Microbiology

For the students of Pharmacy Technicians (Category-B)

Compiled By
Syed Bilal Hussain
Lecturer
Lahore College of Pharmaceutical Sciences

Under Supervision of

Dr. Saima Rehmat
Lecturer
Lahore College of Pharmaceutical Sciences

Dedication

To

Prof. Dr. Naim Anwar Muzaffar “The Father of Pharmacy”

Whose Dedications Toward Pharmacy Education Are Priceless.

Acknowledgement

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Syed Bilal Hussain
Lecturer
Lahore College of Pharmaceutical Sciences

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Introduction To Microbiology

A microorganism or microbe is a microscopic organism, which may be a single celled or multi-cellular organism. Microorganisms can be found everywhere and in close association with every type of multi-cellular organism. They populate the healthy human body by the billions and even as participants in bodily functions.

Most infectious disease is initiated by microorganisms. Many diseases caused by introduction of organisms directly into the bloodstream or internal organs. Infectious disease occurs when the organism causes tissue damage and impairment of body function.

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 micro-organisms and their effects on other living organisms”

Common microbes include…

- Bacteria *(simple single celled organisms)*
- Virus *(need to invade cells)*
- Archaea *(single celled microorganisms)*
- Fungi *(yeasts and molds)*
- Protozoa *(complex single celled microorganisms)*

Types of Microorganisms

The cellular world is divided into two major groups, based on whether or not the cells have a nucleus. Cells that have a well-defined nucleus are called eukaryotic, whereas cells that lack a nucleus are called prokaryotic.

All prokaryotic organisms are classified as bacteria, whereas eukaryotic organisms include fungi, protozoa, and helminthes, as well as humans.

1. **Prokaryotes**
2. **Eukaryotes**

PROKARYOTES

The prokaryotes are a group of organisms whose cells lack a membrane-bounded nucleus

EUKARYOTES

A eukaryote is any organism whose cells contain a nucleus and other structures (organelles) enclosed within membranes.

SCOPE OF MICROBIOLOGY

- The scope of microbiology involves the study of organisms that are not visible to the naked eye.
- Viruses, bacteria, algae and fungus are all consider microorganisms that are studied in microbiology.
- Foods such as bread, cheese, and beer use microorganisms to be formed properly, while vaccinations, vitamins, and antibiotics use the same microorganisms to be effective.
- In the 1970s new discoveries in microbiology led to the development of recombinant DNA technology and genetic engineering.
- Microbiology has played a major role in molecular biology and has been closely tied to the determination of the genetic code, mechanisms of DNA, ribonucleic acid (RNA), and protein synthesis and in studies on the regulation of gene expression and the control of enzyme activity
- Microorganisms are able to grow rapidly and in large amounts in the lab at reasonable cost
- Interest in how micro-organism affects human existence has been the foundation of microbiology.
- With the harmless organisms which convert complex material into simple substances life would be uncertain.
- For a sterile product criterion of quality is simple there should be no living detective micro-organism what so ever.

- 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.
- 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.
- Microbiology has an impact on medicine, agriculture, food science, ecology, genetics, biochemistry, immunology, and many other fields.
- Medical Microbiology deals with diseases of humans and animals; identify and plan measures to eliminate agents causing infectious diseases.
- Immunology is the study of the immune system that protects the body from pathogens.
- Agricultural Microbiology is the study of impact of microorganisms on agriculture; combat plant diseases that attack important food crops.
- Food and Dairy Microbiology is the study of how to prevent microbial spoilage of food & transmission of food-borne diseases, use microorganisms to make food such as cheeses, yogurts, pickles, beer, etc.
- Industrial Microbiology tells us about using microorganisms to make products such as antibiotics, vaccines, steroids, alcohols & other solvents, vitamins, amino acids, enzymes, etc.
- Genetic Engineering: describes the microorganisms used to make hormones, antibiotics, vaccines and other products.

Bacteria

The microscopic, unicellular, prokaryotic organisms characterized by the lack of membrane bounded nucleus and membrane bounded organelles.

Classification of Bacteria on the Basis of Cellular Morphology

The general appearance of an individual cell as seen under bright field of the compound microscope is known as cellular morphology.

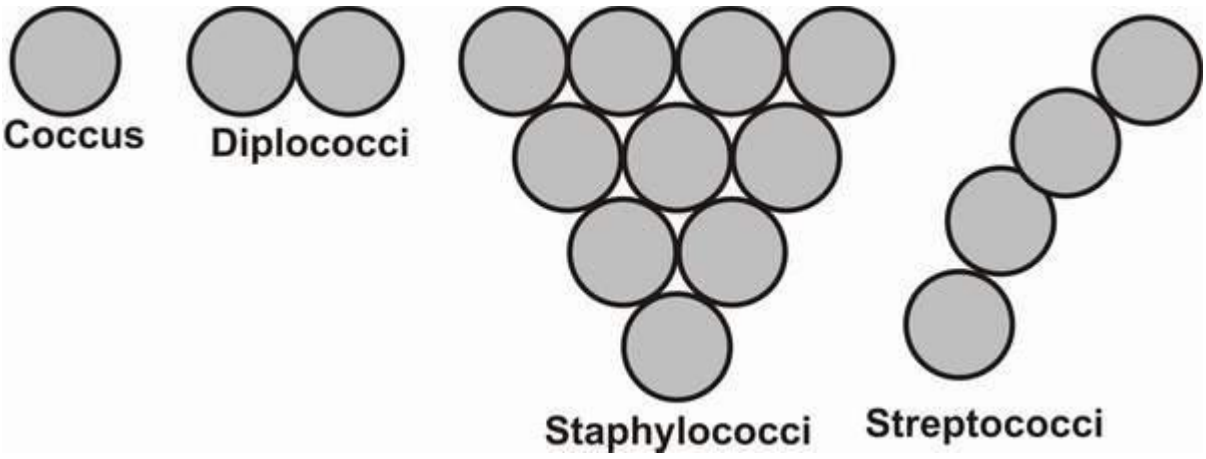
- Coccus (spherical)
- Bacilli (rod like)
- Spirals (winding like)

Cocci

Cocci are round or oval in shape. (Singular coccus)Cocci tend to be quite small being only 0.5 µm to 1.0 µm in diameter.

Cocci are further classified in to following groups on the basis of their microscopic arrangement.

- **Diplococci**
- **Streptococci**
- **Staphylococci**



Diplococci

Those cocci that remain in pairs after reproduction are called Diplococci, e.g. *Neisseria gonorrhoeae*.

Streptococci

Cocci that remain in chains called Streptococci, e.g. *Streptococcus pyogens*.

STAPHYLOCOCCUS

The cocci which divide randomly and form irregular grapes like cluster of cells is called as Staphylococcus, e.g. *Staphylococcus aureus*.

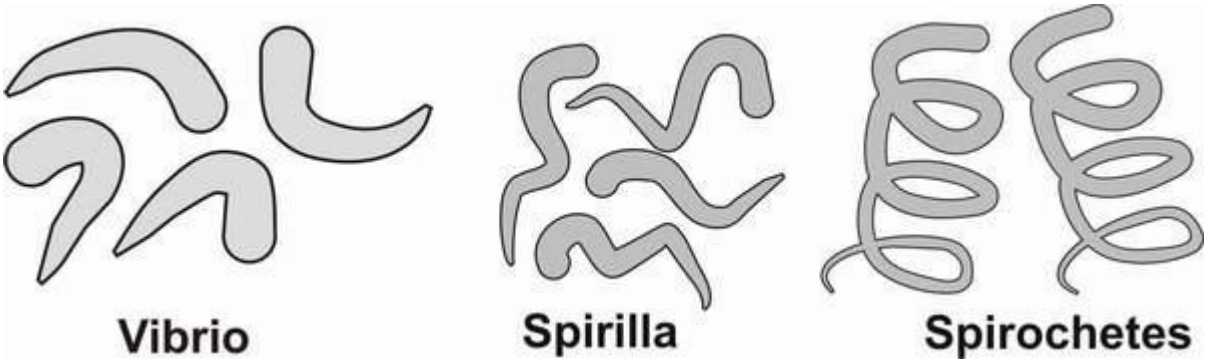
BACILLI

Bacilli are rod shaped bacteria, e.g. E. coli, Salmonela, C. Tatani. B. anthrax etc. the range is 20 µm or as short as 0.5 µm

Spirals

Spirals may have three forms

- **Vibrio:** Curved rods that resemble commas, e.g. *Vibrio cholerae*
- **Spirilla:** Helical shaped with a thick cell wall, e.g. *Spirillum volutan*
- **Spirochetes:** Spiral shaped with a thin cell wall, e.g. *Treponema pallidum*



Classification of Bacteria On the basis Of Staining

The most widely used staining procedure in microbiology is the Gram stain. Gram staining is a differential staining technique that differentiates bacteria into two groups.

1. **Gram Positive Bacteria**
2. **Gram Negative Bacteria**

Gram Positive Bacteria

Gram positive bacteria are those which retain the crystal violet dye after being washed with the alcohol. They are stained dark purple in color. They have this property because they contain higher quantity of peptidoglycan in their cell wall for example *Streptococci, Clostridium, Bacillus anthrax* etc.

Gram Negative Bacteria

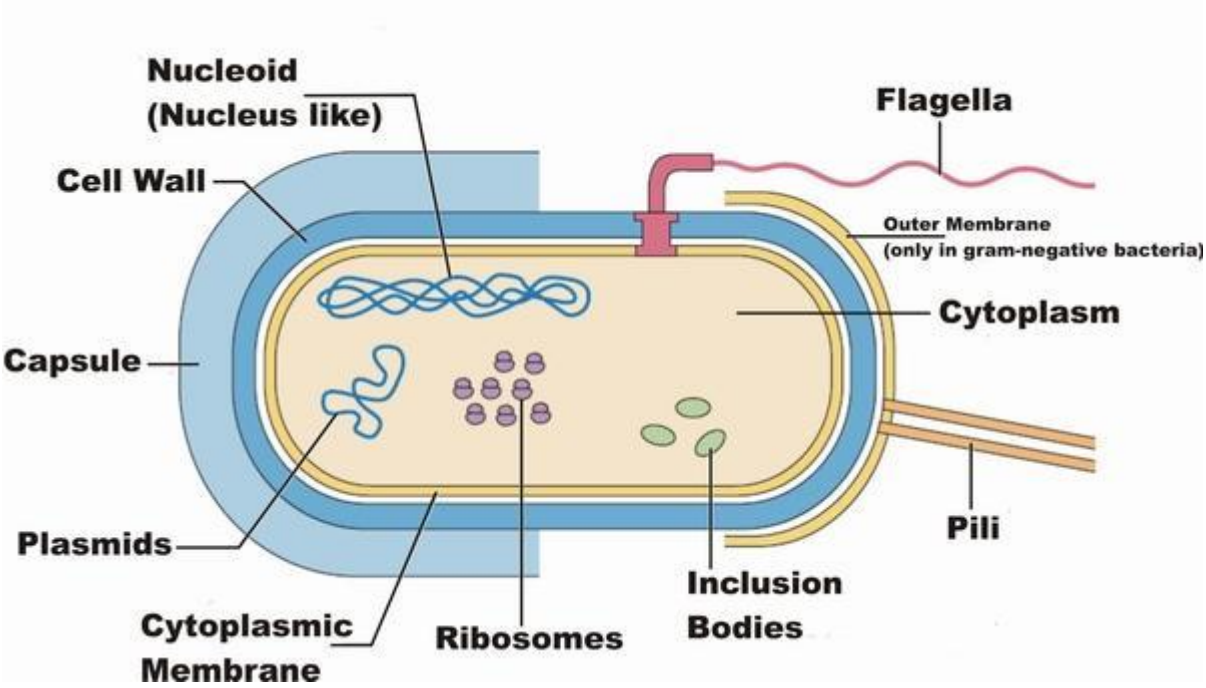
Gram negative Bacteria lose the crystal violet dye when they are washed with alcohol. Then they are counter stained and give pink or red color in Gram staining technique. For example *E. coli, Salmonella Typhi* etc.

Basic Bacterial Cell Structure

Bacterial cells are between 0.3 and 5 μm in size. They have three basic forms: cocci, straight rods, and curved or spiral rods.

Fine Structures of Bacteria consist…

1. **Capsule**
2. **Cell Wall**
3. **Cytoplasmic Membrane**
4. **Cytoplasm**
5. **Nucleoid (Nucleus like)**
6. **Plasmids**
7. **Ribosomes**
8. **Flagella**
9. **Pili**
10. **Inclusion Bodies**



Capsule

Many pathogenic bacteria produce extracellular enzymes to synthesize a polymer that forms a layer around the cell, this layer is called capsule. The capsule protects bacterial cells from phagocytosis.

(Phagocytosis is the ingestion of bacteria by phagocytes, where as phagocytes are the cells that protect the body by ingesting harmful foreign particles e.g. bacteria)

Cell Wall

The bacterial cell wall determines the shape of the cell. It is composed of peptidoglycan and lipopolysaccharides. Peptidoglycan is also known as murein.

Peptidoglycan or Murein

Murein is the most important structural element of bacteria, a netlike polymer material surrounding the entire cell. It is made up of polysaccharide chains cross linked by peptides.

Cell wall of Gram-positive & Gram-Negative Bacteria

Gram Positive Bacteria	Gram Negative Bacteria
Cell wall is thick , 25-30μm	Cell wall is thin , 10-15μm
Remain always rigid	May be rigid or flexible
Lipid content is 1-4%	Lipid content is 11-12%
Lipopolysaccharide is present	Lipopolysaccharide is absent
Teichoic acid is present	Teichoic acid is absent
Produce exotoxin	Produce endotoxin

Cytoplasmic Membrane

The Cytoplasmic membrane or cell membrane is composed of phospholipids. It is basically a double layer of phospholipids with numerous proteins integrated into its structure. The membrane acts as a permeability barrier, restricting the kind and amount of molecules that enter and leave the cell.

Cytoplasm

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

Nucleoid (Nucleus like)

The nucleoid is a region of cytoplasm where the chromosomal DNA is located. It is not a membrane bound nucleus, but simply an area of the cytoplasm where the strands of DNA are found.

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.

Flagella

Flagella give bacteria the ability to move actively. The flagella (singular flagellum) are made up of a class of linear proteins called flagellins.

Pili

Pili are shorter and thinner than flagella and function as attachment organs that promote specific cell-to-cell contact. The attachment can be between the bacterial cell and the host cell, or between one bacterial cell and another.

Inclusion bodies

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

Cell Envelope

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

Culture

Culture is the term given to microorganisms that are cultivated in the lab for the purpose of studying them.

Media

Media is the term given to the combination of ingredients that will support the growth and cultivation of microorganisms by providing all the essential nutrients required for the growth in order to cultivate these microorganisms in large numbers to study them. Among the different kinds of microorganisms the two groups that can be grown in cultures are bacteria and fungi.

Culture Media

Culture media is a media in which the inoculation of microorganisms has been successfully done.

It is a liquid or gel designed to support the growth of microorganisms or cells. Microbiological culture is used for growing microorganisms, such as bacteria or yeast.

Like all other organisms, microorganisms also require food or nutrients for living, on which microorganisms are grown in the laboratory. These nutrients are known as culture medium and the growth itself is culture.

Culture media can be classified on the basis of physical state, chemical composition, and use.

1. **On the Basis of Physical State**
2. **On the Basis of Chemical Composition**
3. **Classification Based on Functional Use or Application**

Classification On the Basis of Physical State

- **Liquid Media**
- **Solid Media**
- **Semi-Solid Media**

Liquid Media

These are available for use in test-tubes, bottles or flasks. Liquid media are sometimes referred as “broths” (e.g. nutrient broth). The modern form of liquid medium is called nutrient broth. There is no agar in liquid media.

Solid Media

This form of media is mainly used in Petri dishes as plate cultures. They are used to observe the colony characteristics, size and shape of microorganisms. 'Agar' is most commonly used to prepare solid media. It contains 1% agar.

Semi-Solid Media

These media are gelatinous in nature with jelly like consistency. These are prepared by adding 0.2-0.5%agar to a fluid medium. These media are used for motility test and for different biochemical test.

Classification On the Basis of Chemical Composition

- **Natural Media**
- **Synthetic Media**
- **Semisynthetic Media**

Natural Media

Those media whose chemical composition is not known are called natural media. It contains all necessary ingredients for growth of microorganisms, but they are in crude form. They are more useful for cultivating unknown bacteria, as it usually provides full range of growing factors such as amino acids, polypeptides, vitamins and minerals.

Synthetic Media

Media prepared by adding precise amount of highly purified inorganic or organic chemicals to distilled water are synthetic media. Chemical composition is known in this media. This media are of great importance in studying the metabolic activity of microorganisms, e.g. enrichment media.

Semisynthetic Media

The media of which chemical composition is partially known is as semisynthetic media .e.g. nutrient agar, PDA etc.

Classification Based on Functional Use or Application

- **Basal Media**
- **Enriched Media**
- **Selective Media**
- **Enrichment Media**
- **Differential Media**
- **Transport Media**
- **Anaerobic Media**

Basal Media

Basal media are basically simple media that supports most non-fastidious bacteria, e.g. Peptone water, nutrient broth and nutrient agar etc.

Enriched Media

Addition of extra nutrients in the form blood, serum, egg yolk etc. to basal medium makes them enriched media, e.g. Chocolate agar, Blood agar etc.

Selective Media

Selective media contain ingredients to inhibit the growth of certain bacteria in a mixture while permitting the growth of others. Any agar media can be made selective by addition of certain inhibitory agents, e.g. Mannitol salt Agar, Eosin Methylene Blue Agar: (EMB) etc.

Differential Media

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, e.g. MacConkey's agar, CLED agar, TCBS agar, XLD agar etc.

Transport Media

Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms. This can be achieved by using transport media e.g. Cary Blair medium, alkaline peptone water medium etc.

Anaerobic Media

Anaerobic bacteria need special media for growth because they need low oxygen content and extra nutrients. Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Boiling the medium serves to expel any dissolved oxygen, e.g. Thioglycollate medium.

Bacterial Cultures

Bacterial culture is growing bacteria on a culture medium in a controlled environment. Different methods are used for bacterial culture. Bacterial culture is used to identify and isolate pure bacterial colonies from a mixed population. Microbiologists use bacterial and other microbial culture methods to identify microorganisms and to diagnose infection.

Pure Culture

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

Following methods are commonly use for bacterial culture

1. **The Streak Plate Method**
2. **Pour Plate Method**
3. **Spread Plate Method**

The Streak Plate Method

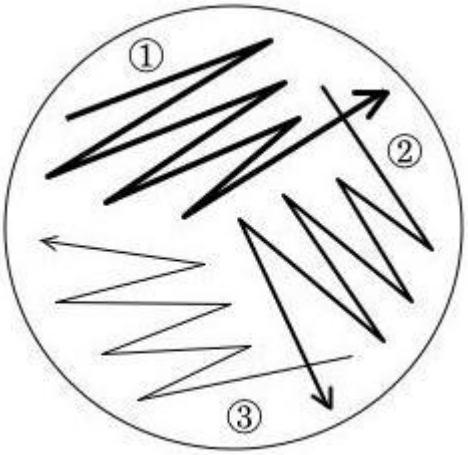
The procedure of streaking a plate with an inoculating loop is used to spread millions of cells over the surface of a solid medium so that some individual cells are deposited at a distance from all others. These cells grow and reproduce forming an isolated colony. One or more colonies will be well separated from all others and represent a source of a pure culture.

Material

- Streak Plates
- Nutrient Agar Plates (NA)
- Bunsen Burner
- Bacteriological Loop

To Streak A Plate...

- Examine the streak plates 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 use inoculating loop.
- Sterilize the loop in the burner flame let cool 3-5 seconds then touch the end of the loop

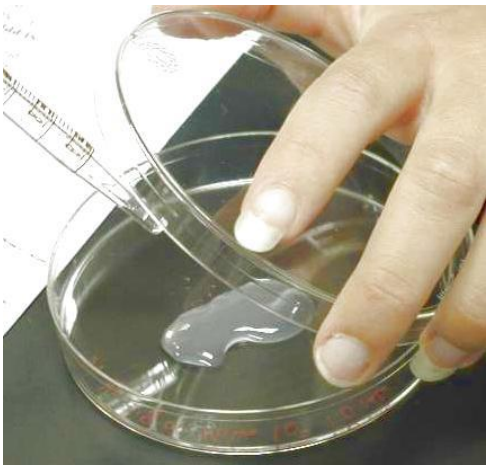


to the isolated colony picking up the micro-organisms from the colony.

- Now cover the streak plate and pick up one NA plate.
- 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 of inoculate another plate from different colonies.
- Try to use colonies that are visibly different in morphology.
- Incubate the plates in the 37°C incubator.

Pour Plate Method

A practical and common laboratory technique used in isolating pure cultures or enumerating the living microorganisms in water, milk, foods, and other materials is the pour plate technique.



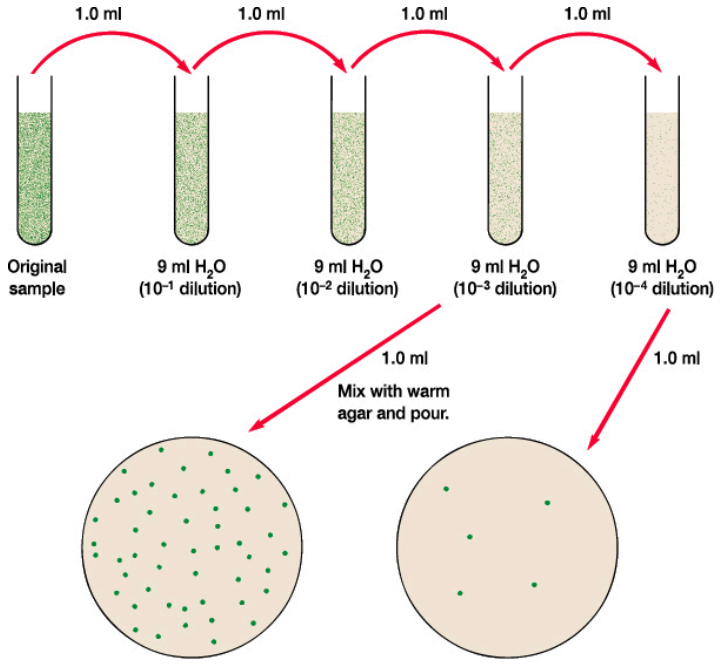
Procedure

To transfer liquid into a pour plate, raise one side of a Petri plate lid only just enough to allow access of the sample (from a tube or pipette). Transfer a known amount of the sample to the dish and cover immediately with the lid. Then pour 15-20 ml of sterile agar culture medium which has been melted and cooled to 45-50°C into the plate. The inoculum and medium are mixed by gentle rotation ten times in one direction and ten times in the other direction. The agar must be allowed to solidify completely before the plates are inverted for incubation. After incubation both surface and subsurface colonies will be observed.

Spread Plate Method

The spread plate technique is a specific streaking technique used in microbiology to grow bacteria on agar plates. The spread plate technique is mostly used to quantify how many bacteria are present in a sample.

Various particle-counting devices, spectrophotometric methods and microscopic techniques have been used to count cells.



Procedure

- Drop 0.1 ml from serial dilutions onto the surface of an agar plate.
- Spread it across the surface using an inoculating loop.
- Incubate plates agar inverted in appropriate conditions.
- Count colonies and calculate the number of microorganisms in the original suspension.

Staining Techniques

Gram staining

Gram staining (or Gram's Method) is a method of differentiating bacterial species into two large groups (gram-positive and gram-negative). The name comes from its inventor, Hans Christian Gram.

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls by detecting peptidoglycan, which is present in a thick layer in gram-positive bacteria.

In a Gram stain test, gram-positive bacteria retain the crystal violet dye, while a counterstained (commonly safranin) added after the crystal violet gives all gram-negative bacteria a red or pink coloring.

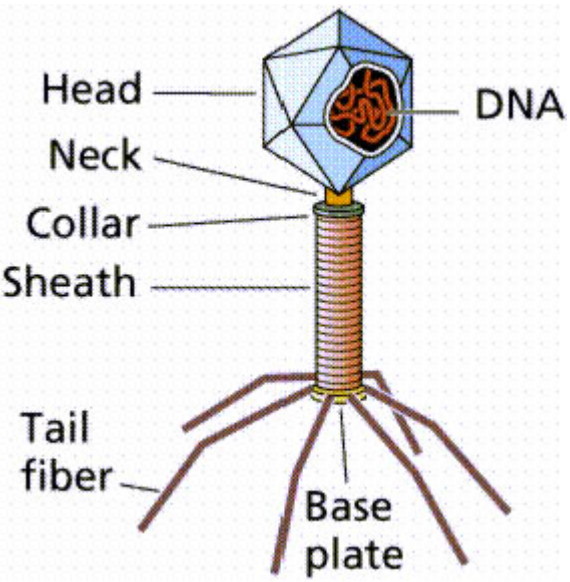
Gram staining Procedure

- Make a thin smear of the material or culture; let it dry at room temperature

- 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 for one minute
- Wash off the stain with gram's or Lougol's iodine and leave the slide covered with iodine for one minute
- Wash gently in water
- Pour on acetone or alcohol till no more violet color comes from the slide for 5-seconds.
- Wash gently in water again
- Stain with one of the following counter stains. Safranin, Neutral red or 1:10 Carbolfuchsin
- Wash gently in water and allow it to dry by standing it vertically
- All slides of bacteria must be examined under the oil immersion lens

VIRUSES

Viruses are complexes consisting of protein and an RNA or DNA genome. They lack both cellular structure and independent metabolic processes. They replicate solely by exploiting living cells based on the information in the viral genome.

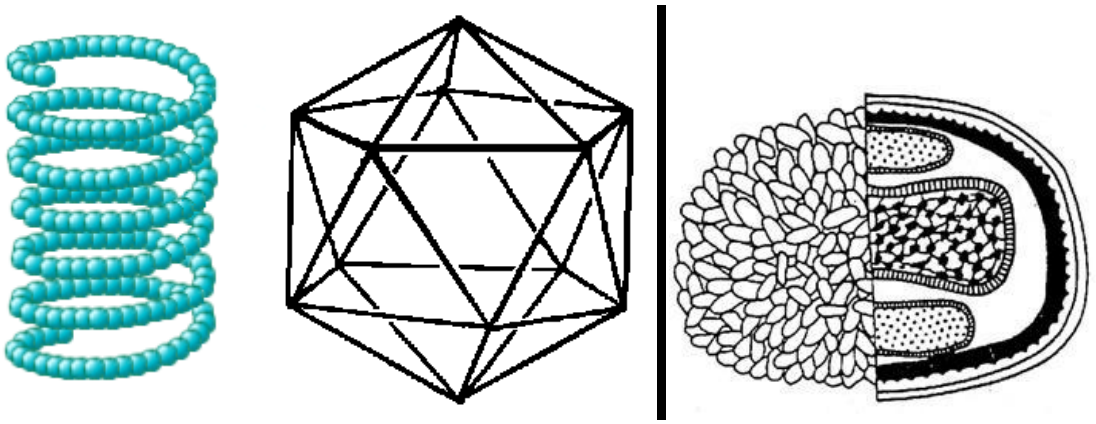


Viruses are autonomous infectious particles that differ widely from other microorganisms in a number of characteristics…

- They have no cellular structure.
- Viruses consisting only of proteins and nucleic acid (DNA or RNA).
- Viruses have no metabolic systems of their own, but rather depend on the synthetic mechanism of a living host cell.
- Viruses exploit normal cellular metabolism by delivering their own genetic information, i.e., nucleic acid, into the host cell. The host cell accepts the nucleic acid and proceeds to produce the components of new viruses in accordance with the genetic information it contains.
- Viruses infect bacteria (so-called bacteriophages), plants, animals, and humans.

SHAPES OF VIRUS

- **Helical Symmetry**
- **Cubic Symmetry**
- **Complex Symmetry**



Complex Symmetry Virus

Cubic/ Icosahedrons Symmetry Virus

Helical Symmetry Virus

Helical Symmetry

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

Cubic Symmetry

The cubic or icosahedrons a polyhedron with 20 triangular faces and 12 corners. Herpes simplex and polio viruses have icosahedral symmetry.

Complex Symmetry

A combination of helical and icosahedral symmetry is described as complex. Complex structural patterns are found in bacteriophages and the smallpox virus

Structure of Virus

A mature virus particle is also known as a virion. It consists of either two or three basic components

1. **Genome**
2. **Capsid**
3. **Envelope**

Genome

The viral genome is either DNA or RNA, and viruses are hence categorized as DNA or RNA viruses. The nucleic acid of DNA viruses is usually double-stranded (ds) and linear or circular depending on the family; the nucleic acid of RNA viruses is usually single-stranded (ss).

Capsid

The capsid is the “shell” of virus-coded protein that encloses the nucleic acid. The combination of these two components is often termed the nucleocapsid. The capsid protects the nucleic acid from degradation.

Envelope

The envelope, which surrounds the capsid, in several virus families is always dependent on cellular membranes. Both cell-coded and viral proteins are integrated in the membrane when these elements are transformed into the envelope, frequently in the form of “spikes”.

Classification of Virus

(Reference: Color Atlas of Medical Microbiology)

The taxonomic system used for viruses is artificial. it does not reflect virus evolution and is based on the following morphological and biochemical criteria.

- **On The Basis Of Genome**
- **On The Basis Of Capsid Symmetry**
- **On The Basis Of An Envelope (Presence Or Absence)**
- **On The Basis Of Diameter Of The Virion**

On The Basis Of Genome

Viruses can be classified on the basis of DNA or RNA genome as well as configuration of nucleic acid structure, for example single-stranded (ss) or double-stranded (ds). RNA viruses are further sub classified according to plus and minus polarity.

On The Basis Of Capsid Symmetry

On the basis of capsid symmetry, viruses can be classified as cubic, helical, or complex symmetry.

On The Basis Of An Envelope (Presence Or Absence)

This classification is on the basis of envelope Presence or absence in virus.

On The Basis Of Diameter Of The Virion

Diameter of the virion, or of the nucleocapsid (the combination of capsid and nucleic acid) with helical symmetry

Fungi

Fungi (singular = fungus) is a member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms.

Only about 200 of the thousands of species have been identified as human pathogens, and among these known pathogenic species fewer than a dozen are responsible for more than 90% of all human fungal infections.

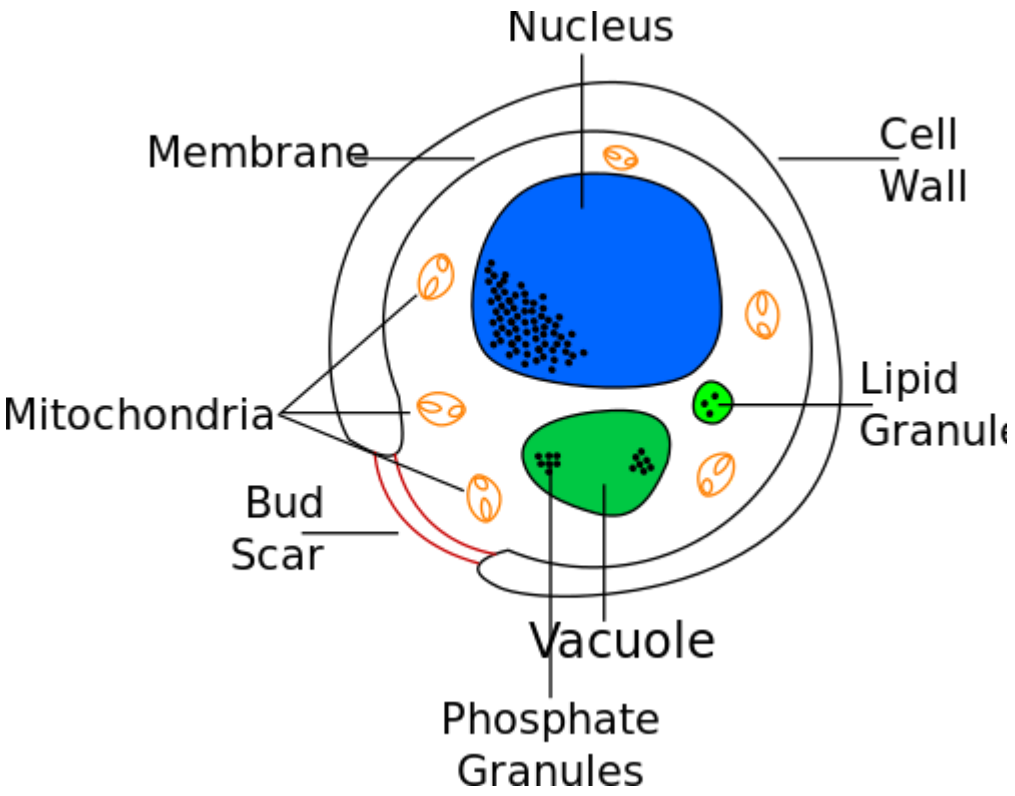
Some nonpathogenic fungi have useful advantages for human beings that are listed below.

Advantages/ Uses of Fungi

- The human use of fungi for food preparation or preservation and other purposes is extensive and has a long history.
- Fungi are used extensively to produce industrial chemicals like citric, gluconic, lactic, and malic acids, & industrial enzymes, such as lipases.
- Mushroom farming and mushroom gathering are large industries in many countries.
- Certain mushrooms enjoy usage as therapeutics in folk medicines, such as Traditional Chinese medicine.
- Many species produce metabolites that are major sources of pharmacologically active drugs.
- Baker's yeast or some unicellular fungus is used to make bread and other wheat-based products, such as pizza dough and dumplings.
- In agriculture, fungi may be useful if they actively compete for nutrients and space with pathogenic microorganisms.
- Several fundamental discoveries in biology were made by researchers using fungi as model organisms, that is, fungi that grow and sexually reproduce rapidly in the laboratory.

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 Of Yeast Cell

The size of yeast cell vary greatly depending on the species, typically measuring 3-4 µm in diameter, although some yeast can reach over 40 µm.

Importance of Yeast

Fermentation

This ethanol is almost always produced by fermentation, the metabolism of carbohydrates by certain species of yeasts under anaerobic or low-oxygen conditions. Beverages such as mead (an alcoholic drink of fermented honey and water), wine, beer, or distilled spirits all use yeast at some stage of their production.

Research

It is also extremely important as a model organism in modern cell biology research and is one of the most thoroughly researched eukaryotic micro-organisms.

Baking

Yeast is used in baking as a leavening agent, where it converts the food/ fermentable sugars present in dough into the gas carbon dioxide. This causes the dough to expand or rise as gas forms pockets or bubbles.

Nutritional Supplements

Yeast is used in nutritional supplements popular with health-conscious individuals. Nutritional yeast is deactivated yeast; it is an excellent source of protein and vitamins, especially the B-complex vitamins, as well as other minerals and cofactors required for growth.

Science

Several yeasts have been widely used in genetics and cell biology largely.

Yeast Extract

Yeast extract is the common name for various forms of processed yeast products that are used as food additives or flavors.

Biofuel Industry

Yeasts have recently been used to generate electricity in microbial fuel cells and produce ethanol for the biofuel industry.

Aquarium Hobby

Yeast is often used by aquarium hobbyists to generate carbon dioxide (CO_2) to nourish plants in planted aquaria.

Mold

Molds are multicellular fungi. It grows in the form of multicellular filaments called hyphae. Molds are neither plants nor animals; they are part of the kingdom Fungi.

Molds are organisms that may be found indoors and outdoors. They are part of the natural environment and play an important role in the environment.

Molds cause biodegradation of natural materials, which can be unwanted when it becomes food spoilage or damage to property. They also play important roles in biotechnology and food science in the production of various foods, beverages, antibiotics, pharmaceuticals and enzymes.

Health Effects

Some diseases of animals and humans can be caused by certain molds. Disease may result from allergic sensitivity to mold spores, from growth of pathogenic molds within the body, or from the effects of ingested or inhaled toxic compounds produced by molds.
Molds produce and release millions of spores in air, water, or insect-borne. They can also produce toxic agents known as mycotoxins. Spores and mycotoxins can have negative effects on human health.

Growth In Buildings And Homes

Mold growth in buildings can lead to a variety of health problems. Various practices can be followed to mitigate mold issues in buildings, the most important of which is to reduce moisture levels that can facilitate mold growth.

Introduction To Microbiology Of Air Water And Soil

Microbiology has revolutionized the study of microorganisms in the environment and improved our understanding of the composition, phylogeny, and physiology of microbial communities. Environmental microbiology is the study of the composition and physiology of microbial communities in the environment. The environment in this case means the air, soil and water.

Microbiology or Air

The microbial flora of air is temporary and variable. Air is not a medium in which micro-organisms can grow but a carrier of particulate matter, dust, and droplets.

Transmission Of Air Borne Micro-Organisms

Organisms are sprayed by coughing and sneezing from the human respiratory tract; and dust particles are circulated by air from the earth's surface. Air micro-organisms may be carried on dust particles or on droplets. Organisms introduced into the air may be transported a few feet or many miles; some die in a matter of seconds, others survive for weeks or months.

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.

- Humidity
- Sunlight
- Temperature
- Size of the particles bearing the microorganisms
- 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

Indoor Air

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

- Ventilation rates
- Crowding
- Nature and degree of activity of the individuals occupying the accommodation

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.

Examples Of Indoor Microbial Content

- Tubercle bacilli
- Diphtheria bacilli
- Hemolytic streptococci

Out Door Air

- Algae, protozoa, yeasts, molds and bacteria have been isolated from the air near the surface of earth.
- Mold spores constituted the largest portion of the airborne micro flora.
- 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.

Airborne Bacterial Diseases

- Diphtheria
- Tuberculosis
- Meningitis

Airborne Viral Diseases

- Small Pox
- Measles
- Influenze
- Common Cold

Airborne Fungal Diseases

- Systemic Mycosis
- Histoplasmosis
- Cryptococcosis

Control Of Micro-Organisms In Air

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.

Listed below are some common and effective methods to controlling the level of air contamination

- Ultraviolet Radiation
- Chemical Agents
- Filtrations
- Air Filters
- Unidirectional Air Flow
- Vacuum Cleaning

Microbiology of Water

Water is essential for the well being of all living organisms. All microbes live in an aqueous environment. Without water they either perish or become inactive.

There are three major types of water

1. Ground Water
2. Surface Water
3. Atmospheric Water

Ground Water

It is subterranean (under the earth's surface) water. It is filtered water, from which bacteria and suspended particles are removed by filtration. Nature has provided a natural mechanism of water purification in this form.

Surface Water

Water of lakes, streams, rivers and oceans represent surface water. Surface water may be contaminated with microbes from Atmospheric water, soil and any wastes dumped into them.

Atmospheric Water

It is the moisture contained in clouds, and precipitated as snow, sleet, hail and rain. Air is washed by atmospheric water. The microbial flora of this water is contributed by air.

Factors Affecting Microbial Flora Of Natural Water

- Nutrients
- Temperature
- pH.
- Oxygen and other gases (CO_2, etc)
- inorganic and organic constituents
- Hydrostatic pressure
- Light
- Salinity

Microorganisms In Water That Cause Diseases

Sometimes microorganisms that cause health effects can be found in drinking water. However, as drinking water is thoroughly disinfected today, disease caused by microorganisms is rarely caused by drinking water. People that swim in swimming pools will find that the water they swim in is disinfected with chlorine, ozone, UV or chlorine dioxide.

Purification of Drinking Water

Following methods are frequently used to purify the drinking water

1. Sedimentation
2. Filtration
3. Chlorination

Sedimentation

Some chemicals (Alum, iron sulfate etc) are added to coagulate suspended particles, in the form of flocks (groups) which cling to organic particles and microbes, while falling through water, drag a major portion to bottom.

Filtration

Different types of filters are used to purify the water. A water filter removes impurities from water by means of a fine physical barrier, a chemical process or a biological process.

Chlorination

Chlorine gas is added to water. Chlorine is continuously added until a residue is present. In this method, most micro-organisms die within 30 minutes.

Microbiology Of 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. Soils differ in depth, chemical composition, physical properties and origin.

Soil Constituents

There are 5 major categories

1. Mineral Particles
2. Organic Residue
3. Water

4. Gases
5. Biological Systems

Microbial Flora of Soil

Bacteria

Bacterial population is highest in both number and variety than all the other groups of microbes.

Fungi

They are most abundant near the surface, where aerobic condition is likely to prevail. Fungi are active in decomposing major constituents of plant tissues such as cellulose and lignin.

Algae

Algae are predominant on the surface or below surface layer of soil. Major types present are Green algae, Blue-green algae, Diatoms (a single-celled alga which has a cell wall of silica).

Protozoa

Most soil protozoa are flagellates or amebas. They are of significance since their dominant mode of nutrition involves ingestion of bacteria.

Viruses

Bacteriophages, as well as some plant and animal viruses are present in soil.

The Rhizosphere

This is the region where the soil and roots make contact. Its microbial population is considerably higher than that of root free soil.

Conditions Influencing Microbial Population

- Amount And Type Of Nutrients
- Available Moisture
- Degree Of Aeration (exposing to air)
- Temperature
- pH

Activity Of Micro-Organisms In Soil

The most important role of soil micro-organisms is their function as biogeochemical agents (The term "biogeochemical" represents that biological, geological and chemical factors are all involved in these functions), for the mineralization of organic carbon, nitrogen, phosphorus, and sulfur compounds, so that these biologically important elements are available for reuse.

STERILIZATION

Sterilization is the process of killing or removing bacteria and all other forms of living organism and their spores from preparation or articles. Sterilization is an absolute term, i.e. the article must be sterile meaning the absence of all microorganisms.

Disinfection

Disinfection is the killing of many, but not all microorganisms. It is a process of reduction of number of contaminating organisms to a level that cannot cause infection, i.e. pathogens must be killed. Some organisms and bacterial spores may survive.

METHOD OF STERILIZATION

There are three common method of sterilization

1. **Physical Methods of Sterilization**
2. **Chemical Methods of Sterilization**
3. **Mechanical Methods of Sterilization**

Physical Methods of Sterilization

- Dry Heat Sterilization *(Flaming, Hot Air Oven, Incineration)*
- Moist Heat Sterilization *(Autoclaving, Heating with a bactericide, Sterilization by boiling water, Tyndallization)*
- Sterilization By Radiations *(Electron beams, X-rays, Gamma rays, Subatomic particles)*

Chemical Methods of Sterilization

- Gaseous Sterilization
- Sterilization By Disinfectants

Mechanical Methods of Sterilization

- Sterilization By Filtration

PHYSICAL METHODS OF STERILIZATION

DRY HEAT STERILIZATION

Substances which are destroyed by moist heat may be sterilized dry heat. Dry heat can be used to sterilized 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 force ventilation of the hot air is used. The standard setting for a hot air oven is at least two hours at 160°C (320F).

A rapid method heats air to 190°C (374F) for 6 minutes for unwrapped objects and 12 minutes for wrapped objects.

Advantages

- Suitable for dry substances
- Suitable for substances that destroyed by moisture
- Glass wares like flasks, test tubes and pipettes can be sterilized

Disadvantages

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

FLAMING

It is simplest method of dry heat sterilization in which the material to be sterilized is kept in the hot part 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.

HOT AIR OVEN

It consists of metallic chamber of aluminum or stainless steel, which is electrically heated and thermostatically controlled

They are of two types

1. **Gravity convection type**, in which air is circulated by gravity convection to all parts of the chamber.
2. **Mechanical convection type**, in which air is circulated by fan.

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

INCINERATION

Incineration is a waste treatment process. It is used to sanitize medical and other hazardous waste before it is discarded with non-hazardous waste.

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 micro-organism are destroyed by denaturizing and coagulation of some of the essential proteins present in the micro-organisms.

Advantages

- Microbe 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 cannot be sterilized.

Method

- Autoclaving
- Heating With Bactericide
- Heating With Boiling Water
- Tandalization

AUTOCLAVING

Autoclave is an apparatus used for sterilization by steam under pressure. 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 121°C.

Working

- Autoclaves commonly use steam heated to 121°C or 134°C.
- To achieve sterility a holding time of at least 15-20 minutes at 121°C or 3minutes 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.

HEATING WITH A BACTERICIDE

In this method bactericide (a substance which kills bacteria) is added to the solutions to be sterilized which are then sealed.

The sealed containers are then heated at 100°C for 30 minutes in water bath.

Commonly used bactericide includes benzalkonium chloride chlorocresol.

STERILIZATION BY BOILING WATER

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

TYNDALLIZATION

This 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

1. Boiling for a period (typically 20 minutes) at atmospheric pressure, Cooling, incubating for a day
2. Boiling, cooling, incubating for a day
3. Boiling, cooling, incubating for a day and finally boiling again

The three incubation periods are to allow heat-resistant spores surviving the previous boiling period to germinate to form the heat-sensitive vegetative (growing) stage, which can be killed by the next boiling steps.

STERILIZATION BY RADIATION

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

Mechanism

By radiations, alternation 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 to the persons operating.
- Radiations may lead to change in colour , texture and solubility.

Methods

Methods exist to sterilize using radiation are

- Electron beams
- X-rays
- Gamma rays
- Subatomic particles

Chemical Methods Of Sterilization

Chemicals are also used for sterilization. Although heating provides the most reliable way to get rid 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.

Common Chemicals Used In This Sterilization Process Are

- Ethylene Oxide
- Ozone
- Chlorine Bleach
- Formaldehyde
- Hydrogen Peroxide

Mechanical Methods Of Sterilization

It includes filtration

Filtration

Filtration involves the physical removal of all cells in a liquid or gas. It is especially important for sterilization of solutions which would be denatured by heat, e.g. antibiotics, injectable drugs, amino acids, vitamins, etc.

Preservatives

Static agents used to inhibit the growth of microorganisms, most often in foods. If eaten they should be nontoxic. Examples are calcium propionate, sodium benzoate, formaldehyde, nitrate and sulfur dioxide.

Antimicrobial Agents

Antimicrobial agents are chemicals that kill or inhibit the growth microorganisms. These agents include chemical preservatives and antiseptics, as well 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.

Applications Of Sterilization In Pharmacy

The success of pharmaceutical manufacturing facility is often dependent upon an efficient sterilization process. Sterilization is critical to the integrity of finished product.

Every hospital uses sterilizing equipment. Autoclaves are used primarily for the sterilization. Sterilization is a very important process actually a must and should process to keep ourselves safe form the infectious atmosphere. Things that are to enter the already sterile body have to be properly sterilized. It is important to keep them germ free and safe to secure our health.

All the culture media should be kept sterile in order to avoid the growth of unwanted organisms. Sterilization is very important in case of injectables as it may cause many blood borne deadly infections like AIDS. It is important to keep all the instruments in the laboratory neat and dust free to avoid any problems pertaining to production.

Fermentation

The conversion of sugar or sugar substitutes into CO_2 and alcohol with the help of microorganisms is called fermentation

Two Key Aspects Of Fermentation Process

The first key aspect is related to production of valuable microbial products via fermentation processes. These include…

- Fermented Foods
- Beverages
- Bread
- Beer
- Cheese
- Wine
- Energy Sources
- Enzymes
- Food Ingredients
- Pharmaceutical Products

The second aspect is the role of microorganisms in waste treatment and pollution control, which utilizes their abilities to degrade virtually all natural and man-made products.

Fermentation Products

- Food, beverages, food additives and supplements
- Health-care products
- Microbial enzymes
- Industrial chemicals and fuels

FOOD, BEVERAGES, FOOD ADDITIVES AND SUPPLEMENTS

A wide range of fermented foods and beverages have been produced throughout recorded history. Dairy products, for example, result from the activities of lactic acid bacteria in milk, which modify flavor and texture, and increase long-term product stability. Yeasts are exploited in the production of alcoholic beverages. Most of the amino acids and vitamins used as supplements in human food and animal feed are produced most economically by microorganisms. In addition, some microorganisms contain high levels of protein with good nutritional characteristics suitable for both human and animal consumption.

HEALTH-CARE PRODUCTS

In terms of providing human benefit, antibiotics are probably the most important compounds produced by industrial microorganisms. Over 4000 antibiotics have now been isolated, but only about 50 are used regularly in antimicrobial chemotherapy. The best known and probably the most medically useful antibiotics are the β-lactams, penicillin and cephalosporin, along with amino-glycosides (e.g. streptomycin) and the tetracycline.

Other important pharmaceutical products derived from microbial fermentation and biotransformations are alkaloids, steroids and vaccines. More recently, therapeutic recombinant human proteins such as insulin, interferon and human growth hormone have been produced by a range of microorganisms.

MICROBIAL ENZYMES

Microbial enzymes, particularly extracellular hydrolytic enzymes, have numerous roles as process aids or in the production of a wide range of specific food and nonfood products.

INDUSTRIAL CHEMICALS AND FUELS

Industrial feedstock chemicals supplied through fermentation include various alcohols, solvents such as acetone, organic acids, polysaccharides, lipids and raw materials for the production of plastics. Some of these fermentation products also have applications in food manufacture.

IMMUNITY

It is a natural or acquired resistance of the body to a certain disease or pathogenic micro-organism 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.

ANTIGENS

Chemical substances capable of mobilizing the immune system and provoking an immune response are called Antigens.

TOLERANCE

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

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 B-lymphocytes.

ANTIGEN-ANTIBODY REACTIONS

A reaction that occurs when an antigen combines with a corresponding antibody to produce an immune complex is called antigen-antibody reaction (Ag-Ab reaction).

TYPES OF ANTIGEN-ANTIBODY (AG-AB) REACTIONS

1. **Agglutination Reactions**
2. **Precipitation and Flocculation Reaction**
3. **Complement Fixation**
4. **Opsonization**

AGGLUTINATION REACTIONS

Aggregation of cells due to antibody binding is known as "Agglutination"

The antibodies that cause agglutination of cells are called "agglutinins" and the antigens aggregated are called "agglutinates".

Precipitation And Flocculation Reaction

When an antibody binds to a soluble antigen, the antigen becomes insoluble and it may precipitate or float in the fluids. If Ag-Ab complex precipitates, it is referred as precipitation reaction.

Complement Fixation

Antibody binding with antigen is not sufficient to remove the antigen from body. Hence Ag-Ab complex initiates activation and binding of complement system to it. Binding of complement system to Ag-Ab complex is known as complement fixation.

Opsonization

Antibodies coat antigen and promote their ingestion by phagocytes. The process of coating particles for subsequent phagocytosis is called opsonization.

Hypersensitivity

Hypersensitivity or hypersensitivity reaction refers to undesirable reactions produced by the normal immune system, including allergies and autoimmunity. These reactions may be damaging, uncomfortable, or often fatal.

Allergy

An allergy is a hypersensitivity disorder of the immune system. Allergic reactions occur when a person's immune system reacts to normally harmless substances in the environment. A substance that causes a reaction is called an allergen.

Vaccine

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

Vaccine improves immunity to a particular disease. Typically it contains an agent that resembles a disease causing microorganism, and is often made from weakened or killed forms of the microbe.

The agent stimulates the body’s immune system to recognize the agent as non-safe, destroy it, and remember it so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters.

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.

Types of Vaccines

- Live, Attenuated Whole-Agent Vaccines
- Inactivate Whole-Agent Vaccines
- Toxoids
- Subunit Vaccine
- Conjugated Vaccines
- Nucleic Acid Vaccine

Live, Attenuated Whole-Agent Vaccines

Live, Attenuated whole-agent Vaccines are made from living but weakened microbes, e.g. Measles, Typhoid vaccines (oral), Sabin polio vaccine (oral) etc.

Inactivate Whole-Agent Vaccines

Inactivate Whole-agent Vaccines are made from microbes that have been killed, e.g. vaccines against Rabies, Influenza, Cholera etc.

Toxoids

Toxoid vaccines are made from inactivated toxic compounds that cause illness rather than the micro-organism. Examples of toxoid-based vaccines include tetanus and diphtheria.

Subunit Vaccine

Subunit vaccines are made from those antigenic fragments of a microorganism that best stimulates an immune response e.g. vaccine against Hepatitis B virus.

Conjugated Vaccines

Certain bacteria have polysaccharide outer coats that are poorly immunogenic. By linking these outer coats to proteins (e.g., toxins), the immune system can be led to recognize the polysaccharide as if it were a protein antigen. This approach is used in the Haemophilus influenzae type B vaccine.

Nucleic Acid Vaccine

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

SERA

Sera or serum, in blood, the serum is the component that is neither a blood cell (serum does not contain white or red blood cells) nor a clotting factor. It is the blood plasma with the fibrinogens removed. Serum includes all proteins not used in blood clotting (coagulation) and all the electrolytes, antibodies, antigens, hormones, and any exogenous substances (e.g., drugs and microorganisms).

ANTISERA

Antisera or Antiserum is blood serum containing polyclonal antibodies (Polyclonal antibodies are antibodies that are secreted by different B cell lineages within the body. They are a collection of immunoglobulin molecules that react against a specific antigen).

Antiserum is used to pass on passive immunity to many diseases. Antibodies in the antiserum bind the infectious agent or antigen. The immune system then recognizes foreign agents bound to antibodies and triggers immune response. The use of antiserum is particularly effective against pathogens which are capable of evading (escape) the immune system.

Course Outlines

Microbiology

1. Introduction and scope of Microbiology.
2. Nomenclature and classification of Microorganisms.

i) The Bacteria.

a) Classification of Bacteria.

b) Culture media, Bacterial cultures and staining methods.

ii) The viruses: Nomenclature and classification of viruses.

iii) Introduction to Fungi, Yeast, Molds.

3. Introduction to Microbiology of air water and soil.
4. Sterilization and Disinfection.

a) Introduction to sterile area and clean area.

b) Methods and application in pharmacy.

5. Fermentation, Pharmaceutical productions produced by fermentation process.
6. Definition of the following:

Immunity, autoimmunity and tolerance, Antigen, Antibodies, Antigen-Antibody reactions. Hypersensitivity and allergy.

7. Vaccines and Sera: Introduction and aims. Types of vaccines.