environment**

<u>Dimorphic Hyphae in plants</u>:

In plant-associate; fungi the opposite type of dimorphism exists: the mycelial form occurs in the plant and the yeast form in the external environment.

Mycelial form ---------------------> Yeast form
Plant **Environment**

Cell wall:

The cell wall of fungi is composed of chitin. Chitin is more resistant to cellulose and lignin which make up plant cell wall

Nutrition: **1-**

Saprotrophs:

They get energy by decomposition of dead organic substances. Like many bacteria, fungi release hydrolytic exoenzymes that digest external substrates. They then absorb the soluble products. They are **chemo-organo-heterotrophs** and use organic compounds as a source of carbon, electrons, and energy.

2-Parasites

They may be either Obligate parasites or Facultative Parasites.

Obligate parasites: They can grow only on the host cell through special hyphal tips called haustoria.

Facultative: Besides living on their hosts they can also survive on the growth media. **Symbionts**

i. Lichens: It is symbiotic association between a fungus and alga.
ii. Mycorrhizae: It is symbiotic association between fungus and the roots of vascular plants.

Respiration:

Fungi usually are **aerobic**. Some yeast, however, are **facultatively anaerobic** and can obtain energy by fermentation, such as in the production of ethyl alcohol from glucose. Obligatory anaerobic fungi are found in the rumen of cattle.

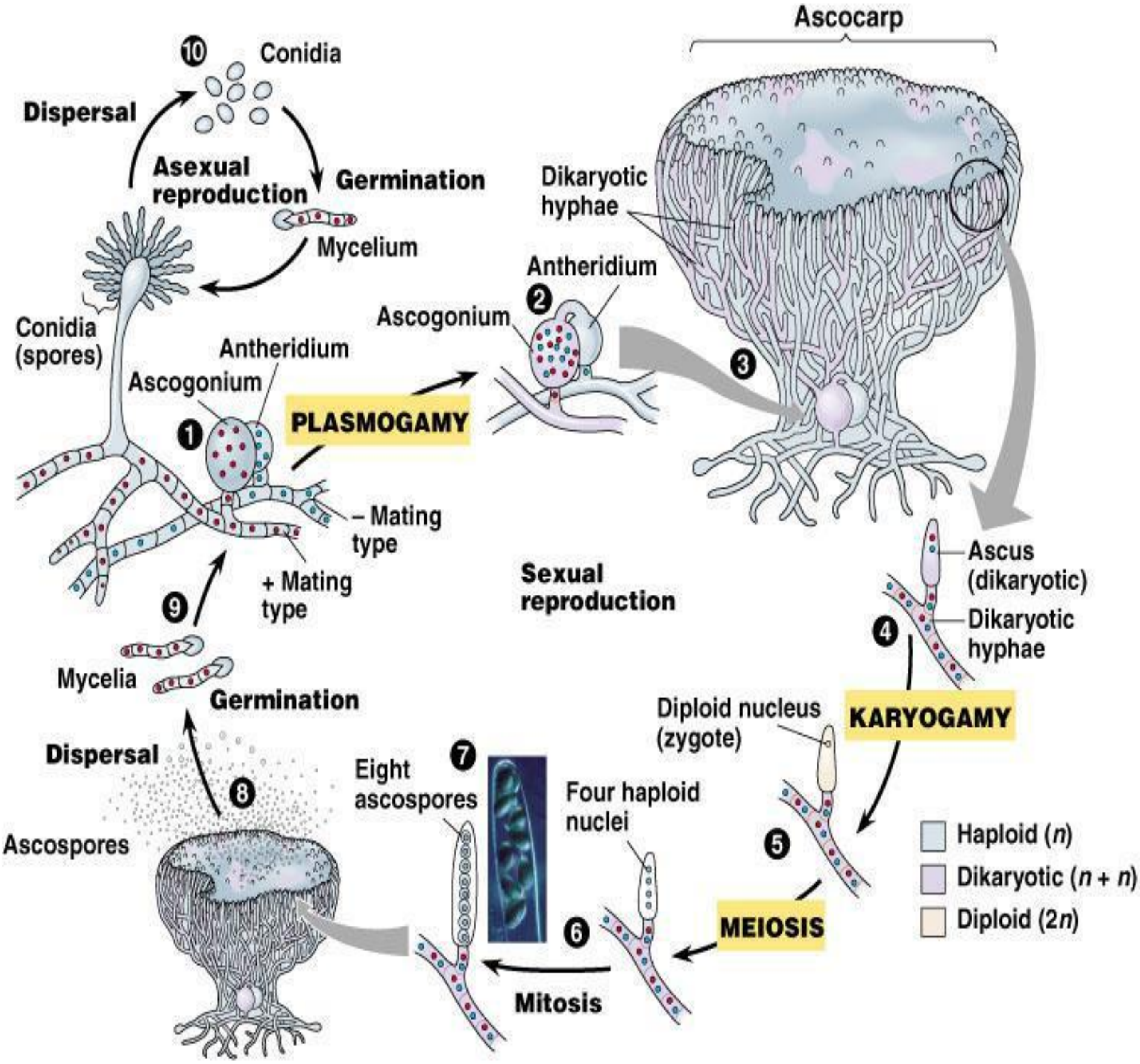
Reproduction:

Yeast: Budding

Asexual Reproduction in Molds:

i- Spores ii-Conida iii- Fragmentation

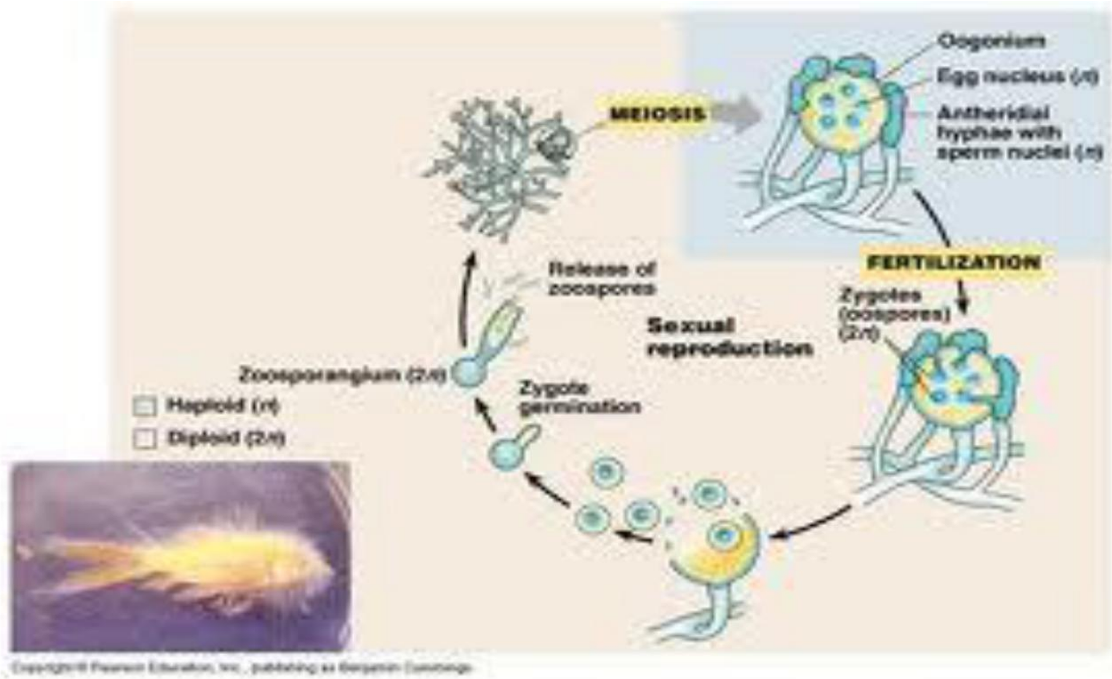
Sexual Reproduction

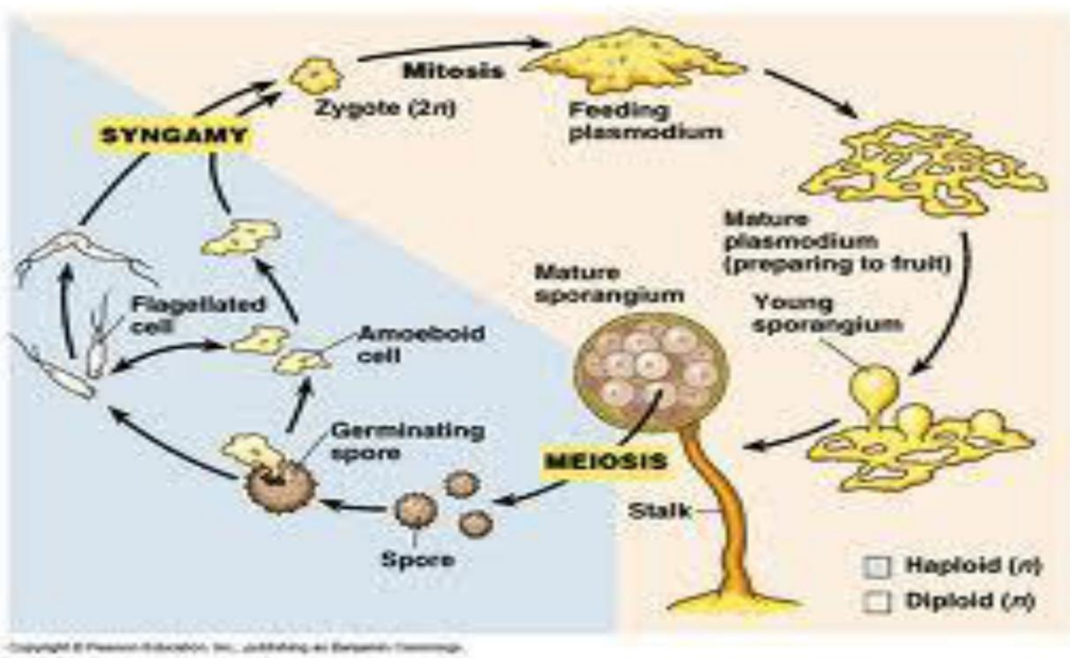


Ascocarp
10 Conidia
Dispersal
Asexual reproduction
Germination
Mycelium
Dikaryotic hyphae
Antheridium
2
Ascogonium
Conidia (spores)
Antheridium
Ascogonium
3
1
PLASMOGAMY
– Mating type
+ Mating type
Sexual reproduction
Ascus (dikaryotic)
9
4
Dikaryotic hyphae
Mycelia
Germination
Diploid nucleus (zygote)
KARYOGAMY
Dispersal
7
Eight ascospores
Four haploid nuclei
8
Ascospores
5
Haploid (n)
Dikaryotic (n + n)
Diploid (2n)
6
MEIOSIS
Mitosis

Slime Molds and Water Molds

The slime molds and water molds resemble fungi in only appearance and life-style. In their cellular organization, reproduction, and life cycles, they are phylogenetically distinct





(Slime mold) (Water mold)

Division Myxomycota (A cellular Slime Molds)

Under appropriate conditions plasmodial (a cellular) slime molds exist as streaming masses of colorful protoplasm that creep along in an amoeboid fashion over moist, rotting logs, leaves, and other organic matter. Feeding is by phagocytosis. Because this streaming mass lacks cell walls, it is called a plasmodium .The plasmodium contains many nuclei, and as the organism grows, the diploid nuclei divide repeatedly.

Division Acrasiomycota (Cellular Slime Molds)

The vegetative stage of cellular slime molds consists of individual amoeboid cells termed myxamoebae the myxamoebae feed phagocytically on bacteria and yeasts. When food is plentiful, they divide repeatedly by mitosis and cytokinesis, producing new daughter myxamoebae. A fruiting body called a sorus or sorocarp forms and matures and then produces spores The spores are eventually released, and when conditions become favorable, they germinate (o release haploid amoebae and repeat the cycle.

Division Oomycota

Members of the division *Oomycota* are collectively known as oomycetes or water molds. Oomycetes resemble true fungi only in appearance, consisting of finely branched filaments called hyphae. However, oomyceles have cell walls of cellulose, whereas the walls of most fungi are made of chitin. Oomycetes are also unlike the true fungi in that they have tubular mitochondria cristae.

Oomvcota means "egg fungi." a reference to the mode of sexual reproduction in water molds.

Nutrition and Habitat:

Saprotrophs:

Water molds such as *Saprolegnia* and *Achlya* are saprophytes that grow as cottony masses on dead algae and small animals, mainly in freshwater environments.

Decomposers:

They are important decomposers in aquatic ecosystems.

Parasites:

Some water molds are parasitic on the gills of fish.

Diseases:

1-The water mold *Peronospora hyoscyami* is currently responsible for the troublesome "**blue mold**" of tobacco plants throughout the world producing millions of dollars of damage yearly to tobacco crops.

2-Other oomycetes cause late blight of potatoes and grape downy mildew.

Microbiology of Water

There are two major types of water.

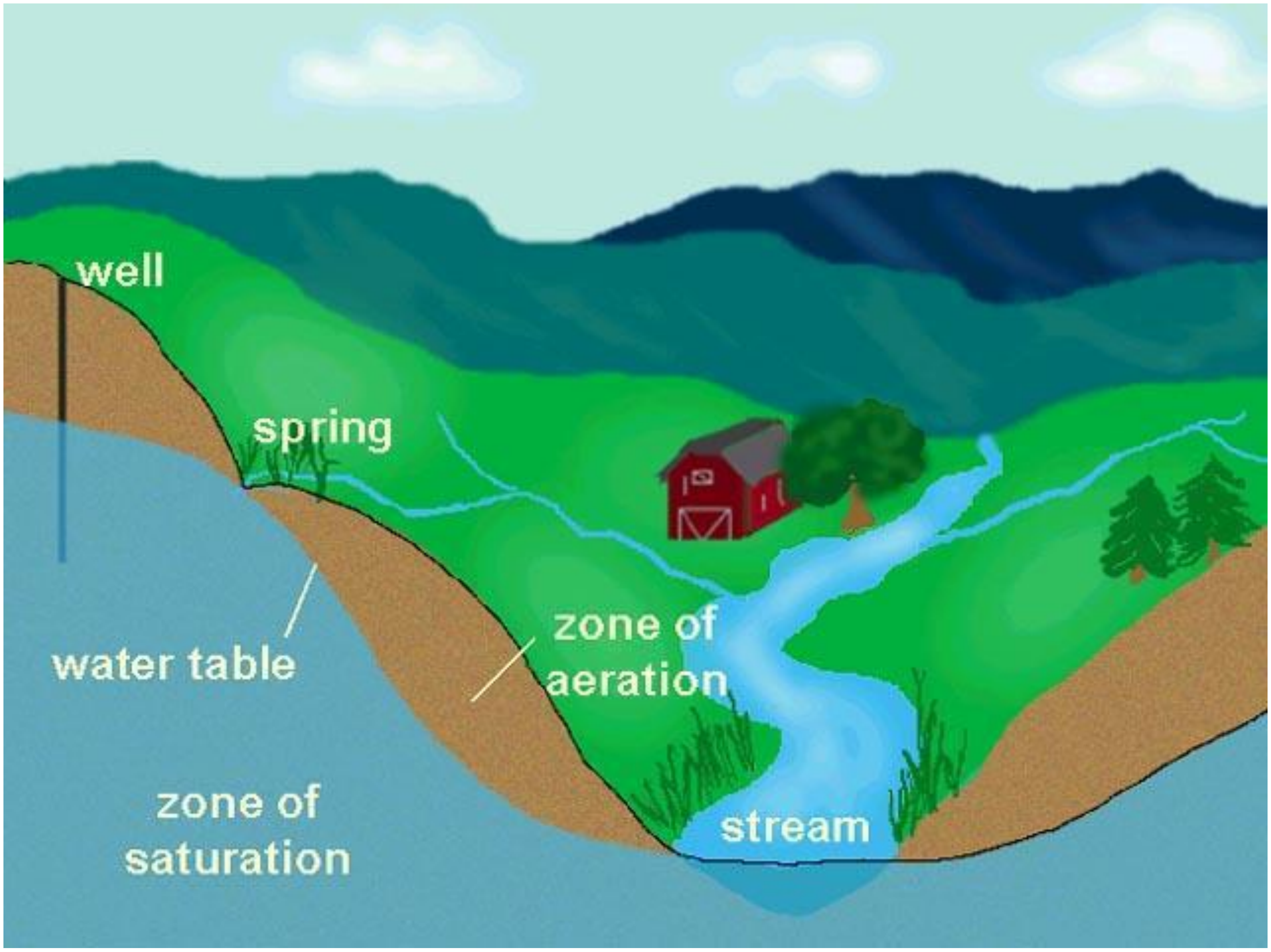
Ground water

It originates from deep wells and subterranean springs. This is virtually free of bacteria due to filtering action of soil, deep sand and rock. However, it may become contaminated when it flows along the channels.

Surface water

It is found in streams, lakes, and shallow wells. The air through which the rain passes may contaminate the water. Other sources are the various types of establishments and agricultural farms etc by the sides the water flows.
Possible sources of microbial contamination of a body of water are soil and agricultural run off, farm animals, rain water, industrial waste, discharges from sewage treatment plants and storm water run off from urban areas.
"Water microbiology is concerned with the microorganisms that live in water, or can be transported from one habitat to another by water."

Many microorganisms are found naturally in fresh and saltwater. These include bacteria, cyanobacteria, protozoa, algae, and tiny animals such as rotifers. These can be important in the food chain that forms the basis of life in the water. For example, the microbes called cyanobacteria can convert the energy of the sun into the energy it needs to live. The plentiful numbers of these organisms in turn are used as food for other life. The algae that thrive in water are also an important food source for other forms of life.



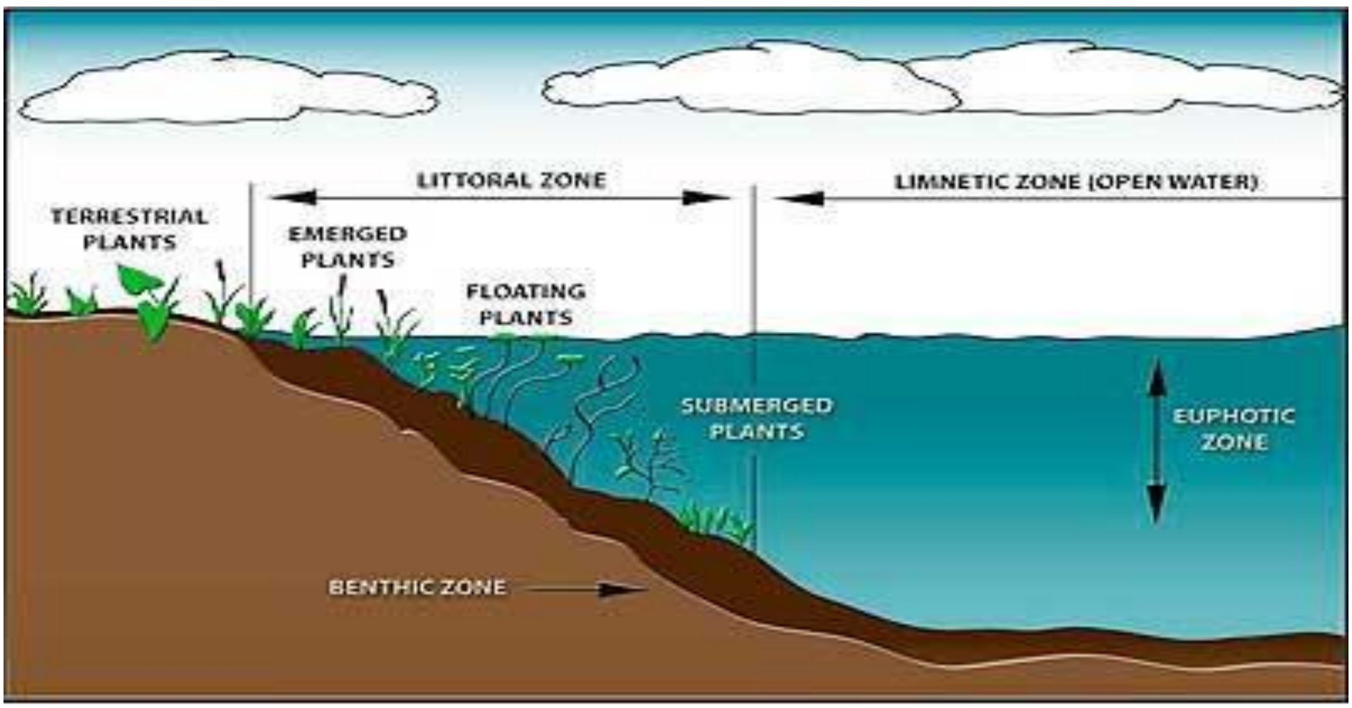
Water can support the growth of many types of microorganisms. This can be advantageous However, the presence of other disease causing microbes in water is unhealthy and even life threatening. For example, the chemical activities of certain strains of yeasts provide us with beer and bread. As well, the growth of some bacteria in contaminated water can help digest the poisons from the water.

. For example, bacteria that live in the intestinal tracts of humans and other warm blooded animals, such as Escherichia coli, Salmonella, Shigella, and Vibrio, can contaminate water if feces enter the water. Contamination of drinking water with a type of Escherichia coli known as O157:H7 can be fatal. The contamination of the municipal water supply of Walkerton, Ontario, Canada in the summer of 2000 by strain O157:H7 sickened 2,000 people and killed seven people.

The intestinal tract of warm-blooded animals also contains viruses that can contaminate water and cause disease. Examples include rotavirus, entero viruses, and coxsackievirus.

Another group of microbes of concern in water microbiology are protozoa. The two protozoa of the most concern are Giardia and Cryptosporidium

Fresh Water Microbiology



The Littoral Zone:

A variety of microorganisms live in fresh water. The region of a water body near the shoreline () is well lighted, shallow, and warmer than other regions of the water. Photosynthetic algae and bacteria that use light as energy thrive in this zone.

The Limnitic Zone:

Further away from the shore is the. Photosynthetic microbes also live here. As the water deepens, temperatures become colder and the oxygen concentration and light in the water decrease. Now, microbes that require oxygen do not thrive. Instead, purple and green sulfur bacteria, which can grow without oxygen, dominate.

The Benthic Zone

Finally, at the bottom of fresh waters (), few microbes survive. Bacteria that can survive in the absence of oxygen and sunlight, such as methane producing bacteria, thrive.

Saltwater Microbiology

Presents a different environment to microorganisms. The higher salt concentration, higher pH, and lower nutrients, relative to freshwater, are lethal to many microorganisms. But, salt loving (halophilic) bacteria abound near the surface, and some bacteria that also live in freshwater are plentiful (i.e., Pseudomonas and Vibrio). Also, in 2001, researchers demonstrated that the ancient form of microbial life known as archaebacteria is one of the dominant forms of life in the ocean. The role of archaebacteria in the ocean food chain is not yet known, but must be of vital importance.

Another microorganism found in saltwater is a type of algae known as dinoflagellelates. The rapid growth and multiplication of dinoflagellates can turn the water red. This "red tide"

depletes the water of nutrients and oxygen, which can cause many fish to die. As well, humans can become ill by eating contaminated fish.
Water can also be an ideal means of transporting microorganisms from one place to another. For example, the water that is carried in the hulls of ships to stabilize the vessels during their ocean voyages is now known to be a means of transporting microorganisms around the globe. One of these organisms, a bacterium called **Vibrio cholerae, causes life threatening diarrhea in humans.**

Methods To Mininmize the Microbial-Contamination

Drinking water is usually treated to minimize the risk of microbial contamination. The importance of drinking water treatment has been known for centuries.
1. For example, in pre-Christian times the storage of drinking water in jugs made of metal was practiced. Now, the anti-bacterial effect of some metals is known.
2. Similarly, the boiling of drinking water, as a means of protection of water has long been known.

3.Chemicals such as chlorine or chlorine derivatives has been a popular means of killing bacteria such as Escherichia coli in water since the early decades of the twentieth century.

4. Other bacteria-killing treatments that are increasingly becoming popular include the use of a gas called ozone.

5. The disabling of the microbe's genetic material by the use of ultraviolet light.

6. Microbes can also be physically excluded form the water by passing the water through a filter. Modern filters have holes in them that are so tiny that even particles as miniscule as viruses can be trapped.

Turbidity-Test

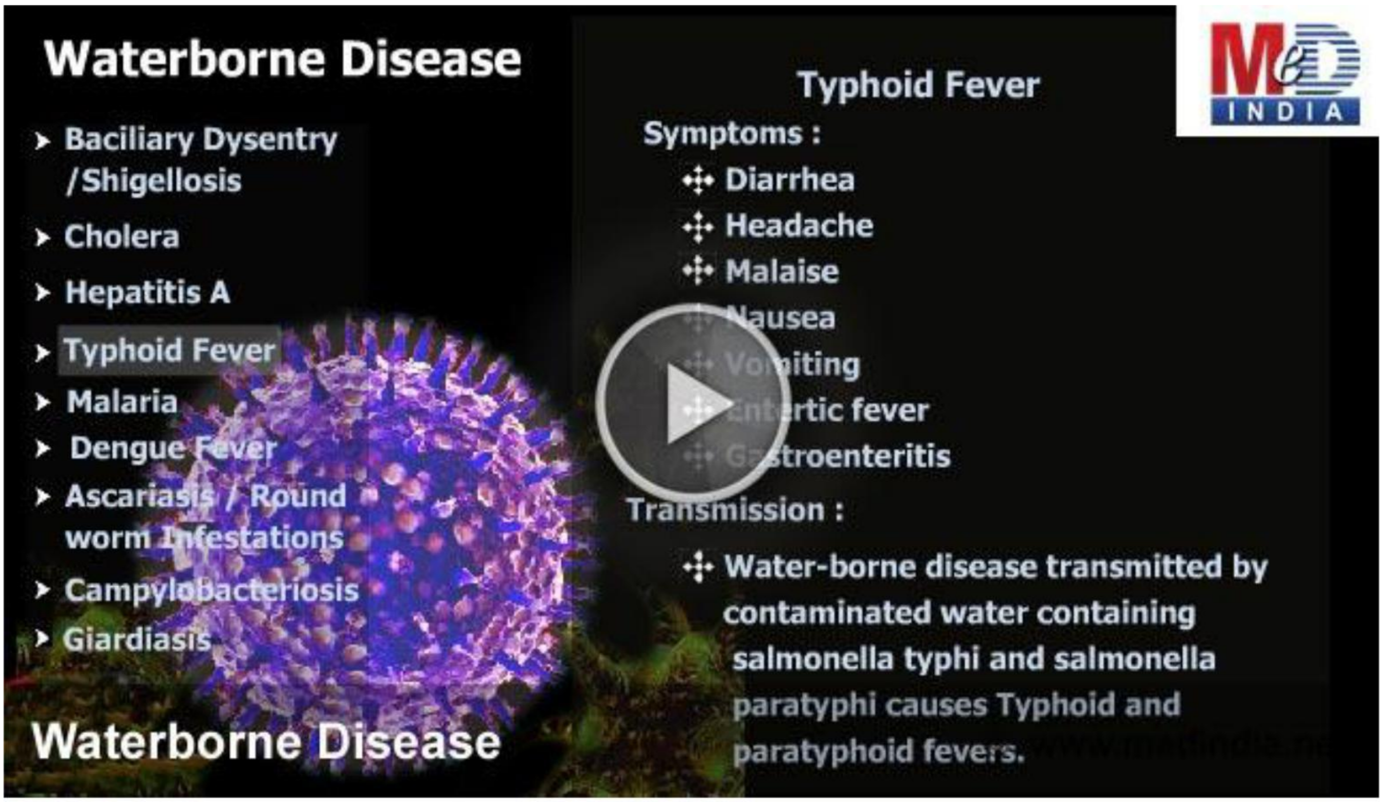
Turbidity-Test

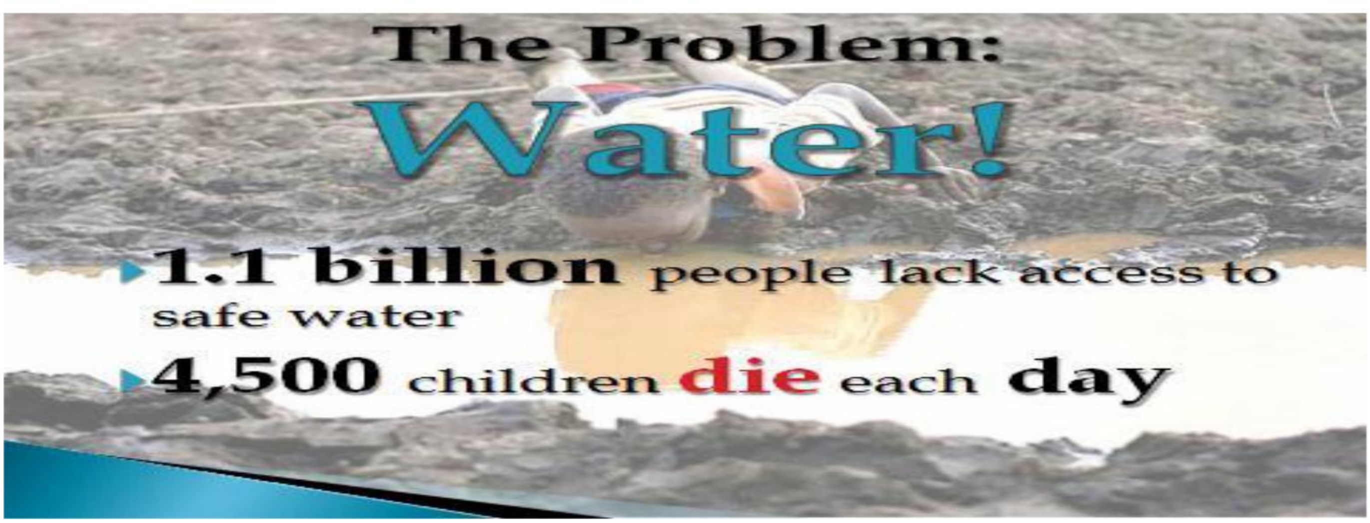
An important aspect of water microbiology, particularly for drinking water, is the testing of the water to ensure that it is safe to drink. Water quality testing can be done in several ways. One popular test measures the turbidity of the water. Turbidity gives an indication of the amount of suspended material in the water. Typically, if material such as soil is present in the water then microorganisms will also be present. The presence of particles even as small as bacteria and viruses can decrease the clarity of the water. Turbidity is a quick way of indicating if water quality is deteriorating, and so if action should be taken to correct the water problem.

In many countries, water microbiology is also the subject of legislation. Regulations specify how often water sources are sampled, how the sampling is done, how the analysis will be performed, what microbes are detected, and the acceptable limits for the target microorganisms in the water sample.

Testing for microbes that cause disease (i.e. Salmonella typhymurium and Vibrio cholerae) can be expensive and, if the bacteria are present in low numbers, they may escape detection. Instead, other more numerous bacteria provide an indication of fecal pollution of the water. Escherichia coli have been used as an indicator of fecal pollution for decades.

The bacterium is present in the intestinal tract in huge numbers, and is more numerous than the disease-causing bacteria and viruses. The chances of detecting Escherichia coli are better than detecting the actual disease causing microorganisms. Escherichia coli also had the advantage of not being capable of growing and reproducing in the water (except in the warm and food-laden waters of tropical countries). Thus, the presence of the bacterium in water is indicative of recent fecal pollution. Finally, Escherichia coli can be detected easily and inexpensively.

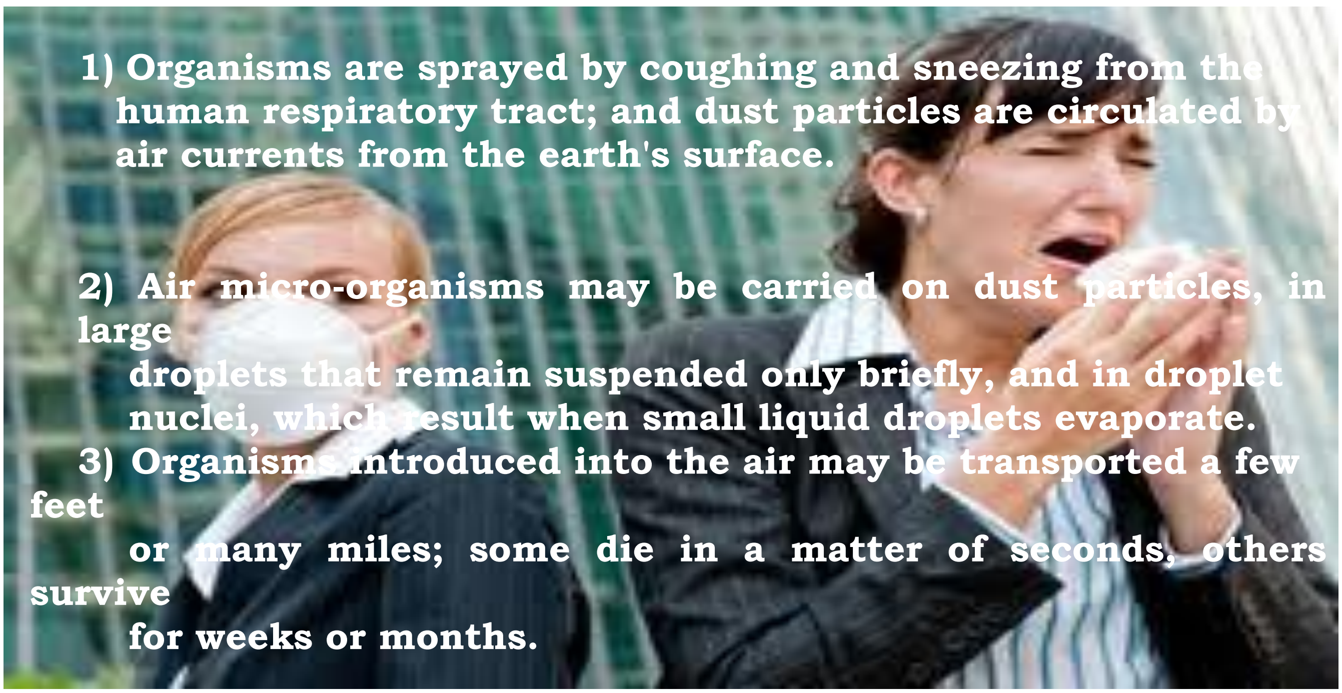




MICROBIOLOGY OF AIR

- The microbial flora of air is transient and variable.
- Air is not a medium in which micro-organisms can grow but a carrier of particulate matter, dust, and droplets, which may be laden with microbes.

Transmission of Air borne Micro-organisms.



Fate of Airborne Micro-organisms

The ultimate fate of airborne micro-organisms is governed by a complex set of circumstances including the atmospheric conditions, e.g.

a) Humidity
b) Sunlight
c) Temperature
d) Size of the particles bearing the microorganisms

e) Nature of the micro-organisms
i.e. the degree of susceptibility or resistance of a particular species to the new physical environment.

The Microbial Content of Air

Although no micro-organisms are indigenous to air, the air in our immediate environment as well as that several miles above the earth's surface, over land as well as over water, contains various species of micro-organisms in large or small numbers.

Indoor Air

The degree of microbial contamination of indoor is influenced by factors such as

1. Ventilation rates
2. Crowding
3. Nature and degree of activity of the individuals occupying quarters.

Sources of Transmission

The airborne micro-organisms are carried on dust particles or in droplets expelled from the nose and mouth during sneezing, coughing or even talking.

Size

- Particles of dust raised from surfaces and droplets expelled from the respiratory tract vary in their dimensions from micrometers to millimeters.
- Those in the low-micrometer range can remain airborne for long periods time, while the larger droplets and dust particles settle rapidly as dust on various surfaces. This dust becomes airborne intermittently during periods activity in the room.

Typical Examples

a) Tubercle Bacilli

Tubercle bacilli have been isolated from the dust of Sanitoria.

b) Diphtheria Bacilli and Hemolytic Streptococci

Diphtheria bacilli and hemolytic streptococci have been isolated from floor dust near patients or carriers harboring these organisms.

Out door: The Atmosphere

1- Algae, protozoa, yeasts, molds and bacteria have been isolated from the air near the surface of earth.
2- Mold spores constituted the largest portion of the airborne micro flora.
3- The predominant mold spores were of the species Cladosporium
4- Among the bacterial types were spore forming and non-spore forming gram positive bacilli, gram positive cocci, and gram negative bacilli,

Occurrence

Bacteria and mold spores have been found high above the earth's surface. The viable bacteria and fungi occur at an altitude of 3,000 m in air masses all the way across the North Atlantic.

Bacterial Species

Bacteria are characterized as:

a. Micrococcus
b. Sarcina
c. Gram-negative rods
d. Gram positive pleomorphic rods

e. Aerobic spore formers

Fungal species:

1. Cladosporium
2. Alternaria
3. Pullularia
4. Penicillium
5. Batrytis
6. Stemphylium

Cladosporium is the most abundant over land as well as sea.

Devices for Microbiological Analysis of Air

The sampling of air to determine its microbial content requires special instruments. Several devices, either

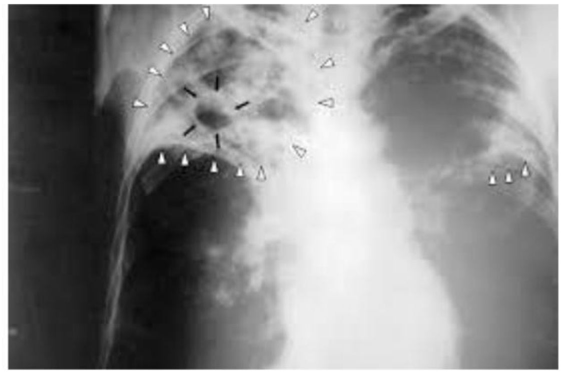
1. Solid impingement devices.
2. Liquid impingement devices have been designed for this purpose.
3. Liquid Impingement Devices:

Techniques Used

Some of the techniques and devices used for microbiological analysis of air are described as below

1) Setting-plate Technique:
2) Sieve and slit-type Samplers:
3) Membrane filter

Air Borne Diseases

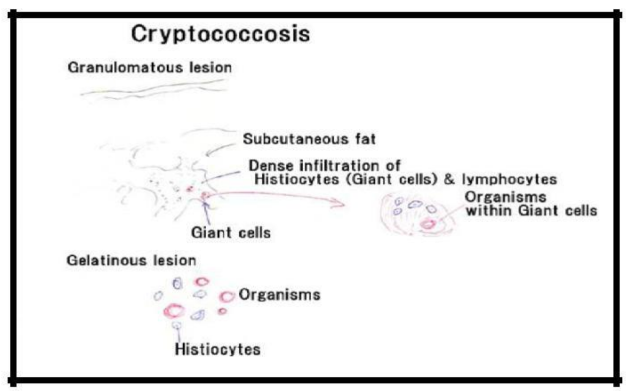


1. Bacterial

- o Diphtheria
- o Tuberculosis
- o Pneumonia
- o Meningitis

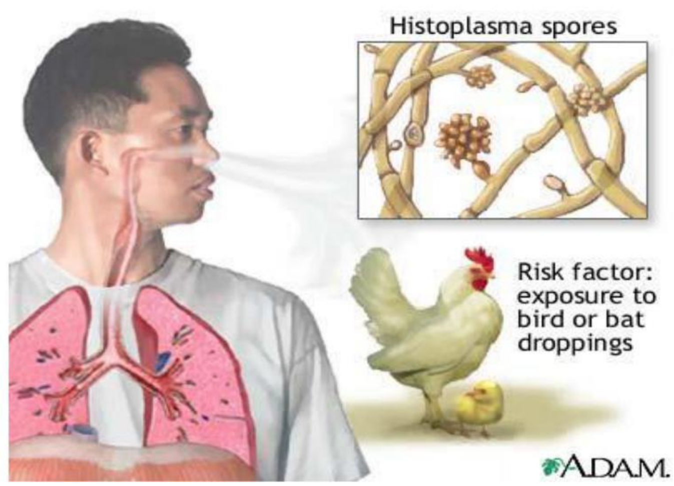
2. Viral

o Small pox
o Measles o Influenza
o Common cold



3. Fungal

- o Systemic Mycosis
- o Histoplasmosis
- o Cryptococcosis



CONTROL OF MICRO-ORGANISMS IN AIR

Since certain infectious agents may be airborne, air hygiene measures to reduce the microbial population of air is of great importance.
The level of air contamination can be reduced, or the air can be sterilized, as the situation demands, by the application of some of the physical and chemical agents.

There are several effective methods of controlling the level air contamination; which are discussed as below:

1) Ultraviolet Radiation

Ultraviolet radiation has great potential value for reducing the microbial flora of air. Practical application of ultraviolet air sanitation can be made in following ways.

a) Direct Irradiation:

b) Indirect Irradiation:

2) Chemical agents

Certain chemical substances vaporized or sprayed into the air of a room are effective in reducing the microbial flora.
Infect the chemical is dispersed as an aerosol and represents its antimicrobial action trough contacts with suspended particles carrying organisms.

Example:

- Triethylene glycol
- Formaldehyde
- Lactic Acid

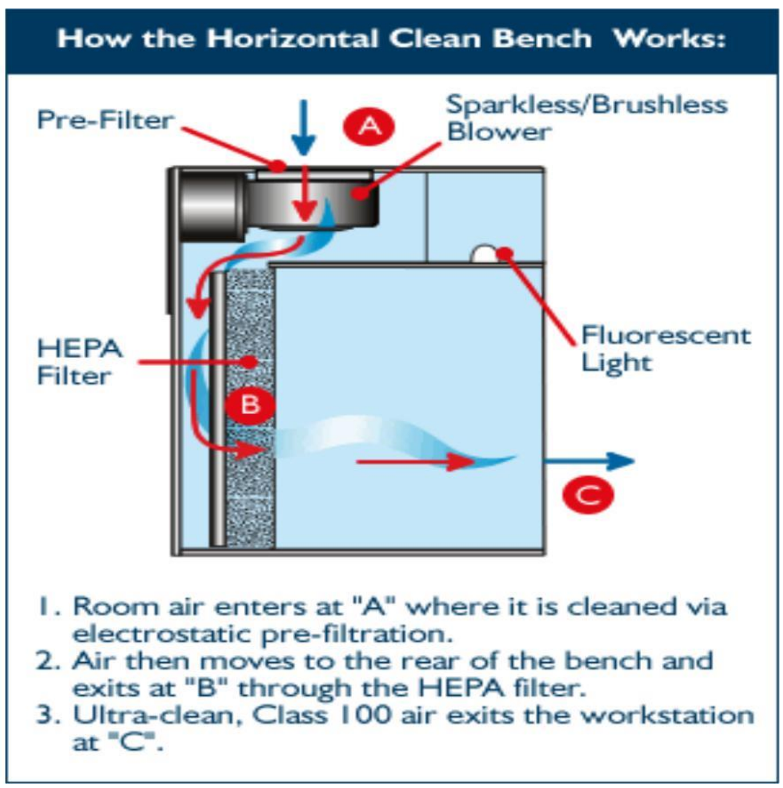
3) Filtration

Following filtration techniques are used for the of air borne organism.

a) Use of cotton plug
b) Air filter

4) Laminar- Airflow system

A new kind of technology for controlling the microbial flora in closed spaces (cabinets or rooms) is known as laminar airflow system.



MICROBIOLOGY OF SOIL

SOIL

The region of earth's crust where geology and biology meet is called soil. The characteristics of the soil environment vary with location and climate soil differs in depth, chemical composition, physical properties and origin.

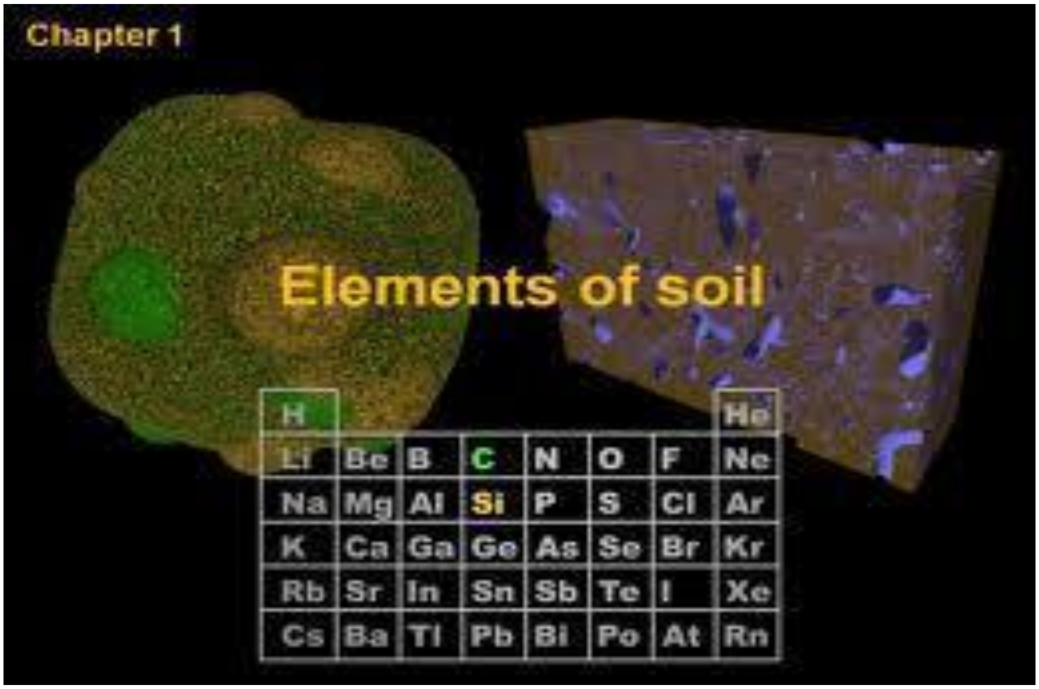
Soil Constituents

There are 5 major categories: Mineral particles, Organic residue, water, Gases, Biological systems.

1) Mineral Particles:

- The dominant mineral particles are compounds of Aluminum, Iron and Silicon.
- Less dominant are Calcium, Magnesium, Potassium, Sodium, Nitrogen, Sulphur, Phosphorous, Titanium, Manganese etc.
- Mineral constituents of soil range in size from small particles (0.002mm or lesser) to large pebbles and gravel.
- Soil can be classified as:

a) Organic soil (having very less inorganic solids but much of organic materials e.g., soil of marshes).



Water

The amount of water depends upon the amount of precipitation and other climate conditions, soil composition, drainage and the living population of soil.

Water is retained as:

- Free water in the spaces between soil particles.
- Absorbed to the surface of particles.
- Various organic and inorganic components of soil are dissolve in soil water and thus are made available as nutrients for soil inhabitants.

Gases

Gaseous phase of soil consists of mainly carbon dioxide, Oxygen and Nitrogen. Small amount of the gases are dissolved in water but much is present primarily in spaces between soil particles.

- The root systems of higher plants.
- Many small animals e.g., rodents, insects, worms etc.
- Tremendous no. of microbes.

Microbial Flora of Soil

1) Bacteria:

- Bacterial population is highest in both number (as several! billions/gm) and variety than all the other groups of microbes.
- Autotrophs and Heterotrophs; mesophiles, thermophiies and Sulphur oxidizers; nitrogen fixer and protein digesters and other kinds of bacteria are all likely to found in soil.

2) Fungi in Soil:

Their numbers range from thousands to hundreds of thousands, Fungi are active in decomposition of cellulose and lignin of plant tissue. The mould mycelium penetrates the soil and forms a net-work which entangles soil particles and forms water stable aggregates. This gives the soil its crumb structure, which is of considerable agricultural importance. Thus fungi a1so improve the physical condition of the soil. Of the other hand, yeasts are generally not found in large numbers except in soils of vineyards and orchards

3) Algae in Soil:

Algae are generally found on the surface of moist soils, where there is sufficient light for their photosynthetic reactions. The major types present are green algae Chlorophyceae, and the diatoms (Bacillariophyceae),
Their total numbers vary from several hundreds to several thousands. In some situations their number is quite large and brings about beneficial changes, especially desert soils. They fix nitrogen in paddy soils used for cultivation of rice.

4) Protozoa in Soil:

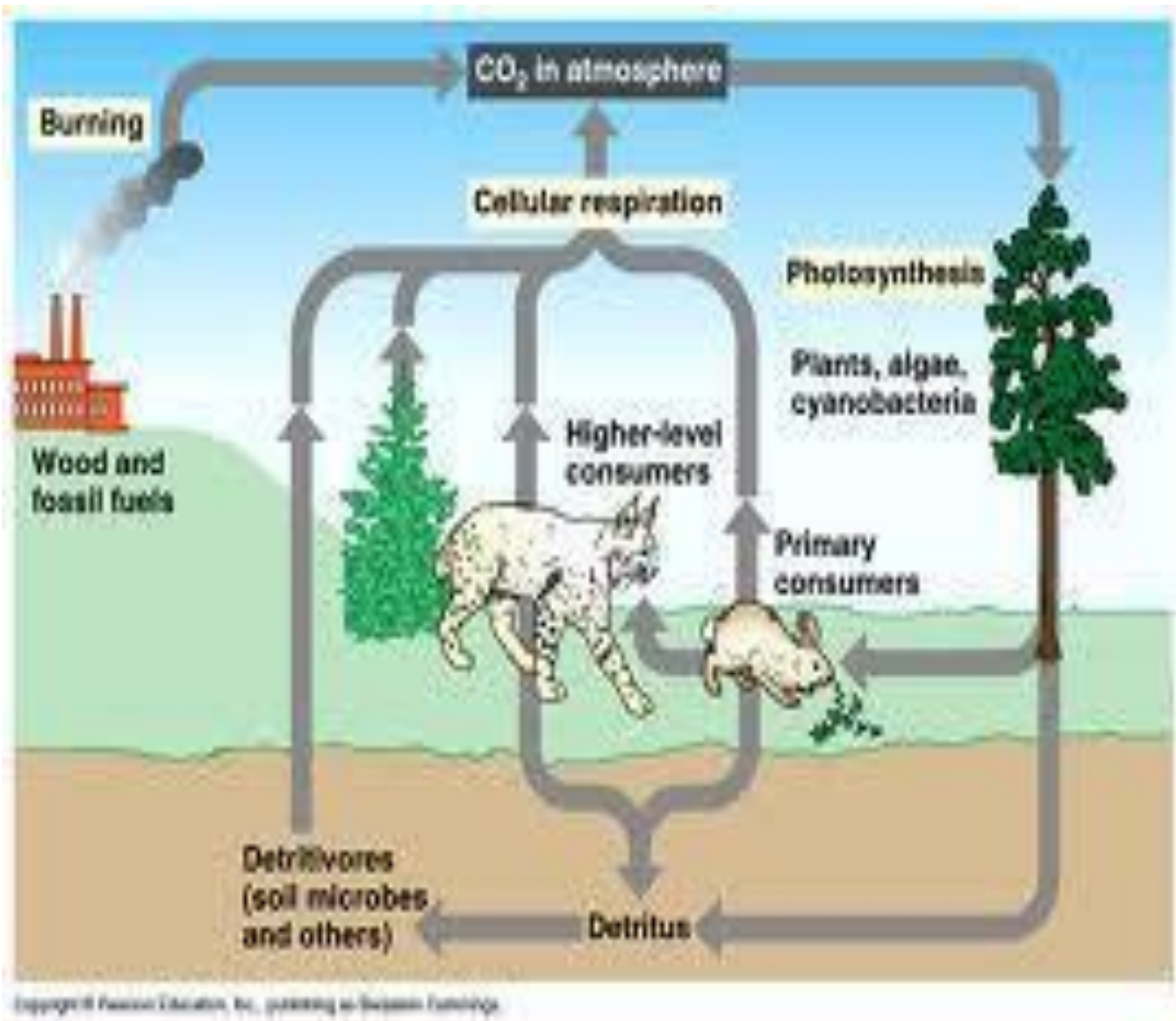
Many types of Protozoa are found in the soil, but flagellates and amoebae usually outnumber the ciliates. Their total numbers may range from a few hundred to several thousands. Depend-ing upon the conditions of the soil the Protozoa may exist as vegetative or cyst forms they use decaying organic matter for food and consume both dead and living bacteria. Since Protozoa do not ingest all bacteria, they maintain some equilibrium of the bacterial flora of the soil.

Roles of Soil Biota:

- Recycling of the energy, carbon, and nutrients in dead plant and animal tissues into forms that are potentially useful for living plants is the key role of soil microorganisms. Thus for the processing of materials that maintain life on earth, these organisms are quite important.
- Human activity has polluted the environment with a wide variety of synthetic or processed compounds. Many of these hazardous or toxic substances can be degraded by soil microorganisms.
- Soil microorganisms are also responsible for transformations of elements between various forms

Carbon Cycle

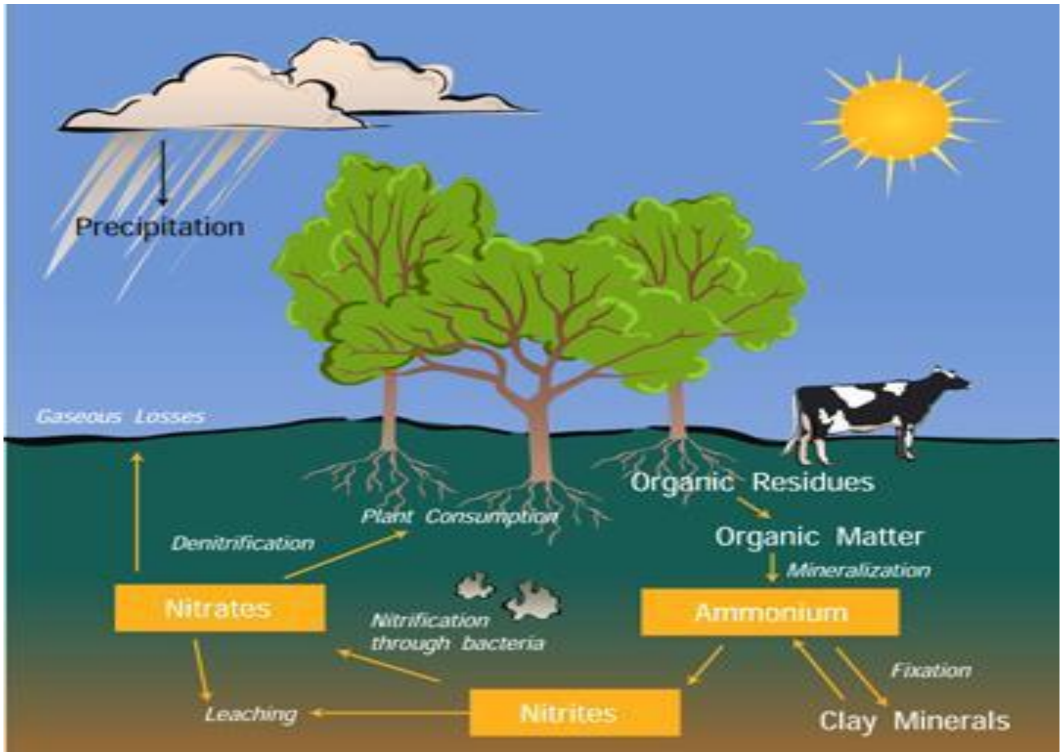
In the carbon cycle, microorganisms transform plant and animal residues into carbon dioxide and the soil organic matter known as humus. Humus improves the water-holding capacity of soil, supplies plant nutrients, and contributes to soil aggregation.



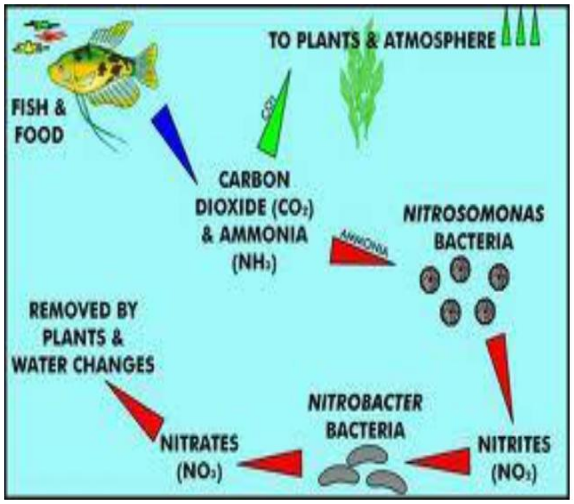
Nitrogen Cycle

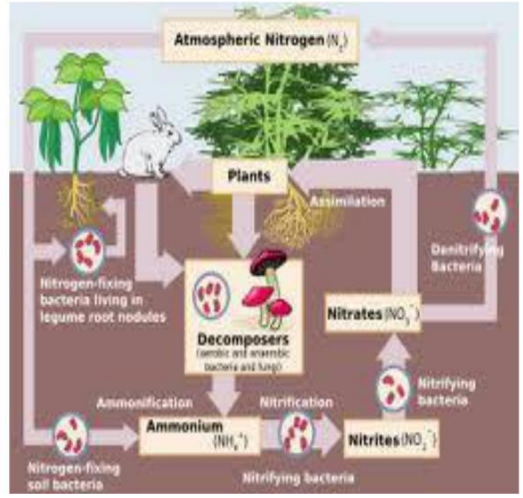
Soil microorganisms play key roles in the .nitrogen cycle. Nitrogen (N2) Fixation

The atmosphere is approximately 80% nitrogen gas (N2), a form of nitrogen that is available to plants only when it is transformed to



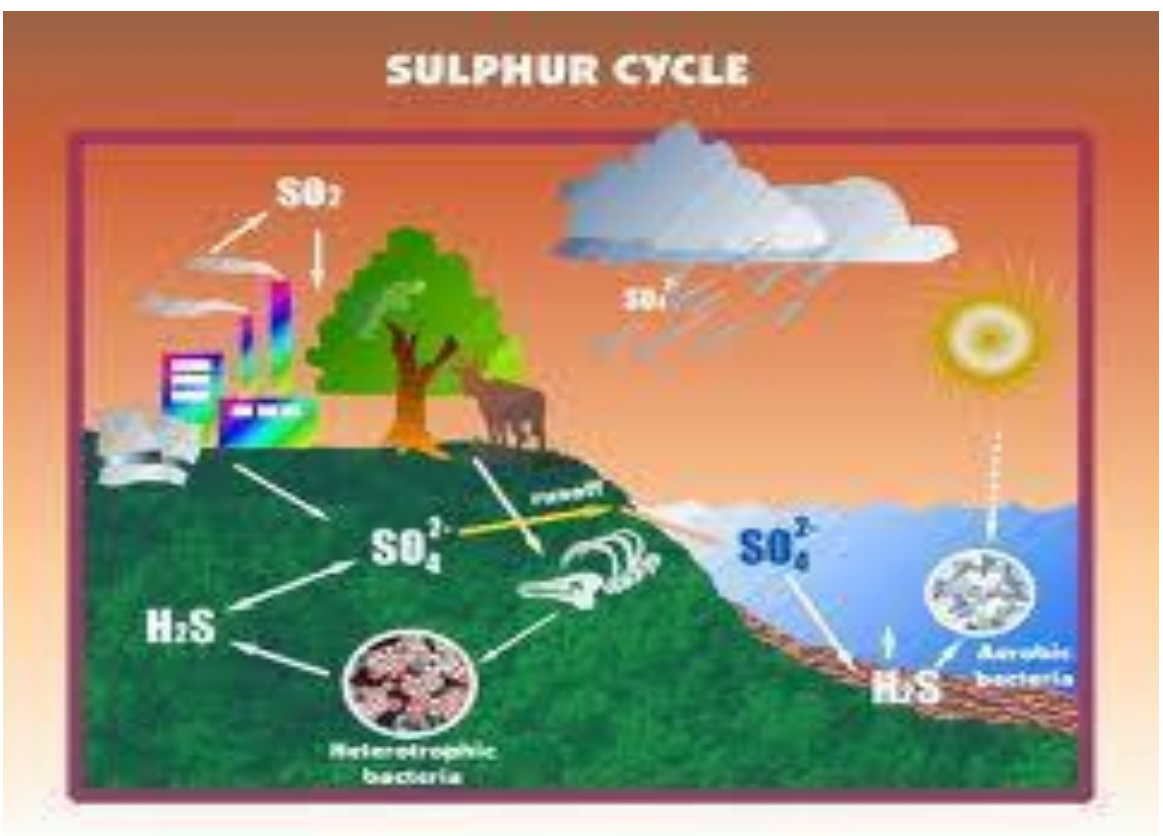
ammonia (NH3) by soil bacteria non-symbiotically by gram positive, spore forming, anaerobic bacillus known as Clostridium, pasteurianum and gram negative, non-spore forming, aerobic pleomorphic coccoid or rod shaped bacteria belonging, to the genus Azotobacter (Azote means nitrogen in French) and symbiotically by members of the genus Rhizobium (Rhizo means root in Greek) fix nitrogen symbiotically. These are gram negative, motile, aerobic, non spore forming bacteria. They are, mainly rod shaped, but when isolated from nodules a variety of morphological shapes are observed.





Sulphur Cycle

Cycling of sulphur is similar to that of nitrogen. Transformations between organic and elemental states and between oxidized and reduced states of sulphur are accomplished by various types of microorganisms, especially bacteria.



STERILIZATION

Sterilization is the process of killing or removing bacteria and all other forms of living organism and their spores from preparation or articles.

Method of Sterilization

Method of sterilization have been divided into following categorie

1) Physical Methods

- Dry heat sterilization
- Moist heat sterilization
- Sterilization by radiations

2) Chemical Methods

- Gaseous sterilization
- Sterilization by disinfectants

3) Mechanical Methods

- Sterilization by filtration

Physical Methods

a) Dry Heat Sterilization

Substances which are destroyed by moist heat may be sterilization moist heat. Dry heat can be used to sterilize items but as the heat takes much longer to be transferred to the organism both the time and the temperature must usually be increased unless forced ventilation of the hot air is used .The standard setting for a hot air oven is at least two hours at 160 C (320 0F)

A rapid method heats air to 190 0C (374 0F) for 6 minutes for unwrapped objects and 12 minutes for wrapped objects Dry heat has the advantage that it can be used on powders and other heat-stable items that are adversely affected by steam.

Substances Sterilized:

Substances which are sterilized by dry heat include fixed oils liquid paraffin, petroleum, propylene glycol and powders in addition to this it is also applied to sterilize glass ware many surgical instruments and surgical catgut. Volatile preparations or substances and surgical dressing can not be sterilized by this method.

Mechanism:

During dry heat sterilization the microorganism and bacteria spores are killed by oxidation since dry heat is less effective than moist heat higher temperature and longer period of exposure are required Exposure at 160 degree for one hour is required for dry heat sterilization

Advantages

- Suitable for substances destroyed by moisture
- Glass wares like flasks test tubes pipettes can be sterilized
- Less damaging to glad and metal equipments than moist

Disadvantages

- Can not used for volatile and thermolabile substances.
- Required long heating time and high temperature
- Not suitable for surgical dressings.

Methods

1. Flaming

It is simplest method of dry heat sterilization in which the material to be sterilized is kept in the hot part of of the Bunsen burner flame for few seconds and the process is repeated several times This method is generally used for those articles which are to be used immediately for example forceps ,blades, knives,needles wire loops ,metal spatulas.

2. Hot Air Oven

It consists of metallic chamber of aluminium or stainless steel, which is electrically heated and thermostatically controlled they are of two types.

i. In which air is circulated by gravity convection to all parts of the chamber.
ii. Mechanical convection type in which air is circulated by fan. The later type is more satisfactory because sterilizing temperature is controlled.

Glass ware conical flasks test tubes pippets etc. are sterilized by this method they should be plugged with non absorbent cotton wool because absorbent cotton wool becomes saturated during process.

3. Incineration

Incineration will also burn any organism to ash. It is used to sanitize medical and other biohazardus waste before it is discarded with non- hazardous waste.

b) Moist heat sterilization

It is the most reliable method of sterilization because in the presence of moisture bacteria are destroyed at a considerably lower temperature rather than dry heating.

Mechanism

By this method the microorganism are destroyed by denaturizing and coagulation of some of the essential proteins present in the microorganisms.

Advantages

- Microbes are killed more effectively
- Ampoules are readily sterilized by this method
- Bulk quantities surgical dressing and surgical instrument are effectively sterilized

Disadvantages

- Thermolabile substances and ointments can not be sterilized.

Method

i. Autoclaving
ii. Heating with bactericide
iii. Heating with boiling water
iv. Tantalization

1. **Autoclaving**

Autoclaving is the process of heating in an autoclave in which saturated steam under pressure is allowed to penetrate through the material for 20 minutes at temperature of 121C.

Autoclave

It is an apparatus used for sterilization by **steam under pressure.**

Working

- ✓ Autoclaves commonly use steam heated to 121 C or 134C.
- ✓ To achieve sterility, a holding time of at least 15-20 minutes at 121 C or 3 minutes at 134 C is required.
- ✓ Additional sterilizing time is required for liquid and instruments packed in layer of cloth as they may take longer to reach the require temperature.
- ✓ Proper autoclave treatment will in activate all fungi, bacteria, viruses and also bacterial spores which can be quite resistant.
- ✓ It will no necessarily eliminate are prions.
- ✓ For prion elimination, various recommendation states 121-132C for 60 minute or 134C for at least 18 minutes.

Precautions:

- ✓ For effective sterilization steam needs to penetrate the autoclave load uniformly.
- ✓ During the initial heating of the chamber, residual air must be removed.
- ✓ For autoclaving, as for all disinfection of sterilization methods cleaning is critical.
- ✓ Cleaning can also removed large number of organisms.
- ✓ Cleaning instruments or utensils inorganic matters, cool water must be used.
- ✓ Treatment with ultrasound or pulsed air can also be used to removed debris.

2. Heating With A Bactericide

- In this method bactericide is added to the solutions to be sterilized which are the sealed.
- The sealed containers are then heated at 100C for 30 minutes in water bath.
- Commonly used bactericide includes benzalkonium chloride, clorocresol.

3. Sterilization by boiling water

- The boiling water bath is most use full for sterilizing instruments like syringes, knives, blades, scissors and others. They are completely dipped in boiling water for 20 minutes.

4. Tyndallization

Tyndallization named after john Tyndall is a lengthy process designed to reduce the level of activity of sporulating bacteria that are left by a simple boiling water method.

- The process involves boiling for a period at atmospheric pressure, cooling, incubating for a day and finally boiling again.
- The three incubation periods are to allowed heat-resistant spores surviving the previous boiling period to germinate to form the heat sensitive vegetative stage which can be killed by next boiling steps.
- Tyndallization ineffective against prions.

c) Sterilization by Radiation

Sterilization by radiation is also known as cooled sterilization because no heat is used in this method. The microorganisms are very susceptible to lethal effects of radiation.

Mechanism

- By radiations, alteration of chemicals takes place present in microorganisms with the formation of new compounds which destroy the microbes.

- ✓ The vital structures of cells such as nucleoproteins are destroyed by radiations which kill the microbes.

Advantages

- ✓ Used in the preservation of food and parenterals containing antibiotics.
- ✓ Used for the sterilization of some bacterial and viral vaccines.
- ✓ No aseptic handling is required because sterilization can be done after packing.

Disadvantages

- ✓ High cost.
- ✓ Radiations are harmful the persons operating.
- ✓ Radiations may lead to change in color, texture and solubility.

Methods

Methods exist to sterilize using radiation such as electron beams, X- rays, gamma rays, or subatomic particles.

Gamma rays

- ✓ Gamma rays are very penetrating and are commonly used for sterilization of disposable medical equipment, such as syringes, needles, cannulas and IV sets.

Electron beam processing

- ✓ Electron beam processing is also commonly used for medical device sterilization, Electron beams use an on-off technology and provide a much higher dosing rate then gamma or x-rays.
- ✓ Due to the higher dose rate, less exposure time is needed and thereby any potential degradation to polymers is reduced.
- ✓ A limitation is that electron beams are less penetrating than either gamma or x-rays.

X-Rays

- ✓ X-rays are less penetration than gamma rays and tend to require longer exposure times, but require less shielding, and are generated by an x-ray machine that can be turned off for servicing and when not in use.

Ultraviolet light

- ✓ Ultraviolet light irradiation (UV, from a germicidal lamp) is useful only for sterilization of surfaces and some transparent objects.
- ✓ Many objects that are transparent to visible light absorbed UV.
- ✓ It also damages many plastics, such as polystyrene foam.

2. Chemical Sterilization

- ✓ Chemical are also used for sterilization. Although heating provides the most reliable way to rid objects of all transmissible agents, it is not always appropriate, because it will damage heat-sensitive materials such biological materials, fiber optics, electronics, and many plastics.

Chemical sterilization includes:

1. Ethylene oxide
2. Ozone
3. Chlorine bleach
4. Glutaraldehyde
5. Formaldehyde
6. Hydrogen peroxide
7. Peracetic acid
8. Prions

Chlorine bleach

- Chlorine bleach is another accepted liquid sterilizing agent.

- House hold bleach consists of 5.25% Sodium hypochlorite.
- It is usually diluted to 1/10 immediately before use; however to kill Mycobacterium tuberculosis. It should be diluted only 1/5.
- The dilution factor must take into account the volume of any liquid waste that it is being used to sterilized.
- Bleach will kill many organisms immediately, but for full sterilization it should be allowed to react for 20 min.
- Bleach will kill many, but not all spores.
- It is highly corrosive and may corrode even stainless steel, surgical instruments.

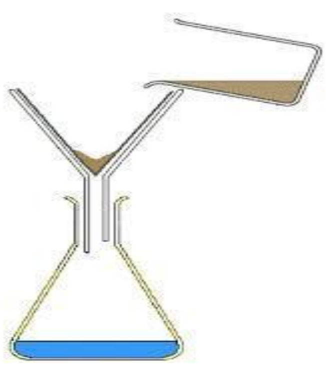
Ozone

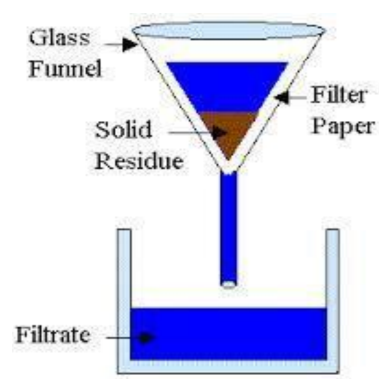
Ozone is used in industrial setting to sterilize water and air, as well as a disinfectant for surfaces.
It has the benefit of being able to oxidize most organic matter. On the other hand, it is a toxic and unstable gas that must be produced on sight, so it is not practical to use in may settings.

3. Mechanical methods

It includes filtration.

Filtration





Involves the physical removal (exclusion) if all cells in a liquid or gas. It is especially important for sterilization of solutions which would be denatured by heat (e.g. antibiotics, inject able drugs, amino acids, vitamins, etc.) portable units can be used in the field for water purification and industrial units can be used to "pasteurize" beverages. Essentially, solutions or gasses are passed through a filter of sufficient pore diameter to remove the smallest known bacterial cells.

Preservatives:

Static agents used to inhibit the growth of microorganisms, most often in foods. If eaten they should be nontoxic and sulfur dioxide.

Antimicrobial agents

Are chemicals that kill or inhibit the growth microorganisms.

- ✓ Antimicrobial agents include chemical preservatives and antiseptics, as will as drugs used in the treatment of infectious diseases of plants and animals.
- ✓ Antimicrobial agents may be of natural or synthetic origin, and they may have a static or cidal effect on microorganisms.

Types of antimicrobial agents

Antiseptics:

Microbial agents harmless enough to be applied to the skin and mucous membrane; should not be taken internally.

Examples

Include alcohols, mercurial, and silver nitrate, and iodine solution, alcohols, detergents.

Disinfectants:

Agents that kill microorganisms but not necessarily their spores but are not safe for application to living tissues, they are used on inanimate objects such as tables, floors, utensils etc.

Example

Includes hypochlorite chlorine compound dye, copper sulfate, quaternary ammonium compounds, and formaldehyde and phenolic compounds.

Application of sterilization processes

The pharmacist uses sterilization process in pharmaceutical tries for sterilization of glassware and other equipments; the preparation of injectables, ophthalmic, irrigating and sterile dosage forms ; and for the dilution of sterile medicaments. However, it must be kept in mind that all types of medicaments and other articles cannot be sterilized by any one method; the pharmacist should have thorough knowledge of sterilization processes that for certain kind of medicament or article, whether dry heat, moist heat, filtration method or any other method is to be used.

In hospital practice the sterilization processes are used for the preparation of sterile gauzes and dressings; for sterilization of glass equipment like test tubes, petri dishes, pipettes, all glass syringes etc. for sterilization of operation theatre, and all types of articles used in operation theatre including aprons, gloves, face masks, hoods, forceps, scissors, knives etc.
The following articles/medicaments require sterilization:

1) Glassware

The glassware and apparatus like flasks, beakers, funnels, dropper bottles, glass rods, pipettes, petridishes, glass tube pestle & mortar, tiles, ointment tubes, ampoules, vials syringes, needles etc. may be sterilized either by moist or dry heat but dry heat method is preferred. .

2) Equipment

Apparatus or devices made up of metal and surgical instruments, spatulas, metal part of bacterial filters such as millipore filter, Seitz filter etc may be sterilized by steam under pressure at a temperature of 121°C for 15 to 30 minutes, depending on the size of instruments. The materials should be wrapped with muslin so that steam may penetrate to the surface of the metal parts.ss Sharp edged instruments should not be sterilized in hot air because long heating at high temperatures in hot air lead to oxidation which reduces the sharpness of the blades.

(3) Rubber and plastics

Rubber articles such as stoppers, gloves, certain catheters and special feeding tubes may be sterilized in the autoclave at 121°C for 20 minutes they must not be subjected to dry heat because high temperatures will spoil the rubber articles. Some synthetic rubbers such as silicon rubber has good heat resistance power therefore may be sterilized either by dry heat or moist heat.

(4) Aqueous/non-aqueous solutions and suspensions

Thermostable aqueous solutions should be sterilized by steam under pressure in an autoclave at a temperature of 115°C to 118°C for 30 minutes but medicaments which are sufficiently thermostable may be sterilized at a temperature of 121°C for 15 minutes. Intravenous solutions such as dextrose and electrolytes; irrigating solution and all thermostable injections in ampoules or vials should be first clarified by filtration and then distributed to final containers under aseptic conditions which are then sealed and sterilized by steam under pressure

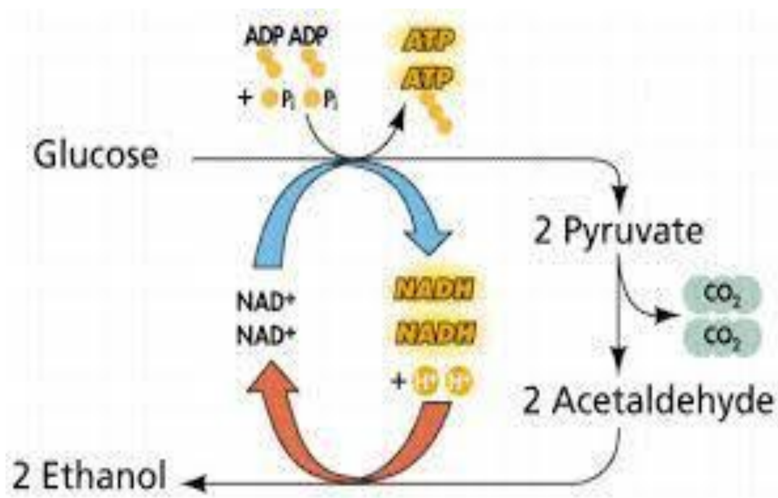
6) Surgical dressings and fabrics

The materials used in surgery and in the treatment of wounds and infections include cotton wool, gauze, gauze swabs, bandages, operation gowns, caps and masks, towels, trolley cloths and rubber sheets. These materials may be sterilized by dry heat, moist heat, ethylene oxide or ionizing radiations.

Fermentation

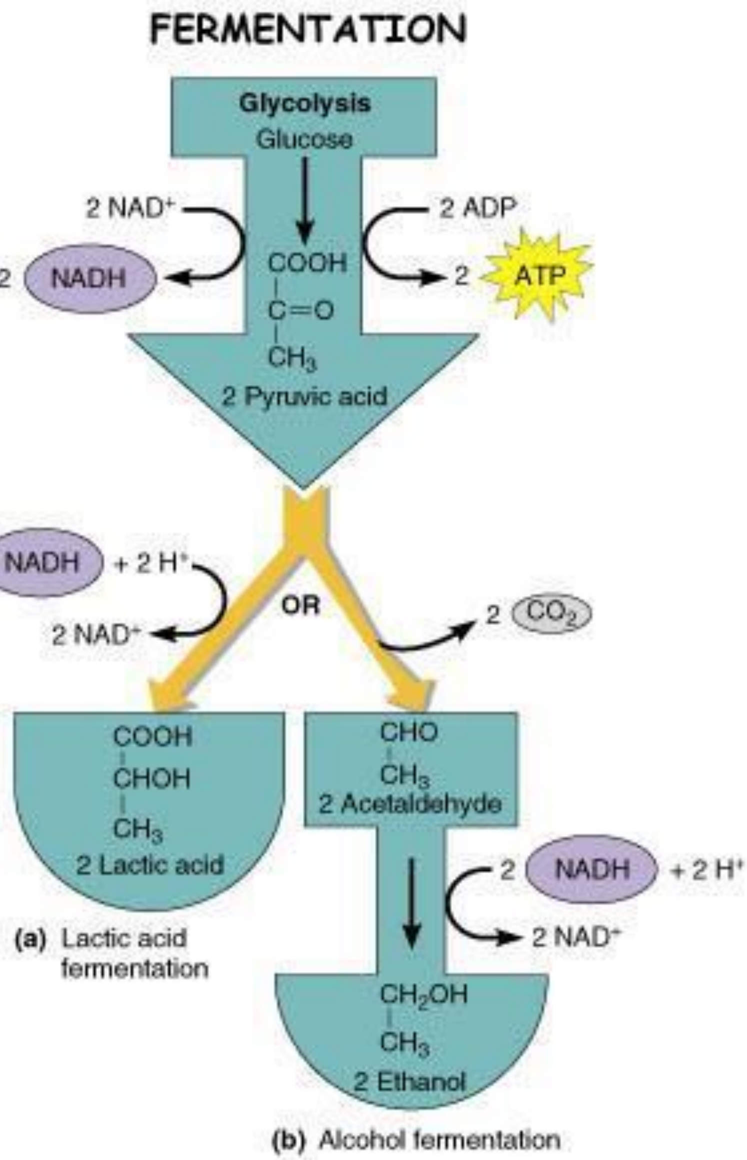
Definition

The chemical process of fermentation is a type of anaerobic respiration because it does not use oxygen as final electron acceptor. Fermentation is a unique process because an organic molecule, usually an intermediary of the chemistry, accepts the electrons.



Process

For example, in the fermentation of glucose by certain bacteria and viruses an intermediately accepts the electrons and proton from NADH formed in reaction of glycolysis. This regenerates the molecules for reuse as electron acceptors. NAD exists in limited supply in the cytoplasm and must be continually regenerated so the glycolysis may proceed. (When oxidative phospborylation is taking place, the NAD is regenerated by giving up its electrons and proton to FAD.)



The bacterium Streptococcus lactis practices fermentation by using pyruvic acid to accept the electrons and proton from NADH. An enzyme reaction converts the pyruvic acid to lactic acid in the process. In a dairy plant, the metabolism is carefully controlled to make buttermilk from fresh milk

. **The fermentation chemistry in yeasts** such as Saccharomyces is somewhat different because yeasts contain a different enzyme. In these cells the pyruvic acid is first converted to acetaldehyde, a process in which carbon dioxide evolves. Acetaldehyde then serves as an acceptor for the electrons and prolong of NADH, and it changes to ethyl alcohol. The liquor industry uses the ethyl alcohol produced in fermentation to make alcoholic beverages such as beer and wine.

Uses

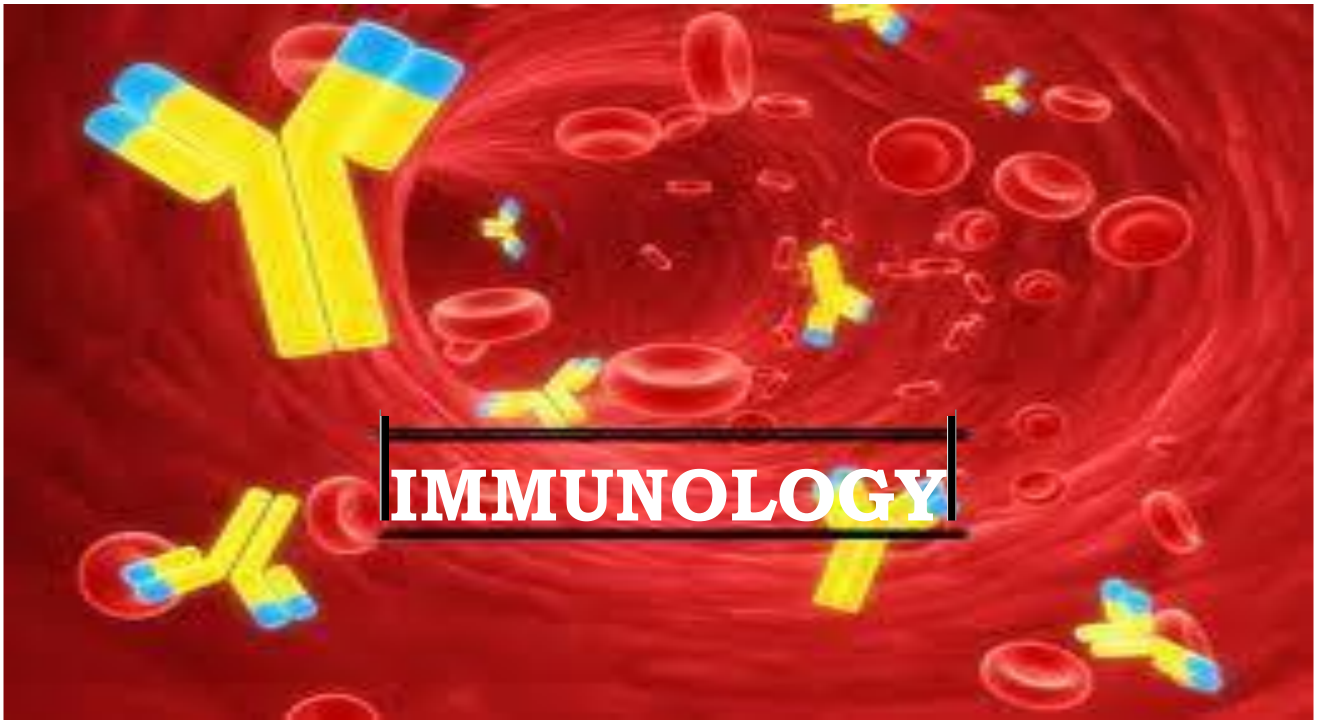
- The food industry, fermentation results in a broad variety of useful product.
- Vinegar, for instance, is a fermentation product of Acetobator species.
- Swiss cheese develops its flavor partly from the propionic acid of fermentation its holes from fermentation gases.
- Pickles and sauerkraut arc- sour because bacteria ferment the carbohydrates in cucumbers and cabbage, respectively.
- Sausage tastes like sausage because bacteria ferment the meat proteins.

- Thus fermentation is useful not only to the microorganisms but also to consumers enjoy the products of fermentation.

Pharmaceuticals and the biotechnology industry

There are 5 major groups of commercially important fermentation:

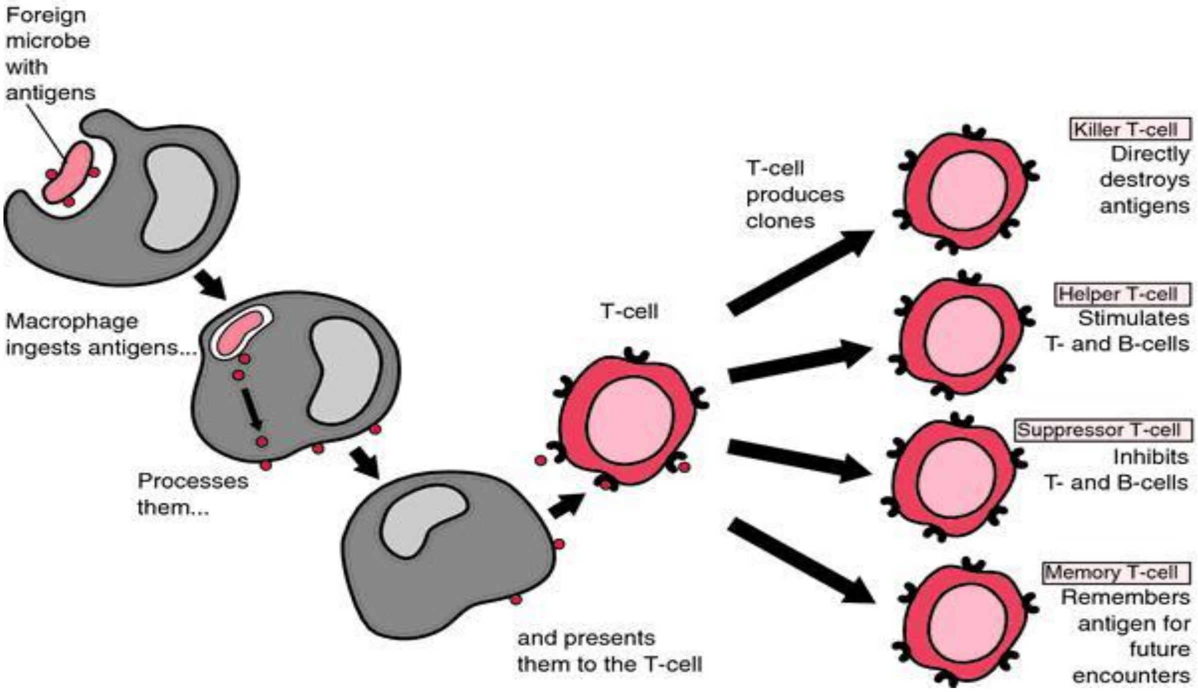
1. Microbial cells or biomass as the product, e.g. single cell protein, bakers yeast, lactobacillus, E. coli, etc.
2. Microbial enzymes: catalase, amylase, protease, pectinase, glucose isomerase, cellulase, hemicellulase, lipase, lactase, streptokinase, etc.
3. Microbial metabolites :
    1. Primary metabolites – ethanol, citric acid, glutamic acid, lysine, vitamins, polysaccharides etc.
    2. Secondary metabolites: all antibiotic fermentation
4. Recombinant products: insulin, HBV, interferon, GCSF, streptokinase
5. Biotransformations: phenyl acetyl carbinol, steroid biotransformation, etc.



IMMUNOLOGY

IMMUNE SYSTEM

Immune system to be a police force of our body. It is a defence system of the body that produces resistance or responce against the foreign particles or micro-organisms. Lymphocytes are the cornerstone of immune system.



IMMUNITY

It is a natural or acquired resistance of the body to a certain disease or pathogenic microorganism or foreign particles produced by immune system.

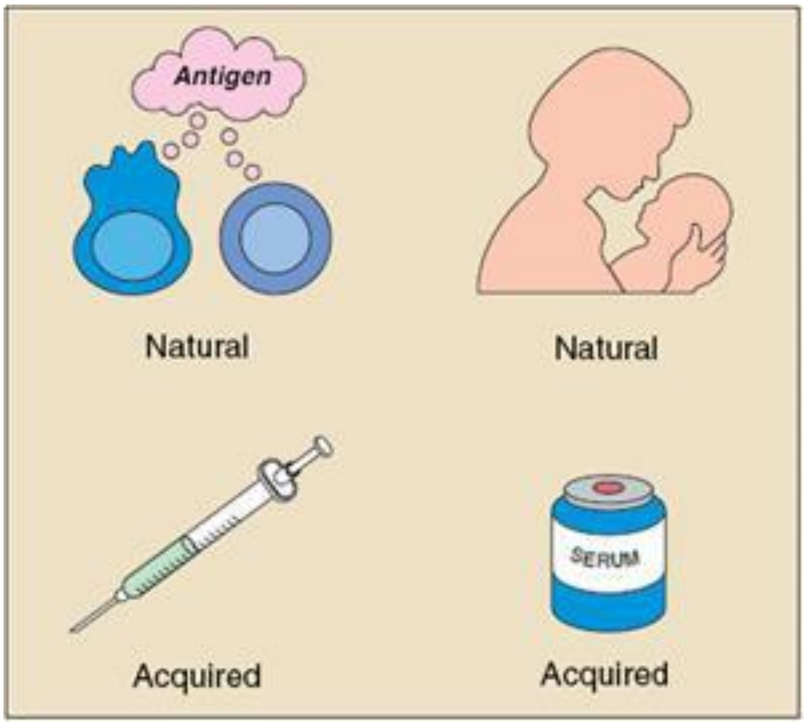
Autoimmunity

The immune system defends the body against infections and certain other diseases. It is made up of different organs, cells, and proteins known as antibodies. It identifies, attacks, and destroys germs and other foreign substances. Sometimes the immune system makes a mistake and attacks the body's own tissues or organs. This is called autoimmunity. One example of an autoimmune disease is type 1 diabetes, in which the immune system destroys the cells in the pancreas that produce insulin.

Types of Immunity

Immunity has two types;

1) Natural Immunity (Non-specific immunity)
2) Acquired Immunity (Specific immunity)



NATURAL IMMUNITY (Non specific immunity)

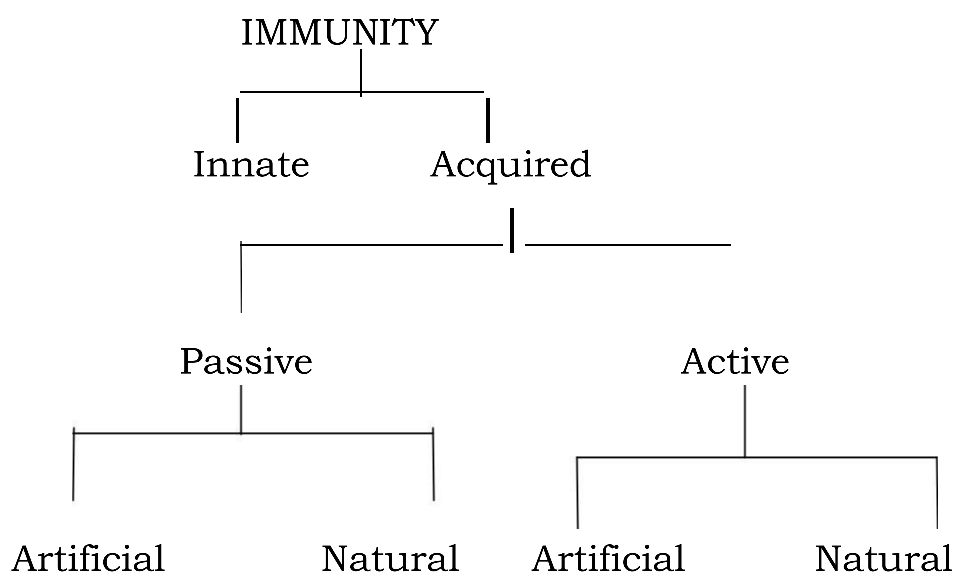
It is the natural resistance of the body against infections by a number of mechanical and chemical stimuli. It is called as nonspecific because it exists in all humans and present from the earliest time of life. Lack of such type of resistance is called as susceptibility.

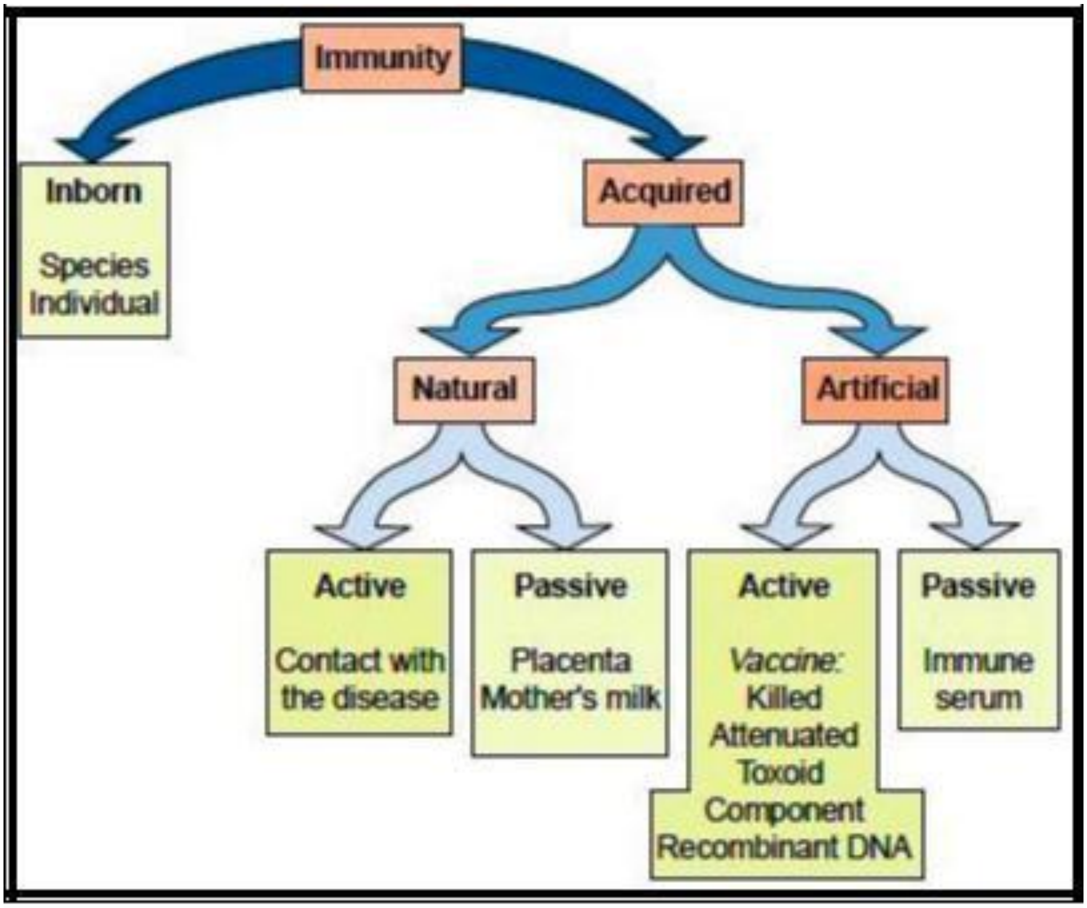
ACQUIRED IMMUNITY (Specific immunity)

It is an acquired resistance of the body against the foreign particles or micro-organisms. It involves the formation of antibodies as a result of stimulation immune system by foreign particles i.e. antigens. They are specific and provide specific resistance

TYPES OF ACQUIRED IMMUNITY (Specific immunity)

[1] Active Immunity
[2] Passive Immunity





Antigens

Definition:

Chemical substances capable of mobilizing the immune system and provoking an immune response are called Antigens. Antigens have two important properties:

Immunogenicity:

The ability to stimulate cells of the immune system.

Reactivity:

The ability to react with products of the immune system cells or the cells themselves.

Antigen Determinants/ epitope

This is area of activity on the molecule called the antigen determinant or epitope. It contains about six to eight amino acid molecules or monosaccharide units.

TOLERANCE

Tolerance is an acquired resistance to foreign particles or drugs Which develops on its repeated administration over prolonged period.

Specific Immunologic Tolerance

Under normal circumstances one's own chemicals do not stimulate an immune response. This failure to stimulate the immune system occurs because substances are interpreted as "self". Prior to birth and during fetal stage the body's proteins and poly saccharides inactivate the immune system cells that otherwise might respond to them. The individual thereby develops a tolerance of "Self" and remains able to respond to "Nonself".

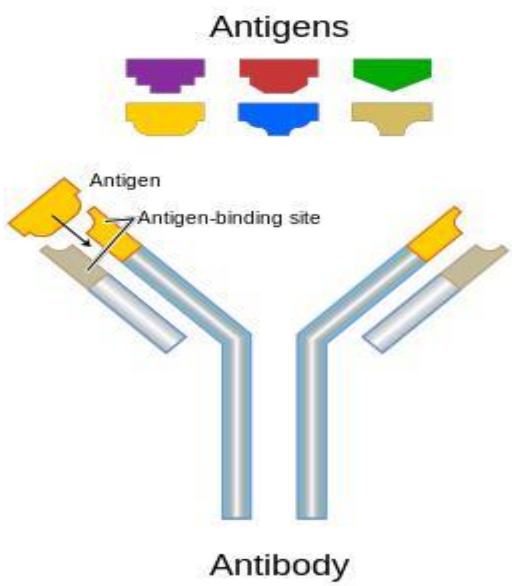
Types of Antigens

Autoantigens:

These are the person's own chemical substances that stimulate an immune response when self-tolerance breaks down. (as in lupus erythematosus)

Allo-antigens:

These are the antigens existing in certain but not all members of a species. The A, B and Rh antigens of humans are typical alloantigens.



Heterophiles:

These are the antigens found in unrelated species. For instance Erythrocytes of horses and the viruses that cause infection mononucleosis have certain identical antigens.

Antibody

A specific substance formed by the body in response to stimulation by specific foreign antigen. Terms antibody (Ab) and immunoglobulin are interchangeable. Antibodies are proteins composed of gamma globulins. These are produced by β-lymphocytes. Antibody mediated immunity s called as HUMORAL IMMUNITY.

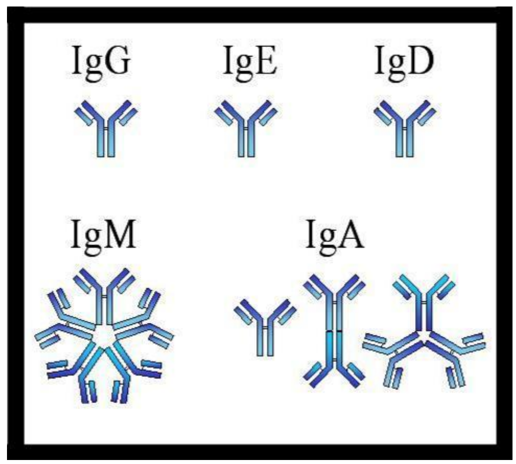
MOMOCLONAL ANTIBODIES

These are the antibodies which are produced from Hybridoma cells. In these antibodies variable regions of each immunoglobulin molecule are same (monoclone).

APPLICATIONS OF MOMOCLONAL ANTIBODIES

Diagnostic kits to detect isolated microorganisms. For specific antiserum therapy and possibly for some forms of cancer. They are used in research for specific identification of components produced in immune reaction, transplantation of organ and bone marrow.

Types of Antibodies



On the basis of differences in the heavy (H) chains in the constant region. Here the Ig stands for Immunoglobulin.

Ig M

It is the antibody of primary antibody response. It is the first antibody to appear in the circulation after stimulation of B-Lymphocytes.

Ig G:

It is the classical gamma globulin. This antibody is the major circulating antibody. IgG appears after 24 to 48 hours after the antigenic stimulation and continues the antigen-antibody reaction begun by IgM.

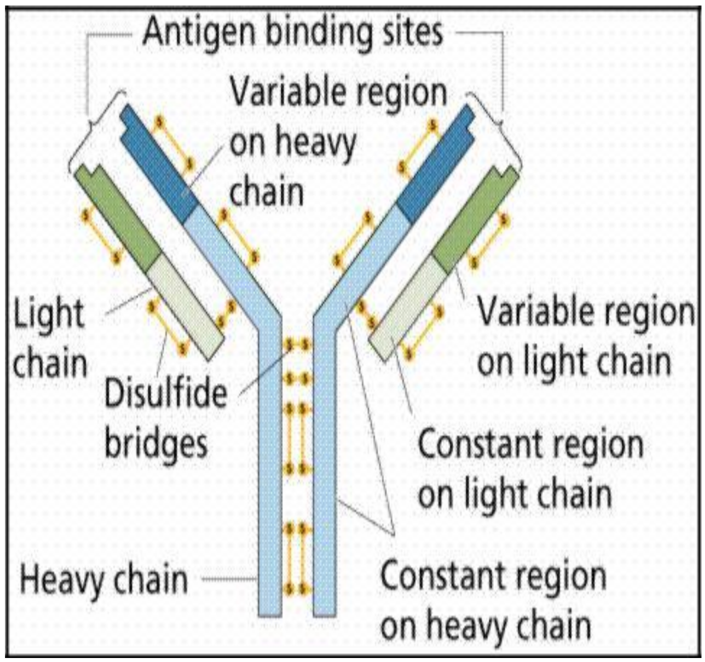
IgA:

It is of two types. Serum Ig A and Secretory IgA. **Serum IgA** is found in serum and is similar to IgG. **Secretory IgA** is found in body secretions. It comes from epithelial cells and helps to move the antibodies into the secretions. It provides resistance in the respiratory and gastrointestinal tracts.

It is also located in tears, saliva and closturm. Colostrums are the first milk secreted by a nursing mother.

IgE:

It plays a major role in allergic reactions by sensitizing cells to certain antigens.



IgD:

Both the functions and significance of the are presently unclear. However it is a cell surface receptor on the B-Lymphocytes together with IgM.

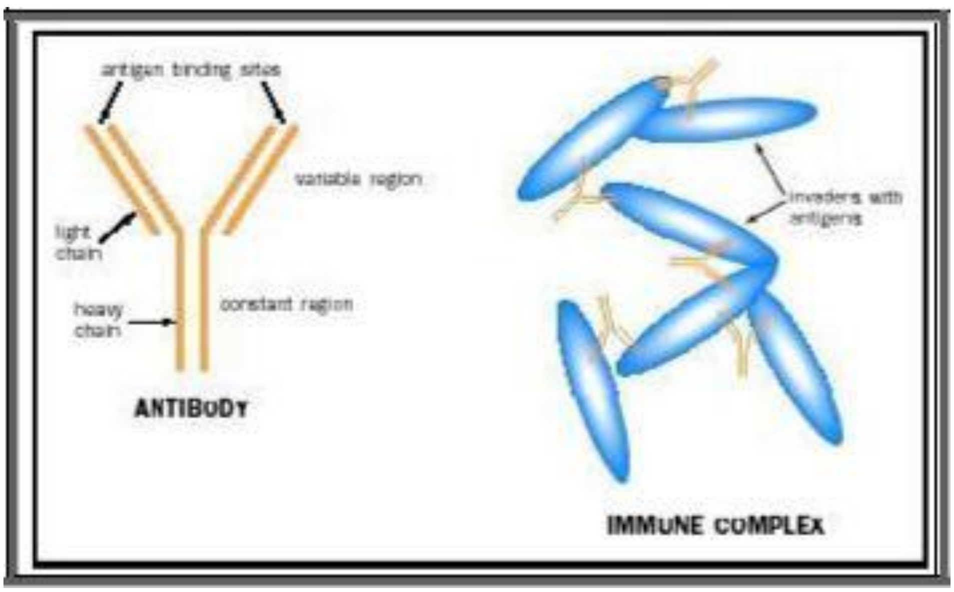
Antigen-Antibody Interactions

In order for specific resistance to develop, antibodies must interact with antigens in such a way that antigen is altered. The alteration may result in:

i) Death to the microorganism that possesses the antigen,

ii) Inactivation of the antigen,

iii) Increased susceptibility of the-antigen to other body defenses.



ANTIGEN-ANTIBODY REACTIONS

(SEROLOGICAL TESTS)

TYPES OF ANTIGEN ANTIBODY REACTIONS

There are three main serological tests used.

A) Agglutination test
B) Precipitation test
C) Complement fixation test

A) Agglutination Test

The antigen in agglutination reactions is a cell or a particle. The addition of homologous antibody will cause clumping or agglutination. Agglutination tests are of following types

1) Tube agglutination test
2) Slide agglutination tests
3) Agglutinin Adsorption test

Diagnostic Applications of Agglutination Tests:

Following Agglutination Test are used for diagnosis of different diseases.

D) Widal Test
E) Weil-Felix Test (Agglutination adsorption Test)
F) TPA Test (Treponema palladium Agglutination Test)
G) COOMB'S TEST (ANTIGLOBULIN TEST)

B) PRECIPITATION TESTS:

In these tests, a reaction takes place between a soluble antigen and a solution of its homologous antibody. The reaction is manifested by the formation of a visible precipitate at the interface of reactants.

There are two types of precipitation reactions.

(1) Ring test.

(2) Agar-diffusion method.

DIAGNOSTIC APPLICATIONS OF PRECIPITATION TESTS:

Following tests of precipitation are important from diagnostic point of view.

- KAHN TEST (VDRL TEST)
- ASCOLI TEST

C) COMPLEMENT FIXATION TEST:

It is a normal thermolabile protein constituent of blood serum that anticipate in antigen-antibody reactions,

DIAGNOSTIC APPLICATIONS OF CFT:

- WASSERMAN TEST FOR SYPHILIS
- T. PALLIDUM CFT

Allergy

According to British Immunological society. The allergy can be defined as;

"Allergy is a specific hypersensitivity of an individual to foreign particles Usually a protein to which a specific individual is exposed."

OR

"An allergy is sensitivity to a normally harmless substance, one that does not bother most people. The allergen (the foreign substance that provokes a reaction) can be a food, dust particles, a drug, insect venom, or mold spores, as well as pollen. Allergic people often have sensitivity to more than one substance."

- Swollen nasal mucous membranes, Complications in bronchi
- Migraine, GIT disturbances, Eczema, Dermatitis, Asthma,
- Runny nose/Stuffy nose Conjunctivitis, Tearing, Vomiting,
- Headache, Fever, Skin Rash.

Hypersensitivity

"It is state of increase sensitivity to an antigen arising from the previous exposure to that antigen"

"It is an exaggerated are in inappropriate reaction of immune system which are harmful to body"

or

An exaggerated response to an antigen that occurs after a prior exposure to the antigen, with consequent tissue damage,

Types of Hyper sensitivity reactions

Hyper sensitivity reaction has been classified by Gell and Coombs in to four major types

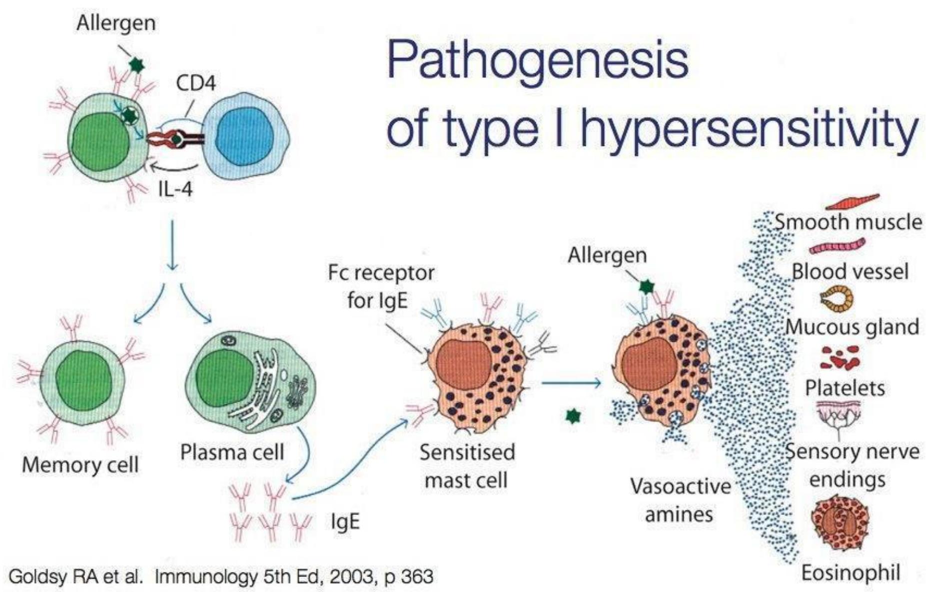
1. Anaphylactic hypersensitivity (immediate hypersensitivity)
2. Cytotoxic Hypersensitivity
3. Complex mediated Hypersensitivity
4. Cell mediated Hypersensitivity

TYPE I- ANAPHYLACTIC HYPERSENSITIVITY

This is a typical allergic response mediated by IgE type of antibodies in response to specific antigen called allergen. This reaction may be life threatening accompanied by Anaphylaxis, in which the mediators induce severe contraction of body's smooth muscles.

Common Allergens:

Plant pollens, Serum proteins, Drugs like penicillin etc.

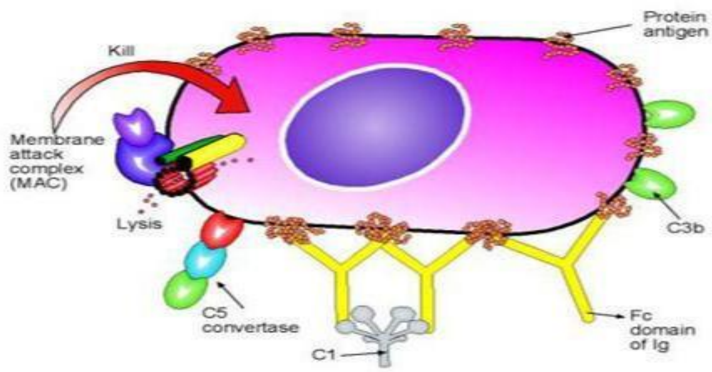


TYPE II, CYTOTOXIC HYPERSENSITIVITY

- These reactions occur when a specific antibody (Typically IgG or IgM) causes destruction of host cells.
- Cells Involved are WBCs, RBCs, and platelets.

Type 2 - antibody-dependent cytotoxicity

Figure 2a: Classical complement pathway



Antibody-dependent activation of the classical complement pathway begins with binding of IgM or IgG to antigen present on the surface of a cell. Recruitment of C1 complement protein that binds the Fc domain of Ig initiates the formation of the membrane attack complex (MAC) that promotes lysis of the cell. In addition, the Fc domain of bound antibody and surface bound complement protein C3b can promote phagocytosis by phagocytes expressing Fc and complement receptors.

immunopaedia.org

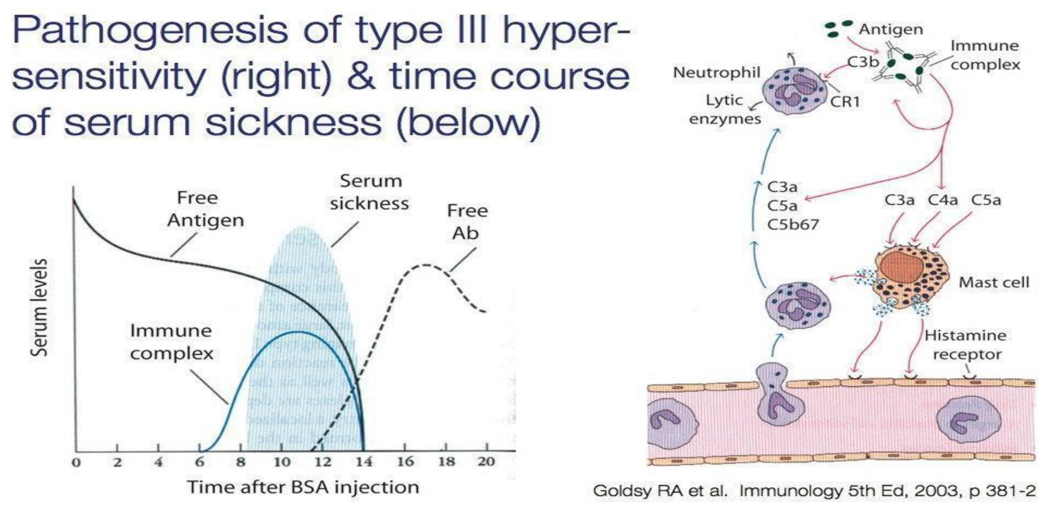
EXAMPLES

1. Blood transfusion reaction.
2. Hemolytic disease of newborn (Erythroblastosis fetalis).
3. Autoimmune disorders: These are the diseases in which body produces antibodies against its own cells.
4. Thrombocytopenia
5. Agranulocytosis

6.Myasthenia gravis

TYPE III, IMMUNE COMPLEX HYPERSENSITIVITY

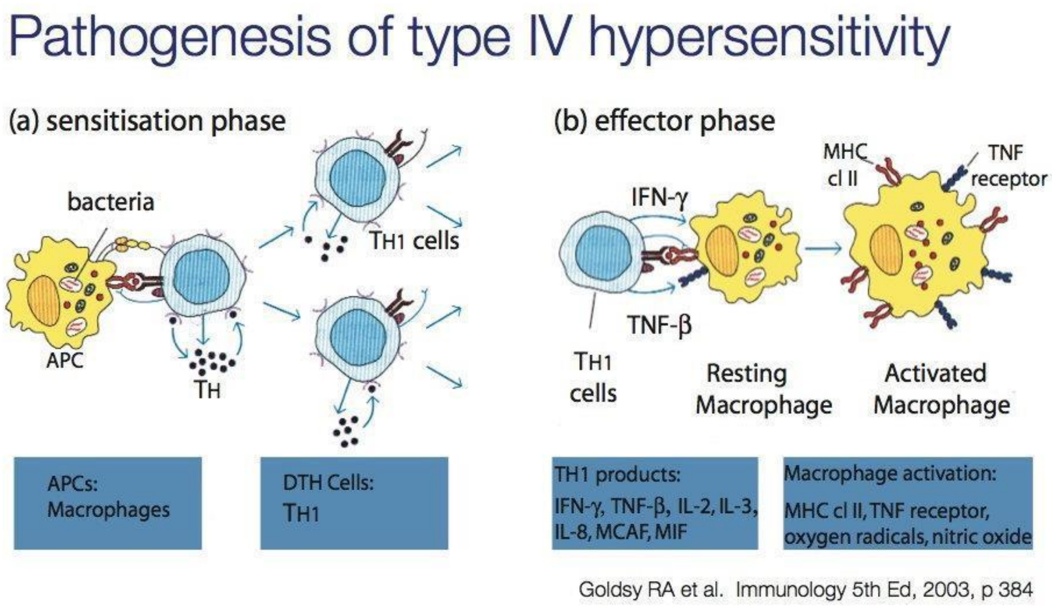
"It is caused by the deposition of immune complex Antigen antibody complex) at various body locations, mainly blood vessels, kidneys, joints, lungs & skin".



- Serum sickness
- Arthus reaction
- Rheumatoid arthritis

TYPE IV, CELL MEDIATED HYPERSENSITIVITY

- "It is an exaggeration of CMI (T-lymphocytes) and develops beginning 18-24 hours following contact with antigen & peaks in 2-3 days".
- Various substances can elicit this type of response. Include metals, cosmetics, microbes & plant products.



TYPES

Two major forms of this allergy are

- Infection allergy
- Contact dermatitis

VACCINES

DEFINITION

"Vaccine is a suspension of living or killed pathogenic microorganism modified to make it non pathogenic and administration of which induce immune response in the recipient sufficient to prevent susceptible disease."

- A vaccine is a preparation which is used to improve immunity to a particular disease. The term derives from Edward Jenners use of cowpox ("vacca" means cow in Latin), which, when administered to humans, provided them protection against smallpox, the work which Louis Pasteur and others carried on.

- Vaccines are based on the concept" of variolation originating in China, in which a person is deliberately infected with a weak form of smallpox.
- Jenner realized that milkmaids who had contact with cowpox did not get smallpox.

- The process of distributing and administrating vaccines is referred to as vaccination.

- Since vaccination was much safer, smallpox inoculation fell into disuse and was eventually banned in England in 1848.

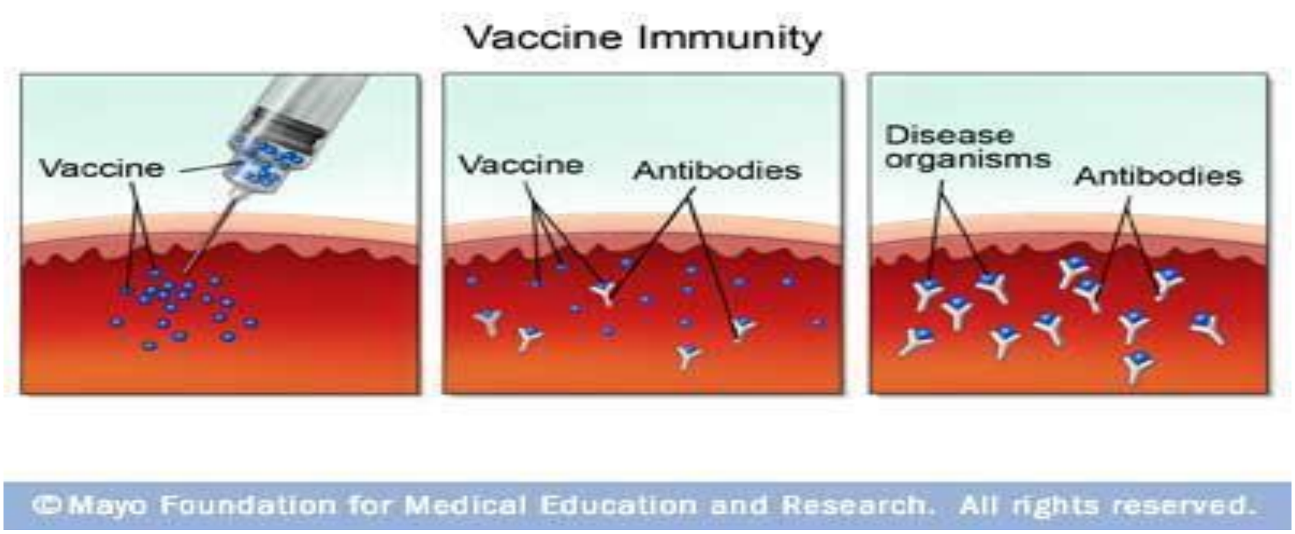
- Vaccines can be prophylactic (e.g. to prevent or ameliorate the effects of a future infection by any natural or "wild" pathogen), or therapeutic (e.g. vaccines against cancer are also being investigated;

- Vaccines may be dead or inactivated organisms or purified products derived from them.

DEVELOPING IMMUNITY

The immune system recognizes vaccine agents as foreign, destroys them, and 'remembers them. When the virulent version of an agent comes along the body recognize the protein coat on the virus, and thus prepared to respond by.

(1) Neutralizing the target agent before it can enter cells,
(2) By recognizing and destroying infected cells before that agent can multiply to vast numbers.



Vaccine have contributed to the eradication of small pox, one of the most contagious and deadly diseases known to man. Other diseases such as rubella, polio, measles, mumps, chicken pox, and typhoid are No where near as common as they where just hundreds years ago.

AIM OF VACCINATION:

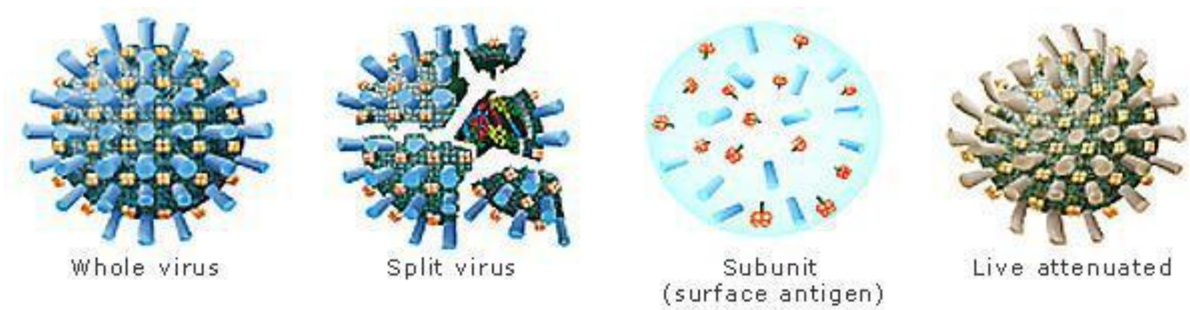
The aim of vaccination in the individual is to induce a prime state such that on contact with the relevant infection a more rapid and effective secondary response could be mounted leading to the prevention of disease thus the primary aim is to eliminate the disease.

IDEAL VACCINE:

Some important requirements (features) of an ideal vaccine are:

- **Non- pathogenicity:** Non pathogenic not cause original disease.
- **Immunogenicity:** Strongly immunogenic to produce antibodies.
- **Efficacy:** Greater than 90%.
- **Effective:** When given orally.
- **Produce long lived immunity:** (hope full life long)
- **Low cost**.
- Induce a wide range of appropriate responses.
- Compatible with co administrated vaccine.
- Stable genetically and thermally.

TYPES OF VACCINES



1. LIVE ATTENUATED VACCINES

There are conventionally used live viral bacterial vaccines, consist of mutants of wild type microbes which are limited in their ability to infect body e.g. BCG (bacterial vaccines), Measles, polio, yellow fever small pox (viral vaccines). They are prepared by same method as killed vaccines except the process of sterilization.

2. KILLED VACCINES.

These consist of killed or inactivated microorganisms are used where living vaccines are not available e.g. rabies, influenza, polio (viral vaccines), cholera, pertusis, plague, typhoid (bacterial vaccines).

3. HETEROLOGOUS VACCINES.

"These vaccines are based on the principle that if the antigenic site is available on some other organism inspite of using actual molecule, we can use this heterosite to induce some immune - response" e.g. monkey derived rotavirus has been tried with some success in human infants.

4.SUB-UNIT VACCINES (2nd GENERATION VACCINES).

"These ere vaccines which contain one or more pure or semi pure part or subunits of microorganisms i.e. pilli capsule, coat etc. e.g. Haemophilus meningitis vaccine produced from capsule polysaccharide molecule.

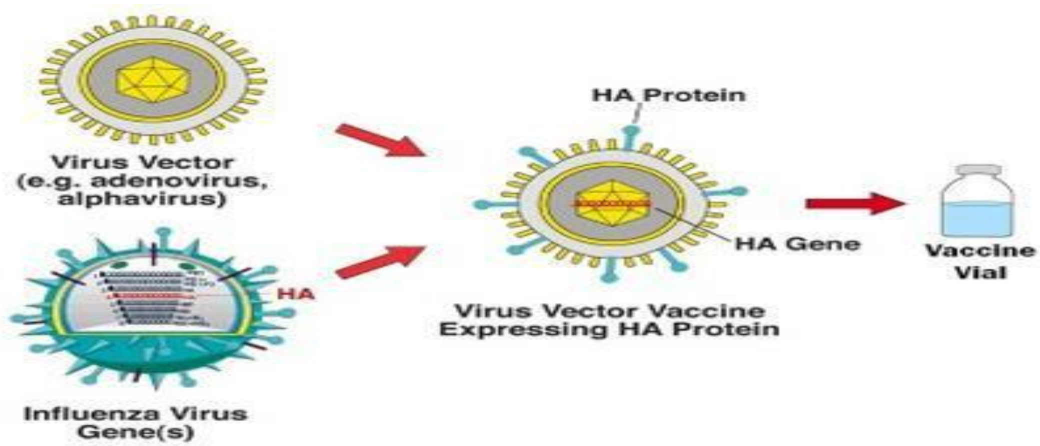
5. MARKER VACCINES.

These are vaccines which can be used in conjunction with a diagnostic test to differentiate a vaccinated animal from a carrier animal e.g. used for Herpes viruses of pigs & cattle's.

6. DNA VACCINES (3rd GENERATION VACCINES).

These are the vaccines that contain microbial fraction produced by genetic engineering. These are also called polynucleotide or genetic vaccines.

6. VECTORED VACCINES.



In this type of vaccines, the vector completed with inserted gene itself act as vaccine.
Viruses used as vector are adenovirus, Herpes virus.

Bacteria used as vector are salmonella, BCG.

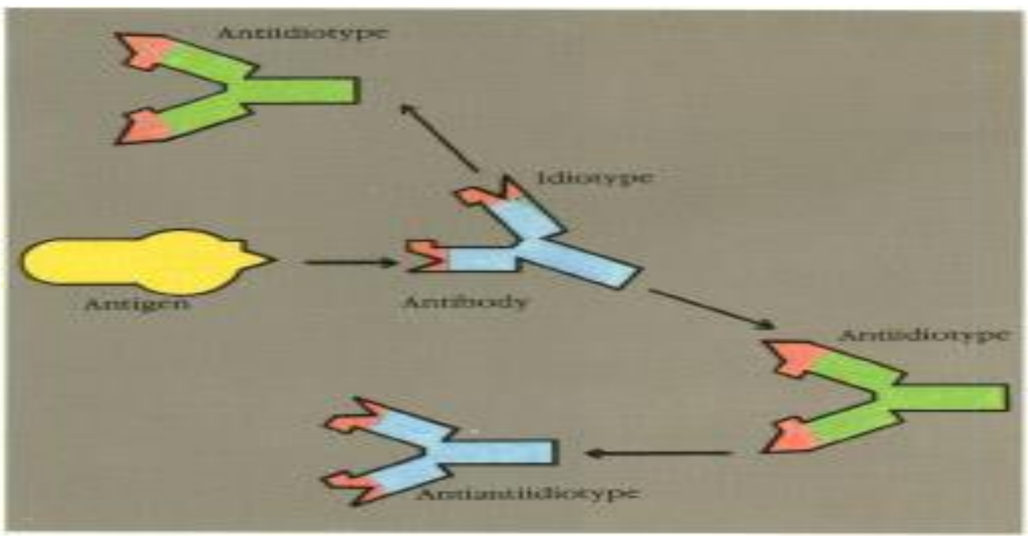
8. ONE-SHOT VACCINE.

It will be the vaccine of future consisting of viral or bacteria vector containing genes for all required vaccines (i.e. against all type of diseases) appeared in weekly review of "The New Scientist" in London.

9. PEPTIDE VACCINES.

These are the subunit vaccines prepared by chemical synthesis of short immunogenic peptides"

10. ANTI-IDIOTYPE VACCINES.



It is a vaccine in which antibody molecules are used to prepare antigen such that these antibody molecules themselves are copies of antigen. The antibody, which recognizes the antigen is called idiotype, which is use to raise second antibodies (antiideotype antibodies) e.g. this technique is successfully used against streptococci.

11. GENE-DELETED VACCINES.

"These are genetically engineered vaccines which involve the removal or mutation of virulence gene of the pathogen"

ANTISERA (IMMUNE SERA, SEROTHERAPY)

These are preparations containing antibodies introduced into the body of patient to provide passive immunity.

- They are used with advantage that they provide immediate and effective supply of antibodies.
- Antisera are used both prophylactically and therapeutically.

Types:

1. Non specific Antisera e.g. Normal human imrnunoglobulins.
2. Specific Antiseia e.g. antitoxins, antibacterial and Antiviral sera.

NON-SPECIFIC ANTISERA

HUMAN NORMAL IMMUNOGLOBULINS

Y - Globulin injection contains almost all globulins of human plasma together with smaller amounts of other plasma proteins.Y - Globulin consists of three distinct components IgA, IgG, IgM.It is given I/m and is useful in number of viral and life threatening bacteria! Infections diseases.Since they are prepared from humans, so are well tolerated.

SPECIFIC ANTISERA ANTITOXINS:

These are antibodies to toxins of microorganisms which combine with toxins to neutralize its toxicity."

EXAMPLES

Diphtheria antitoxins, Gas gangrene antitoxins, tetanus antitoxins, staphylococcus antitoxins.

ANTI-BACTERIAL SERA

These provide passive immunity against diseases caused by endotoxin producing bacteria.

They are prepared in the same way as antitoxins except.

A. I/V routes are used for injection.

B. Methods of refining the sera are different because antibacterial antibodies are associated with y-globulin fractions.

EXAMPLE

Leptospira antiserum (BPC)

ANTI-VIRAL SERA

Antiviral antibodies are believed to act differently because viruses are intracellular parasites and antibodies cannot penetrate cells therefore inactivation must take place in body fluids.

PREPARATION

Most of antiviral sera are prepared in humans since horses are not susceptible to several viral diseases.
However, Rabies antiserum is prepared in horses.