South Valley University
Faculty of Science
Botany & Microbiology
Department

GENERAL MICROBIOLOGY

prepared by

Dr. Amany Atta El-Shahir

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CHAPTER I

1) INTRODUCTION

INTRODUCTION

Microbiology: study of microscopic organisms (that are too small to be seen with necked eye) .

1- Study of microorganisms:

i. The science of microbiology naturally rests upon the existence of *microorganisms*. Not surprisingly, the field of *microbiology* began simultaneously with the discovery of microorganisms.

ii. Depending upon how you would like to argue the point, *microbiology* as a science either did not exist, or existed in an only theoretical sense (e.g., postulated, unseen organisms) prior to the discovery of microorganisms.

2- The reliance of the field of *microbiology* on such a loose definition of microorganism does not make for a terribly cohesive discipline:

i. "The difficulty in setting the boundaries of microbiology led Roger Stanier to suggest that the field be defined not only in terms of the size of its subjects but also in terms of its techniques. A microbiologist usually first isolates a specific microorganisms from a population and then cultures it. Thus microbiology employs techniques--such as sterilization and the use of culture media--that are necessary for the successful isolation and growth of microorganisms." (p. 3, Prescott *et al.*, 1997).

3- Microorganism

Very small organisms**:**

i. Living things which individually are too small to be seen with the naked eye.

ii. Something studied using characteristic techniques including:
   1. aseptic technique.
   2. pure culture technique.
   3. microscopic observation of whole organisms.

4- **All of the following** may be considered microorganisms:

i. bacteria (eubacteria, archaebacteria).
ii. fungi (yeasts, molds).
iii. protozoa.
iv. microscopic algae.
v. viruses.
vi. various parasitic worms.

5- **See "lecture review"** below for an overview of these types:

organism:	types:	description:	nutrition type:	durable state:	some diseases:
algae:	brown, red, green, diatoms, dinoflagellates, euglenoids	photosynthetic aquatic eucaryotes, cell walls, unicellular and multicellular	photoautotrophs	---	---
bacteria:	eubacteria, archaeabacteria, Gram-negative, Gram-positive, acid fast, cyanobacteria	procaryotes, absorbers, wet conditions, animal decomposers, cell walls, unicellular	chemoheterotrophs, photoheterotrophs, chemoautotrophs, photoautotrophs	endospores (some)	tetanus, botulism, gonorrhea, chlamydia, tuberculosis, etc., etc., etc.
cyanobacteria:	blue-green algae	photosynthetic aquatic procaryotes, green lake scum, cell walls	photoautotrophs	---	---
fungi:	yeasts (unicellular fungi), molds (filamentous fungi)	eucaryotes, absorbers, dry conditions, plant decomposers, cell walls, ~100 human pathogens	chemoheterotrophs	spores	mycoses: candida, ringworm, athlete's foot, jock itch, etc.
helminths:	Flatworms (platyhelminths), roundworms (nematodes)	metazoan (multicellular animal) parasites, engulfers and absorbers	chemoheterotrophs	---	tape worm, trichinosis, hook worm, etc.
protozoa:	Unicellular and slime molds, flagellates, ciliates	eucaryotes, parasites, engulfers and absorbers, wet conditions, no cell wall, ~30 human pathogens	chemoheterotrophs	cysts (some)	malaria, giardiasis, amoebic dysentery, etc.
viruses:	Enveloped, non-enveloped	acellular, obligate intracellular parasites	not applicable	virion particles, encased in durable state host	common cold, flu, HIV, herpes, chicken pox, etc.

6- Environmental microbiology

Basic environmental processes:

i. The existence and functioning of microbes is absolutely crucial to environmental health.

ii. By health, I don't simply mean the cleaning up of pollution. Instead, microbes are absolutely necessary for such basic things as:

   1. making nutrients available from non-living sources.
   2. providing energy to ecosystems.
   3. freeing up nutrients from no longer living sources.

iii. Without microorganisms we would:

   1. have no oxygen to breath.
   2. nothing to eat .
   3. not be able to utilize the energy in food even if we could eat it .
   4. not be able move about without constantly tripping over the bodies of dead organisms .

iv. In more technical terms, a sampling of what microorganisms are do to environment:

   1. microbes are producers.
   2. microbes are nitrogen fixers .
   3. microbes are decomposers .
   4. microbes are symbionts .

7- Industrial microbiology

a. Microbes have played important roles in manufacturing products for as long as there has been history.

b. Microorganisms are used to:

- ferment useful chemicals (ethanol, acetone, etc.)
- produce certain food stuffs (wine, cheese, yogurt, bread, half sour pickles, etc.)
- produce of recombinant products (recombinant insulin, human growth hormone, etc.)
- destroy wastes (sewage, oil spills, bioremediation)

8- Medical microbiology

a. Microbes both cause and prevent disease.

b. Microbes produce antibiotics used to treat disease.

c. The single most important achievement of modern medicine is the ability to treat or prevent microbial disease.

d. Most of this course will consider the physiology of microbes and their role in disease.

9- Pathogen

a. A microorganism is considered to be a *pathogen* or *pathogenic* if it is capable of producing disease.

b. Though only a minority of microorganisms are *pathogenic*, practical knowledge of microbes is necessary for their treatment so is highly relevant to medicine and related health sciences.

10- Normal flora [normal microbiota]

a. Not disease-causing:

- *Normal flora* are those not-typically-disease-causing microorganisms normally found in and on healthy individuals.

- Also known as normal microbiota.

b. Very abundant:

- *Normal flora* are extremely abundant in terms of absolute numbers.
- A normal human has approximately 10^{13} body cells and 10^{14} individual *normal flora*!
- However, microorganisms also tend to be very small, bacteria especially are much smaller than are our own cells.

c. All found externally:

- *Normal flora* are found mostly:
    1. on the skin
    2. in the eyes
    3. in the nose
    4. in the mouth
    5. in the upper throat
    6. in the lower urethra
    7. in the lower intestine
    8. especially in the large intestine

•

11- Divisions of microbiology:

a- Organisms studied:

1- Bacteriology (study of bacteria)
2- Mycology (study of fungi)
3- Phycology (study of algae)
4- Virology (study of virus)
5- Parasitology (study of parasites)

b- Health related:

1- Epidemiology (study of spread of diseases)
2- Immunology (study of immune system)
3- Chemotherapy (treatment of diseases with chemical compounds)

12- Binomial nomenclature

d. Each "species" or organism is assigned two names corresponding the genus and "specific epithet" (i.e., species).
e. When employing binomial nomenclature, the following conventions are employed:
    - the genus name (e.g., *Escherichia*) is always capitalized
    - the species name (e.g., *coli*) is never capitalized
    - the species name is never used without the genus name (e.g., *coli* standing alone)
    - the genus name may be used without the species name (e.g., *Escherichia* may stand alone, though no longer actually describes a species)
    - when both genus and species name are present, the genus name always comes before the species name (e.g., "*coli* of *Eschichia*" does not work)
    - when both genus and species name are present, the species name is always placed directly after the genus name (e.g., *Escherichia coli*, not *coli Escherichia*)
    - genus and species are always italicized (or underlined) (e.g., Escherichia coli is a no-no)

Prokaryotes, Eukaryotes

Microorganisms and all other living organisms are classified as **prokaryotes** or **eukaryotes.** Prokaryotes and eukaryotes are distinguished on the basis of their cellular characteristics. For example, prokaryotic cells lack a nucleus and other membrane-bound structures known as organelles, while eukaryotic cells have both a nucleus and organelles (Figure 1).

- **Prokaryotes:** Organisms whose cells lack a nucleus and therefore have DNA floating loosely in the liquid center of the cell. Prokaryotes divide, and thus reproduce, by simple mitosis.
- **Eukaryotes:** Organisms that have a well-defined nucleus to house and protect the DNA. Eukaryotes divide by meiosis for sexual reproduction.

Prokaryotes: Cells without a nucleus

Organisms composed of cells without nuclei are classified as *prokaryotes,* which means "before nucleus." Prokaryotes are the most common forms of life on earth. You are, at this very moment, covered in and inhabited by millions of prokaryotic cells: bacteria. Much of your life and your body's processes depend on these arrangements; for example, the digestion going on in your intestines is partially powered by bacteria that break

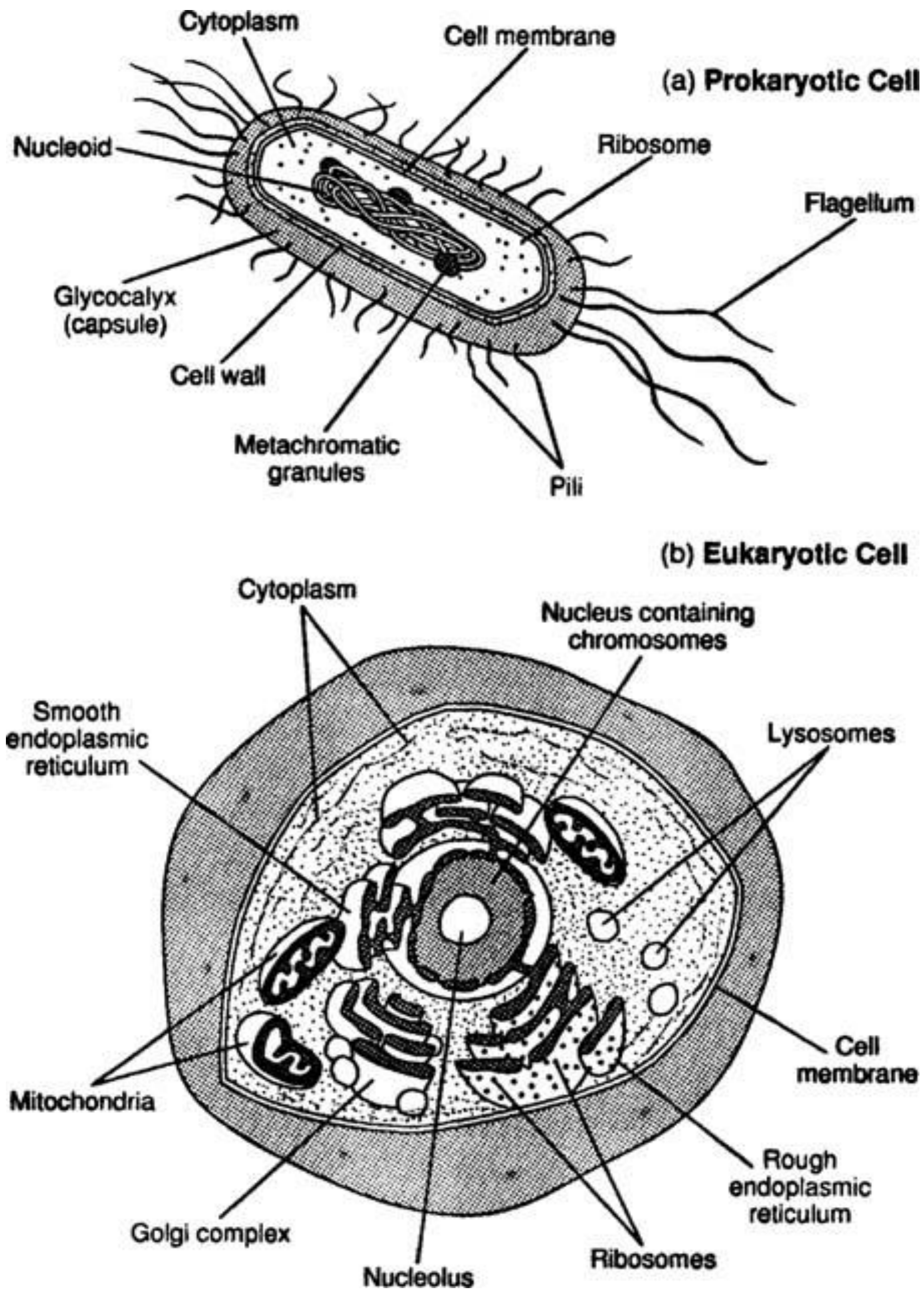


Figure 1 *The important cellular features of (a) a prokaryotic cell (a bacterium) and (b) a eukaryotic cell*

down the food you eat. Most of the bacteria in your body are completely harmless to you. Other species of bacteria, however, can be vicious and deadly, causing rapidly transmitted diseases such as cholera.

All bacteria, regardless of temperament, are simple, one-celled prokaryotic organisms. None have cell nuclei, and all are small cells with relatively small amounts of DNA.

The exterior of a prokaryotic cell is encapsulated by a *cell wall* that serves as the bacteria's only protection from the outside world. A *plasma membrane* (*membranes* are thin sheets or layers) regulates the exchange of nutrients, water, and gases that nourish the bacterial cell. DNA, usually in the form of a single hoop-shaped piece (segments of DNA like this one are called *chromosomes*), floats around inside the cell. The liquid interior of the cell is called the *cytoplasm.* The cytoplasm provides a cushiony, watery home for the DNA and other cell machinery that carries out the business of living. Prokaryotes divide, and thus reproduce, by simple mitosis.

Eukaryotes: Cells with a nucleus

Organisms that have cells with nuclei are classified as *eukaryotes* (meaning "true nucleus"). Eukaryotes range in complexity from simple one-celled animals and plants all the way to complex multicellular organisms like you. Eukaryotic cells are fairly complicated and have numerous parts to keep track of. Like prokaryotes, eukaryotic cells are held together by a *plasma membrane,* and sometimes a *cell wall* surrounds the membrane (plants, for example have cell walls). But that's where the similarities end.

The most important feature of the eukaryotic cell is the *nucleus* — the membrane-surrounded compartment that houses the DNA that's divided into one or more chromosomes. The nucleus protects the DNA from damage during day-to-day living. Eukaryotic chromosomes are usually long, string-like segments of DNA instead of the hoop-shaped ones found in prokaryotes. Another hallmark of eukaryotes is the way the DNA is

packaged: Eukaryotes usually have much larger amounts of DNA than prokaryotes, so to fit all that DNA into the tiny cell nucleus, it must be tightly wound around special proteins.

Unlike prokaryotes, eukaryotes have all sorts of cell parts, called *organelles,* that help carry out the business of living. The organelles are found floating around in the watery cytoplasm outside the nucleus.

Two of the most important organelles are the following:

- **Mitochondria:** The powerhouses of the eukaryotic cell, mitochondria pump out energy by converting glucose to ATP (adenosine triphosphate). ATP acts like a battery of sorts, storing energy until it's needed for day-to-day living. Both animals and plants have mitochondria.

- **Chloroplasts:** These organelles are unique to plants. They process the energy of sunlight into sugars that then are used by plant mitochondria to generate the energy that nourishes the living cells.

Compare prokaryotic and eukaryotic cells.

SIMILARITIES:
1. They both have DNA as their genetic material.
2. They are both membrane bound.
3. They both have ribosomes .
4. They have similar basic metabolism .
5. They are both amazingly diverse in forms.

DIFERENCES:
1. eukaryotes have a nucleus, while prokaryotes do not
2. eukaryotes have membrane-bound organelles, while prokaryotes do not. The organelles of eukaryotes allow them to exhibit much higher levels of intracellular division of labor than is possible in prokaryotic cells.
3. Eukaryotic cells are, on average, ten times the size of prokaryotic cells.
4. The DNA of eukaryotes is much more complex and therefore much

more extensive than the DNA of prokaryotes.

5. Prokaryotes have a cell wall composed of peptidoglycan, a single large polymer of amino acids and sugar . Many types of eukaryotic cells also have cell walls, but it is not made of peptidoglycan.

6. The DNA of prokaryotes floats freely around the cell; the DNA of eukaryotes is held within its nucleus and associated with histones (proteins).

7. Eukaryotes undergo mitosis; prokaryotes divide by binary fission (simple cell division).

2) Sterilization

Sterilization

STERILIZATION is a term referring to any process that eliminates

(removes) or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. Sterilization can be achieved by applying the proper combinations of heat, chemicals, irradiation, high pressure, and filtration.

COMMERCIAL STERILIZATION—term used for canned food—it must be heated enough to kill endospores of *Clostridium botulinum*. More resistant endospores may survive.

DISINFECTION—destruction of vegetative pathogens. Most often this term refers to application of a chemical called a disinfectant to an inert surface (floors, countertops, etc.), but ultraviolet radiation and boiling are other methods used. Endospores are not necessarily killed.

ANTISEPSIS—chemical called an antiseptic is applied to living tissue. Some microbes are killed, but not all.

DEGERMING—mechanical removal of microbes from a limited area, such as wiping an injection site with alcohol.

SANITIZATION—high-temperature washing or washing and then dipping into a chemical disinfectant. This is used to lower microbial counts on eating or drinking utensils.

Treatments that kill microbes end in ---cide.

Germicide—kills microbes but possibly not endospores

Fungicide—kills fungi

Virucide—kills viruses

Bacteriocide—kills bacteria

Treatments ending in ----static stop growth (no increase in numbers) but do not kill.

Bacteriostatic

Microbistatic

Sepsis—indicates bacterial contamination.

Asepsis—absence of significant contamination.

FACTORS THAT INFLUENCE THE EFFECTIVENESS OF STERILIZATION:

1. Number of microbes the more there are to begin with, the longer it takes to eliminate them all.

2. Environmental factors:

a. Organic matter (blood, feces, etc.) often interferes with chemical antimicrobials, and also to a lesser extent with heat treatment. Any medium containing fats or proteins tends to protect bacteria.

b. Disinfectants work better in warmer temperatures

c. Biofilms protect microbes

d. Presence of fats & proteins protect microbes

e. pH—heat is more effective in an acid pH.

3. Time of exposure

a. The lower the temperature used for heat treatment, the longer the time required.

b. Effects of irradiation are also related to time.

c. Chemical antimicrobials require a certain amount of exposure time; extended exposure to kill resistant bacteria or endospores.

4. Microbial characteristics susceptibility to different agents varies among microbes. Also, endospores are much more difficult to eliminate.

ACTIONS OF MICROBIAL CONTROL AGENTS

1. ALTERATION OF MEMBRANE PERMEABILITY—the plasma membrane regulates the entry of materials into and the exit of wastes out of

the cell. Damage to the plasma membrane causes leakage of cell contents into the surroundings, killing the cell or at least preventing cell division. Remember, death in microbes is loss of the ability to reproduce.

2. DAMAGE TO PROTEINS—enzymes and other proteins are essential for cell function.

a. Hydrogen bonds hold proteins in the characteristic 3-dimensional shape required for their functions. Heat and certain chemicals break these bonds and the shape is lost. This is called denaturation.

b. Covalent bonds, which are also part of protein structure, may be broken by chemicals or heat even though they are stronger than hydrogen bonds.

3. DAMAGE TO NUCLEIC ACIDS—DNA and RNA carry the cell's genetic information and function in protein synthesis. Damage to these by heat, radiation, or chemicals usually kills the cell.

METHODS OF STERILIZATION

- Physical methods.
- Chemical methods.
- Filtration.

1)Physical methods

Drying and salting were the earliest methods of food preservation, so these were also the earliest means of controlling bacterial growth. Physical means of control include:

Heat Osmotic pressure Radiation

I- HEAT

Canned foods are an example of control of microbial growth by heating. In the medical world, lab media, glassware, and

surgical instruments are examples of things usually sterilized by heat. Heat resistance varies among microbes.

Thermal death point is the lowest temperature at which all microbes in a liquid culture will be killed in 10 minutes.

Thermal death time is the minimum length of time in which all bacteria in a liquid culture will be killed at a given temperature.

A . MOIST HEAT—all moist heat methods kill microbes by coagulating or denaturing their proteins, including enzymes. This occurs faster in the presence of water, so moist heat requires lower temperatures and less time of exposure than dry heat.

a. BOILING (100° C)—kills all vegetative bacterial pathogens, almost all viruses, and fungi and their spores in 10 minutes or less (often much less). Endospores and a few viruses are much more resistant to boiling.

Hepatitis A virus—30 minutes

Endospores—up to 20 hours

Although boiling will kill most pathogens, it is not a dependable means of sterilization.

b. AUTOCLAVE—this device uses steam under pressure for effective sterilization. Items to be sterilized are placed in a chamber, which is then sealed. All air is exhausted and steam under pressure is injected. This achieves higher temperatures than boiling. It is the preferred means of sterilization for all materials that can withstand it.

The most common setting uses steam under 1.5 atm.pressure and reaches a temperature of 121° C. This kills all organisms and their endospores in about 15 - 20 minutes.

(Different autoclave shapes)

Here we use an autoclave to sterilize culture media. In hospitals, doctors' offices, dentists' offices, etc. this methods is used to sterilize medical equipment.

Materials being autoclaved are often wrapped in paper, so that after sterilization the outside of the package can be handled without contaminating the sterile item inside.

To ensure the autoclaving process was able to cause sterilization, most autoclaves have meters and charts that record or display pertinent information such as temperature and pressure as a function of time. Indicator tape is often placed on packages of products prior to autoclaving. A chemical in the tape will change color when the appropriate conditions have been met. Some types of packaging have built-in indicators on them.

The size of the container, the volume of a liquid, and the type of wrapping can influence the time and temperature required for sterilization.

c. PASTEURIZATION—Pasteur used mild heat to kill microbes in beer and wine ingredients before fermentation. The same general idea is now used for milk. Pasteurization kills pathogenic bacteria and viruses, although some harmless bacteria do survive. (Heat sufficient to kill all microbes changes the character of the milk.) Bacteria that are left eventually cause the milk to spoil, but the life is greatly prolonged.

Originally, milk was heated to 63° C for 30 minutes. Today, new methods use 72° C for 15 seconds (high-temperature short-time pasteurization). Milk can be sterilized so that it can be sealed in a carton and stored without refrigeration. This requires a temperature of 140° C for about 3 seconds (ultra-high temperature treatment). This process gives the milk an “off” taste.

B. DRY HEAT—this kills by burning to ashes or by oxidation

a. FLAMING—we use this on loops in the lab. Leaving the loop in the flame of a Bunsen burner or alcohol lamp until it glows red ensures that any infectious agent gets inactivated. This is commonly used for small metal or glass objects.

A variation on flaming is to dip the object in 70% ethanol (or a higher concentration) and merely touch the object briefly to the Bunsen burner flame, but not hold it in the gas flame. The ethanol will ignite and burn off in a few seconds. 70% ethanol kills many, but not all, bacteria and viruses, and has the advantage that it leaves less residue than a gas flame. This method works well for the glass "hockey stick"-shaped bacteria spreaders.

b. INCINERATION—burning contaminated paper disposables in a controlled chamber.

c. HOT-AIR STERILIZATION—items are placed in an oven. Typical procedure would be 170º C for 4-6 hours. It takes much longer for dry heat to kill than moist heat. This method sterilizes by oxidation. It is mainly used for items that the autoclave is not suitable for.

(Hot air ovens)

This may be due to size or quantity--lots of lab glassware might be sterilized in a hot-air oven.

11- 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 typically 20 minutes at atmospheric pressure, cooling, incubating for a day, boiling, cooling, incubating for a day, 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 step. This is effective because many spores are stimulated to grow by the heat shock. The procedure only works for media that can support bacterial growth - it will not sterilize plain water. Tyndallization is ineffective against prions.

II- RADIATION:

1. IONIZING RADIATION—this includes X rays, gamma rays, and high-energy electron beams. These all have very short wavelengths and high levels of energy. They cause ionization of water within cells, which results in formation of hydroxyl radicals. These destroy cell components, especially DNA. This process is used to sterilize wrapped plastic disposables such as syringes, catheters, gloves, suture materials, vials of injectables, disposable Petri dishes, pipettes, etc. It is also used to sterilize spices. Recently, approval has been granted for use of low level radiation

of fruits and meats. The post office is now using this method to sterilize some mail.

This process does involve the use of dangerous radiation and can only be used in a properly shielded room, so it is mostly used in factories where wide scale use of the setup makes it economical.

2. NONIONIZING RADIATION—this has a longer wavelength and less energy. Ultraviolet (UV) light is the common example. It causes the formation of thymine dimers, which interferes with DNA replication and formation of mRNA.

UV lamps are used in hospitals and in food service. This method does not sterilize, but it does reduce bacterial growth. Penetrating power is very low, so any type of covering protects microbes.

Sunlight has some weak antimicrobial effects, but the wavelengths of sunlight are too long to work well.

3. MICROWAVES—little effect on microbes but the heat may kill them.

2) Chemical methods:

Most chemical agents are disinfectants or antiseptics, although there are a few chemical sterility.

"Disinfectants' are those chemicals that destroy pathogenic bacteria from inanimate surfaces.

Those chemicals that can be safely applied over skin and mucus membranes are called ' Antiseptics'.

An ideal antiseptic or disinfectant should have the following properties

1- Should have wide spectrum of activity.
2- Should be able to destroy microbes within practical period of time.
3- Should be active in the presence of organic matter.
4- Should be active in any pH.
5- Should be stable.
6- Should be speedy.
7- Should be non toxic, non allergenic.
8- Should not have bad odor.

TYPES OF DISINFECTANTS

1. PHENOLS and PHENOLICS

a. PHENOL (carbolic acid) was used by Lister to reduce the incidence of surgical infections. It is irritating to skin and mucous membranes and has a bad odor, so it is rarely used today. Its main use now is in throat lozenges and sprays, but the concentration is so low that there is little antimicrobial effect, although there is some local anesthetic action. Some throat sprays may have a concentration above 1% and these may show antibacterial action.

b. PHENOLICS—chemicals derived from phenol—the molecule has been chemically altered to make it less irritating and more effective. These agents act in several ways, damaging plasma membranes, inactivating enzymes, and denaturing proteins. Phenolics are often used as disinfectants because they remain active in the presence of organic matter. Original

Lysol products contain O-phenyl phenol, but many of the newer ones do not.

2. BIGUANIDES—chlorhexidine is an example. These are similar to phenolics, but are less toxic. Biguanides act by disrupting the plasma membrane and are excellent as surgical

scrubs and for patient preps, although they must be kept away from the eyes. They are also used in dental treatments.

They are effective against most vegetative bacteria and fungi, but not against endospores and many viruses.

3. HALOGENS

a. IODINE—one of the oldest and most effective antiseptics. Works against all bacteria and many endospores, fungi, and viruses. It acts by combining with amino acids, especially tyrosine, and inhibits the functions of microbial proteins. It also alters plasma membranes.

1) Tincture—iodine combined with alcohol

2) Iodophor—iodine combined with an organic carrier molecule. These are still quite effective, but they are less toxic and do not stain as badly. Betadine is an example.

In addition to use as a skin disinfectant and wound treatment, iodine can be used to purify drinking water. (5 – 10 drops per quart)

b. CHLORINE—when chlorine is added to water, hypochlorous acid forms. This is a strong oxidizing agent that interferes with cellular enzymes.

1) A liquid form of compressed **chlorine gas** is used for treating water systems, swimming pools, and sewage. When added to water chorine gas forms hypochlorous acid.

2) **Calcium hypochlorite** is used to disinfect dairy equipment and restaurant equipment. It can also be called chloride of lime.

3) **Sodium hypochlorite (Chlorox)** is used as a household disinfectant and bleach. Adding 2 - 4 drops of Chlorox to a quart of water can make it safe as drinking water (let sit 30 minutes before using). A solution of Chlorox is the recommended disinfectant for killing the AIDS virus in households.

4) **Chlorine dioxide**—either in gaseous form or in aqueous solution. Used to fumigate enclosed areas, in water treatment, or as a disinfectant/antiseptic.

5) **Chloramines**—combine chlorine and ammonia, used to sanitize glassware, eating utensils, and food-handling equipment as well as in water treatment.

4. ALCOHOLS—these kill bacteria and fungi but not endospores and most viruses. Alcohol acts mainly by denaturing proteins, but it can also disrupt membranes and dissolve lipids. Alcohols evaporate rapidly, leaving no residue. Alcohols are frequently used as skin degerming agents. Wiping

with alcohol mostly wipes away microbes, skin oils, and dirt, although some microbes may be killed.

Alcohols are not satisfactory for cleaning open wounds, because they coagulate a surface layer of protein and leave bacteria unharmed beneath it. Most hand sanitizers contain alcohol as the active ingredient.

a. ETHANOL (ethyl alcohol)—70 % concentration is ideal, although concentrations of 60 - 95 % are effective. 100% is not effective because some water must be present for denaturation to occur.

b. ISOPROPANOL (isopropyl alcohol or rubbing alcohol)—more commonly used because it is cheaper and more effective. Usual concentration is 90%.

Both of these alcohols may be mixed with other agents to enhance activity. When another agent is mixed with alcohol, the solution is called a tincture.

5. HEAVY METALS—very low concentrations of heavy metals can be effective against microbes. This is called oligodynamic action, and works by denaturing proteins, including enzymes.

a. SILVER NITRATE (1% solution)—this was once used to swab sore throats (in the days before antibiotics). It was also used in the eyes of newborns, to prevent an eye infection caused by the gonorrhea bacteria. Antibiotic ointments are now used for this purpose.

b. PURE SILVER INCORPORATED INTO DRESSINGS (ACTICOAT)--In recent years, dressings containing silver have been used in treating infections caused by antibiotic-resistant bacteria. The silver

seems to shut down energy production in bacteria and little resistance has been found, and tissue damage does not occur as with other means of delivering silver.

c. OTHER FORMS OF SILVER—Cream containing silver combined with a sulfa drug, catheters impregnated with silver, a silver-containing product for surfaces (Surfacine).

d. MERCURY---compounds such as mercuric chloride were probably the earliest disinfectant. They are bacteriostatic. The drawbacks are toxicity, corrosiveness and inactivation by organic matter. Mercurochrome and merthiolate were once widely used, but contained such tiny amounts of mercury that they had little effect. Mercury compounds may be used in paint to prevent mildew.

e. COPPER---copper sulfate is used to kill algae (algicide) in bodies of water or aquariums. Copper compounds may also be used in paint to prevent mildew.

f. ZINC---zinc chloride is found in some mouthwashes and zinc is often used as an antifungal in paint. Zinc lozenges & such are sold as treatment for colds, but their effect is questionable.

6. SURFACE-ACTIVE AGENTS (SURFACTANTS)---these agents decrease surface tension and include soaps and detergents.

a. SOAPS---main value is in causing microbes to be mechanically removed. Washing with soap breaks up the oily film that covers skin and

allows microbes and dirt to be washed away. Deodorant soaps have antimicrobial ingredients added.

7. CHEMICAL FOOD PRESERVATIVES---these are frequently added to retard spoilage, and are believed to be safe for consumption.

a. SULFUR DIOXIDE

b. ORGANIC ACIDS---they interfere with the metabolism of molds or damage their plasma membranes.

1) Sorbic acid, potassium sorbate and sodium benzoate are added to prevent growth of mold in acid foods such as cheese and soft drinks.

2) Calcium propionate---used in bread

c. SODIUM NITRITE OR NITRATE---added to meat products such as ham, bacon, and hot dogs. It preserves the red color of the meat and prevents germination and growth of botulism endospores.

As nitrites react with amino acids, compounds called nitrosamines are formed. These are carcinogens. Because of this, the amount added to meat has been reduced.

9. ANTIBIOTICS---two which are not used in treating disease are used as food preservatives.

a. NISIN---added to cheese to prevent growth of endospores

b. NATAMYCIN---added to foods to prevent growth of fungi

10 ALDEHYDES---these can act very effectively against microbes. They inactivate proteins. Aldehydes are commonly used as embalming fluid.

a. FORMALDEHYDE

1) FORMALDEHYDE GAS---can be used as a disinfectant

2) FORMALIN, a 37% aqueous solution of formaldehyde gas, has been used to preserve biological specimens and inactivate bacteria and viruses in vaccines.

b. GLUTARALDEHYDE---this is more effective and somewhat less irritating than formaldehyde. It is used to disinfect medical equipment that cannot withstand autoclaving. This is the most commonly used chemical sterilant.

A 2 % solution, such as Cidex, kills bacteria including *Mycobacterium tuberculosis* and viruses in 10 minutes. Endospores require 3 - 10 hours. If the 10 hours are allowed, this is considered to be chemical sterilization. However, instruments must be rinsed before use, so unless they are handled aseptically and rinsed with sterile water, this is more likely to be disinfection.

11. GASEOUS CHEMICAL STERILANTS---these are used in a closed container, which is sometimes called a gas autoclave.

ETHYLENE OXIDE---this is the one most frequently used. It acts by denaturing proteins. It kills all microbes and endospores, but has several disadvantages:

1) Long exposure time (4 - 18 hours)

2) Items must then be aired 12 - 24 hours before use

3) Highly toxic and explosive

In spite of these drawbacks, the product is still used because:

1) Can be used on items that cannot withstand autoclaving

2) Some large hospitals have ethylene oxide chambers where even large items such as mattresses can be sterilized

3) Was used to sterilize spacecraft returning to earth

4) Used to sterilize spacecraft that landed on the moon

12. PLASMA STERILIZATION—used to sterilize medical equipment that cannot be autoclaved. Mostly metal or plastic instrument parts that involve long hollow tubes with a small internal diameter. The procedure uses a container with a vacuum, a strong electromagnetic field, and a chemical. High temps not required but very expensive.

13. PEROXYGENS (oxidizing agents)---these oxidize cellular components of microbes.

a. OZONE (O_3)---highly reactive form of oxygen, often used along with chlorine to disinfect water

b. HYDROGEN PEROXIDE---although frequently used, it is a poor choice as an antiseptic for open wounds, because human cells contain the enzyme catalase, which breaks down the peroxide before it has much chance to act. (This is where the bubbles come from).

It is an effective disinfectant for inanimate objects, where it can even kill endospores. It is used to disinfect food packaging (before the food is put in) and contact lenses.

Although peroxide does not directly kill microbes well in wounds, it is used in deep wounds because it releases oxygen as it breaks down, which makes conditions unfavorable for anaerobic bacteria.

3) FILTRATION

This is the passage of a liquid or gas through a screen like material with pores small enough to retain microbes. This method is used to sterilize items that would be destroyed by heat. Some examples are:

Certain culture media

Enzymes

Vaccines

Antibiotics

HEPA (high-efficiency particulate air filters) trap microbes larger than 0.3 µm in diameter. They are sometimes used in operating rooms and rooms of transplant patients to lower numbers of bacteria.

To filter liquids, filters of unglazed porcelain were originally used. Bacteria were trapped but viruses went through. For this reason, they were called filterable viruses. In recent years, membrane filters have been developed. These are made of cellulose or plastic and the size of the pores can be selected, down to a size that will even retain most viruses.

Different types of filters:

1- Membrane filters : used in production processes are commonly made from materials such as mixester cellulose or polyethersulfone (PES).
2- Sintered glass filter : made from finely ground glass that are fused sufficiently to make small.
3- Air filters : air can be filtered using HEPA filters. They are usually used in biological.

The filtration equipment and the filters themselves may be purchased as pre-sterilized disposable units in sealed packaging, or must be sterilized by the user, generally by autoclaving at a temperature that does not damage the fragile filter membranes. To ensure proper functioning of the filter.

CHAPTER II
BACTERIOLOGY

BACTERIOLOGY

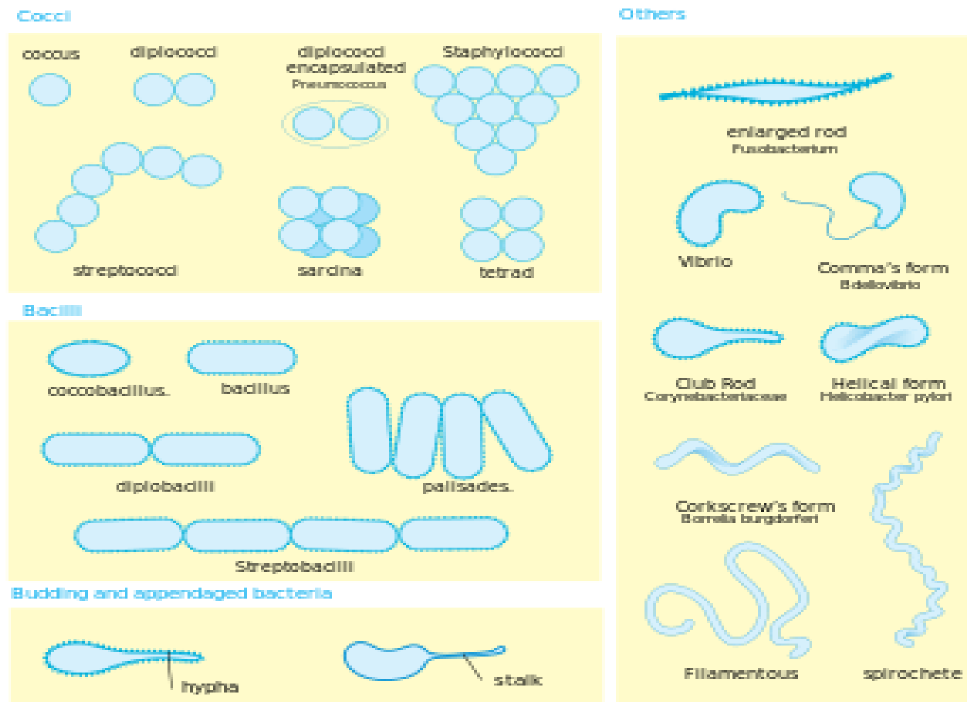
History

Bacteria were first observed by "Antonie van Leeuwenhoek" in 1676, using a single-lens microscope of his own design.[14] He called them "animalcules" and published his observations in a series of letters to the Royal Society. The name *Bacterium* was introduced much later, by Christian Gottfried Ehrenberg in 1828. In fact, *Bacterium* was a genus that contained non-spore-forming rod-shaped bacteria, as opposed to *Bacillus*, a genus of spore-f

The word *bacteria* is the plural of the New Latin *bacterium*, which is the latinisation of the Greek βακτήριον (*baktērion*), meaning "staff, cane", because the first ones to be discovered were rod-shaped.

There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a milliliter of fresh water; in all, there are approximately five nonillion (5×10^{30}) bacteria on Earth, forming a biomass that exceeds that of all plants and animals. Bacteria are vital in recycling nutrients, with many steps in nutrient cycles depending on these organisms, such as the fixation of nitrogen from the atmosphere.

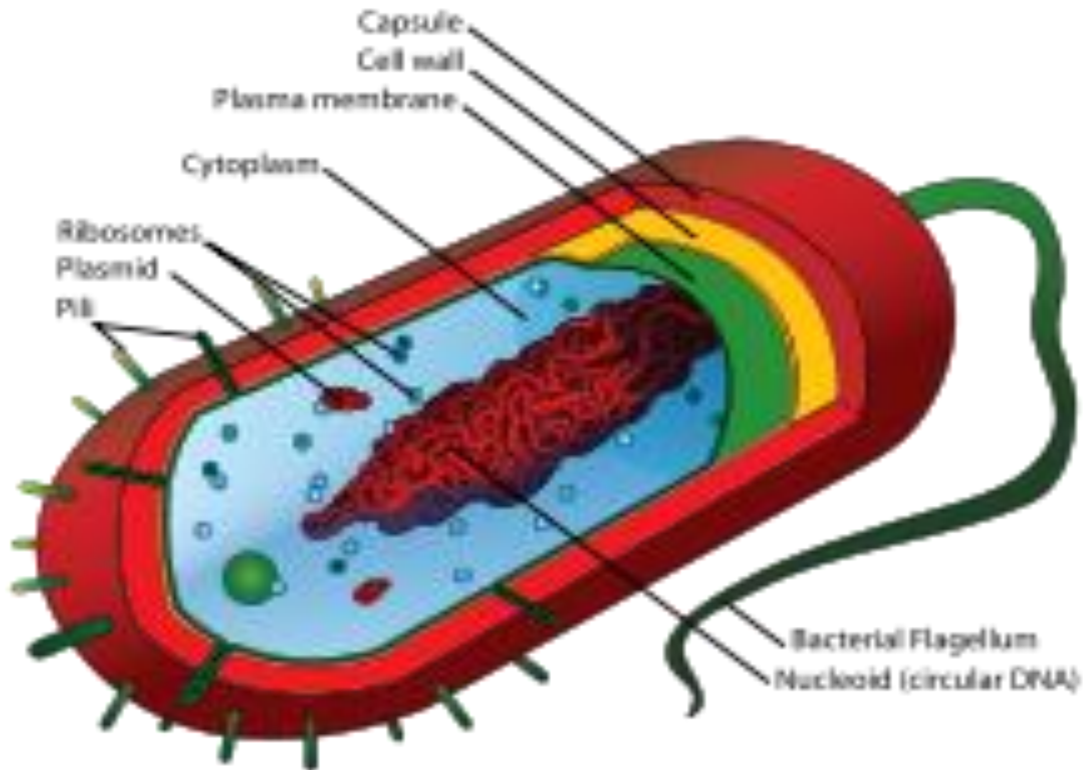
Morphology:



Bacteria display a wide diversity of shapes and sizes, called *morphologies*. Bacterial cells are about one tenth the size of eukaryotic cells and are typically 0.5–5.0 micrometres in length. However, a few species — for example, *Thiomargarita namibiensis* and *Epulopiscium fishelsoni* — are up to half a millimeter.

Most bacterial species are either spherical, called cocci (*sing*. coccus, from Greek *κόκκος-kókkos*, grain, seed), or rod-shaped, called bacilli (*sing*. bacillus, from Latin *baculus*, stick). can be spiral-shaped, called spiral.

Cellular structure:



1)Intracellular structures

- Bacteria do not tend to have membrane-bound organelles in their cytoplasm and thus contain few large intracellular structures.
- Carboxysomes are protein-enclosed bacterial organelles.
- Ggenetic material is typically a single circular chromosome located in the cytoplasm in an irregularly shaped body called the nucleoid.

2) Extracellular structures:

Cell wall

A common bacterial cell wall material is peptidoglycan (called murein in older sources), which is made from polysaccharide chains cross-linked by peptides containing D-amino acids.

There are broadly speaking two different types of cell wall in bacteria, called Gram-positive and Gram-negative. The names originate from the reaction of cells to the Gram stain.

1- Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids.
2- Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins.

Flagella

Flagella are rigid protein structures, about 20 nanometres in diameter and up to 20 micrometres in length, that are used for motility.

Fimbriae

Fimbriae are fine filaments of protein, They are distributed over the surface of the cell, and resemble fine hairs when seen under the electron microscope. Fimbriae are believed to be involved in attachment to solid surfaces or to other cells and are essential for the virulence of some bacterial pathogens.

Nutritional types in bacterial metabolism

Nutritional type	Source of energy	Source of carbon	Examples
Phototrophs	Sunlight	Organic compounds (photoheterotrophs) or carbon fixation (photoautotrophs)	Cyanobacteria, Green sulfur bacteria, Chloroflexi, or Purple bacteria
Lithotrophs	Inorganic compounds	Organic compounds (lithoheterotrophs) or carbon fixation (lithoautotrophs)	Thermodesulfobacteria, *Hydrogenophilaceae*, or Nitrospirae
Organotrophs	Organic compounds	Organic compounds (chemoheterotrophs) or carbon fixation (chemoautotrophs)	*Bacillus*, *Clostridium* or *Enterobacteriaceae*

Endospores:

highly resistant, dormant structures

- Endospore is formed and this is not a reproductive process
- Endospores have a central core of cytoplasm containing DNA and ribosomes surrounded by a cortex layer and protected by an impermeable and rigid coat.

- can survive extreme physical and chemical stresses, such as high levels of UV light, gamma radiation, detergents, disinfectants, heat, freezing and pressure.

Metabolism:

Bacterial metabolism is classified into nutritional groups on the basis of three major criteria:

- The kind of energy used for growth, the source of carbon.
- The electron donors used for growth.
- An additional criterion of respiratory microorganisms are the electron acceptors used for aerobic or anaerobic respiration**,** Carbon metabolism in bacteria is either heterotrophic, where organic carbon compounds are used as carbon sources, or autotrophic, meaning that cellular carbon is obtained by fixing carbon dioxide. Heterotrophic bacteria include parasitic types. Typical autotrophic bacteria are phototrophic cyanobacteria, green sulfur-bacteria and some purple bacteria, but also many chemolithotrophic species, such as nitrifying or sulfur-oxidising bacteria.[96] Energy metabolism of bacteria is either based on phototrophy, the use of light through photosynthesis, or based on chemotrophy, the use of chemical substances for energy, which are mostly oxidised at the expense of oxygen or alternative electron acceptors (aerobic/anaerobic respiration).

Filaments of photosynthetic cyanobacteria

Finally, bacteria are further divided into lithotrophs that use inorganic electron donors and organotrophs that use organic compounds as electron donors. Chemotrophic organisms use the respective electron donors for energy conservation (by aerobic/anaerobic respiration or fermentation) and biosynthetic reactions (e.g. carbon dioxide fixation), whereas phototrophic organisms use them only for biosynthetic purposes. Respiratory organisms use chemical compounds as a source of energy by taking electrons from the reduced substrate and transferring them to a terminal electron acceptor in a redox reaction. This reaction releases energy that can be used to synthesise ATP and drive metabolism. In aerobic organisms, oxygen is used as the electron acceptor. In anaerobic organisms other inorganic compounds, such as nitrate, sulfate or carbon dioxide are used as electron acceptors. This leads to the ecologically important processes of denitrification, sulfate reduction and acetogenesis, respectively.

Another way of life of chemotrophs in the absence of possible electron acceptors is fermentation, where the electrons taken from the reduced substrates are transferred to oxidised intermediates to generate reduced fermentation products (e.g. lactate, ethanol, hydrogen, butyric acid). Fermentation is possible, because the energy content of the substrates is higher than that of the products, which allows the organisms to synthesise ATP and drive their metabolism.[97][98]

These processes are also important in biological responses to pollution; for example, sulfate-reducing bacteria are largely responsible for the production of the highly toxic forms of mercury (methyl- and dimethylmercury) in the environment.[99] Non-respiratory anaerobes use

fermentation to generate energy and reducing power, secreting metabolic by-products (such as ethanol in brewing) as waste. Facultative anaerobes can switch between fermentation and different terminal electron acceptors depending on the environmental conditions in which they find themselves.

Lithotrophic bacteria can use inorganic compounds as a source of energy. Common inorganic electron donors are hydrogen, carbon monoxide, ammonia (leading to nitrification), ferrous iron and other reduced metal ions, and several reduced sulfur compounds. Unusually, the gas methane can be used by methanotrophic bacteria as both a source of electrons and a substrate for carbon anabolism.[100] In both aerobic phototrophy and chemolithotrophy, oxygen is used as a terminal electron acceptor, while under anaerobic conditions inorganic compounds are used instead. Most lithotrophic organisms are autotrophic, whereas organotrophic organisms are heterotrophic.

In addition to fixing carbon dioxide in photosynthesis, some bacteria also fix nitrogen gas (nitrogen fixation) using the enzyme nitrogenase. This environmentally important trait can be found in bacteria of nearly all the metabolic types listed above, but is not universal.[101]

Regardless of the type of metabolic process they employ, the majority of bacteria are only able to take in raw materials in the form of relatively small molecules, which enter the cell by diffusion or through molecular channels in cell membranes. The Planctomycetes are the exception (as they are in possessing membranes around their nuclear material). It has recently been shown that *Gemmata obscuriglobus* is able to take in large molecules via a process that in some ways resembles endocytosis, the process used by eukaryotic cells to engulf external items.

Growth and reproduction:

- Many bacteria reproduce through binary fission.
- bacterial populations can double as quickly as every 9.8 minutes
- Bacterial growth follows three phases**:**
  1- The lag phase, a period of slow growth has high biosynthesis rates.
  2- The logarithmic phase (log phase), also known as the exponential phase. The log phase is marked by rapid exponential growth.
  3- The stationary phase and is caused by depleted nutrients. The cells reduce their metabolic activity and consume non-essential cellular proteins.

Movement:

Many bacteria can move using a variety of mechanisms: flagella are used for swimming through water; bacterial gliding and twitching motility move bacteria across surfaces.

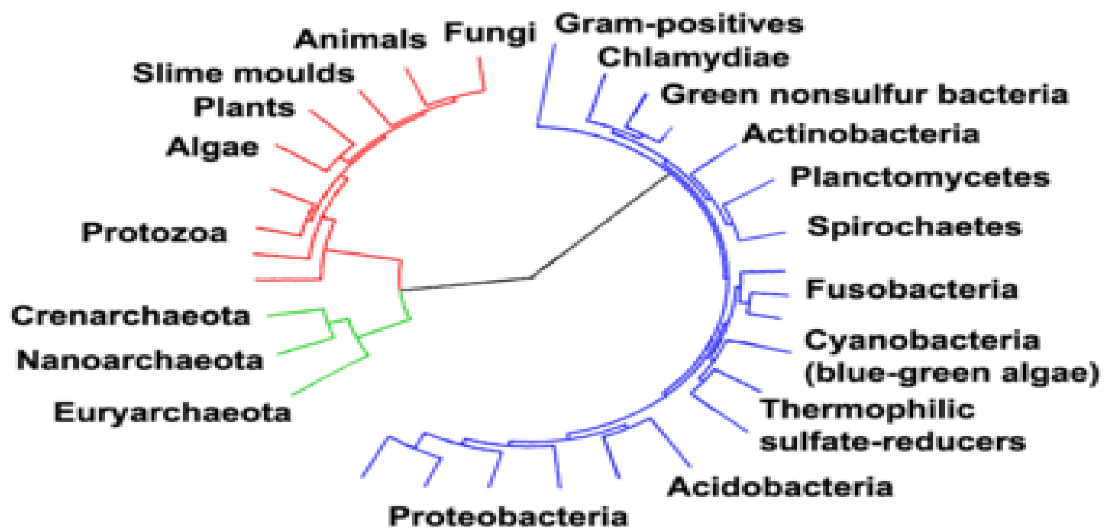
Classification:

Classification seeks to describe the diversity of bacterial species by naming and grouping organisms based on similarities. Bacteria can be classified on the basis of cell structure, cellular metabolism or on differences in cell components such as DNA, fatty acids, pigments, antigens and quinones. While these schemes allowed the identification and classification of bacterial strains, it was unclear. To overcome this uncertainty, modern bacterial classification emphasizes molecular systematic, using genetic techniques**.** The International Committee on Systematic Bacteriology (ICSB) maintains international rules for the naming of bacteria and taxonomic categories and for the ranking of them in the International Code of Nomenclature of Bacteria.

The term "bacteria" was traditionally applied to all microscopic, single-cell prokaryotes. However, molecular systematics showed prokaryotic life to consist of two separate domains, originally called *Eubacteria* and *Archaebacteria*, but now called *Bacteria* and *Archaea* that evolved independently from an ancient common ancestor. The archaea and eukaryotes are more closely related to each other than either is to the bacteria. These two domains, along with Eukarya, are the basis of the three-domain system, which is currently the most widely used classification system in microbiolology. However, due to the relatively recent introduction of molecular systematics and a rapid increase in the number of genome sequences that are available, bacterial classification remains a changing and expanding field.[5][143] For example, a few biologists argue that the Archaea and Eukaryotes evolved from Gram-positive bacteria.

Identification of bacteria in the laboratory is particularly relevant in medicine, where the correct treatment is determined by the bacterial species causing an infection. Consequently, the need to identify human

pathogens was a major impetus for the development of techniques to identify bacteria.



Phylogenetic tree showing the diversity of bacteria, compared to other organisms. Eukaryotes are colored red, archaea green and bacteria blue.

The Gram stain, developed in 1884 by Hans Christian Gram, characterises bacteria based on the structural characteristics of their cell walls. The thick layers of peptidoglycan in the "Gram-positive" cell wall stain purple, while the thin "Gram-negative" cell wall appears pink. By combining morphology and Gram-staining, most bacteria can be classified as belonging to one of four groups (Gram-positive cocci, Gram-positive bacilli, Gram-negative cocci and Gram-negative bacilli). Some organisms are best identified by stains other than the Gram stain, particularly mycobacteria or *Nocardia*, which show acid-fastness on Ziehl–Neelsen or

similar stains. Other organisms may need to be identified by their growth in special media, or by other techniques, such as serology.

Culture techniques are designed to promote the growth and identify particular bacteria, while restricting the growth of the other bacteria in the sample. Often these techniques are designed for specific specimens; for example, a sputum sample will be treated to identify organisms that cause pneumonia, while stool specimens are cultured on selective media to identify organisms that cause diarrhoea, while preventing growth of non-pathogenic bacteria. Specimens that are normally sterile, such as blood, urine or spinal fluid, are cultured under conditions designed to grow all possible organisms. Once a pathogenic organism has been isolated, it can be further characterised by its morphology, growth patterns such as (aerobic or anaerobic growth, patterns of hemolysis) and staining.

As with bacterial classification, identification of bacteria is increasingly using molecular methods. Diagnostics using such DNA-based tools, such as polymerase chain reaction, are increasingly popular due to their specificity and speed, compared to culture-based methods.[148] These methods also allow the detection and identification of "viable but nonculturable" cells that are metabolically active but non-dividing.[149] However, even using these improved methods, the total number of bacterial species is not known and cannot even be estimated with any certainty. Following present classification, there are a little less than 9,300 known species of prokaryotes, which includes bacteria and archaea.[150] but attempts to estimate the true level of bacterial diversity have ranged from 10^7 to 10^9 total species – and even these diverse estimates may be off by many orders of magnitude.

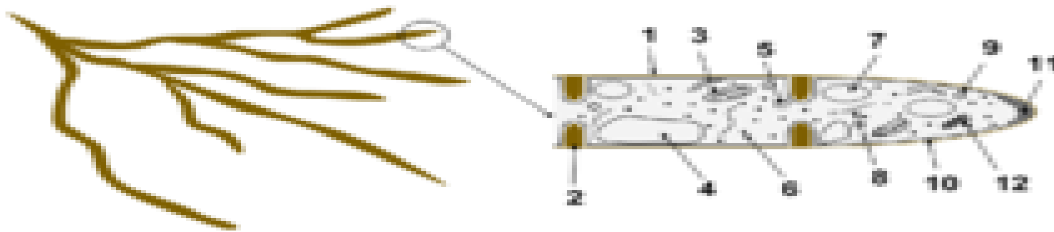
CHAPTER III

1) MYCOLOLOGY

FUNGI

A **fungus** (plural: fungi) in Latin means mushroom is a member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and molds (British English: moulds), as well as the more familiar mushrooms. These organisms are classified as a kingdom, **Fungi**, which is separate from plants, animals, and bacteria. One major difference is that fungal cells have cell walls that contain chitin. The discipline of biology devoted to the study of fungi is known as **mycology**, which is often regarded as a branch of botany, even though genetic studies have shown that fungi are more closely related to animals than to plants.

Characteristics:



FungalHyphaeCells

1- Hyphal wall 2- Septum 3- Mitochondrion 4- Vacuole 5- Ergosterol crystal 6- Ribosome 7- Nucleus 8- Endoplasmic reticulum 9- Lipid body 10- Plasma membrane 11-Golgi apparatus.

Shared features:

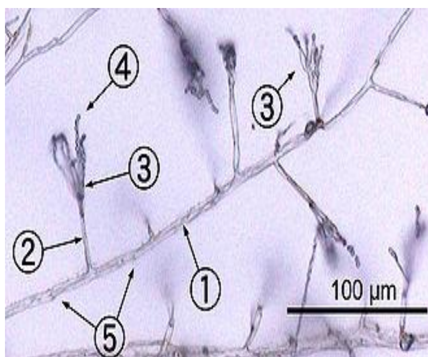
- **With other eukaryotes**: As other eukaryotes, fungal cells contain membrane-bound nuclei with chromosomes that contain DNA.

- **With animals**:, requiring preformed organic compounds as energy sources.
- **With plants:** Fungi possess a cell wall and vacuoles. They reproduce by both sexual and asexual means, and like basal plant groups (such as ferns and mosses) produce spores. Similar to mosses and algae, fungi typically have haploid nuclei.
- **With euglenoids and bacteria:** Higher fungi, euglenoids, and some bacteria produce the amino acid L-lysine in specific biosynthesis steps, called the α-aminoadipate pathway.
- The cells of most fungi grow as tubular, elongated, and thread-like (filamentous) structures and are called hyphae, which may contain multiple nuclei and extend at their tips.

Unique features:

- Some species grow as single-celled yeasts that reproduce by budding or binary fission. Dimorphic fungi can switch between a yeast phase and a hyphal phase in response to environmental conditions.
- The fungal cell wall is composed chitin.

Morphology



An environmental isolate of *Penicillium*

1. hypha 2. conidiophore 3. phialide 4. conidia 5. septa

Most fungi grow as hyphae, which are cylindrical, thread-like structures 2–10 µm in diameter and up to several centimeters in length. The combination of apical growth and branching/forking leads to the development of a mycelium, an interconnected network of hyphae. Hyphae can be either septate or coenocytic: septate hyphae are divided into compartments separated by cross walls.

Nutrition

The fungi are traditionally considered heterotrophs, organisms that rely solely on carbon fixed by other organisms for metabolism.

Fungi are feeding by several relations :

1- Saprophytic (*Aspergillus, Rhizopus, Penicillium*)
2- Parasitic (*Puccinia graminis*)
3- Symbiotic (myccorrhizae, with algae and cyanobacteria Lichens)

Reproduction

1) Asexual reproduction:
   - Fragmentation
   - Fission
   - Budding
   - Spores (conidia)
2) Sexual reproduction:
   - Plasmogamy
   - Karyogamy
   - Meiosis diploid become haploid forming sexual spores (ascospores – basidiospore – zygospores).

Diversity

Fungi have a worldwide distribution, and grow in a wide range of habitats, including air, soil, water, on plants, on humans.

Classification of Fungi by Various Botanists

Article Shared by Neelesh T

Classification of Fungi by I. E. A. Gaumann and B. O. Dodge (1928):

E. A. Gaumann and B. O. Dodge (1928) also did not consider the Myxomycetes as fungi. They classified fungi based on both the phases (i.e., haploid and diploid) of life cycle; structure of thallus and organs of fructifications.

They divided the fungi into four classes are:

1. Archimycetes:

Thallus naked; diploid stage in the life cycle is represented only by the zygote.

2. Phycomycetes:

Thallus with distinct cell wall; diploid stage in the life cycle is represented only by the zygote.

3. Ascomycetes:

Thallus highly developed with distinct cell wall. Dikaryotic mycelium is formed by plasmogamy during sexual reproduction and meiosis takes place in special reproductive organ, the ascus. Ascospores are developed endogenously in the ascus, which remain naked or enclosed in fruit body.

4. Basidiomycetes:

Thallus as in Ascomycetes; Dikaryotic mycelium is formed by plasmogamy; the fruit body is known as basi- diocarp. The basidiospores develop exo- genously on the basidium on/inside the basidiocarp.

The different taxa considered in this system are:

Domain
Kingdom
Phylum
Class
Order
Family
Genus
Species

Further the species are divided into varieties, biological strains, physiological races, etc.

The different taxa considered in this system along with their 'ending' are:

Phylum — mycota

Sub-phylum — mycotina

Class — mycetes

Sub-class — mycetidae

Order — ales

Family — aceae

But the genera and species have no standard ending. In this system, division has been replaced by phylum and all taxa are written in italics.

All the three groups are placed under the Domain Eukaryota. Out of three kingdoms, kingdom Fungi includes only fungi, but the other two kingdoms include non-fungal phyla also.

The earlier consideration of the sub-division Deuteromycotina representing the asexual stages of Ascomycotina and Basidiomycotina (Ainsworth 1973, Hawksworth et al1983) was now

reconsidered as a formal taxon as they are not a monophyletic group. The members are described under Mitosporic fungi.

Phylum Ascomycota:

Important characteristics:

1. Vegetative body is unicellular or commonly well developed, branched septate mycelium with uni- or multinucleate cells having perforated septa.

2. Mostly, the cell wall is composed of chitin and glucans, but in unicellular form, it is composed of glucans and mannans.

3. Vegetative reproduction takes place by fragmentation (in filamentous form), fission and budding (in unicellular form).

4. Asexual reproduction takes place by non-motile spores, such as conidia, oidia and chlamydospores.

5. Sexual reproduction takes place by gametangial copulation (Saccharomyces), gametangial contact (Penicillium), somatogamy (Morchella) or spermatiza- tion (Polystigma).

6. Complete absence of motile structures.

7. The product of sexual reproduction is the ascospores grown inside a small specialised sac-like structure, called ascus.

8. The fruit bodies (inside which ascus developed) are the ascocarps. The asco- carps may be cleistothecium (Penicilliumi), apothecium (Ascobolus), perithecium (Daldenia) or ascostroma (Elsinoe veneta).

Common genera:

Saccharomyces, Penicillium, Daldenia, Ascobolus, Morchella etc.

Phylum Basidiomycota:

Important characteristics:

1. Presence of well-developed, branched and septate mycelium having simple (e.g., Ustlaginales and Uredinales) or dolipore (e.g., Auriculariaceae, aphyllo- phorales and Agaricales) septum (Fig. 4.2B & 4.2D).

2. The mycelial cells contain one nucleus, called monokaryotic i.e., primary mycelium or two nuclei, called dikaryotic i.e., secondary mycelium. The secondary mycelia may organise and form fruit body, called tertiary mycelium.

3. The cell wall is mainly composed of chitin and glucans.

4. Reproduction

(a) Vegetative reproduction takes place by budding and fragmentation.

(b) Asexual reproduction takes place by conidia, oidia or chlamy- dospores. This is lacking in some higher taxa of this subdivision.

(c) Sex organs are absent. During sexual reproduction, the dikaryotic cell is formed by somatogamy, sperma- tisation or by buller phenomenon. The dikaryotic phase persists for long period of time. Karyogamy occurs in basidium mother cell and forms diploid nucleus, which is ephemeral (short lived). 4-haploid basidiospores are formed by meio- sis. Basidiospores are developed exogenously on the horn-shaped structure, the sterigmata (generally 4) on the basidium.

5. Basidia are of two types: Holobasidium (aseptate) e.g., Agaricus, Polyporus etc. (Fig. 4.56A) and Phragmobasidium (septate) e.g., Puccinia, Ustilago (Fig.4.56B) etc.

6. Except in lower forms (Puccinia, Ustilago), secondary mycelia by aggregation form fruit body, the basidiocarp [Agaricus, Polyporus etc.). The number of spores' on each basidium is commonly 4, but 2 or more than 4 are also present.

Common genera:

Agaricus, Polyporus, Puccinia etc.

Class Basidiomycetes Class Teliomycetes Class Ustomycetes

Phylum. Chytridiomycota:

Important characteristics:

1. Vegetative body is coenocytic and thalloid, either globose or ovoid structure, either an elongated simple hypha, or well, developed mycelium.

2. Cell wall is mainly made up of chitin and glucan.

3. Nuclear division is intranuclear and centric type.

4. Members of this group produce motile cells at some stage of their life cycle.

5. Motile cells (zoospores and gametes) possess single posteriorly placed, whiplash type of flagellum except a few polyflagellate cells.

6. Sexual reproduction takes place by planogametes developed in gametangia. The fused gametes form zygote. Zygote on germination develops either into a resting spore or resting sporangium except' a few those develop diploid thallus.

Common genera:

Synchytrium, Monoble- pharis, Rhizophidium etc.

Class Chitridiomycetes.

Phylum Zygomycota:

Important characteristics:

1. The thallus is normally haploid, consisting of coenocytic mycelium and its wall contains chitin and chitosan.

2. The mycelium contains cell organelles like other fungi, except typical golgi bodies and centriole.

3. Asexual reproduction takes place by aplanospores.

4. Sexual reproduction takes place by gametangial copulation results in the formation of zygospore.

Common genera:

Mucor, Rhizopus, Phyco- myces, Cunninghamella etc.

Class Trichomycetes

Class Zygomycetes

Kingdom Straminopila or Chromista (i.e., pseudo- fungi).

Phylum Hyphochytriomycota:

It is a very small group, comprising of about 23 known species.

Important characteristics:

1. The organisms are thalloid, soil-inhabiting or aquatic, chitrid-like.

2. Cell wall contains both chitin and cellulose.

3. Thalli are either holocarpic or eucarpic. The holocarpic thalli are endobiotic and converted into a zoosporangium. In eucarpic forms, the thalli may consist of a single reproductive organ bearing a branched rhizoidal system or may be polycentric with septate and branched hyphae.

4. Motile cells (zoospores) possess single anteriorly placed flagellum, converted with flagellar hairs, which developed inside zoosporangium and are released through discharge tubes.

5. Sexual reproduction has not been demonstrated conclusively in any member. But some evidence suggestive of sexual cycle has been described for Anisolpidium ectocarpi parasitises on Ectocarpus

mitchelIae. Suspected zygote has been reported in some, but meiosis has not been reported.

Common genera:

Rhizidiomyces, Reessia, Hyphochytrium etc.

Phylum Labyrinthulomycota:

(Net Slime Molds)

Important characteristics:

1. Members are found primarily in estuarine (a wide tidal mouth of a river) and near shore habitat; associated with algae, leaves of higher plants and organic debris.

2. Members are mostly saprobic or weak parasites and exhibit nutrition through absorption.

3. Vegetative body is a net slime mold.

4. Presence of an ectoplasmic net-work of anastomosing, branched, wall-less filaments, produced by cells with a specialized cell surface organelle, the sagenogen or bothrosome.

5. Cell walls are composed of scales derived from Golgi.

6. Zoospores are flagellate, heterokont (unequal flagella) type. Flagella are laterally inserted. Larger one is tinsel, directed anteriorly and the shorter one is whiplash, directed posteriorly.

Common genera:

Labyrinthula, Thrausto- chytrium etc.

Phylum Oomycota:

Important characteristics:

1. Members of Oomycetes are found to grow in both fresh water and salt water as well as in terrestrial habitat.

2. They are either unicellular or filamentous, composed of profusely branched and coenocytic hyphae.

3. Septa develop in older region and also at the base of reproductive structures.

4. Cell wall is composed primarily of β- glucans, but also contains hydroxylpro- line, an amino acid; and small amount of cellulose.

5. Cells contain mitochondria with tubular cristae and with various types of biochemical and molecular characteristics.

6. Cell divisions (both mitotic and meiotic) are intranuclear and centric (i.e., the nuclear envelop remains intact until the end of division and centrioles are present at the poles of the dividing nuclei).

7. Unicellular forms are holocarpic, but filamentous forms are eucarpic.

8. Asexual reproduction takes place by means of biflagellate zoospores with shorter whiplash and longer tinsel flagella.

9. Zoospore ultrastructure shows various characteristics.

10. Sexual reproduction is oogamous and takes place by gametangial contact and which produces thick-walled sexual spore, the oospore.

11. Meiosis takes place in the developing gametangia (antheridia and oogonia).

Common genera:

Pythium, Peronospora, Albugo etc.

Kingdom Protozoa (i.e., the slime moulds).

Phylum Plasmodiophoromycota:

(Endoparasitic Slime Molds)

This group is commonly known as endoparasitic slime molds. They are obligate parasites grow on algae, aquatic fungi and higher plants (commonly in the roots).

Important characteristics:

1. Members of this class are obligate parasites (i.e., biotrophic) on fresh water algae, aquatic fungi and higher plants (commonly in the roots).

2. Vegetative body consists of a naked holocarpic plasmodium.

3. Plasmodia are of two types in their life cycle:

Sporangiogenous plasmodium (forms sporangium) and cytogenous-plasmodium (gives rise to cyst i.e., resting spore).

4. Zoospores biflagellate, having equal flagella of whiplash type situated in opposite direction, the shorter one in anterior and longer one in posterior side.

Common genera:

Plasmodiophora, Octo- myxa, Sorodiscus etc.

Phylum Dictyosteliomycota:

(Dictyostelid Cellular Slime Molds)

Important characteristics:

1. These are saprobic slime molds, grow in the middle of organic debris like dung, decaying plants and also in soil.

2. Somatic phase is microscopic and the fructifications are minute, inconspicuous (not easily noticed) and ephemeral (short-lived).

3. The somatic amoebae have filose pseudopodia and a nuclear envelope persisting up to later stage.

4. Somatic amoebae aggregate together to form a pseudoplasmodium.

5. The amoebae never fuse together, but retain their individuality with full cooperation as member of an association till the formation of sorocarp (Gr. sorus, heap; karpos, fruit).

6. The sorocarp is differentiated into two regions: stalk and spores. On germination, spores develop into myxamoeba.

Common genera:

Dictyostelium, Polysphon- dylium etc.

Phylum Acrasiomycota

(Acrasid Cellular Slime Molds)

Members of this group are commonly known as Acrasid Cellular Slime Molds. They are found profusely in the upper layer of humus in deciduous forests and in cultivated lands.

Important characteristics:

1. Somatic phase mainly consists of amoeboid cells or myxamoebae.

2. Myxamoebae aggregate to form a pseudoplasmodium, which develops fruit body.

3. Lack of flagellated cells except in Pocheina rosea.

4. Fruit bodies may be sorocarp (in Dictyostelium) or sporocarp (in Protostelium).

Common genera:

Dictyostelium, Protostelium etc.

Phylum Myxomycota:

They are commonly known as true slime molds or plasmodial slime molds, found in damp places especially on old wood and other decomposing plant parts.

Important characteristics:

1. Somatic body is a free-living plasmodium.

2. They feed on yeast cells, protozoa, fungal spores and other substances.

3. Reproduction takes place by asexual and sexual means.

Asexual reproduction takes place by fragmentation in plasmodium or by binary fission in myxamoebae.

Sexual reproduction takes place by fusion between flagellated zoospores or myxamoeba to form zygote, from which multinucleate plasmodium develops by mitotic divisions. They develop different types of fructification. These are sporangium, aethalium and plasmodiocarp (Fig.4.11 D-G). Meiosis takes place during spore formation in the fructification.

Common genera:

Ceratiomyxa, Physarum etc.

Class Myxomycetes

Class Protosteliomycetes.

CHAPTER III

3) PHYCOLOLOGY

ALGAE

Algae means in Latin "seaweed" are a very large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms. Most are photosynthetic like plants, and "simple" because they lack the many distinct cell and organ types found in The term *algae* is now restricted to eukaryotic organisms. All true algae therefore have a nucleus enclosed within a membrane and plastids bound in one or more membranes.

Accordingly the modern study of marine and freshwater algae is called either phycology.

General Characteristics of Algae:

1- Aquatic algae (Fresh water algae-Marine algae).

2- Terrestrial algae (moist soil).

3- Structure of thallus (Filamentous – Non filamentous).

4- Feeding: Most algae are **photoautotrophic** and carry on photosynthesis. The chlorophyll and other pigments occur in **chloroplasts**.

5- Mobility**:**

a- Most algae are free floating and drift with water currents.

b-Some groups such as green algae have limited mobility, using flagella .

6- Reproduction:

a- Asexual: Fagmentation -Spore formation (zoospores)-Binary fission also takes place (as in bacteria).

b- Sexual: Gametes fuse to form zygot (Isogamy-Anisogamy).

Classification of algae:

Criteria used for classification of algae:

1- Pigments.

The pigments found in algae are categorized in chlorophylls, phycobilins, and carotenoids. Popular carotenoids include astaxanthin, lutein, fucoxanthin, canthaxanthin, zeaxanthin, β-cryptoxanthin and finds application as antioxidant, anti-inflammatory, immunoprophylactic, antitumor activities among others.

Table 1:Pigment composition of several algal groups (after Dring 1982):

DIVISION	COMMON NAME	MAJOR ACCESSORY PIGMENT
Chlorophyta	Green algae	chlorophyll b
Charophyta	Charophytes	chlorophyll b
Euglenophyta	Euglenoids	chlorophyll b
Phaeophyta	Brown algae	chlorophyll c1 + c2, fucoxanthin
Chrysophyta	Yellow-brown or golden-brown algae	chlorophyll c1 + c2, fucoxanthin
Pyrrhophyta	Dinoflagellates	chlorophyll c2, peridinin
Cryptophyta	Cryptomonads	chlorophyll c2, phycobilins
Rhodophyta	Red algae	phycoerythrin, phycocyanin
Cyanophyta	Blue-green algae	phycocyanin, phycoerythrin

2- Storage nutrients

As in land plants, the major carbohydrate storage product of the green algae is usually starch in the form of amylose or amylopectin. These starches are polysaccharides in which the monomer, or fundamental unit, is glucose. Green algal starch comprises more than 1,000 sugar

molecules, joined by alpha linkages between the number 1 and number 4 carbon atoms.

4- **Flagella number**

Most flagellate cells have two flagella, and therefore two basal bodies, each with microtubular roots.

5- **Cell wall structure**

The cell walls of many, but not all, algae contain cellulose. Cellulose is formed from similar glucose molecules but with beta linkages between the number 1 and 4 carbons.

6- **Reproduction**

Algae regenerate by sexual reproduction, involving male and female gametes (sex cells), by asexual reproduction, or by both ways.

- Asexual reproduction

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

- Sexual reproduction

is characterized by the process of meiosis, in which progeny cells receive half of their genetic information from each parent cell. Sexual reproduction is usually regulated by environmental events. In many species, when temperature, salinity, inorganic nutrients (e.g., phosphorus, nitrogen, and magnesium), or day length become unfavourable, sexual reproduction is induced. A sexually reproducing organism typically has two phases in its life cycle. In the first stage,

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

The life cycles of sexually reproducing algae vary; in some, the dominant stage is the sporophyte, in others it is the gametophyte.

In freshwater species especially, the fertilized egg, or zygote, often passes into a dormant state called a zygospore. Zygospores generally have a large store of food reserves and a thick, resistant cell wall. Following an appropriate environmental stimulus, such as a change in light, temperature, or nutrients, the zygospores are induced to germinate and start another period of growth.

According these criteria algae classified into six divisions:

1- Cyanophyta.

2- Chlorophyta.

3- Chrysophyta.

4- Euglenophyta.

5- Phaeophyta.

6- Redohphyta.

Algae important for:

1- Production of oxygen.

2- Biological treatment.

3- Nitrogen fixation (Cyanophyta).

4- Industry (Agar-Agar , Antibiotic , Makeup powder , Tap).

CHAPTER IV
VIROLOGY

VIRUS

Etymology:

A **virus** is a small infectious agent that can replicate only inside the living cells of an organism. Viruses can infect all types of organisms, from animals and plants to bacteria . The word is from the Latin *virus* referring to poison and other noxious substances, first used in English in 1392. *Virulent*, from Latin *virulentus* (poisonous), dates to 1400.

Life properties

Opinions differ on whether viruses are a form of life, or organic structures that interact with living organisms. They have been described as "organisms at the edge of life", since they resemble organisms in that they possess genes and evolve by natural selection, and reproduce by creating multiple copies of themselves through self-assembly. Although they have genes, they do not have a cellular structure, which is often seen as the basic unit of life. Viruses do not have their own metabolism, and require a host cell to make new products. They therefore cannot naturally reproduce outside a host cell.

Structure

Viruses display a wide diversity of shapes and sizes, called *morphologies*. In general, viruses are much smaller than bacteria. Most viruses that have been studied have a diameter between 20 and 300 nanometres. Some filoviruses have a total length of up to 1400 nm; their diameters are only about 80 nm. Most viruses cannot be seen with an optical microscope so

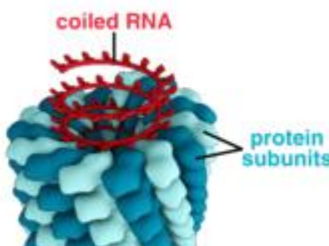
scanning and transmission electron microscopes are used to visualize virions.

A complete virus particle, known as a virion, consists of :

- **nucleic acid** surrounded by a protective coat of protein called a **capsid**. These are formed from identical protein subunits called **capsomeres.**
- Viruses can have **a lipid "envelope"** derived from the host cell membrane.
- **The capsid** is made from proteins encoded by the viral genome and its shape serves as the basis for morphological distinction
- **Nucleoproteins** is proteins associated with nucleic acid .
- **Nucleocapsid** is association of viral capsid proteins with viral nucleic acid.

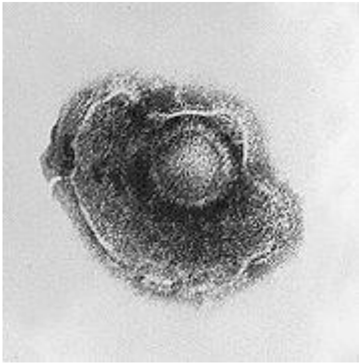
In general, there are four main morphological virus types:

1- **Helix** structure of tobacco mosaic virus: RNA coiled of repeating protein sub-units.



2- **Icosahedral** electron micrograph of adenovirus

3- **Herpes** viruses have a lipid envelope

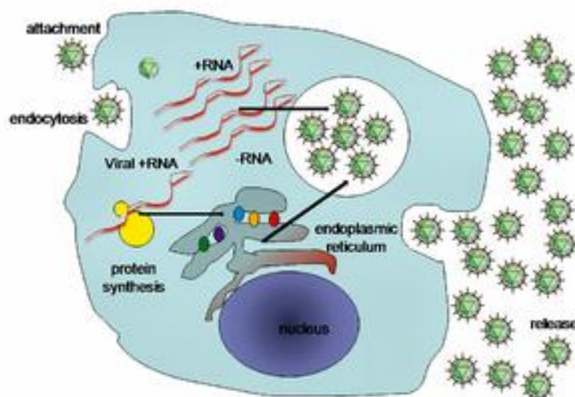


4- Complex see figure

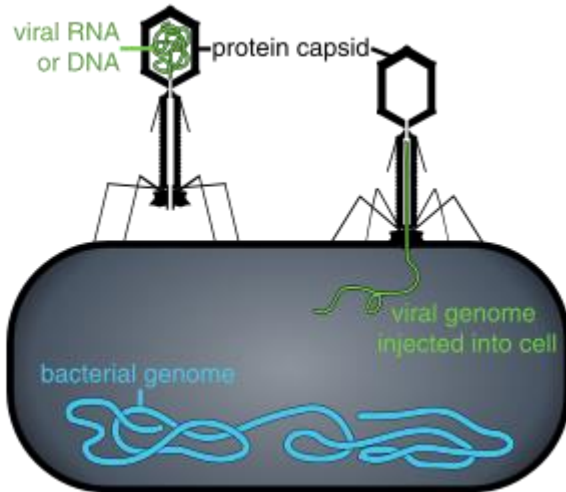
A virus has either DNA or RNA genes and is called a DNA virus or a RNA virus, respectively. The vast majority of viruses have RNA genomes. Plant viruses tend to have single-stranded RNA genomes and bacteriophages tend to have double-stranded DNA genomes.

Replication cycle

Viral populations do not grow through cell division, because they are acellular. Instead, they use the machinery and metabolism of a host cell to produce multiple copies of themselves, and they *assemble* in the cell.



A typical virus replication cycle



Some bacteriophages inject their genomes into bacterial cells (not to scale)

The life cycle of viruses differs greatly between species but there are six *basic* stages in the life cycle of viruses: ?def

1- **Attachment** is a specific binding between viral capsid proteins and specific receptors on the host cellular surface. This specificity determines the host range of a virus.

2- **Penetration** follows attachment: Virions enter the host cell through receptor-mediated endocytosis or membrane fusion. This is often called viral entry.

3- **Uncoating** is a process in which the viral capsid is removed: This may be by degradation by viral enzymes or host enzymes or by simple dissociation; the end-result is the releasing of the viral genomic nucleic acid.

4- **Replication** of viruses involves primarily multiplication of the genome. Replication involves synthesis of viral messenger RNA (mRNA) from "early" genes.

5- **Assembly** of the virus particles, some modification of the proteins often occurs. In viruses such as HIV, this modification (sometimes

called maturation) occurs *after* the virus has been released from the host cell.

6- **Released** viruses can be released from the host cell by lysis, a process that kills the cell by bursting its membrane and cell wall if present: This is a feature of many bacterial and some animal viruses. Some viruses undergo a lysogenic cycle where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosomes.

Host range

different types of viruses can infect only a limited range of hosts and many are species-specific. Other viruses, such as rabies virus, can infect different species of mammals and are said to have a broad range

ICTV classification

The International Committee on Taxonomy of Viruses (ICTV) developed the current classification system and wrote guidelines that put a greater weight on certain virus properties to maintain family uniformity.

The general taxonomic structure is as follows:

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

In the current (2011) ICTV taxonomy, six orders have been established, the Caudovirales, Herpesvirales, Mononegavirales, Nidovirales, Picornavirales

and Tymovirales. A seventh order Ligamenvirales has also been proposed. The committee does not formally distinguish between subspecies, strains, and isolates. In total there are 6 orders, 87 families, 19 subfamilies, 349 genera, about 2,284 species and over 3,000 types yet unclassified.

Species names often take the form of *[Disease] virus*, particularly for higher plants and animals.

The establishment of an order is based on the inference that the virus families it contains have most likely evolved from a common ancestor. The majority of virus families remain unplaced. As of 2012, seven orders, 96 families, 22 subfamilies, 420 genera, and 2,618 species of viruses have been defined by the ICTV.[5][6] The orders are the *Caudovirales, Herpesvirales, Ligamenvirales, Mononegavirales, Nidovirales, Picornavirales,* and *Tymovirales*. These orders span viruses with varying host ranges. The *Ligamenvirales*, infecting archaea, are the most recent addition to the classification system.

Caudovirales are tailed dsDNA (group I) bacteriophages.

Herpesvirales contain large eukaryotic dsDNA viruses.

Ligamenvirales contains linear, dsDNA (group I) archaean viruses.

Mononegavirales include nonsegmented (-) strand ssRNA (Group V) plant and animal viruses.

Nidovirales are composed of (+) strand ssRNA (Group IV) viruses with vertebrate hosts.

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

Tymovirales contain monopartite (+) ssRNA viruses that infect plants.

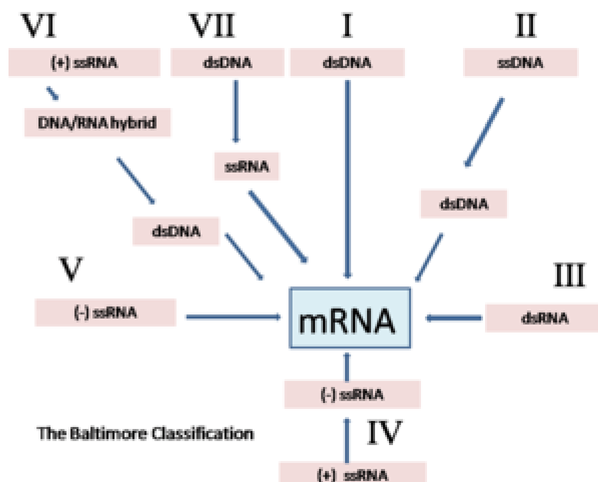
Other variations occur between the orders: *Nidovirales*, for example, are isolated for their differentiation in expressing structural and nonstructural proteins separately.

Structure-based virus classification

It has been suggested that similarity in virion assembly and structure observed for certain viral groups infecting hosts from different domains of life (e.g., bacterial tectiviruses and eukaryotic adenoviruses or prokaryotic Caudovirales and eukaryotic herpesviruses) reflects an evolutionary relationship between these viruses. Therefore, structural relationship between viruses has been suggested to be used as a basis for defining higher-level taxa - structure-based viral lineages - that could complement the existing ICTV classification scheme.

Baltimore classification

Main articles: Baltimore classification and Virus Information Table



The Baltimore Classification

The Baltimore Classification of viruses is based on the method of viral mRNA synthesis

Baltimore classification (first defined in 1971) is a classification system that places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), Sense, and method of replication. Named after David Baltimore, a Nobel Prize-winning biologist, these groups are designated by Roman numerals. Other classifications are determined by the disease caused by the virus or its morphology, neither of which are satisfactory due to different viruses either causing the same disease or looking very similar. In addition, viral structures are often difficult to determine under the microscope. Classifying viruses according to their genome means that those in a given category will all behave in a similar fashion, offering some indication of how to proceed with further research. Viruses can be placed in one of the seven following groups:[10]

- I: **dsDNA viruses** (e.g. Adenoviruses, Herpesviruses, Poxviruses)
- II: **ssDNA viruses** (+ strand or "sense") DNA (e.g. Parvoviruses)
- III: **dsRNA viruses** (e.g. Reoviruses)
- IV: **(+)ssRNA viruses** (+ strand or sense) RNA (e.g. Picornaviruses, Togaviruses)
- V: **(−)ssRNA viruses** (− strand or antisense) RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
- VI: **ssRNA-RT viruses** (+ strand or sense) RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
- VII: **dsDNA-RT viruses** DNA with RNA intermediate in life-cycle (e.g. Hepadnaviruses)

Visualization of the 7 groups of virus according to the Baltimore Classification

DNA viruses

For more details on this topic, see DNA virus.

- **Group I**: viruses possess double-stranded DNA. Viruses that cause chickenpox and herpes are found here.
- **Group II**: viruses possess single-stranded DNA.

Virus family	Examples (common names)	Virion naked/enveloped	Capsid symmetry	Nucleic acid type	Group
1. Adenoviridae	Adenovirus, infectious canine hepatitis virus	Naked	Icosahedral	ds	I
2. Papovaviridae	Papillomavirus, polyomaviridae, simian vacuolating virus	Naked	Icosahedral	ds circular	I
3. Parvoviridae	Parvovirus B19, canine parvovirus	Naked	Icosahedral	ss	II
4. Herpesviridae	Herpes simplex virus, varicella-	Enveloped	Icosahedral	ds	I

	zoster virus, cytomegalovirus, Epstein–Barr virus				
5. Poxviridae	Smallpox virus, cow pox virus, sheep pox virus, orf virus, monkey pox virus, vaccinia virus	Complex coats	Complex	ds	I
6. Hepadnaviridae	Hepatitis B virus	Enveloped	Icosahedral	circular, partially ds	VII
7. Anelloviridae	Torque teno virus	Naked	Icosahedral	ss circular	II

RNA viruses

For more details on this topic, see RNA virus.

- **Group III**: viruses possess double-stranded RNA genomes, e.g. rotavirus.
- **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.

Virus Family	Examples (common names)	Capsid naked/enveloped	Capsid Symmetry	Nucleic acid type	Group
1. Reoviridae	Reovirus, rotavirus	Naked	Icosahedral	ds	III
2. Picornaviridae	Enterovirus, rhinovirus, hepatovirus, cardiovirus, aphthovirus, poliovirus, parechovirus, erbovirus, kobuvirus, teschovirus, coxsackie	Naked	Icosahedral	ss	IV
3. Caliciviridae	Norwalk virus	Naked	Icosahedral	ss	IV
4. Togaviridae	Rubella virus, alphavirus	Enveloped	Icosahedral	ss	IV
5. Arenaviridae	Lymphocytic choriomeningitis virus	Enveloped	Complex	ss(-)	V
6. Flaviviridae	Dengue virus, hepatitis C virus, yellow fever virus	Enveloped	Icosahedral	ss	IV
7. Orthomyxoviridae	Influenzavirus A, influenzavirus B, influenzavirus C, isavirus, thogotovirus	Enveloped	Helical	ss(-)	V
8. Paramyxoviridae	Measles virus, mumps virus,	Enveloped	Helical	ss(-)	V

	respiratory syncytial virus, Rinderpest virus, canine distemper virus				
9. Bunyaviridae	California encephalitis virus, hantavirus	Enveloped	Helical	ss(-)	V
10. Rhabdoviridae	Rabies virus	Enveloped	Helical	ss(-)	V
11. Filoviridae	Ebola virus, Marburg virus	Enveloped	Helical	ss(-)	V
12. Coronaviridae	Corona virus	Enveloped	Helical	ss	IV
13. Astroviridae	Astrovirus	Naked	Icosahedral	ss	IV
14. Bornaviridae	Borna disease virus	Enveloped	Helical	ss(-)	V
15. Arteriviridae	Arterivirus, equine arteritis virus	Enveloped	Icosahedral	ss	IV
16. Hepeviridae	Hepatitis E virus	Naked	Icosahedral	ss	IV
17. Retroviridae	HIV	Enveloped			

Reverse transcribing viruse

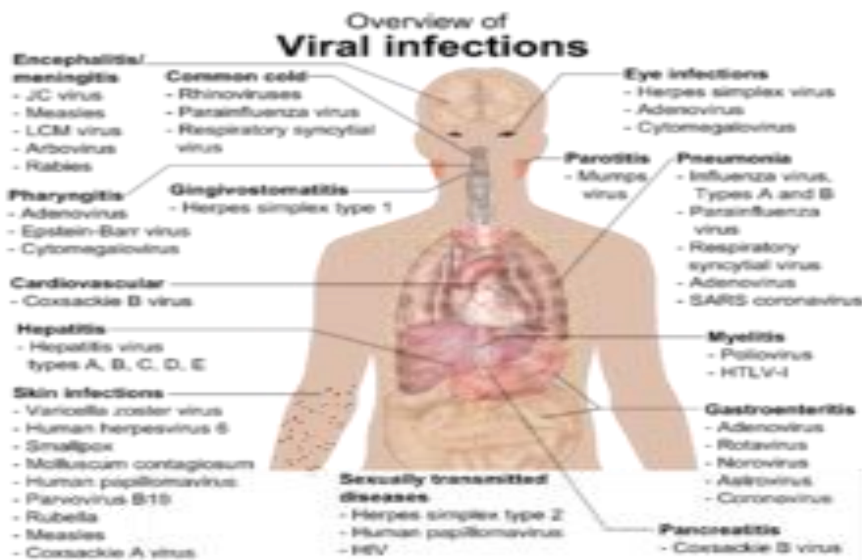
- **Group VI**: viruses possess single-stranded RNA viruses that replicate through a DNA intermediate. The retroviruses are included in this group, of which HIV is a member.
- **Group VII**: viruses possess double-stranded DNA genomes and replicate using reverse transcriptase. The hepatitis B virus can be found in this group.

Holmes classification

Holmes (1948) used Carl Linnaeus's system of binomial nomenclature to classify viruses into 3 groups under one order, Virales. They are placed as follows:

- **Group I:** *Phaginae* (attacks bacteria)
- **Group II:** *Phytophaginae* (attacks plants)
- **Group III:** *Zoophaginae* (attacks animals)

Role in human disease



Examples of common human diseases caused by viruses include the common cold, influenza, chickenpox, and cold sores. Many serious diseases such as ebola, AIDS, avian influenza, and SARS are caused by viruses. Viruses are an established cause of cancer in humans and other species. Viral cancers occur only in a minority of infected persons (or animals). Cancer viruses come from a range of virus families, including both RNA and DNA viruses,

Host defense mechanisms

The body's first line of defense against viruses is the innate immune system. This comprises cells and other mechanisms that defend the host

from infection in a non-specific manner. This means that the cells of the innate system recognize, and respond to, pathogens in a generic way, but, unlike the adaptive immune system, it does not confer long-lasting or protective immunity to the host.

When the adaptive immune system of a vertebrate encounters a virus, it produces specific antibodies that bind to the virus and often render it non-infectious. This is called humoral immunity. Two types of antibodies are important. The first, called IgM, is highly effective at neutralizing viruses but is produced by the cells of the immune system only for a few weeks. The second, called IgG, is produced indefinitely. The presence of IgM in the blood of the host is used to test for acute infection, whereas IgG indicates an infection sometime in the past. IgG antibody is measured when tests for immunity are carried out.

A second defense of vertebrates against viruses is called cell-mediated immunity and involves immune cells known as T cells. The body's cells constantly display short fragments of their proteins on the cell's surface, and, if a T cell recognizes a suspicious viral fragment there, the host cell is destroyed by *killer T* cells and the virus-specific T-cells proliferate.

Prevention and treatment

1- Vaccines

Vaccination is a cheap and effective way of preventing infections by viruses. Vaccines were used to prevent viral infections long before the discovery of the actual viruses. Vaccines are available to prevent over thirteen viral infections of humans, and more are used to prevent viral infections of animals. Vaccines can consist of live-attenuated or killed viruses, or viral proteins (antigens). Live vaccines contain weakened forms

of the virus, which do not cause the disease but, nonetheless, confer immunity. Such viruses are called attenuated. Live vaccines can be dangerous when given to people with a weak immunity, because in these people, the weakened virus can cause the original disease.

2- Antiviral drugs

Aciclovir for Herpes simplex virus infections and lamivudine for HIV and Hepatitis B virus infections.

Epidemiology

Viral epidemiology is the branch of medical science that deals with the transmission and control of virus infections in humans. Transmission of viruses can be vertical, which means from mother to child, or horizontal, which means from person to person. Examples of vertical transmission include hepatitis B virus and HIV, where the baby is born already infected with the virus. Another, more rare, example is the varicella zoster virus, which, although causing relatively mild infections in humans, can be fatal to the foetus and newborn baby.

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GENERAL MICROBIOLOGY PRACTICAL

Prepared by

Dr. Amany Atta El- Shahir

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Safety Procedures for the Microbiology Laboratory

General Laboratory Safety Practices and Procedures

1. If you are **taking immune-suppressants, are pregnant,** or have a known medical condition that would prevent full participation in the laboratory, please contact the course instructor before the first day of lab.
2. Read and understand each laboratory exercise **before** you come to class.
3. Do not eat, drink, smoke, or chew pens in the laboratory.
4. You must wear close-toed shoes while in the laboratory and long pants.
5. No hats of any kind will be allowed in lab, unless allowed by University policy and cleared with the instructor.
6. Long hair should be pulled back to keep it away from bacterial cultures, bacticinerator or open flames.
7. Follow precautionary statements given in each exercise.
8. Personal electronic devices will be turned off and stored while in this laboratory. *The unauthorized use of any electronic device (phone, tablet, computer) in lab will result in a loss of course points.
9. Know where specific safety equipment is located in the laboratory, such as the fire extinguisher, safety shower, and the eyewash station.
10. Recognize the international symbol for biohazards, and know where and how to dispose of all waste materials, particularly biohazard waste. Note that all biohazard waste must be sterilized by autoclave before it can be included in the waste stream.

Figure 1: Biohazard Symbol

11. Keep everything other than the cultures and tools you need OFF the lab bench. Only necessary work material should be at or on the laboratory bench. Coats, backpacks, and other personal belongings will not be allowed on the laboratory bench top. Store them in a place designated by your instructor. This is to prevent cluttering of the workspace and to avoid exposing them to permanent stains, caustic chemicals, and microorganisms used in the exercises.
12. Leave all laboratory facilities and equipment in good order at the end of each class. Before leaving the laboratory, check to make sure the bacticinerator heat sterilizer is turned off.

13. Never, under any circumstances, remove equipment, media, or microbial cultures from the laboratory.
14. No pets are allowed in the laboratory.

Microbiology Specific Laboratory Safety Practices

During the course of the semester in the laboratory you will be taught the methods used in the proper handling of microorganisms. Although you will not be working with any that are human pathogens, exercise caution in handling all material coming in contact with live microbial cultures. All cultures should be handled with respect and proper aseptic technique *as if they were potential pathogens.* This is called **"universal precaution"**. Specific instructions that should be followed:

1. Remember that all bacteria are potential pathogens that may cause harm under unexpected or unusual circumstances. If you as a student have a compromised immune system or a recent extended illness, you should share those personal circumstances with your lab instructor.
2. Wear gloves when working with cultures, and when your work is completed, dispose of the gloves in the biohazard garbage. Lab coats, safety glasses or goggles are also required. These will be stored in the laboratory each week in a ziplock bag.
3. Disinfect your work area both BEFORE and AFTER working with bacterial cultures.
4. Cultures of live microorganisms and any material coming in contact with live cultures must be properly sterilized after use in the laboratory. Your instructor will inform you of specific procedures. Follow the general rules outlined below.

   a. Glassware such as test tubes, bottles, and flasks may be reused and washed after sterilization. These are normally placed on a cart at the front of the laboratory after you have finished an experiment or exercise. BE SURE TO REMOVE LABELS before placing any glassware on the cart. Your instructor will sterilize and then wash these items.

   b. Some materials, such as plastic petri dishes, plastic pipettes, microscope slides, and swabs, are considered disposable. These are used once and if they become contaminated by contact with live microorganisms are sterilized and discarded. All of these disposable contaminated materials should be placed in the designated waste container containing a BIOHAZARD autoclave bag.

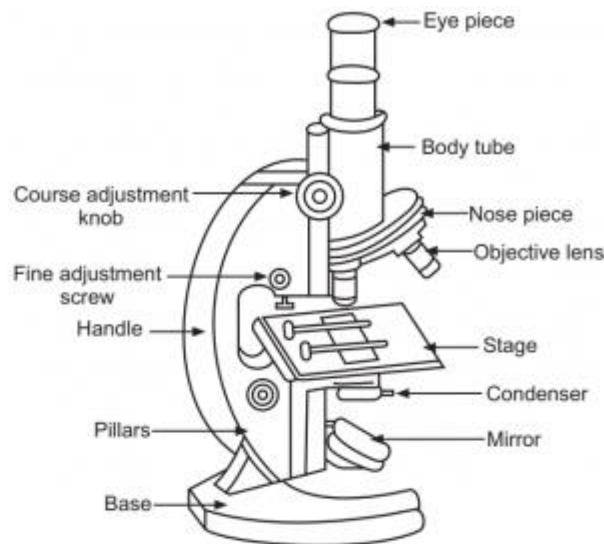
5. Never place contaminated pipette tips (or pipettes), inoculating loop, or any other contaminated material on the bench top. Sterilize loops before and after each use. Place contaminated pipette tips in the orange biohazard buckets on

your bench. Place all other contaminated materials in their designated waste containers. Do not place or put anything containing live microorganisms in the sink.

6. Aerosols should be avoided by the use of proper technique for sterilizing the inoculating loops and by performing any mixing of cultures and reagents in such a way as to avoid splashing.
7. Cultures or reagents should always be transferred with an automatic pipettor that will be provided. In no case should one employ mouth pipetting.
8. Always keep cultures capped and in proper storage racks when not being used during an exercise.
9. In the event of an accidental spill involving a bacterial culture, completely saturate the spill area with disinfectant, then cover with paper towels and allow the spill to sit for 10 minutes. Then carefully remove the saturated paper towels, dispose of them in the biohazard waste, and clean the area again with disinfectant. Notify your instructor about the spill. If the chemical is marked "danger" or "caustic" you should notify the instructor who will handle this type of spill.
10. Immediately report all accidents such as spills, cuts, burns, or other injuries to the instructor
11. Make sure that lab benches are completely cleared (everything either thrown away or returned to storage area) before you leave the lab.
12. Clothing worn in the microbiology laboratory should be washed before being subsequently worn in a facility such as a hospital, clinic or nursing home, or in an area of public food preparation.
13. In the event of a fire alarm, follow the directions of your instructor, and meet at the place designated by your instructor.

Parts of a Compound Microscope

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



Compound Microscope Definitions for Labels

- **Eyepiece (ocular lens) with or without Pointer**: The part that is looked through at the top of the compound microscope. Eyepieces typically have a magnification between 5x & 30x.
- **Monocular or Binocular Head**: Structural support that holds & connects the eyepieces to the objective lenses.
- **Arm**: Supports the microscope head and attaches it to the base.
- **Nosepiece**: Holds the objective lenses & attaches them to the microscope head. This part rotates to change which objective lens is active.
- **Base**: Bottom base of the microscope that houses the illumination & supports the compound microscope.
- **Objective lenses**: There are usually 3-5 optical lens objectives on a compound microscope each with different magnification levels. 4x, 10x, 40x, and 100x are the most common magnifying powers used for the objectives. The total magnification of a compound microscope is calculated by multiplying the objective lens magnification by the eyepiece magnification level. So, a compound microscope with a 10x eyepiece magnification looking through the 40x objective lens has a total magnification of 400x (10 x 40).

- **Specimen or slide**: The object used to hold the specimen in place along with slide covers for viewing. Most slides & slide covers are thin glass rectangles.
- **Stage or Platform**: The platform upon which the specimen or slide are placed. The height of the mechanical stage is adjustable on most compound microscopes.
- **Stage clips or mechanical stage**: Clips on the stage that hold the slide in place on the mechanical stage.
- **Aperture - Disc or Iris Diaphragm**: Circular opening in the stage where the illumination from the base of the compound microscope reaches the platform of the stage.
- **Abbe Condenser**: This lens condenses the light from the base illumination and focuses it onto the stage. This piece of the compound microscope sits below the stage & typically acts as a structural support that connects the stage to arm or frame of the microscope.
- **Coarse and fine adjustment controls**: Adjusts the focus of the microscope. These knobs increase or decrease the level of detail seen when looking at the slide or specimen through the eyepiece of the compound microscope.
- **Stage height adjustment**: Adjusts the position of the mechanical stage vertically & horizontally. It is important to adjust these knobs so that the objective lens is never coming into contact with the slide or specimen on the stage.
- **Mirror**: Reflects light into the base of the microscope. Earlier microscopes used mirrors that reflected light into the base of the microscope instead of halogen bulbs as their source of illumination.
- **Illumination**: Light used to illuminate the slide or specimen from the base of the microscope. Low voltage halogen bulbs are the most commonly used source of illumination for compound microscopes.
- **Bottom Lens or Field Diaphragm**: Knob used to adjust the amount of light that reaches the specimen or slide from the base illumination.

Sterilization

STERILIZATION is a term referring to any process that eliminates

(removes) or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. Sterilization can be achieved by applying the proper combinations of heat, chemicals, irradiation, high pressure, and filtration.

COMMERCIAL STERILIZATION—term used for canned food—it must be heated enough to kill endospores of *Clostridium botulinum*. More resistant endospores may survive.

DISINFECTION—destruction of vegetative pathogens. Most often this term refers to application of a chemical called a disinfectant to an inert surface (floors, countertops, etc.), but ultraviolet radiation and boiling are other methods used. Endospores are not necessarily killed.

ANTISEPSIS—chemical called an antiseptic is applied to living tissue. Some microbes are killed, but not all.

DEGERMING—mechanical removal of microbes from a limited area, such as wiping an injection site with alcohol.

SANITIZATION—high-temperature washing or washing and then dipping into a chemical disinfectant. This is used to lower microbial counts on eating or drinking utensils.

Treatments that kill microbes end in ---cide.

Germicide—kills microbes but possibly not endospores

Fungicide—kills fungi

Virucide—kills viruses

Bacteriocide—kills bacteria

Treatments ending in ----static stop growth (no increase in numbers) but do not kill.

Bacteriostatic

Microbistatic

Sepsis—indicates bacterial contamination.

Asepsis—absence of significant contamination.

FACTORS THAT INFLUENCE THE EFFECTIVENESS OF STERILIZATION:

1. Number of microbes the more there are to begin with, the longer it takes to eliminate them all.

2. Environmental factors:

a. Organic matter (blood, feces, etc.) often interferes with chemical antimicrobials, and also to a lesser extent with heat treatment. Any medium containing fats or proteins tends to protect bacteria.

b. Disinfectants work better in warmer temperatures

c. Biofilms protect microbes

d. Presence of fats & proteins protect microbes

e. pH—heat is more effective in an acid pH.

3. Time of exposure

a. The lower the temperature used for heat treatment, the longer the time required.

b. Effects of irradiation are also related to time.

c. Chemical antimicrobials require a certain amount of exposure time; extended exposure to kill resistant bacteria or endospores.

4. Microbial characteristics susceptibility to different agents varies among microbes. Also, endospores are much more difficult to eliminate.

ACTIONS OF MICROBIAL CONTROL AGENTS

1. ALTERATION OF MEMBRANE PERMEABILITY—the plasma membrane regulates the entry of materials into and the exit of wastes out

of the cell. Damage to the plasma membrane causes leakage of cell contents into the surroundings, killing the cell or at least preventing cell division. Remember, death in microbes is loss of the ability to reproduce.

2. DAMAGE TO PROTEINS—enzymes and other proteins are essential for cell function.

a. Hydrogen bonds hold proteins in the characteristic 3-dimensional shape required for their functions. Heat and certain chemicals break these bonds and the shape is lost. This is called denaturation.

b. Covalent bonds, which are also part of protein structure, may be broken by chemicals or heat even though they are stronger than hydrogen bonds.

3. DAMAGE TO NUCLEIC ACIDS—DNA and RNA carry the cell's genetic information and function in protein synthesis. Damage to these by heat, radiation, or chemicals usually kills the cell.

METHODS OF STERILIZATION

i. Physical methods.
ii. Chemical methods.
iii. Filtration.

1)Physical methods

Drying and salting were the earliest methods of food preservation, so these were also the earliest means of controlling bacterial growth. Physical means of control include:

Heat   Osmotic pressure   Radiation

I- HEAT

Canned foods are an example of control of microbial growth by heating. In the medical world, lab media, glassware, and

surgical instruments are examples of things usually sterilized by heat. Heat resistance varies among microbes.

Thermal death point is the lowest temperature at which all microbes in a liquid culture will be killed in 10 minutes.

Thermal death time is the minimum length of time in which all bacteria in a liquid culture will be killed at a given temperature.

A . MOIST HEAT—all moist heat methods kill microbes by coagulating or denaturing their proteins, including enzymes. This occurs faster in the presence of water, so moist heat requires lower temperatures and less time of exposure than dry heat.

a. BOILING (100° C)—kills all vegetative bacterial pathogens, almost all viruses, and fungi and their spores in 10 minutes or less (often much less). Endospores and a few viruses are much more resistant to boiling.

Hepatitis A virus—30 minutes

Endospores—up to 20 hours

Although boiling will kill most pathogens, it is not a dependable means of sterilization.

b. AUTOCLAVE—this device uses steam under pressure for effective sterilization. Items to be sterilized are placed in a chamber, which is then sealed. All air is exhausted and steam under pressure is injected. This achieves higher temperatures than boiling. It is the preferred means of sterilization for all materials that can withstand it.

The most common setting uses steam under 1.5 atm.pressure and reaches a temperature of 121° C. This kills all organisms and their endospores in about 15 - 20 minutes.

(Different autoclave shapes)

Here we use an autoclave to sterilize culture media. In hospitals, doctors' offices, dentists' offices, etc. this methods is used to sterilize medical equipment.

Materials being autoclaved are often wrapped in paper, so that after sterilization the outside of the package can be handled without contaminating the sterile item inside.

To ensure the autoclaving process was able to cause sterilization, most autoclaves have meters and charts that record or display pertinent information such as temperature and pressure as a function of time. Indicator tape is often placed on packages of products prior to autoclaving. A chemical in the tape will change color when the appropriate conditions have been met. Some types of packaging have built-in indicators on them.

The size of the container, the volume of a liquid, and the type of wrapping can influence the time and temperature required for sterilization.

c. PASTEURIZATION—Pasteur used mild heat to kill microbes in beer and wine ingredients before fermentation. The same general idea is now used for milk. Pasteurization kills pathogenic bacteria and viruses, although some harmless bacteria do survive. (Heat sufficient to kill all microbes changes the character of the milk.) Bacteria that are left eventually cause the milk to spoil, but the life is greatly prolonged.

Originally, milk was heated to 63° C for 30 minutes. Today, new methods use 72° C for 15 seconds (high-temperature short-time pasteurization). Milk can be sterilized so that it can be sealed in a carton and stored without refrigeration. This requires a temperature of 140° C for about 3 seconds (ultra-high temperature treatment). This process gives the milk an “off” taste.

B. DRY HEAT—this kills by burning to ashes or by oxidation

a. FLAMING—we use this on loops in the lab. Leaving the loop in the flame of a Bunsen burner or alcohol lamp until it glows red ensures that any infectious agent gets inactivated. This is commonly used for small metal or glass objects.

A variation on flaming is to dip the object in 70% ethanol (or a higher concentration) and merely touch the object briefly to the Bunsen burner flame, but not hold it in the gas flame. The ethanol will ignite and burn off in a few seconds. 70% ethanol kills many, but not all, bacteria and viruses, and has the advantage that it leaves less residue than a gas flame. This method works well for the glass "hockey stick"-shaped bacteria spreaders.

b. INCINERATION—burning contaminated paper disposables in a controlled chamber.

c. HOT-AIR STERILIZATION—items are placed in an oven. Typical procedure would be 170° C for 4-6 hours. It takes much longer for dry heat to kill than moist heat. This method sterilizes by oxidation. It is mainly used for items that the autoclave is not suitable for.

(Hot air ovens)

This may be due to size or quantity--lots of lab glassware might be sterilized in a hot-air oven.

8- **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 typically 20 minutes at atmospheric pressure, cooling, incubating for a day, boiling, cooling, incubating for a day, 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 step. This is effective because many spores are stimulated to grow by the heat shock. The procedure only works for media that can support bacterial growth - it will not sterilize plain water. Tyndallization is ineffective against prions.

II- RADIATION:

1. IONIZING RADIATION—this includes X rays, gamma rays, and high-energy electron beams. These all have very short wavelengths and

high levels of energy. They cause ionization of water within cells, which results in formation of hydroxyl radicals. These destroy cell components, especially DNA. This process is used to sterilize wrapped plastic disposables such as syringes, catheters, gloves, suture materials, vials of injectables, disposable Petri dishes, pipettes, etc. It is also used to sterilize spices. Recently, approval has been granted for use of low level radiation of fruits and meats. The post office is now using this method to sterilize some mail.

This process does involve the use of dangerous radiation and can only be used in a properly shielded room, so it is mostly used in factories where wide scale use of the setup makes it economical.

2. NONIONIZING RADIATION—this has a longer wavelength and less energy. Ultraviolet (UV) light is the common example. It causes the formation of thymine dimers, which interferes with DNA replication and formation of mRNA.

UV lamps are used in hospitals and in food service. This method does not sterilize, but it does reduce bacterial growth. Penetrating power is very low, so any type of covering protects microbes.

Sunlight has some weak antimicrobial effects, but the wavelengths of sunlight are too long to work well.

3. MICROWAVES—little effect on microbes but the heat may kill them.

2) Chemical methods:

Most chemical agents are disinfectants or antiseptics, although there are a few chemical sterility.

"Disinfectants' are those chemicals that destroy pathogenic bacteria from inanimate surfaces.

Those chemicals that can be safely applied over skin and mucus membranes are called ' Antiseptics'.

An ideal antiseptic or disinfectant should have the following properties

1- Should have wide spectrum of activity.
2- Should be able to destroy microbes within practical period of time.
3- Should be active in the presence of organic matter.
4- Should be active in any pH.
5- Should be stable.
6- Should be speedy.
7- Should be non toxic, non allergenic.
8- Should not have bad odor.

TYPES OF DISINFECTANTS

1. PHENOLS and PHENOLICS

a. PHENOL (carbolic acid) was used by Lister to reduce the incidence of surgical infections. It is irritating to skin and mucous membranes and has a bad odor, so it is rarely used today. Its main use now is in throat lozenges and sprays, but the concentration is so low that there is little antimicrobial effect, although there is some local anesthetic action. Some throat sprays may have a concentration above 1% and these may show antibacterial action.

b. PHENOLICS—chemicals derived from phenol—the molecule has been chemically altered to make it less irritating and more effective. These agents act in several ways, damaging plasma membranes, inactivating enzymes, and denaturing proteins. Phenolics are often used as disinfectants because they remain active in the presence of organic matter. Original Lysol products contain O-phenyl phenol, but many of the newer ones do not.

2. BIGUANIDES—chlorhexidine is an example. These are similar to phenolics, but are less toxic. Biguanides act by disrupting the plasma membrane and are excellent as surgical

scrubs and for patient preps, although they must be kept away from the eyes. They are also used in dental treatments.

They are effective against most vegetative bacteria and fungi, but not against endospores and many viruses.

3. HALOGENS

a. IODINE—one of the oldest and most effective antiseptics. Works against all bacteria and many endospores, fungi, and viruses. It acts by combining with amino acids, especially tyrosine, and inhibits the functions of microbial proteins. It also alters plasma membranes.

1) Tincture—iodine combined with alcohol

2) Iodophor—iodine combined with an organic carrier molecule. These are still quite effective, but they are less toxic and do not stain as badly. Betadine is an example.

In addition to use as a skin disinfectant and wound treatment, iodine can be used to purify drinking water. (5 – 10 drops per quart)

b. CHLORINE—when chlorine is added to water, hypochlorous acid forms. This is a strong oxidizing agent that interferes with cellular enzymes.

1) A liquid form of compressed **chlorine gas** is used for treating water systems, swimming pools, and sewage. When added to water chorine gas forms hypochlorous acid.

2) **Calcium hypochlorite** is used to disinfect dairy equipment and restaurant equipment. It can also be called chloride of lime.

3) **Sodium hypochlorite (Chlorox)** is used as a household disinfectant and bleach. Adding 2 - 4 drops of Chlorox to a quart of water can make it safe as drinking water (let sit 30 minutes before using). A solution of Chlorox is the recommended disinfectant for killing the AIDS virus in households.

4) **Chlorine dioxide**—either in gaseous form or in aqueous solution. Used to fumigate enclosed areas, in water treatment, or as a disinfectant/antiseptic.

5) **Chloramines**—combine chlorine and ammonia, used to sanitize glassware, eating utensils, and food-handling equipment as well as in water treatment.

4. ALCOHOLS—these kill bacteria and fungi but not endospores and most viruses. Alcohol acts mainly by denaturing proteins, but it can also

disrupt membranes and dissolve lipids. Alcohols evaporate rapidly, leaving no residue. Alcohols are frequently used as skin degerming agents. Wiping with alcohol mostly wipes away microbes, skin oils, and dirt, although some microbes may be killed.

Alcohols are not satisfactory for cleaning open wounds, because they coagulate a surface layer of protein and leave bacteria unharmed beneath it. Most hand sanitizers contain alcohol as the active ingredient.

a. ETHANOL (ethyl alcohol)—70 % concentration is ideal, although concentrations of 60 - 95 % are effective. 100% is not effective because some water must be present for denaturation to occur.

b. ISOPROPANOL (isopropyl alcohol or rubbing alcohol)—more commonly used because it is cheaper and more effective. Usual concentration is 90%.

Both of these alcohols may be mixed with other agents to enhance activity. When another agent is mixed with alcohol, the solution is called a tincture.

5. HEAVY METALS—very low concentrations of heavy metals can be effective against microbes. This is called oligodynamic action, and works by denaturing proteins, including enzymes.

a. SILVER NITRATE (1% solution)—this was once used to swab sore throats (in the days before antibiotics). It was also used in the eyes of newborns, to prevent an eye infection caused by the gonorrhea bacteria. Antibiotic ointments are now used for this purpose.

b. PURE SILVER INCORPORATED INTO DRESSINGS (ACTICOAT)--In recent years, dressings containing silver have been used in treating infections caused by antibiotic-resistant bacteria. The silver seems to shut down energy production in bacteria and little resistance has been found, and tissue damage does not occur as with other means of delivering silver.

c. OTHER FORMS OF SILVER—Cream containing silver combined with a sulfa drug, catheters impregnated with silver, a silver-containing product for surfaces (Surfacine).

d. MERCURY---compounds such as mercuric chloride were probably the earliest disinfectant. They are bacteriostatic. The drawbacks are toxicity, corrosiveness and inactivation by organic matter. Mercurochrome and merthiolate were once widely used, but contained such tiny amounts of mercury that they had little effect. Mercury compounds may be used in paint to prevent mildew.

e. COPPER---copper sulfate is used to kill algae (algicide) in bodies of water or aquariums. Copper compounds may also be used in paint to prevent mildew.

f. ZINC---zinc chloride is found in some mouthwashes and zinc is often used as an antifungal in paint. Zinc lozenges & such are sold as treatment for colds, but their effect is questionable.

6. SURFACE-ACTIVE AGENTS (SURFACTANTS)---these agents decrease surface tension and include soaps and detergents.

a. SOAPS---main value is in causing microbes to be mechanically removed. Washing with soap breaks up the oily film that covers skin and allows microbes and dirt to be washed away. Deodorant soaps have antimicrobial ingredients added.

7. CHEMICAL FOOD PRESERVATIVES---these are frequently added to retard spoilage, and are believed to be safe for consumption.

a. SULFUR DIOXIDE

b. ORGANIC ACIDS---they interfere with the metabolism of molds or damage their plasma membranes.

1) Sorbic acid, potassium sorbate and sodium benzoate are added to prevent growth of mold in acid foods such as cheese and soft drinks.

2) Calcium propionate---used in bread

c. SODIUM NITRITE OR NITRATE---added to meat products such as ham, bacon, and hot dogs. It preserves the red color of the meat and prevents germination and growth of botulism endospores.

As nitrites react with amino acids, compounds called nitrosamines are formed. These are carcinogens. Because of this, the amount added to meat has been reduced.

9. ANTIBIOTICS---two which are not used in treating disease are used as food preservatives.

a. NISIN---added to cheese to prevent growth of endospores

b. NATAMYCIN---added to foods to prevent growth of fungi

10 ALDEHYDES---these can act very effectively against microbes. They inactivate proteins. Aldehydes are commonly used as embalming fluid.

a. FORMALDEHYDE

1) FORMALDEHYDE GAS---can be used as a disinfectant

2) FORMALIN, a 37% aqueous solution of formaldehyde gas, has been used to preserve biological specimens and inactivate bacteria and viruses in vaccines.

b. GLUTARALDEHYDE---this is more effective and somewhat less irritating than formaldehyde. It is used to disinfect medical equipment that cannot withstand autoclaving. This is the most commonly used chemical sterilant.

A 2 % solution, such as Cidex, kills bacteria including *Mycobacterium tuberculosis* and viruses in 10 minutes. Endospores require 3 - 10 hours. If the 10 hours are allowed, this is considered to be chemical sterilization. However, instruments must be rinsed before use, so unless they are handled aseptically and rinsed with sterile water, this is more likely to be disinfection.

11. GASEOUS CHEMICAL STERILANTS---these are used in a closed container, which is sometimes called a gas autoclave.

ETHYLENE OXIDE---this is the one most frequently used. It acts by denaturing proteins. It kills all microbes and endospores, but has several disadvantages:

1) Long exposure time (4 - 18 hours)

2) Items must then be aired 12 - 24 hours before use

3) Highly toxic and explosive

In spite of these drawbacks, the product is still used because:

1) Can be used on items that cannot withstand autoclaving

2) Some large hospitals have ethylene oxide chambers where even large items such as mattresses can be sterilized

3) Was used to sterilize spacecraft returning to earth

4) Used to sterilize spacecraft that landed on the moon

12. PLASMA STERILIZATION—used to sterilize medical equipment that cannot be autoclaved. Mostly metal or plastic instrument parts that involve long hollow tubes with a small internal diameter. The procedure uses a container with a vacuum, a strong electromagnetic field, and a chemical. High temps not required but very expensive.

13. PEROXYGENS (oxidizing agents)---these oxidize cellular components of microbes.

a. OZONE (O_3)---highly reactive form of oxygen, often used along with chlorine to disinfect water

b. HYDROGEN PEROXIDE---although frequently used, it is a poor choice as an antiseptic for open wounds, because human cells contain the enzyme catalase, which breaks down the peroxide before it has much chance to act. (This is where the bubbles come from).

It is an effective disinfectant for inanimate objects, where it can even kill endospores. It is used to disinfect food packaging (before the food is put in) and contact lenses.

Although peroxide does not directly kill microbes well in wounds, it is used in deep wounds because it releases oxygen as it breaks down, which makes conditions unfavorable for anaerobic bacteria.

3) FILTRATION

This is the passage of a liquid or gas through a screen like material with pores small enough to retain microbes. This method is used to sterilize items that would be destroyed by heat. Some examples are:

Certain culture media

Enzymes

Vaccines

Antibiotics

HEPA (high-efficiency particulate air filters) trap microbes larger than 0.3 μm in diameter. They are sometimes used in operating rooms and rooms of transplant patients to lower numbers of bacteria.

To filter liquids, filters of unglazed porcelain were originally used. Bacteria were trapped but viruses went through. For this reason, they were called filterable viruses. In recent years, membrane filters have been developed. These are made of cellulose or plastic and the size of the pores can be selected, down to a size that will even retain most viruses.

Different types of filters:

1- Membrane filters : used in production processes are commonly made from materials such as mixester cellulose or polyethersulfone (PES).

2- Sintered glass filter : made from finely ground glass that are fused sufficiently to make small.
3- Air filters : air can be filtered using HEPA filters. They are usually used in biological.

The filtration equipment and the filters themselves may be purchased as pre-sterilized disposable units in sealed packaging, or must be sterilized by the user, generally by autoclaving at a temperature that does not damage the fragile filter membranes. To ensure proper functioning of the filter.

Classification of culture media based on the basis of composition

1. Synthetic or chemically defined medium
   A chemically defined medium is one prepared from purified ingredients and therefore whose exact composition is known.
2. Non synthetic or chemically undefined medium

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

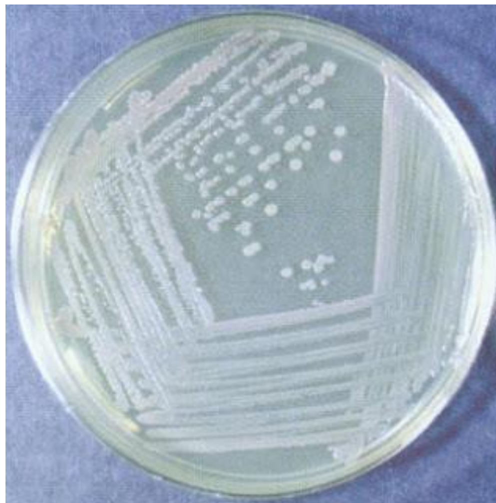
Synthetic medium may be simple or complex depending up on the supplement incorporated in it. A simple non-synthetic medium is capable of meeting the nutrient requirements of organisms requiring relatively few growth factors where as

complex non-synthetic medium support the growth of more fastidious microorganisms.

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

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

1. General purpose media/ Basic media
Basal media are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar are considered as basal medium. These media are generally used for the primary isolation of microorganisms.


Nutrient Agar

2. Enriched medium (Added growth factors):

Blood Agar

Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes them enriched media. Enriched media are used to grow nutritionally exacting (fastidious)

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

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

antibiotics, dyes, chemicals, alteration of pH or a combination of these.

a. Selective medium

Principle: Differential growth suppression Selective medium is designed to suppress the growth of some microorganisms while allowing the growth of others. Selective medium are agar based (solid) medium so that individual colonies may be isolated.

Examples of selective media include:

1. Thayer Martin Agar used to recover *N.gonorrhoeae* contains antibiotics; vancomycin, colistin and nystatin.
2. **Mannitol Salt Agar** and Salt Milk Agar used to recover *S.aureus* contains 10% NaCl.
3. Potassium tellurite medium used to recover *C.diphtheriae* contains 0.04% potassium tellurite.

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

5. Pseudosel Agar (Cetrimide Agar) used to recover *P. aeruginosa* contains cetrimide (antiseptic agent).
6. Crystal Violet Blood Agar used to recover *S. pyogenes* contains 0.0002% crystal violet.
7. **Lowenstein Jensen Medium** used to recover *M.tuberculosis* is made selective by incorporating malachite green.
8. Wilson and Blair's Agar for recovering *S. typhi* is rendered selective by the addition of dye brilliant green.
9. Selective media such as **TCBS Agar** used for isolating *V. cholerae* from fecal specimens have elevated pH (8.5-8.6), which inhibits most other bacteria.

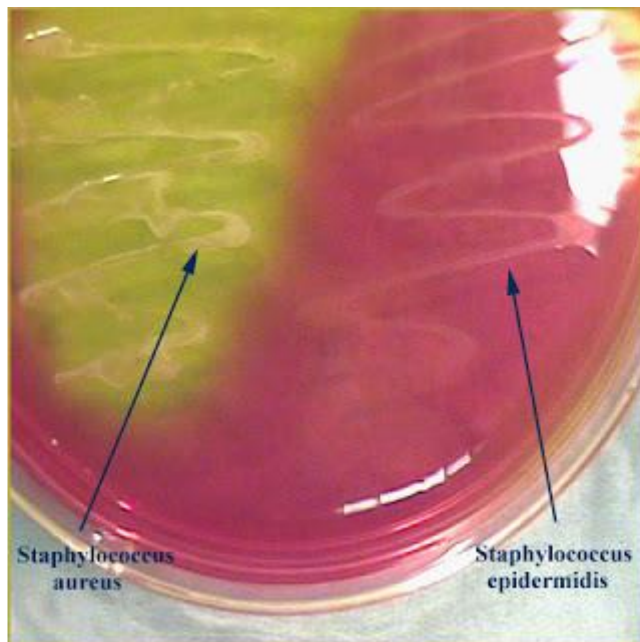
b. Enrichment culture medium

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

4. Differential/ indicator medium: differential appearance: Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates

etc, so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.

Examples of differential media include:



1. **Mannitol salts agar** (mannitol fermentation = yellow)
2. **Blood agar** (various kinds of hemolysis i.e. α, β and γ hemolysis)
3. **Mac Conkey agar** (lactose fermenters, pink colonies whereas non- lactose fermenter produces pale or colorless colonies.
4. **TCBS** (*Vibrio cholerae* produces yellow colonies due to fermentation of sucrose)

5. Transport media:

Clinical specimens must be transported to the laboratory

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

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

6. Anaerobic media:

Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients.

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

Robertson Cooked Meat (RCM) medium that is commonly used to grow *Clostridium* spps contains a 2.5 cm column of bullock heart meat and 15 ml of nutrient broth. Thioglycollate broth contains sodium thioglycollate, glucose, cystine, yeast extract and casein hydrolysate.

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

7. Assay media

These media are used for the assay of vitamins, amino acids and antibiotics. E.g. antibiotic assay media are used for determining antibiotic potency by the microbiological assay technique. Other types of medium includes

- Media for enumeration of Bacteria,
- Media for characterization of Bacteria,
- Maintenance media etc.

Common Fungal Culture Media and their use

JANUARY 26, 2014 BY TANKESHWAR ACHARYA IN CULTURE MEDIA USED IN MICROBIOLOGY, MYCOLOGY

Two general types of culture media are essential to ensure the primary recovery of all clinically significant fungi from clinical specimens. One medium should be non-selective (such as Brain Heart Infusion Agar; i.e., one that will permit the growth of virtually all clinically relevant fungi) and other medium should be selective, specially tailored to isolate specific pathogenic fungi of interest.

For optimal recovery of fungal pathogen, a battery of media should be used, and the followings are recommended:

1. Media with or without cyclohexamide (Cycloheximide is added to inhibit the growth of rapidly growing contaminating molds.)
2. Media with or without an antibacterial agent (Chloramphenicol, Gentamicin and Ciprofloxacin are commonly used antibacterial for this purpose).

Antibacterial agents are used to kill the contaminating bacterial species. **If the sample is taken from sterile site, it is not necessary to use media containing antibacterial agents.**

1. **Brain-heart infusion (BHI) agar**: It is a non-selective fungal culture medium that permits the growth of virtually all clinically relevant fungi. It is used for the primary recovery of saprophytic and **dimorphic fungi**
2. **Czapek's agar**: It is used for the subculture of *Aspergillus* species for their differential diagnosis.

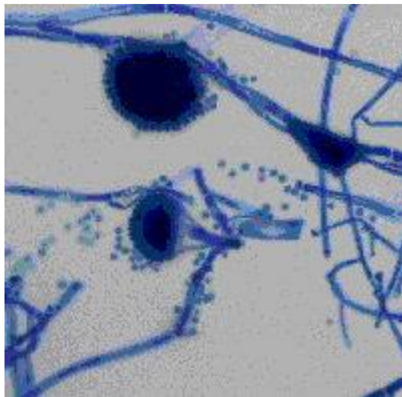
3. **Inhibitory mold agar (IMA):** Primary recovery of dimorphic pathogenic fungi. Saprophytic fungi and dermatophytes will not be recovered.
4. **Mycosel/Mycobiotic agar:**
   1. It is generally Sabouraud's dextrose agar with cycloheximide and chloramphenicol added.
   2. It is used for the primary recovery of dermatophytes.
   3. Niger Seed Agar: It is used for the identification of *Cryptococcus neoformans*.
5. **Potato Dextrose Agar (PDA):** It is a relatively rich medium for growing a wide range of fungi.
6. **Sabouraud's Heart Infusion (SABHI) agar:** Primary recovery of saprophytic and dimorphic fungi, particularly fastidious strains.

Penicillium notatum on Sabouraud agar
Image source: ASM

7. **Sabouraud's dextrose agar (SDA):**
   1. Sabouraud's agar is sufficient for the recovery of dermatophytes from cutaneous samples and yeasts from vaginal cultures.

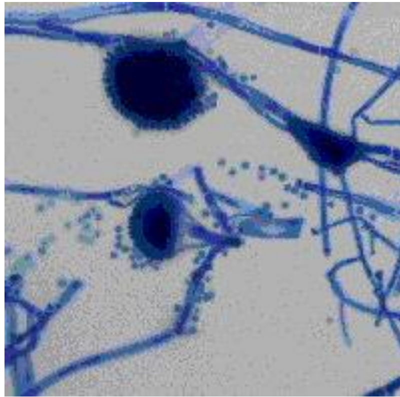
2. Not recommended as a primary isolation medium because it is insufficiently rich to recover certain fastidious pathogenic species, particularly most of the dimorphic fungi.
3. Sabouraud's dextrose agar (2%) is most useful as a medium for the subculture of fungi recovered on enriched medium to enhance typical sporulation and provide the more characteristic colony morphology.

Detection of Fungi using Stains/Staining Reactions



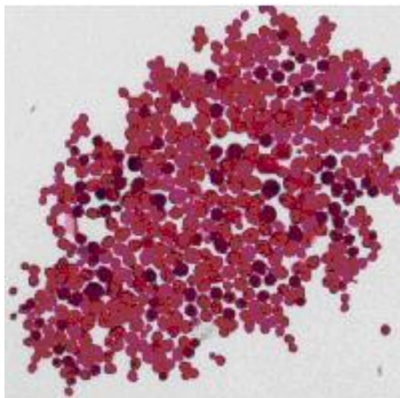
Fungi, yeasts and molds are widespread throughout the environment. For healthy persons they are not a serious problem as long as certain standards of hygiene are maintained. If the immune system is compromised, however, through chronic illness or tumor, they may pose an infection risk or result in manifest illness.They can, for instance, infect the nails, hair, skin, lungs, kidneys or lymph nodes. Detecting them is an essential prelude to instituting targeted remedial measures.Detection of fungi with various stains is quick and simple, and can be optimized through the use of ready-to-use Depending on the consistency of the

specimen material, it may be necessary to perform a simple pre-treatment step with alkali prior to staining.



Lactophenol blue stain

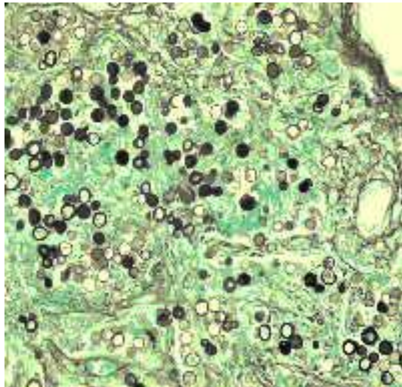
Use of a ready-to-use lactophenol blue solution enables the specimen to be stained in a single step. The fungi are stained dark blue and stand out well against the light blue background.



PAS (Periodic Acid Schiff) reaction

Fungi are very easily visualized in a PAS reaction with Schiff's reagent, this method now being a standard application.Schiff's reagent is available ready-to-use as an individual reagent or as part of the PAS Kit, which also contains the periodic acid required for the oxidation reaction. As Schiff's reagent/PAS Kit is stored at room temperature, no time is required to warm the solution, so the result is obtained more quickly.In the case of specimen materials that need alkali pre-treatment it is

important to make sure that they are not allowed to react too long, otherwise they may take on a soup-like or gelatinous consistency. The pre-treated specimen should be neutralized with 10% lactic acid and adjusted to pH 3-5. The PAS reaction involves first oxidizing the specimen and then reacting it with Schiff's reagent until the fungal elements take on a bright red color.



PAS and methenamine silver staining for histological specimens
When histological material is examined for fungi, the procedure may likewise be a PAS reaction or a silver staining method. In the case of the PAS reaction the fungi are turned bright red in the tissue. Among the silver staining methods, the Gomori methenamine silver (GMS) stain is the method of choice. The fungi are seen as brown to black on a light green background that is created by counterstaining with Light Green SF. The methenamine silver stain is a tried and tested method that is simply, safely and reliably performed with a kit containing ready-to-use reagents or suitably prepared reagents.

Ready-to-use staining solutions and kits are a recommended way of applying standard methods reproducibly according to the standards of quality management required in medical laboratories. For all of the products mentioned, instructions are provided in the kit or, in the case of

individual staining solutions, are available on the Internet or on request. These products count as IVDs and are CE certified.

For further information about the wide range of products available from Merck Millipore for fungal detection, please contact Merck Millipore (see details above) or EMD Chemicals in the USA or click the link below.

Product code	Description	Presentation
113741	Lactophenol blue solution	100 mL
109033	Schiff's reagent	500 mL
101646	PAS Kit	2 x 500 mL
100820	Methenamine silver plating kit acc.to Gomori	sufficient for 50 prepared specimens

LACTOPHENOL COTTON BLUE STAIN

Cat. no. Z68	Lactophenol Cotton Blue Stain	15ml

INTENDED USE

Hardy Diagnostics Lactophenol Cotton Blue Stain is recommended for mounting and staining yeast and molds.

SUMMARY

Lactophenol Cotton Blue Stain is formulated with lactophenol, which serves as a mounting fluid, and cotton blue. Organisms suspended in the stain are killed due to the presence of phenol. The high concentration of the phenol deactivates lytic cellular enzymes thus the cells do not lyse.

Cotton blue is an acid dye that stains the chitin present in the cell walls of fungi.

REAGENT FORMULA

Ingredients per liter:*

Phenol	200.0gm
Cotton Blue	0.5gm
Glycerol	400.0ml
Lactic Acid	200.0ml
Deionized Water	200.0ml

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Upon receipt store at 2-30ºC. Product should not be used if there are any signs of contamination, deterioration, or if the expiration date has passed. Product is light sensitive; protect from light.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "Storage" on the Hardy Diagnostics Technical Document website for more information.

PROCEDURE

Specimen Collection: This product is intended to be used primarily with pure cultures, although certain specimens may be examined directly using this stain. Consult appropriate references for further information concerning the use of Lactophenol Cotton Blue Stain with specimens. [5,7,8]

Method of Use: Place a drop of Lactophenol Cotton Blue Stain in the center of a clean slide. Remove a fragment of the fungus colony 2-3mm from the colony edge using an inoculating or teasing needle or MycoMount™ adhesive strips (Cat. no. MM40). Place the fragment in the drop of stain and tease gently. Apply a coverslip. Do not push down or tap the cover slip as this may dislodge the conidia from the conidiophores. Examine the preparation under low and high, dry magnification for the presence of characteristic mycelia and fruiting structures. Consult appropriate references for diagnostic features of fungi isolated in clinical and non-clinical specimens. [1-6]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Lactophenol Cotton Blue Stain is useful in the recognition and presumptive identification of fungi. Additional characteristics including colony morphology and biochemical tests should be used where appropriate for final identification. For further information, consult the appropriate references.[1-6]

Refer to the document "Limitations of Procedures and Warranty" on the Hardy Diagnostics Technical Document website for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Results
Aspergillus brasiliensis formerly *A. niger* ATCC ® 16404	Delicate blue hyphae and fruiting structures with a pale blue background.
Trichophyton mentagrophytes ATCC ® 9533	Delicate blue hyphae and fruiting structures with a pale blue background.

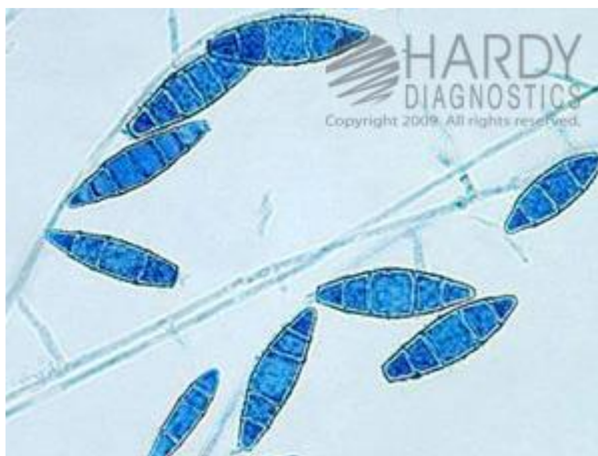
User Quality Control

It is recommended that each new lot or shipment of reagent be tested with known positive and negative controls. [3,9]

Physical Appearance

Lactophenol Cotton Blue Stain should appear clear, and blue in color.

Lactophenol Cotton Blue Stain (Cat. no. Z68).



Microscopic image of *Microsporum gypseum* stained with Lactophenol Cotton Blue Stain (Cat. no. Z68).

1. Algal Culture Media

In their natural habitats algae obtain all the nutrients, minerals and vitamins they require from the water in which they live. To grow them in the lab however, you must provide them with all of these essential resources i.e. you will need to make up some growth media.

Algae media refers to the solution or culture in which algae grow. All the media have several components in common: sources of nitrogen (in the from of nitrate, nitrite and ammonia), phosphorus, vitamins and trace metals. However the specific types of these nutrients, their concentrations and ratios vary between the media.

F/2 Medium There are many recipes for such algal growth media, F/2 medium however, is the most common and widely used general enriched seawater medium designed for growing marine algae used in the phycological and aquaculture studies.

Stock solutions and salts

In any recipes you will see two types of stocks "working stocks" and "primary stocks".

Working stocks are those whose aliquots contribute directly to making the final media.

Primary stocks are normally made where several single substance solutions are then combined to form the working stock.

Stock solutions are made up by accurately weighing the prescribed amount of nutrient and dissolving in a specified volume of distilled water, if possible in a volumetric flask. Some nutrients will readily dissolve, others need heat and stirring to fully dissolve. In contrast vitamin stocks are heat sensitive and should not be subjected to heat treatment and should also be kept in the dark. Failure to fully dissolve the primary stocks of some nutrients such as EDTA can lead to gross precipitation when these stocks are combined to make the media.

Nutrients come with different **salts** and hydration. For example, while copper and zinc may be two desired active constituents they are readily obtained from suppliers with either SO_4 or Cl_2 salts (ie $CuSO_4$ or $CuCl_2$ and $ZnSO_4$ or $ZnCl_2$). Some nutrients also come with different hydrations, ie the .*n*H2O suffix. Substituting one form for another may have no effect on the growth of some microalgae species, but it can lead to poor growth in others and also lead to unwanted and time consuming precipitation problems as the overall ratio of salts in the medium has changed. Therefore deviating from the prescribed recipes is to be avoided and ordering the correct form is recommended.

Seawater source and treatment

The marine microalgae species should be grown using unpolluted oceanic seawater or artificial seawater. Artificial seawater media is composed of marine salts and nutrients added to pure freshwater. Artificial seawater is only necessary where a clean natural seawater source is unavailable or in particular research studies where the exact composition needs to be controlled. Off-shore sites have very low concentrations of metal and organic pollutants therefore it suitable as the base medium for a wide range of marine microalgae species.

The seawater should be collected in clean black polyethylene containers and then stored until needed (preferred at 4 ^{0}C). Then it is treated using a filtration.

F/2 Medium Stock Solutions:

Quantity per liter of Media	Compound	Stock Concentration	
1.0 ml	$NaNO_3$	75.0 g/L dH_2O	Add to Media
1.0 ml	NaH_2PO_4.H2O	5.0 g/L dH_2O	Autoclave separately
1.0 ml	Na_2SiO_3.9H2O	30.0 g/L dH_2O	Autoclave separately
1.0 ml	f/2 Trace Metal Solution	(see recipe below)	Autoclave separately
0.5 ml	f/2 Vitamin Solution	(see recipe below)	Filter sterilized and added to autoclaved Media

Mineral salts working solution

1.	$NaNO_3$	75.0 g/L dH_2O	Add to Media

Add Nitrate directly to filtered seawater and autoclave.

Use: 1 ml per litre of seawater medium.

2.	NaH_2PO_4.H2O	5.0 g/L dH_2O	Autoclave separately
3.	Na_2SiO_3.9H2O	30.0 g/L dH_2O	Autoclave separately

Put the salts into one 1-I screw-capped oven-resistant glass bottle and fill with DW to the mark. If not available, deionized water can also be employed. When fully dissolved, autoclave separately aliquots of the phosphate and silicate. Store at ambient temperature, avoiding direct light. Add these aseptically to your media once it has cooled to room temperature. Store non-sterile stocks in the refrigerator.

Use: 1 ml per litre of sterilized seawater medium.

If the alga to be grown does not require silica e.g diatom, then it is recommended that the silica be omitted.

4. Trace Metal Solution

To 950 mL distilled H_2O add:

Quantity	Compound	Stock Solution
1.3 g	$FeCl_3.6H_2O$	-
8.7 g	$Na_2EDTA.2H_2O$	-
1.0 ml	$CuSO_4.5H_2O$	980 mg / 100 ml dH_20
1.0 ml	$Na_2MoO_4.2H_2O$	630 mg / 100 ml dH_20
1.0 ml	$ZnSO_4.7H_2O$	2.2 g / 100 ml dH_20
1.0 ml	$CoCl_2.6H_2O$	1.0 g / 100 ml dH_20
1.0 ml	$MnCl_2.4H_2O$	18.0 g 100 ml dH_20

Solution will initially be cloudy. Add 1N NaOH to adjust pH to about 4.5; solution should clear unless too much NaOH has been added. Bring final volume to one liter.

Use: 1 ml per litre of sterilized seawater medium.

5. Vitamin Solution

First, prepare primary stock solutions for vitamin B_{12} and biotin (Vitamin H) according to the proportions indicated below. To prepare final vitamin solution, begin with 950 mL of dH_2O, dissolve the thiamine, add the amounts of the primary stocks as indicated in the quantity column below, and bring final volume to 1 liter with dH_2O.

Warning: do not autoclave any vitamin solution but Filter sterilize.

Quantity	Compound	Stock Solution	Tip
1.0 ml	Vitamin B_{12}	10 mg / 10 ml dH_2O	
1.0 ml	Biotin	10 mg / 10 ml dH_2O	Dissolve in a little 0.1N NaOH first
200.0 mg	Thiamine HCl	-	

Store primary stock solutions in freezer. Store completed vitamin solution in the refrigerator or freeze small aliquots.

Use: 0.5 ml per litre of sterilized seawater medium.

Fig. Working solutions ready to use

Isolation and Culture of Algae

Algal cultures are essential when conducting competition studies, bioassays, assessment of zooplankton food preferences, and determination of algal life histories. They are also necessary for molecular systematic work. Algal cultures may be "unialgal," which means they contain only one kind of alga, usually a clonal population (but which may contain bacteria, fungi, or protozoa), or cultures may be "axenic," meaning that they contain only one alga and no bacteria, fungi or protozoa. There are four major techniques for obtaining unialgal isolates: streaking, spraying, serial dilution, and single-cell isolations. Streaking and spraying are useful for single-celled, colonial, or filamentous algae that will grow on an agar surface; cultures of some flagellates, such as *Chlamydomonas* and *Cryptomonas* may also be obtained by these procedures. Many flagellates, however, as well as other types of algae must be isolated by single-organism isolations or serial-dilution techniques. We will practice spraying and single-organism isolations.

Spraying. In this technique, a stream of compressed air is used to disperse algal cells from a mixture onto the surface of a petri plate containing growth medium solidified with agar. Hold a petri plate about 18 inches from the touching tips of two Pasteur pipettes. One of these is attached to an airline via a hose, and mounted onto a ringstand. The other pipette is suspended tip-up into a container holding the algal mixture. The airflow from the first pipette creates a vacuum that draws a stream of algae-containing liquid up from the container through the second pipette. The airflow also sprays the suspended algae through the air, where they can be intercepted by the agar plate.

Single-cell/colony/filament isolations. The first step in this procedure is to prepare a number of "micropipettes" (very fine-tipped pipettes) from glass Pasteur pipettes. Hold a pipette in both hands; the tip end is held with a forceps so that the glass near the tip is within the flame of a bunsen burner (gas flame). The pipette is held in the flame only until the glass becomes slightly soft. This is determined by testing for flexibility by moving the tip with the forceps. Then the pipette is removed from the flame and pulled out straight. or at an angle so that there is a bend. **If you pull the pipette while it is still in the flame, it will seal up, so don't do this.** Always remove the pipette before pulling it! Use the forceps to break the tip. You can vary the diameter of the finely pulled tip by changing the speed of pulling; the diameter of a slowly-pulled tip will be greater than that of a rapidly-pulled tip. You would want a narrow diameter tip if you are trying to isolate very small algae, but a larger diameter tip is required for large cells. Try to match the diameter of the pipette tip to the size of the algal cells to be isolated.

Prepare a multiwell plate with sterilized media in each well. Place multiple drops of sterilized media or water onto the inside surface of a sterile petri plate. Attach a micropipette to a length of rubber tubing, attach a ethanol-sterilized mouthpiece to the the other end of the tubing, and put the mouthpiece in your mouth. Place a petri dish of algae on the stage of a dissecting microscope and locate the single cell/colony/filament to be isolated. Then find the tip of the micropipette and move it to the vicinity of the alga, then suck it up into the pipette tip, then stop the suction. Try to avoid sucking up any other algae. Now remove the pipette from the dish, then blow the liquid+alga into one of the drops of water on a petri plate). Break off and dispose of the portion of the micropipette tip that contained liquid; this has been contaminated. The micropipette can continue to be used until all of the pulled portion has been consumed. Now use the micropipette

to transfer the isolated alga from the first drop into a series of fresh drops. This is a washing procedure that helps remove contaminants. After transfer through 5-10 drops, transfer the alga into a well of the multiwell plate holding liquid growth medium suitable for that particular species. Repeat the procedure. Usually several attempts are made because not all isolated algae will continue to grow, or some may be contaminated with other algal cells.

A particularly effective means of obtaining unialgal cultures is isolation of zoospores immediately after they have been released from parental cell walls, but before they stop swimming and attached to a surface. Recently-released zoospores are devoid of contaminants, unlike the surfaces of most algal cells. But catching zoospores requires a steady hand and experience.

Filaments can be grabbed with a slightly curved pipette tip and dragged through soft agar (less than 1%) to remove contaminants. It is best to begin with young branches or filament tips which have not yet been extensively epiphytized.

Antibiotics can be added to the growth medium to discourage growth of contaminating cyanobacteria and other bacteria. Addition of germanium dioxide will inhibit growth of diatoms.

Axenic cultures (beyond the scope of this course) can be obtained by treating isolated algae to an extensive washing procedure, and/or with one or more antibiotics. Resistant stages such as zygotes or akinetes can be treated with bleach to kill epiphytes, then planted on agar for germination. It is usually necessary to try several different concentrations of bleach and times of exposure to find a treatment that will kill epiphytes without harming the alga.

Place the tubes/dishes with isolated algae into the culture room and allow growth to occur for 3-4 weeks. Examine them with the dissecting scope for signs of growth or contamination.

Freshwater Growth Media used in this class:

1) BBM is Bold's basal medium, chemically defined; good for many green algae.

2) Soil-water is undefined and used for algae whose nutritional requirements are unknown, or which will not grow on simple inorganic media. The soil should be loam from a site where herbicides have not recently been used. Sometimes it is advisable to add a dried pea to the medium before autoclaving.

3) SD11 is a defined medium that is somewhat more complex than BBM; it contains a vitamin mixture. Good for many green algae.

4) DYIII is a defined medium to which vitamins are often added, used for culture of chrysophytes and cryptomonads as well as some dinoflagellates.

Reference: Stein (ed.) 1973. Handbook of Phycological Methods. Culture methods and growth measurements. Cambridge University Press.

10

METHODS OF ISOLATION OF BACTERIA

10.1 INTRODUCTION

We have learned in earlier chapters that there exist so many bacteria that cause human disease.so now our task is to isolate these bacteria and identify them. The identification is required so as to cure the illness or the infection caused due to these bacteria, using appropriate antibiotics. Identification also holds significance for epidemiological purposes.

This chapter would focus on various methods used for isolation of bacteria. While in subsequent chapters we would learn about identification of bacteria and the ways to contain the infections caused by them.

OBJECTIVES

After reading this chapter, you will be able to :

- Expalin the steps involved in the isolation of bacteria.
- describe the significance of Specimen collection.
- describe the significance of Preservation and transportation of specimen.
- explain the role of microscopy in isolation of bacteria.
- explain various methods for isolation of bacteria.

10.2 ISOLATION OF BACTERIA

Isolation of bacteria forms a very significant step in the diagnosis and management of the illness. Isolation of bacteria involves various steps –

- Specimen collection
- Preservation and transportation of specimen

Notes

- Microscopic examination of sample
- Various methods used for isolation of bacteria

Specimen collection

Many different specimens are sent for microbiological examination from patients with suspected bacterial infection. Common specimens include urine, faeces, wound swabs, throat swabs, vaginal swabs, sputum, and blood. Less common, but important specimens include cerebrospinal fluid, pleural fluid, joint aspirates, tissue, bone and prosthetic material (e.g. line tips).

Some types of specimen are normally sterile e.g. blood, CSF. These samples are usually obtained via a percutaneous route with needle and syringe, using appropriate skin disinfection and an aseptic technique. The culture of bacteria from such specimens is usually indicative of definite infection except if they are skin contaminants (bacteria inhabitants of normal skin).

Fig. 10.1: Universal container.

In contrast, many microbiological specimens are obtained from non-sterile sites e.g. vaginal or throat swabs, urine sample, stool sample. Such samples often contain bacteria of no clinical relevance in addition to possible pathogens, making the interpretation of culture results more difficult. In general it is preferable to send samples from sterile sites if available.

It is preferred to obtain the samples for bacteriological culture before antibiotic therapy is started. This maximizes the sensitivity of the investigations and reduces false-negative results. Similarly, samples of tissue or pus are preferred over swabs, to maximize the recovery of bacteria in the laboratory.

Specimens must be accurately labelled and accompanied by a properly completed requisition form, indicating the nature of the specimen, the date of sample collection, relevant clinical information, the investigations required, and details of antibiotic therapy, if any.

Notes

This allows the laboratory to perform the correct range of tests, and helps in the interpretation of results and reporting. Along with clinical specimens, medical microbiology laboratories also process samples of food, water and other environmental samples (e.g. air sampling from operating theatres) as part of infection control procedures.

High-risk samples

Certain bacterial infections are a particular hazard to laboratory staff, and specimens that might contain these pathogens should be labelled as 'high risk' to allow for additional safety measures if necessary. For example - blood cultures from suspected typhoid *(Salmonella typhi)* or brucellosis (*Brucella* species), and samples from suspected *Mycobacterium tuberculosis*.

Preservation and Transport of specimen

Most specimens are sent to the laboratory in sterile universal containers. Swabs are placed in a suitable transport medium (eg. charcoal medium) otherwise it leads to false negative reporting.

Fig. 10.2: Charcoal laden transport media

Specimens should be transported as soon as possible to the laboratory. In case a delay is anticipated the specimen should be stored at 4° C.

Immediate transport is necessary in order to:

(i) Preserve the viability of the 'delicate' bacteria, such as *Streptococcus pneumoniae* or *Haemophilus influenzae* (delays in processing can cause false-negative culture results);

(ii) Minimize the multiplication of bacteria (e.g. coliforms) within specimens before they reach the laboratory. In particular urine and other specimens that utilize a semiquantitative culture technique for thier detection, as delays in transport can give rise to falsely high bacterial counts when the specimen is processed.

Microscopy

A Gram stain helps with the visualization of bacteria, and gives an indication of the type of bacteria present, based on the shape of the bacteria and the staining

properties (Gram positive: purple; Gram negative: pink/red). A Gram stain also helps to identify mixtures of bacteria, helps to determine the appropriate range of agar plates to be used for subsequent culture, and helps with the interpretation of culture results.

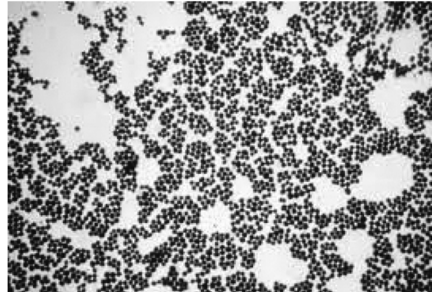


Fig. 10.3: Gram positive cocci

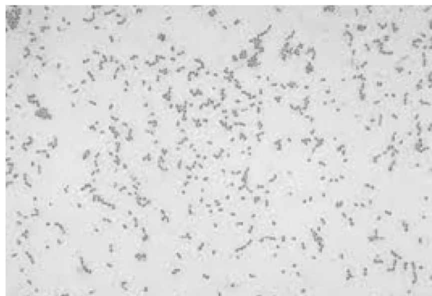


Fig. 10.4: Gram negative bacilli

For liquid specimens e.g. CSF, the sample is first centrifuged to concentrate any bacterial cells in the deposit, and Gram stain and culture is performed from the deposit after the supernatant is decanted. This helps increase the sensitivity of both microscopy and culture.

Ziehl-Neelsen (ZN) stain is used to demonstrate the presence of Mycobacteria. Mycobacteria can also be visualized using the fluorescent dye auramine and a fluorescence microscope. Direct immunofluorescence is employed to detect certain pathogens (e.g. *Legionella, Pneumocystis*) using specific antibodies conjugated to a fluorescent dye.

Another microscopic technique is dark ground microscopy. This is mainly used to detect the thin spirochaetal cells of *Treponema pallidum* (syphilis bacteria).

INTEXT QUESTIONS 10.1

1. Specimens that contain pathogens which are hazardous to laboratory staff should be labeled as ....
2. Swabs are sent to laboratory in .... medium

3. If delay is anticipated in transporting the specimen, it should be stored at .... temperature
4. .... gives an indication of bacteria present in the sample
5. .... stain is used in demonstration of mycobacteria
6. .... microscopy is used to detect syphilis organism

10.3 METHODS OF ISOLATION OF BACTERIA

Methods of isolation of bacteria can be broadly classified into two

- Culture methods
  - On Solid media
  - On Liquid media
  - Automated systems
- Non-culture methods

Culture methods

The specimens received in the laboratory are plated on the culture media. The appropriate culture media is selected depending upon the bacteria suspected. The following precautions need to be taken into consideration when the culture methods are processed

- Optimal atmospheric conditions
- Optimal temperature
- Growth requirement of the bacteria

Atmospheric conditions:

Colonies of bacteria are usually large enough to identify after 18–24 hours of incubation (usually at 37°C), but for some bacteria longer incubation times are required (from 2 days to several weeks). Culture plates are incubated (1) in air, (2) in air with added carbon dioxide (5%), (3) anaerobically (without oxygen) or (4) micro-aerophilically (a trace of oxygen) according to the requirements of the different types of bacteria that may be present in specimens.

In case of Mycobacteria especially the scotochromogen the culture bottles are placed in dark or the bottles are covered with black paper and kept for incubation at 37°C.

Temperature:

Most of the bacteria requires a temperature of 37°C for optimal growth. This temperature is provided placing the inoculated culture plates in the incubator set at 37°C temperature.

Notes

Fig. 10.5: Incubator

Growth requirement of the bacteria

Different bacteria have different growth requirements. For eg Streptococcus pneumoniae requires factor V and factor X for its growth, which are found in chocolate agar. Thus for sample suspected of S. pneumoniae the samples are plated on chocolate agar. Similarly depending upon the growth requirements the appropriate culture media are used.

INTEXT QUESTIONS 10.2

1. .... & .... methods are commonly used methods for bacterial isolation
2. Colonies of bacteria can be identified after .... hours of incubation
3. The optimum temperature most bacteria require to grow are ....
4. Chocolate agar has .... & .... which is used in the diagnosis of streptococci Pneumonia

10.4 CULTURE ON SOLID MEDIA

The principal method for the detection of bacteria from clinical specimens is by culture on solid culture media. Bacteria grow on the surface of culture media to produce distinct colonies.

Different bacteria produce different but characteristic colonies, allowing for early presumptive identification and easy identification of mixed cultures. There are many different types of culture media. Agar is used as the gelling agent to which is added a variety of nutrients (e.g. blood, peptone and sugars) and other factors (e.g. buffers, salts and indicators).

Some culture media are nonselective (e.g. blood agar, nutrient agar) and these will grow a wide variety of bacteria. While some e.g. MacConkey agar are more selective (in this case through the addition of bile salts selecting for the 'bile-tolerant' bacteria found in the large intestine such as *Escherichia coli* and *Enterococcus faecalis*). MacConkey agar also contains lactose and an indicator system that identifies lactose-fermenting coliforms (e.g. *Escherichia coli, Klebsiella*) from lactose-non fermenting coliforms (e.g. *Morganella Salmonella*). Media can be made even more selective by the addition of antibiotics or other inhibitory substances, and sophisticated indicator systems can allow for the easy detection of defined bacteria from mixed populations.

Method of inoculating the solid culture media

Method used for inoculating the solid media depends upon the purpose of inoculation- whether to have isolated colonies or to know the bacterial load of the sample (quantitative analysis).

For obtaining the isolated colonies streaking method is used, the most common method of inoculating an agar plate is streaking.

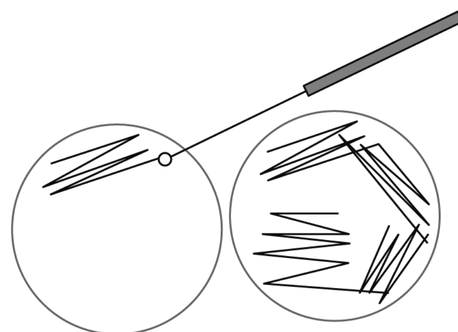


Fig. 10.6: Streaking method

Notes

Streak plates

1. A small amount of sample is placed on the side of the agar plate (either with a swab, or as a drop from an inoculating loop).
2. A sterile loop is then used to spread the bacteria out in one direction from the initial site of inoculation. This is done by moving the loop from side to side, passing through the initial site.
3. The loop is then sterilised (by flaming) again and the first streaks are then spread out themselves.
4. This is repeated 2-3 times, moving around the agar plate as shown in the figure.

In this method single bacterial cells get isolated by the streaking, and when the plate is incubated, forming discrete colonies that will have started from just one bacterium each.

For quantitative analysis or semi quantitative analysis of the sample for example in case of urinary tract infection. In fact E.*coli* is implicated as the causative organism in urinary tract infection only if there are $>10^5$Colony forming units per millilitre of urine. The method of inoculating the solid culture media is as shown in the figure.

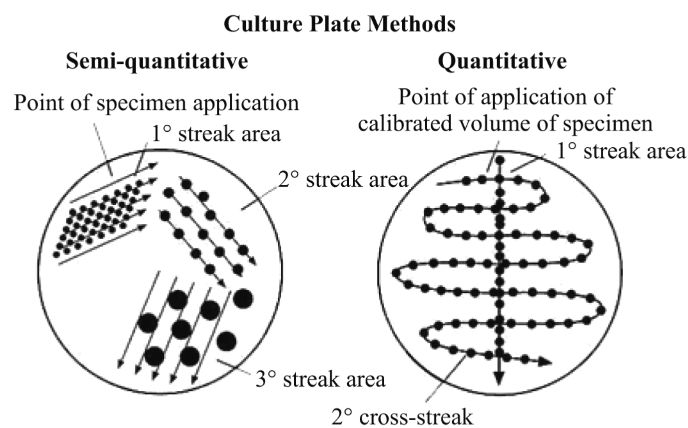


Fig. 10.7: Inoculation methods

Fig. 10.8: Uninoculated Mac conkey Agar and Blood agar plate

Notes

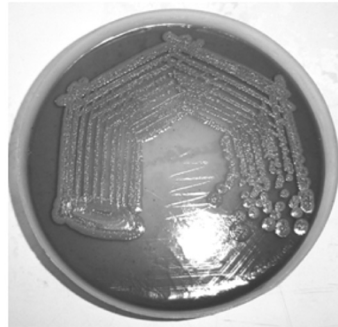


Fig. 10.9: Lactose fermenting (pink coloured) colonies on mac conkey agar

Culture in liquid media

Bacteria can also be grown in liquid media (broth). Like agar plates, broth cultures may be non selective or selective. Bacterial growth is easy to detect as the clear liquid turns turbid, usually within 24–48 hr, but incubation may need to be extended to 14 days or more.

The advantage of broth culture is that it is significantly more sensitive than direct culture on agar. The disadvantage is that, by itself, it is not easy to determine the type of bacteria present or whether a mixed growth has occurred, and in most cases the broth must be subcultured onto solid agar plates. This causes an additional delay in culture results. Broth cultures are also prone to contamination.

Broth enrichment media are used when high sensitivity is required e.g. for detection of bacteria from CSF, or to detect small numbers of *Salmonella* in a stool sample containing many millions of other bacteria.

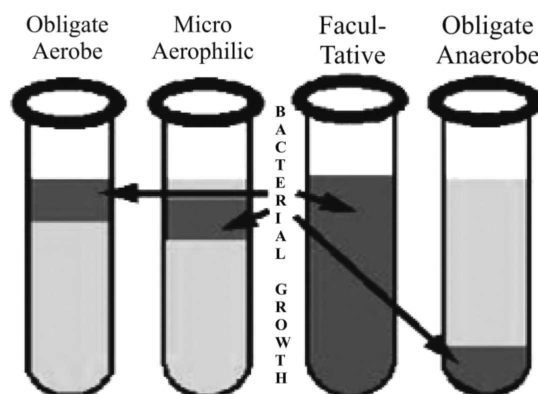


Fig. 10.10: Liquid media

Automated system

Automated blood culture systems eg. BACTEC, BacteAlert utilize liquid culture. Bacterial growth may be detected by a variety of methods (e.g. detection of bacterial CO_2 production).

Notes

Fig. 10.11: Bactec

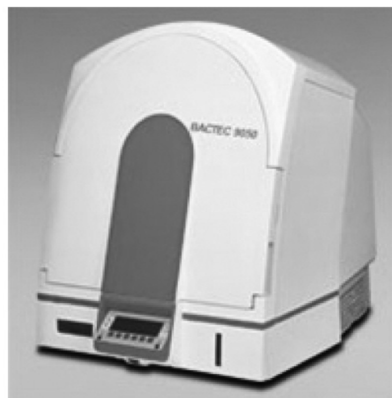


Fig. 10.12: Bactec

Automated liquid culture systems are also available for the culture of Mycobacteria, and similar technology can be used to automate sensitivity

The advantage of automated system are

Rapidity : they aid in faster growth of bacteria. Thus less time consuming.

The incidence of contamination during the processing of sample are minimised

Real time monitoring of the growth

One of the main limitations is the commercial viability.

Non culture methods

Isolation of bacteria can also be carried out by non-culture methods. In particular the more advanced Amplification techniques like Polymerase chain reaction (PCR), ligase chain reaction (LCR), strand displacement amplification (SDA), and nucleic acid sequence based amplification (NASBA) are being used in clinical laboratories for isolation and identification of bacteria.

The following are some of the factors that are considered in interpreting bacteriological culture results:

- type of specimen
- any delays in processing
- types of bacteria recovered
- knowledge of the normal human flora at different sites
- clinical information provided on the request form
- details of recent antibiotic therapy

There must be good liaison between healthcare workers and the microbiology laboratory, in order to ensure that the most appropriate investigations are performed, results are interpreted correctly, and clinically relevant bacteriological reports are produced.

INTEXT QUESTIONS 10.3

1. .... is used as gelling agar in culture media
2. .... culture media grow a wide variety of bacteria
3. .... is an example of selective media
4. For obtaining the isolated colonies .... method is common method of inoculating
5. .... is the liquid medium in which bacteria may be grown
6. Examples of Amplication techniques are ...., ... & ....

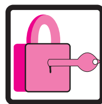
WHAT YOU HAVE LEARNT

- Isolation of bacteria forms a very significant step in the diagnosis and management of the illness. Isolation of bacteria involves various steps – Specimen collection, Preservation and transportation of specimen, Microscopic examination of sample.Various methods used for isolation of bacteria culture methods which includes culture on solid or liquid media and automated system. Non culture methods include the molecular techniques eg PCR, SDA, NASBA.

Notes

TERMINAL QUESTIONS

1. What is the need for isolation of bacteria?
2. Describe in brief various steps involved in the isolation of bacteria.
3. What is difference between blood agar and chocolate agar
4. Explain the term selective and non selective media with proper examples.
5. Draw a labeled diagram of inoculation of solid culture media for isolation of bacteria.
6. Draw a labeled diagram for inoculation of solid media for processing the urine sample of a patient suspected of urinary tract infection.
7. Describe in brief the advantages and the limitation of use of liquid culture media for isolation of bacteria.
8. Mention the advantages and the disadvantages of automated system for isolation of bacteria.
9. Name some non culture methods for isolation of bacteria



ANSWERS TO INTEXT QUESTIONS

10.1

1. High-risk
2. Charcoal
3. 4°C
4. Gram stain
5. Ziehl-Neelson
6. Dark Ground

10.2

1. Direct culture & Non-culture
2. 18-24
3. 37°C
4. Factor V & Factor X

Notes

10.3

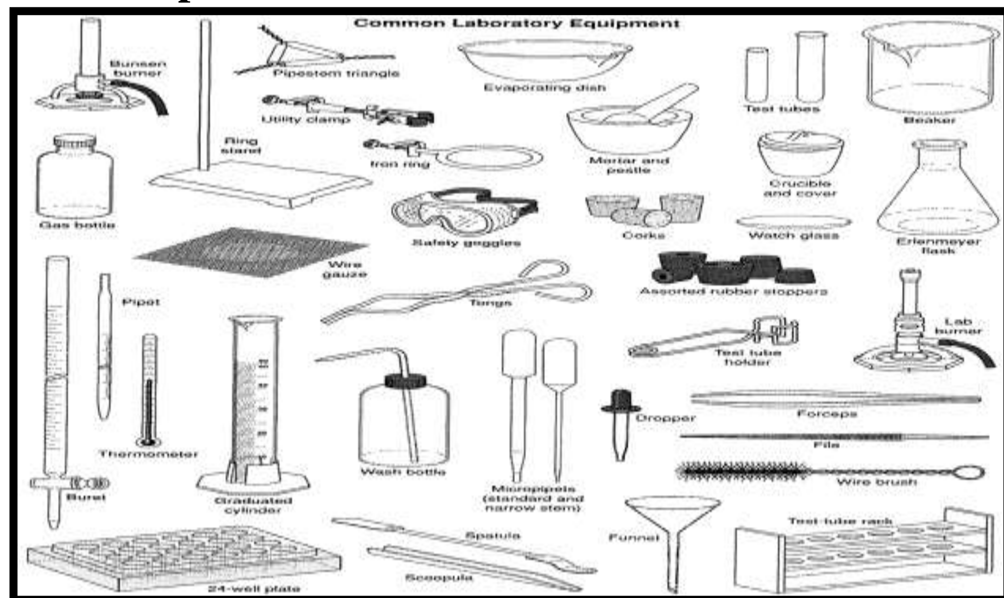
1. Agar
2. Non-selective
3. MacConkey
4. Streaking
5. Broth
6. Polymerase Chain Reaction, Ligase Chain Reaction, Nucleic Acid Sequence Based Amplification

Fungi Isolation:

Fungus spread in many environments heavily where there is no place free of the presence of one or more type of fungus or spores،Which can be isolated from soil, air or water, fungi parasitize humans and plants and less on animal causing diseases and economic losses, so it is necessary to isolation and Diagnosis these fungi to reduce the danger.

Materials and tools:

- ❖ **Slides and cover slips**
- ❖ **Petri dishes**
- ❖ **Burner**
- ❖ **Gloves**
- ❖ **Lactophenol cotton blue (LPCB)**
- ❖ **Pipettes**
- ❖ **Inoculating needle**
- ❖ **Flask and bakers**
- ❖ **Test tube**
- ❖ **Media**
- ❖ **Microscope**



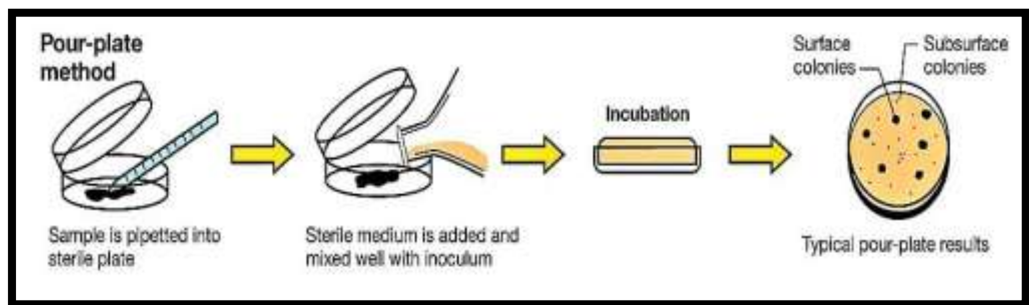
Materials and tools in laboratory

1- Isolation fungi from soil

a- Pour plate methods (Direct Isolation)

- Weighted 0.1 gm of soil and placed it in Petri dish.
- Poured the culture media in Petri dish near flame .
- Moved the Petri dish with a circular movement right and left for mixing the soil with media .
- Leave the plate until it solid , then incubated for 5 days at 25-28 °C.

  Not: Can distribution the soil sample on the surface of solid culture media .



Pour plate method

b- Indirect isolation :

It's a modified method of poured plate methods, use to Purification fungi from bacteria .

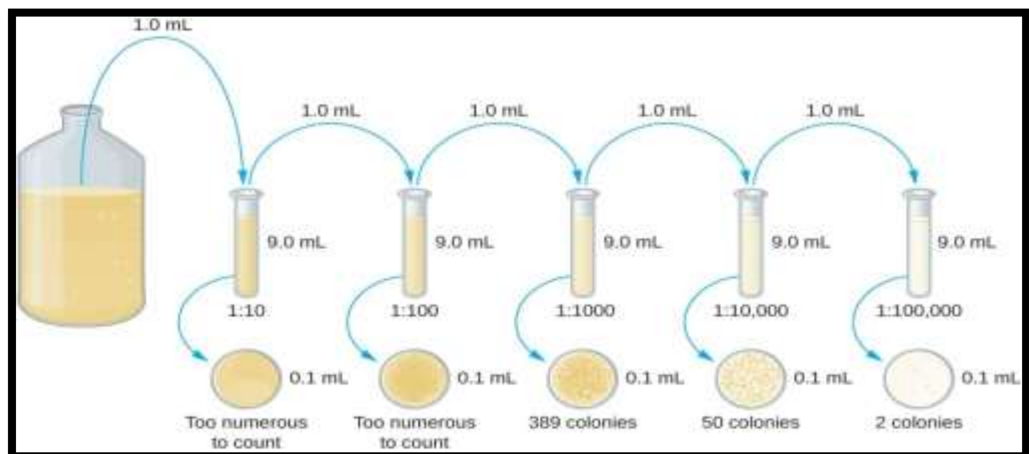
- Add 10 ml of culture media in Petri dish and left to solid.
- Placed 0.1 gm of soil sample above the culture media .
- Poured 5 ml of culture media on the plate and left to solid.

In this case hyphal fungi grow to high because it **Aerobic** organisms while the bacteria (**anaerobic**) stay within the culture media .

c- Dilution method:

- Weighted 1 gm of soil and placed it in test tube contain 10 ml Distilled water(DW) (stoke), Mixed it well.
- Prepare 5 test tube each contain 9 ml of (DW).
- Take 1ml of stoke to the first tube that will be first dilution 1/ 10 (10^{-1}).
- Take 1ml of the first dilution to the second tube that will be the second dilution 1/ 100 (10^{-2}).
- Take 1ml of second dilution to the third tube that will be the third dilution 1/ 1000 (10^{-3}).
- Take 1ml of third dilution to the fourth tube that will be the fourth dilution 1/ 10000 (10^{-4}).
- Take 1ml of fourth dilution to the fifth tube that will be the fifth dilution 1/ 100000 (10^{-5}).
- Placed 1 ml of each filtrate dilution in a Petri dish and then poured the culture media, moved the dish for mixing the sample with the culture media.
- Leaf it until solid , then incubated for 5 days at 25-28°C.

Not: The dilution (1ml) can be distributed on surface of solid culture media .



Dilution method

2- Isolation fungi from air:

- Poured the media on Petri dishes and leave it until solid .
- Open the dished in several different places for 30 min.
- Incubated for 5 days at 25-28 °C.

3- Isolation fungi from water:

a- Dilution method:

- Take 1ml of water sample placed it in test tube contain 9ml of (D.W) , that will be first dilution 1/ 10 (10^{-1}).
- Take 1ml of the first dilution to the second tube that will be the second dilution 1/ 100 (10^{-2}).
- Take 1ml of second dilution to the third tube that will be the third dilution 1/ 1000 (10^{-3}).
- Take 1ml of third dilution to the fourth tube that will be the fourth dilution 1/ 10000 (10^{-4}).
- Take 1ml of fourth dilution to the fifth tube that will be the fifth dilution 1/ 100000 (10^{-5}).
- Placed 1 ml of each filtrate dilution in a Petri dish and then poured the culture media, moved the dish for mixing the sample with the culture media.
- Leaf it until solid , then incubated for 5 days at 25-28°C.

  Not: The dilution (1ml) can be distributed on surface of solid culture media .

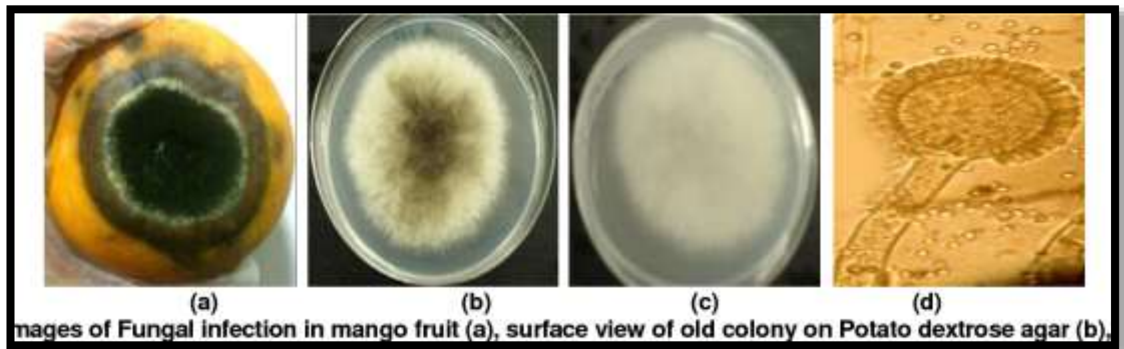
b- Filtration methods:

- Filtrate the water sample using cellulose filter Placed in a sterile suppression.
- After the passage the sample, Filter paper taken by sterile forceps and placed on surface of culture media then incubated the dishes for 5 days at 25-28 °C.

Filtration methods

4- Isolation fungi from fruits and vegetables:

- Placed the media into the plate and left it until solid .
- Surface sterilization to rotten part by **Sodium Hypochlorite or** Potassium Permanganate (2%) .
- Washing the sample by (D.W) 2-3 times .
- Transfer part rotten to the Petri dish by a sterile needle.
- Incubated the dishes for 5 days at 25-28 °C.



Isolation fungi from fruits

Not: Sometimes **baits** used to attract certain types of fungi, where a growth appear clear on these baits then transferred to the culture media , the baits may be some type of fruits , seeds, skin, hair .