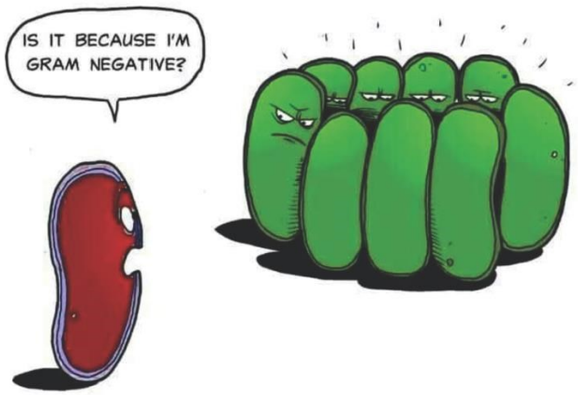
23. Asexual reproduction by simple cell division/fission is seen in
(1) filamentous fungi and colonial filamentous cyanobacteria. (2) unicellular protists and filamentous fungi.
(3) colonial filamentous cyanobacteria and unicellular fungi.
(3) bacteria and colonial unicellular cyanobacteria.
(5) colonial non filamentous cyanobacteria and bacteria. 38/2022

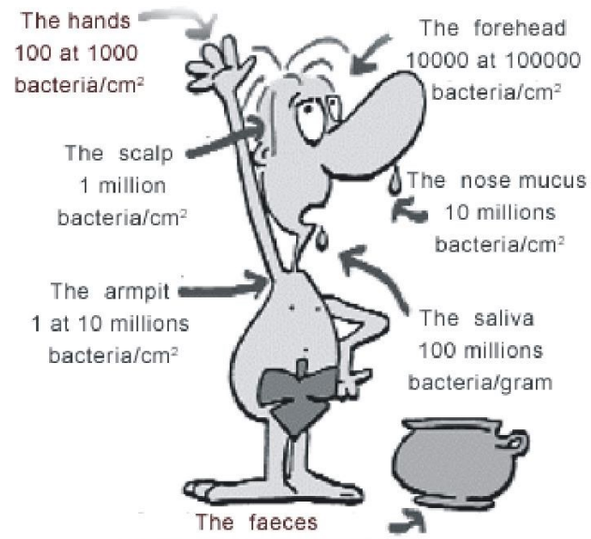
24. Which of the following statements regarding viroids and prions are correct?
A - Creutzfeldt-Jakob disease is a human disease caused by prions.
B - Viroids carry signals for their multiplication in host plant cells.
C - Viroids have a short piece of DNA protected by a protein coat.
D - Nucleic acids in prions replicate with the help of host genes.
(1)A and B only. (3) A and D only. (5) B and D only. B (2) A and C only. (4) B and C only. 36/2023



Microbiology

LANKA ADVANCED LEVEL BIOLOGY ENGLISH MEDIUM

1



Unit 09 Microbiology

9.1.0 : Investigates diversity and handling of microorganisms
9.1.1: Explores the diversity and nature of microorganisms
9.1.2: Explores some basic laboratory techniques in microbiology

SAMPATH LANKADHEERA
Voice/SMS: 0777211384 B.Sc. (Hons), M.Sc.

12. Which of the following diseases is caused by anaerobic bacteria?
(1) Cholera (2) Tetanus (3) Rabies (4) Tuberculosis (5) AIDS

13. What is the name given to spherical shape bacterial cells arranged in an array?
(1) *Bacillus* (2) *Diplococcus* (3) *Streptococcous* (4) *Staphylococcus* (5) *Streptobacillus*

14. Which of the flowing is incorrect regarding microorganisms?
(1) They are the most abundant group of organisms in the biosphere
(2) They are the fastest reproducing organisms
(3) They play an important role as primary producers in land ecosystems
(4) They show four different types of nutrition (5) They are the major decomposes on earth.

15. Which of the following statements is incorrect regarding viruses?
(1) Most plant viruses contain DNA (2) Animal viruses contains either DNA or RNA
(3) Some viruses contain enzymes (4) All viruses are obligate parasites
(5) Viruses are used in DNA recombinant technology.

16. Which of the following methods is suitable to sterilize a liquid culture medium containing blood serum?
(1) Pasteurization (2) Autoclaving at 121^{0}C for 10 minutes (3) Boiling at 100^{0}C for 10 minutes
(4) Filtration using a sterile membrane filters (5) Freezing at 20^{0}C

17. Which of the following is correct regarding chemo-autotrophic bacteria?
(1) They use organic compounds as the source of energy (2) They obtain carbon from organic compounds
(3) They use light as the source of energy (4) Some use nitrate as the source of energy
(5) All fix atmospheric nitrogen

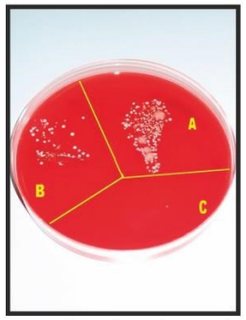
18. Which of the following is incorrect regarding viruses?
(1) They do not show a cellular organization (2) They are obligate parasites
(3) DNA or RNA may exist as a double stranded or single stranded form in the viral genome
(4) Some viruses contain enzyme polymerase in the Capsid
(5) They are not found in the natural habitats like soil or water

19. Fungi differ from bacteria because fungi
(1) are saprophytic (2) have absorptive nutrition (3) produce antibiotics (4) are eukaryotic
(5) reproduce asexually

20. Various steps involved in the simple staining procedure of microorganisms in a sample of toddy are given below in an incorrect sequence. Find the correct sequence
A - Preparation of a thin smear on a slide B - Heat fixing of the smear
C - Addition of methylene blue stain and leaving for 30 second D - Air drying of the smear
E - Washing the smear with water, drying and microscopic examination
(1) A, B, C, D (2) A, D, B, C, E (3) A, B, D, C, E (4) A, D, C, B, E (5) E, C, D, B, A

21. Which of the following statements is correct regarding the culture media used to grow microbes in the laboratory?
(1) Agar in culture media provides the suitable pH range for the growth of microorganisms.
(2) Glucose is generally used to prepare culture media to grow fungi.
(3) Culture media for bacteria are prepared using potatoes.
(4) Any microorganism can be cultured in a culture medium.
(5) Sodium chloride is usually added to all culture media. 39/2019 new

22. Which of the following statements regarding microorganisms is correct?
(1) Almost all mycoplasmas are parasites of animals and plants.
(2) Fungi are chemoheterotrophs which show saprophytic or parasitic modes of nutrition.
(3) Purple non-sulphur bacteria utilize light as the source of energy and CO, as the source of carbon.
(4) Streptococcus bacteria divide in multiple planes.
(5) In cyanobacteria, nitrogen fixation is catalyzed by nitrogenase enzyme present in akinetes. 37/2020



Microbial growth on a cultivation plate without procedures (A),
after washing hands with soap (B) and after disinfection with alcohol (C).

MCQ

1. Which of the following sentence is incorrect regarding viruses?
(1) Viruses can transmit from one host to another (2) Viruses particles produce energy from respiration
(3) Viruses can be cultured in live culture media (4) Viruses can be crystallized (5) Viruses are host specific.

2. What is incorrect regarding bacterial cell and fungal cell?
(1) Ribosomes are present in both cells (2) Mitosis present in both cells
(3) Cell walls present in both cells (4) Fungal cell contain mitochondria and it's absent in bacterial cell
(5) Nucleus present in fungi not absent in bacteria

3. Which of the followings are not inhabited by bacteria?
(1) Surface of human skin (2) Healthy person's blood (3) Sea water (4) Surface of leaves (5) Inspiratory air

4. Which of the following feature is present in both bacteria and cyanobacteria?
(1) Phycocyanin pigments (2) Prokaryotic (3) Heterotrophic (4) Cell wall containing chitin (5) Bear flagella

5. Viruses are deviated from other living organisms because they do not contain
(1) Proteins (2) Nucleic acids (3) Cellular organization (4) Hereditary material
(5) No reproductive mechanism

6. Which of the following best to sterilization of Petri dishes?
(1) Autoclave (2) U.V rays (3) Steam (4) Exposure to chemical fumes (5) Hot dried air

7. Which of the flowing statement is incorrect regarding viruses?
(1) Cannot observe under L.M. (2) Contain DNA or RNA as nucleic acids (3) Can undergo mutation
(4) Prokaryotic organisms (5) Contain protein capsule

8. Which of the flowing methodologies are use to sterilize agar medium in the laboratory
(1) Intermittent heating to 100^0C for 3 days (2) Heating under normal pressure at 100^oC 10min
(3) Heat at 121^0C under a pressure of 15 pounds per square inch (4) Heat in an oven for 2 hrs at 160^0C
(5) Filtering through a mili-pore filter

9. Which of the following is incorrect regarding viruses?
(1) DNA or RNA present as genetic material (2) Nucleic acids are covered by a protein capsule
(3) They can replicate only within living organisms (4) At low temperatures they lose the ability of infection
(5) Some viruses are spread with droplets while sneezing

10. Which of the following is incorrect regarding cyanobacteria?
(1) They act as indicators of water pollution (2) They are important as indicators of water pollution
(3) They contain chlorophyll "a" as the core pigment (4) Mucopeptides are present on the cell wall
(5) They reproduce asexually by heterosists.

11. Which of the following sterilization method can be used to sterilize laboratory pipettes?
(1) Dry heat (2) Wet heat (3) Open flame (4) U.V. (5) Ethylene oxide

9.1.1: Explores the diversity and nature of microorganisms

Nature of microorganisms

- Microbiology is the study of organisms that are too small and are not …… ……..…….. to the naked eye or …….. eye when they exist individually.
- These organisms are referred to as microorganisms.
- Microorganisms include ……..……..…., ……..., ……../ …… (BGB), …… and ……...
- ……. such as ……. and ……., ……..., …….. and …….. are also studied under microbiology.

Microscopic nature of microorganisms

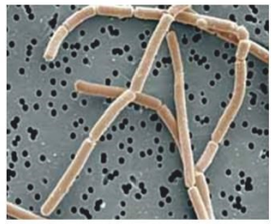
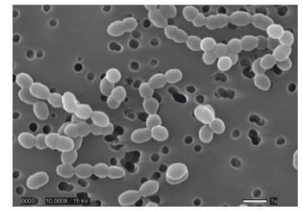
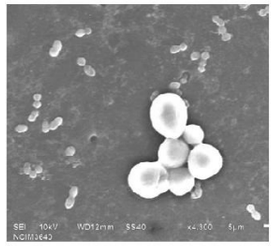
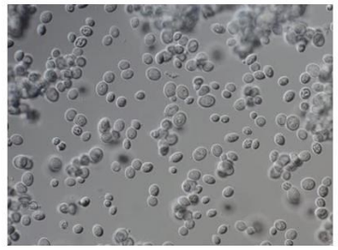
- In general, microorganisms are less than ……... in size and cannot be observed with unaided eye. Therefore, they must be observed with a microscope.
- Microorganisms and their structural components are measured in micrometers and nanometers.

  1 micrometer (μm) = 10^{-6} m

  1 nanometer (μm) = 10^{-9} m
- Some microorganisms are more readily visible than other because of their larger size.

Ubiquitous nature of microorganisms:

- Microorganisms are ……. on earth. They are found in ……..., ……..……., ……... and ……. and …….. surfaces of other organisms. Marine and freshwater microorganisms form the basis of food chain in oceans and freshwaters.
- Some of them do ……... and are ……... in aquatic environments. Soil microorganisms help …….. of chemical elements between soil, water, air and living organisms.

For yoghurt/curd sample	
 Lactobacillus	 Streptococcus
For toddy sample	
 Sample of Toddy	 Bakers Yeast

PRACTICAL NO.38

- Use of alcohol and other disinfectants to control microorganisms

Objectives

- Students should be able to,
- develop the skills to control microorganisms
- explain what disinfectants are
- Materials and equipment
  1. Nutrient agar medium.
  2. Sterilized petri dishes.
  3. Bunsen burner.
  4. Phenol/ Lysol/ chlorine compound.
  5. Alcohol

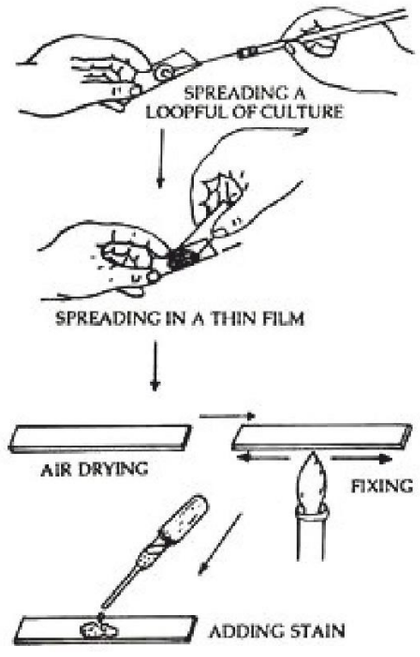
Instructions

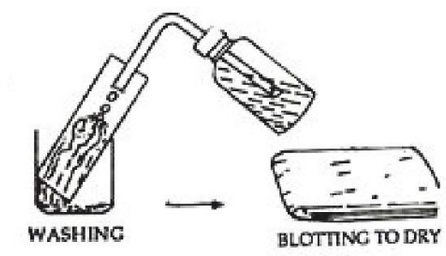
- Assist students to do the following;
  1. Prepare two sets of sterilized Petri dishes with solidified nutrient agar medium.
  2. Expose them to the air for about 10 minutes.
  3. Close one set of Petri dishes after rinsing with alcohol/ phenol/ Lysol/ Chlorine compounds
  4. Other set without detergent should be kept closed.
  5. Observe both for number of colonies after 24-48 hours of incubation
  6. Ask students to record their observations and comment.

- Emulsify the scrapings in the drop of water and spread the suspension in the shape of a circle (the smear should be very thin)

B - For toddy.
- Do not use water as the microorganisms are already suspended in water.
- Follow other steps as above
- Let the smear air dry

Common to both A and B
- Heat fix the smear by passing the slide through a flame two or three times. (Do not heat fix until the smear is completely air dried)
- Flood the prepared, heat – fixed smear with 2 or 3 drops of Methylene Blue and allow 30-60 seconds for the stain to act.
- Wash with tap water to remove the excess stain and gently blot the smear with blotting paper and let it dry.
- Examine the stained smears under the microscope
- Make the students to observe and note the colour of the stained bacteria and yeast
- Instruct them to make appropriate diagrams of bacteria and yeast.
- Direct the students to distinguish between bacteria and yeast.





- Microorganisms suspended in air as ……..., have the opportunity to travel long distances with the …….. current and ……....
- Pathogenic bioaerosols, cause opportunities for disease spreading. Only a ……. of microorganisms that associate with other organisms such as plants, animals and human are …….
- ……... of them are ……..… or ……....
- **However, ……. viruses are ……...to the organisms they attached to.**
- Some microorganisms are capable of inhabiting ……... environmental conditions that are unfavorable or even lethal for other organisms.
- Such microorganisms are known as ……..
- Extremophiles have been found inside the Earth's crust, deep sea at …… ……., …… …… or …….. …… basic conditions, …… ……., …….. sea water and anaerobic conditions.
- Extremophiles are classified according to the conditions in which they grow.

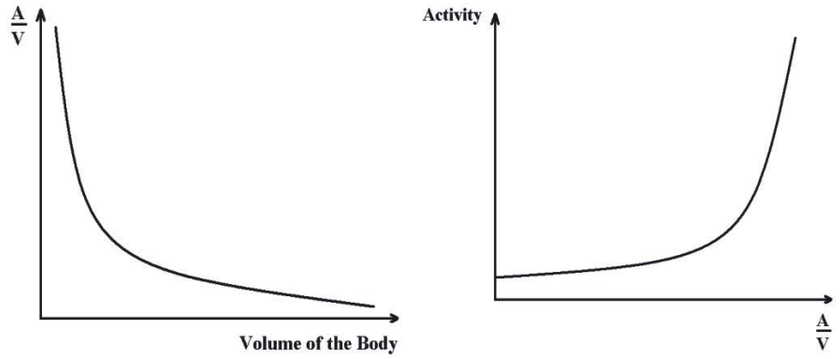
Type of Extremophile	Condition
Thermophiles (40^0C—70^0C)	high temperatures
Psychrophiles	low temperatures
Acidophiles	acid pH
Alkaliphiles	basic pH
Halophiles	require NaCI
Barophies	high pressure

Table 9.1: Types of Extremophiles

- Some of these extreme environments consist of more than one extreme condition. For example, Many hot springs are acidic or alkaline in nature, at the same time deep seas are cold and remain in high pressure.
- Microorganisms live in such environments are adapted to live with more than one extreme condition.

High growth rate of microorganisms:

- Rates of growth and reproduction of microorganisms are high.
- Microorganisms possess a ……... surface area/volume ratio due to their ……...…….. size.
- This means that they have large surface area available for exchange of materials from external environment.
- As a result, ……. rate of materials in to the inside of cells and the exit of waste materials to the outside of the cells increases and results in high metabolic rate.
- Therefore, average …… ……. or the time required to double the population of microorganisms is relatively less.



Morphological, nutritional, and physiological diversity of microorganisms:

- Microorganisms possess diverse morphological forms.
- Bacteria possess diversity in their shapes, basically three distinct shapes;
  1. Rod shape - …….
  2. Spherical shape - …….
  3. Spiral shape - …….
- The Coccus bacteria may arranged in different forms;
  1. …….
  2. …….
  3. …….
  4. …….
  5. …….
  6. …….
- Bacillus bacteria may arranged in to either

Observing Microorganisms

- Most microorganisms appear almost ……. when viewed through a standard …… …….. we have to prepare them for observations. One of the ways this can be done is by staining, means coloring the microorganisms with a dye.
- However, before the microorganisms stain, they must be …….(attached) to the microscopic slide. A simple stain is an ……... or ……solution of a single ……... dye.
- The primary purpose of a simple stain is to ……. the entire microorganisms so that ……..., ……... and basic structures are visible. Some of the simple stains commonly used in the laboratory are …… ……..., ……. and ……....

PRACTICAL NO. 37

- Staining of microorganisms found in toddy and yoghurt using a simple stain (Methylene Blue)

Objectives

- Students should be able to

1. prepare smears from solid and liquid samples,
2. stain the smear using a simple staining technique,
3. observe the stained microorganisms under the microscope.
4. Materials and equipment
5. Toddy and yoghurt /curd sample
6. Methylene Blue (dilute solution)
7. Slides and cover slips
8. Inoculating loops
9. Bunsen burner
10. Distilled water
11. Light microscopes
12. Marking pen or wax pencil

Instructions

- Instruct the students to carry out the following procedure.

1. Preparation of smear

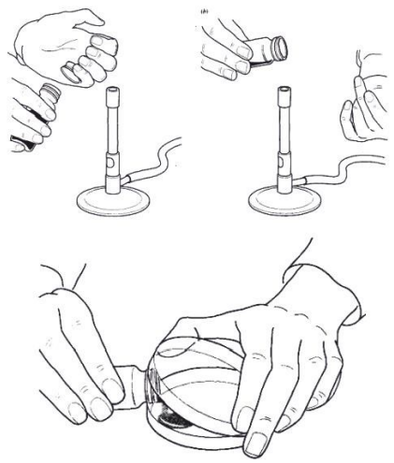
- Clean slides with cleanser, rinse and air dry
- Handle the clean slides by their edges, preferably using a pair of forceps
- Use marker pen or pencils to label each slide according to the sample used (toddy, yoghurt / curd).

A - For yoghurt/curd

- Place 1 or 2 loops full of distilled water on the centre of one slide using the sterilized inoculating loop.
- Heat the loop until it is red hot and allow to cool.
- Scrape a small amount of the sample using the cooled loop.

Instructions

1. Prepare Nutrient agar and PDA from the given material.
2. Let students sterilize the solutions by autoclaving at 121^0C for 15 min (15lb/sq in.)
3. Assist students to prepare nutrient agar and PDA plates as given below.
4. Pour about 15 ml of the sterilized Nutrient Agar and PDA into sterilized Petri dishes, using aseptic techniques.
5. Set aside to solidify.

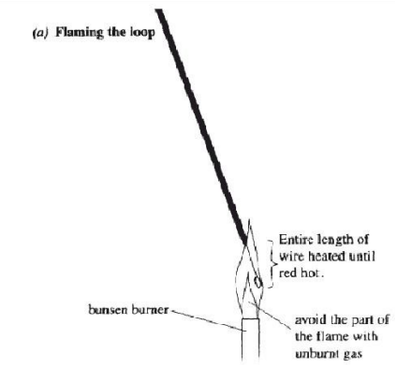


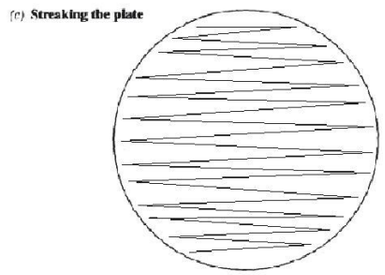
Inoculation of the plates:

1. Label the bottom of each agar plate using a marker pen.
2. Flame the inoculating loop to redness, allow it to cool and aseptically obtain a loop full of the sample. Eg. toddy and yoghurt
3. Place a loop full of sample on the agar plate near the edge of the dish and streak on the agar surface in a zig zag pattern
4. Incubate for 24-48 hr. at room temperature

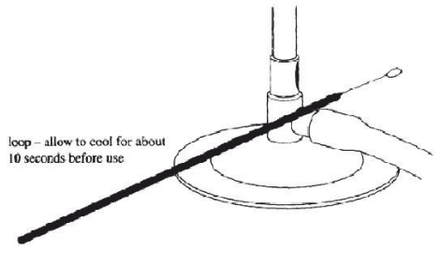
Observe the colonies on incubated plates

1. Instruct the students to record the practical highlighting the following.
2. Preparation of microbiological culture medium
3. Inoculation and incubation of culture plates
4. Observation of colony types

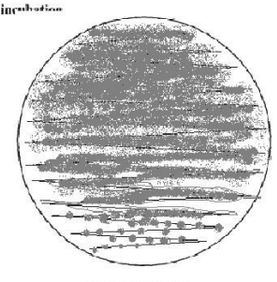




(b) Cooling the loop



(d) Final appearance after incubation



individual colonies formed from individual bacteria

1. ……
2. ……

- Spiral bacteria may arranged in to either
  1. ……
  2. ……
  3. ……
- Cyanobacteria exhibit a great variety of shapes and arrangements,
  1. ……
  2. ……
- Multi cellular Cyanobacteria may appear as either
  1. ……
  2. ……
- Filamentous appear as …….. and the non-filamentous appear as …….. or colonies forming spherical, cubical, square or irregular shape.
- Two morphological varieties are found in viruses based on their symmetry of protein coats;
  1. ……
  2. ……
- In fungi, some of them are unicellular and others multicellular, consists of a mass of fine tubular branching threads known as hyphae, collectively form mycelium. Hyphae may be …… or ……..
- ……are smaller proteinaceous particles.
- Unicellular protists possess wide range of morphological diversity.
- ……..are ……...(variable shapes).
- Microorganisms show a diversity of nutritional types. Based on the sources of carbon and energy, nutritional types of microbes are classified.
- There are four major nutritional types seen among microorganisms; ……., ……., ……... and ……..
- Based on the utilization of O_2(g), microorganisms can be classified in to four physiological groups; ……. aerobes, ……. anaerobes, ……. anaerobes and ……..
- Some microbes capable of fixing atmospheric molecular nitrogen, show physiological diversity; free-living nitrogen fixing microbes and symbiotic nitrogen fixing microbes.

Types of microorganisms

1. **Bacteria**

- Bacteria (singular, bacterium) are single-celled (unicellular) prokaryotic organisms.
- They show different morphological forms and arrangements.
- The most obvious structural feature of bacteria is the shape of individual cells. There are three basic shapes.
  1. Spherical; Coccus (plural – Cocci)
  2. Rod shape; Bacillus (plural - Bacilli)
  3. Spiral shape; (plural – Spirilli)
- During cell division, cells can remain attached to each other and form different forms of cell arrangements.

1. **Different forms of cell arrangement of coccus bacteria (Table9.2)**

Coccus	
Diplococcus	
Streptococcus	
Tetrad	
Sarcinae	
Staphylococcus	

Preparation of culture media

- Microorganisms cannot be studied in their natural habitat such as in soil, water or air. Therefore, we need to bring them to the laboratory and provide similar conditions for their growth and reproduction.
- A nutrient material prepared for providing nutrition and anchorage essential to the growth of microorganisms at laboratory condition is called a culture medium.
- Not all microorganism can be grown on a laboratory culture media.
- They are called non-culturable microorganisms. Some microorganisms grow well on any culture medium whereas other microorganisms require special medium.
- Suppose we want to grow a culture of microorganisms present in a certain soil sample.
- The culture medium should contain necessary nutrients, sufficient moisture and suitable PH.
- This medium must initially be sterile which means it should not contain any living microorganisms. Therefore, when preparing a culture medium all glassware and liquid nutrient solutions should be sterilized.
- Nutrient agar (NA) and potato dextrose agar (PDA) are two general media, commonly used to grow bacteria and fungi respectively.
- Nutrient agar is made up of peptone, meat extract, sodium chloride, agar and distilled water. Whereas, PDA is made up of potato, glucose, agar and distilled water.
- Here, agar is used as a solidifying agent. Agar solidifies at temperatures below 40 ^{0}C which means a culture medium containing agar is a solid medium. For growing microorganisms, solid culture medium is usually contained in Petri dishes or test tubes.

PRACTICAL NO. 36

- Preparation of a microbiological culture medium (Nutrient Agar/ Potato Dextrose Agar), inoculation with a sample of toddy and yoghurt and observation of microbial colonies.

Objectives

- Students should be able to

1. Prepare a microbiological culture medium,
2. Inoculate the culture medium with an inoculum,
3. Distinguish various types of colonies of microorganisms after incubation.

Materials and equipment

- 150 ml flask with screw cap or cotton wool plug
- 100 ml graduated cylinder
- Sterilized glass rod
- Sterilized Petri dishes
- Inoculating loop
- Bunsen burner
- Autoclave

For nutrient agar	**For potato Dextrose agar**
• Peptone 10 g • Beef extract 10 g • Sodium chloride 05 g • Agar 15 g • Distilled water 1000 ml	• Potato 200g • Glucose 20g • Agar 15g • Distilled water 1000ml

- Toddy and yoghurt sample

PRACTICAL NO. 35

Sterilization of water, culture media, glassware, heat labile substances and inoculating needles

Objectives;

- Students should be able to identify suitable technique for sterilization of a given material
- describe the procedure for different sterilization techniques

Materials and equipment

1. Autoclave/ Pressure cooker
2. Oven
3. Culture media
4. Inoculating needles
5. Cotton wool
6. Pipettes
7. Conical flasks
8. Petri dishes
9. Beakers

Instructions

- Instruct the students to follow the techniques used in sterilization.

a) Sterilization by dry heat (using direct flame)

i. For inoculating needles, loops and such materials which will not be damaged by heat. Hold in flame of Bunsen burner until red hot.

ii. In the case of scalpels, metal spatulas and glass rods dip in methylated spirits or ethyl alcohol. Allow excess spirit to drip off and flame the instrument in the Bunsen flame.

b) Sterilization by dry heat (in the hot air oven)

- For sterilization of dry glassware such as Petri dishes, flasks and pipettes.
- Prepare glassware for sterilization as follows: -
- Wash glassware, clean and wipe dry thoroughly.
- Wrap the glassware in Aluminum foil or wrap in a paper or store in a suitable container (canister).
- For conical flasks, plug the mouth with clean cotton wool and cover the plugs with Aluminum foil.
- For pipettes, plug mouth with cotton wool and burn the cotton fibres sticking out using a Bunsen flame.
- Wrap the pipettes individually in Aluminium foil or paper or store in a suitable container (canister).
- Store all prepared glassware in an oven, at a temperature of 170 0C for 1-2 hrs depending on the amount of glassware in the oven. Keep the oven door tightly closed.

c) Sterilization in an autoclave (wet heat).

For sterilization of water/ culture media

- Prepare the glassware for autoclaving according to the procedure outlined above.
- Place the prepared liquid culture media or water in test tubes, flasks or bottles as appropriate.
- Plug the containers with cotton wool and cover with Aluminium foil or paper.
- If bottles with screw caps are used, loosen the screw cap slightly.
- Place the containers/ glassware in the autoclave.
- Close the lid of the autoclave tightly and open the valve.
- Set the pressure at 15 lb/sq inch and heat
- Close the valve when water vapour is released.
- Autoclave for 15 - 20 minutes at 121^0C

d) Sterilization by filtration using membrane filter apparatus.

- For sterilization of heat labile substances.

1. Sterilize the components of the membrane filter apparatus separately.
2. Filter the liquid using membrane filters.
3. Collect the filtrate into sterile bottles/test tubes
4. Direct the students to record their observations highlighting the following:
5. different types of apparatus used in sterilization.
6. procedures followed

Guide the students

- comment on the selection of suitable technique for sterilizing different materials

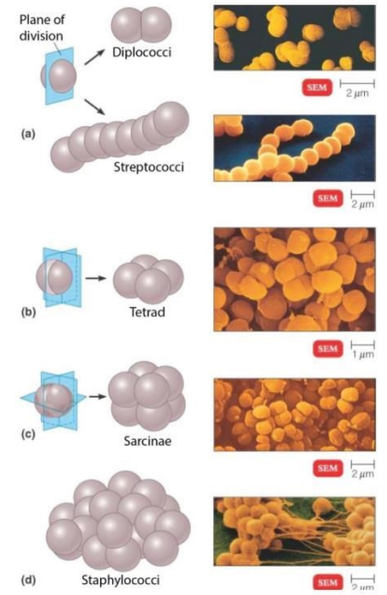


Figure 9.1. Cell arrangement of coccus bacteria. Shown are diagrammatic representations of planes of cell division and various cell arrangements (left) and their scanning electron microscopic (SEM) views (right).

2. Different forms of cell arrangement of bacillus bacteria (Figure 9.2)

- Bacilli divide only across their short axis. Therefore, there are few cell arrangement forms.

Single bacillus	
Diplobacillus	
Streptobacillus	

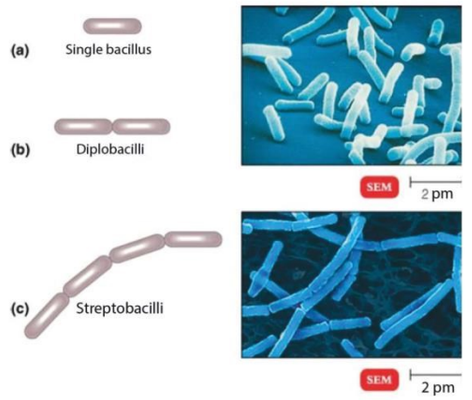
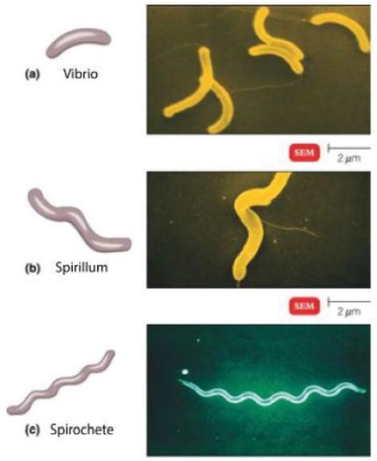


Figure 9.1. Cell arrangement of coccus bacteria. Shown are diagrammatic representations of planes of cell division and various cell arrangements (left) and their scanning electron microscopic (SEM) views (right).

3. Different forms of cell arrangement of spiral bacteria (Table 3)

- Spiral bacteria have one or more twists, they are never straight.

Vibrio	
Spirillum	
Spirochete	



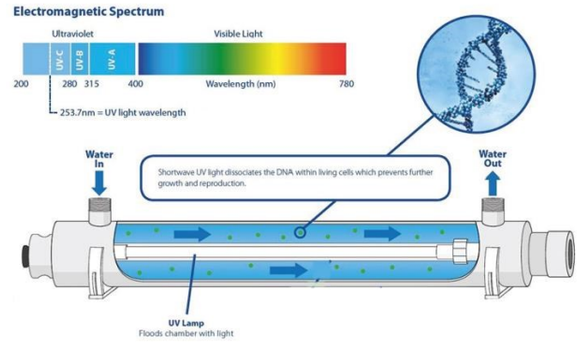
- Bacteria show a diversity of nutritional types. Four major nutritional types can be identified among bacteria. They are classified based on the source of energy and carbon.

Nutritional type	Source of energy	Source of carbon	Example
Photoautotrophs		Carbon dioxide (Inorganic Carbon)	
Photoheterotrophs	Light		
Chemoautotrophs		(Inorganic Carbon)	
Chemoheterotrophs	Organic chemicals		

- Microorganisms can be classified into 4 groups on the basis of their tolerance to oxygen.

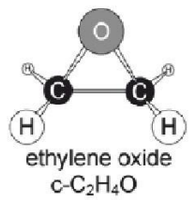
UV Radiation

- UV Radiation kills microorganisms that falls in to direct exposure, either through destruction or damaging DNA.
- However, a major disadvantage of UV is that radiation does not penetrate through solid surfaces and coverings such as paper, glass and textile.
- Therefore, anything to be sterilized should have direct contact with radiation.
- UV radiation is commonly used to sterilize air in hospital rooms such as operating theaters and nurseries.



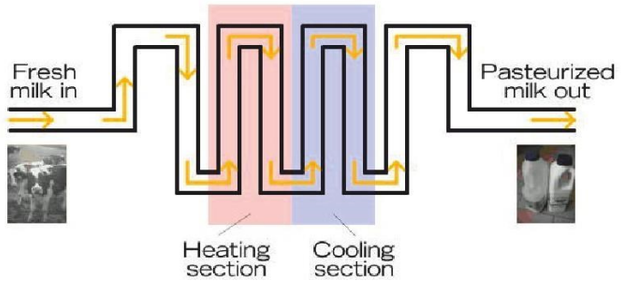
II. Chemical methods of sterilization

- Few chemicals such as ethylene oxide and chlorine dioxide (both are gases) are currently used as chemical sterilizing agents.
- Majority of chemical agents reduce microbial populations to a safe levels or remove vegetative forms of pathogens.
- **Ethylene oxide** kills microorganisms and endospores.
- It is also highly penetrating. Therefore ethylene oxide is used to sterilize mattresses in hospitals.



- **Chlorine dioxide** has been used to fumigate enclosed building areas contaminated with endospores of Bacillus anthracis.
- It is most commonly used in water treatment prior to chlorination.

- The objectives of milk pasteurization are to eliminate pathogenic microorganisms and reduce microbial number which prolongs milk quality under refrigeration.
- High temperature short-time (HTST) pasteurization, which uses temperature of at least 72^0C for 15 seconds and low temperature long time (LTLT) 63^0C for 30 minutes are the two main methods of pasteurization.
- Milk can also be sterilized by ultra-high-temperature (UHT) pasteurization.
- Here, milk is heated to about 140^0C in less than 5 seconds by flashing steam. This milk can be stored for several months without refrigeration.

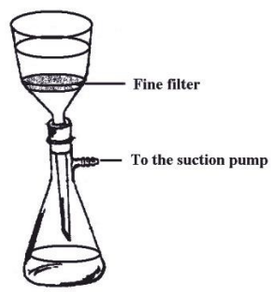


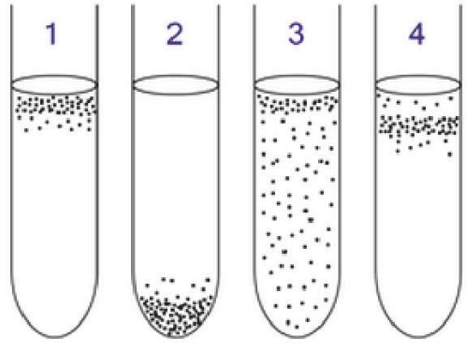
Boiling

- Boiling the materials such as surgical instruments to 100°c. Most of the pathogenic microorganisms are killed at boiling temperature.

Filtration (Eg: Membrane filters)

- Filtration is used to sterilize heat sensitive liquids such as solutions containing enzymes, vitamins, antibiotics, vaccines and some culture media.
- Material to be sterilized is passed through a filter by using vacuum.
- Filter retains microorganism and the liquid is passed through the filter.
- Membrane filters are widely used to sterilize heat sensitive solutions.
- Pores of membrane filters are from 0.01 µm to 0.45 µm size, retain almost all the microorganisms including viruses and some large protein molecules.





Physiological Group	Description	Example
Obligate aerobic		*Acetobacter sp*
Obligate anaerobic	They cannot survive in the presence of oxygen. These microorganism generate energy by fermentation	
	These microorganisms prefer to grow in the presence of oxygen producing energy by oxidative phosphorylation, but they can also grow in anaerobic environments using fermentation	
Microaerophilic		*Lactobacillus sp.*

- Some bacteria are able to fix atmospheric nitrogen. They show diversity in nitrogen fixation.

1. Free-living nitrogen fixing bacteria: Azotobacter sp.
2. Symbiotic nitrogen fixing bacteria: Rhizobium sp. with legume root

- Mostly bacteria undergo asexual reproduction by ……..., and in some occasion, ……... or ……...
  In rare occasions, bacteria of two strains share a portion of genetic material through the process of '……...'. (Exchange of DNA between 2 living bacterial cells)

2. Cyanobacteria

- Cyanobacteria are named for their characteristic blue-green (cyan) pigmentation.
- Cyanobacteria also exhibit a great variety of shapes and cell arrangements, unicellular to colonial forms (Figure 9.4).

Unicellular form

- Cells separate after cell division. However, in nature majority of unicellular forms stay together by copious (abundant) secretion of mucilage by daughter cells.

Colonial form

- Cells remain attached by walls or held in a common gelatinous matrix forming a colony of cells. Colonies may either be non-filamentous or filamentous.

Non-filamentous colonial form

- Depending on the plane of division and direction there are different arrangements such as spherical, cubical, square or irregular shape.

Dry heat sterilization

- Here, dry heat is used to destroy the microorganisms present in the desired materials such as glassware, Petri dishes, pipettes, inoculation loops, inoculation needles, scalpels, etc.

Direct flaming

- It is a simple method of dry heat sterilization.
- This is used in laboratories to sterilize inoculating loops, inoculating needles and scalpel blades by heating them on the flames of Bunsen burners/hot spirit lamp until they reach red hot.

Incineration

- It is mostly done in an incineration oven. Incineration is used to sterilize hospital waste.
- Microorganisms are burned to ash during direct flaming and Incineration.

Hot-air sterilization

- Microorganism are killed by oxidation. Items to be sterilized are heated to about 170^0C and maintain for 2 hours in a dry air oven.
- This type of sterilization is often used to sterilize glassware such as Petri plates, flasks, beakers, bottles and glass pipettes.

Pasteurization

- Louis Pasteur found spoilage of beer and wine can be prevented by applying mild heat that kills organisms causing spoilage without seriously damaging the taste, texture and nutritional content of the product.
- Later, the same principle was applied to milk products, now known as pasteurized milk.

Filamentous colonial form

- Is the result of cell division in a single plane and a single direction forming a chain or thread like structure.

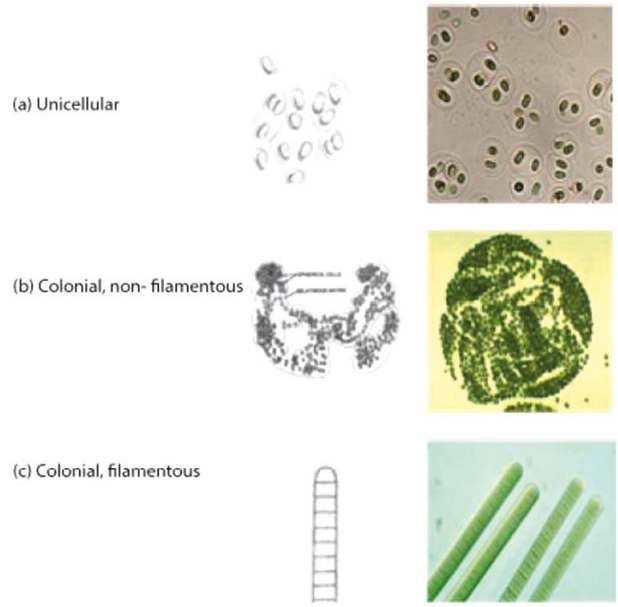


Figure 9.4. Cell arrangement of cyanobacteria. Shown are diagrammatic representations (left) and light microscopic views (left) of cell arrangement.

- Cyanobacteria are …….that carryout oxygenic photosynthesis similar to plants and algae.
- Many of cyanobacteria are capable of fixing atmospheric nitrogen. Eg: …….. is a free living nitrogen fixing cyanobacteria, …….. - …….. symbiotically fix nitrogen with its partner, *Azolla sp.* (water fern).
- In most cases nitrogen fixation takes place in special cells called …….. Nitrogen fixation is catalyzed by the enzyme called ……. in the heterocyst.
- Nitrogenase is sensitive to ……... Heterocyst carry ……...cell wall to protect nitrogenase from oxygen that could diffuse from neighboring photosynthetic cells and from air or water.
- Cyanobacteria carry another specialized cell type called ……... They are thick walled resting spores with stored food. Akinete is resistant to drought and high temperature.

- Therefore, akinete is able to survive during unfavorable environmental conditions although ……. cells dries out.
- Cyanobacteria reproduce only by asexual methods.
- ……... unicellular and ……... non- filamentous types undergo simple cell division while ……..filamentous and colonial ……. forms reproduce by fragmentation.

Fungi

- Fungi (singular, fungus) are eukaryotes. They may be ……... (yeast) or ……...…….(molds). Some multicellular fungi form mushrooms.
- Molds form visible masses called ……., which are composed of long filament like structures called hyphae. Many molds contain cross-walls called ……. (singular, septum).
- Septa divide hyphae into distinct single nucleate …….. Some molds do not contain septa in their hyphae resulting in long continuous cells with many nuclei. These are called …… hyphae.
- The …….growths sometimes found on bread and fruit are mycelia of molds. Fungi are chemoheterotrophs and acquire food by ……..
- They possess …….mode of nutrition. They play important role in food chain by decomposing dead plant materials by secreting enzymes and thereby recycle vital elements.
- ……... (plant and animal pathogens) and …….. (lichens and mycorrhizae) modes of nutrition also found among fungi.
- Unicellular fungi reproduce asexually by ……... or ……., on the other hand, filamentous fungi (molds) reproduce ……. and/or ……... by producing spores.

9.1.2: Explores some basic laboratory techniques in microbiology

- For the study of morphology and biochemical properties of microorganisms, it is essential to culture them on artificial media.
- There are some basic laboratory techniques such as preparation of artificial culture media and sterilization techniques, to be followed in culturing of the microorganism of interest without any contamination.

Methods of sterilization

- Sterilization is the process of removal or destruction of all forms of microbial life including endospores. There are two types of sterilization, physical and chemical.

I. Physical methods of sterilization

- Sterilization by moist heat, dry heat, filtering using membrane filters, exposure to UV radiation are some of the physical methods used in sterilization.

Moist heat sterilization

- Here, moist heat is used to destroy the microorganisms present in the desired materials such as culture media, temperature labile reagents/ fluids and various laboratory utensils.
- This is done by denaturing of proteins by high temperature and pressure.
  Eg. autoclaving- In an autoclave, steam with 121^0C temperature at the pressure of 1 atm/15 psi is used for sterilization. Extending the above condition for 15 minutes is sufficient to kill all microorganisms (except prions) and their endospores.
- Autoclaving is used to sterilize culture media, solutions, syringes and needles, healthcare instruments and various other items that can withstand high temperatures and pressure.
- Glassware can also be sterilized with an autoclave if care is taken to ensure that the steam contacts all surfaces. Pressure cooker also can be used for moist heat sterilization.

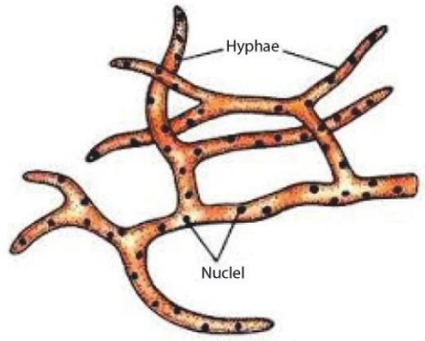
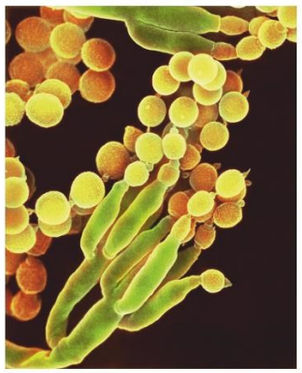
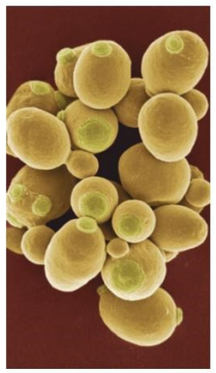


Figure 9.5 Fungi filamentous thallus, branched mycelium



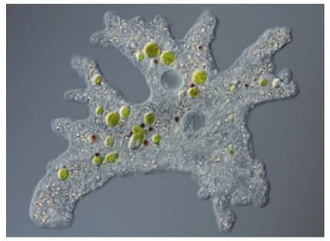
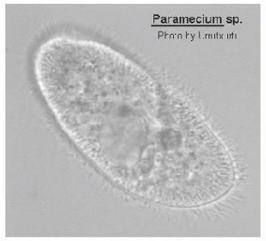
Reproductive method of Penicillium (Spore formation)



Reproductive method of Yeast (budding)

Unicellular Protists

- Unicellular Protists are ……..., vary in their shapes and possess locomotive structures such as pseudopods, cilia or flagella. They exist either individually or form colonies. Some join together and form filaments.
- ……., …….. and ……. modes of nutrition are found among protists.
- There are ……..., ……... and …….. anaerobic respiratory modes found among protists.
- Some algae contribute to the symbiotic interactions in ……...
- They reproduce sexually by gametes and asexually by fission.

Mollicutes

- Mollicutes are …….. included in the ……
  ……..
- …….. and ……..., are considered as unique due to absence of cell walls.

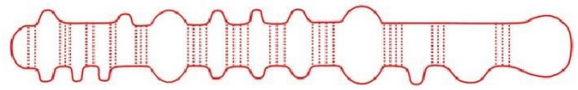
Mycoplasma and Phytoplasma

- Mycoplasma are pleomorphic, vary in shape from spherical to filamentous. They are the smallest prokaryotic group of organisms invisible under light microscope. Mycoplasma do not contain flagella. Almost all mycoplasma are parasites of humans and animals. Mycoplasma require high amount of organic growth factors. They reproduce by budding and binary fission and do not produce spores. Mycoplasma are aerobic or facultative anaerobic.
- Phytoplasma resembles to mycoplasma in many ways. They are similar in size to mycoplasma. Both can only be seen under electron microscope. Shape varies from spherical to filamentous. Phytoplasma only infect plants and are generally present in the phloem sap. They cannot grow in artificial media. They are transmitted mostly by leaf-hoppers. Therefore, they reproduce in both leafhoppers as well as plant body. They reproduce by budding and binary fission. They possess aerobic or facultative anaerobic mode of respiration.

Mycoplasma	Phytoplasma

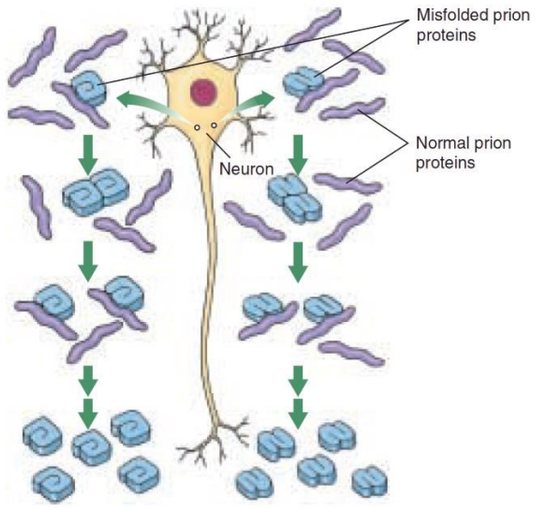
Viroids

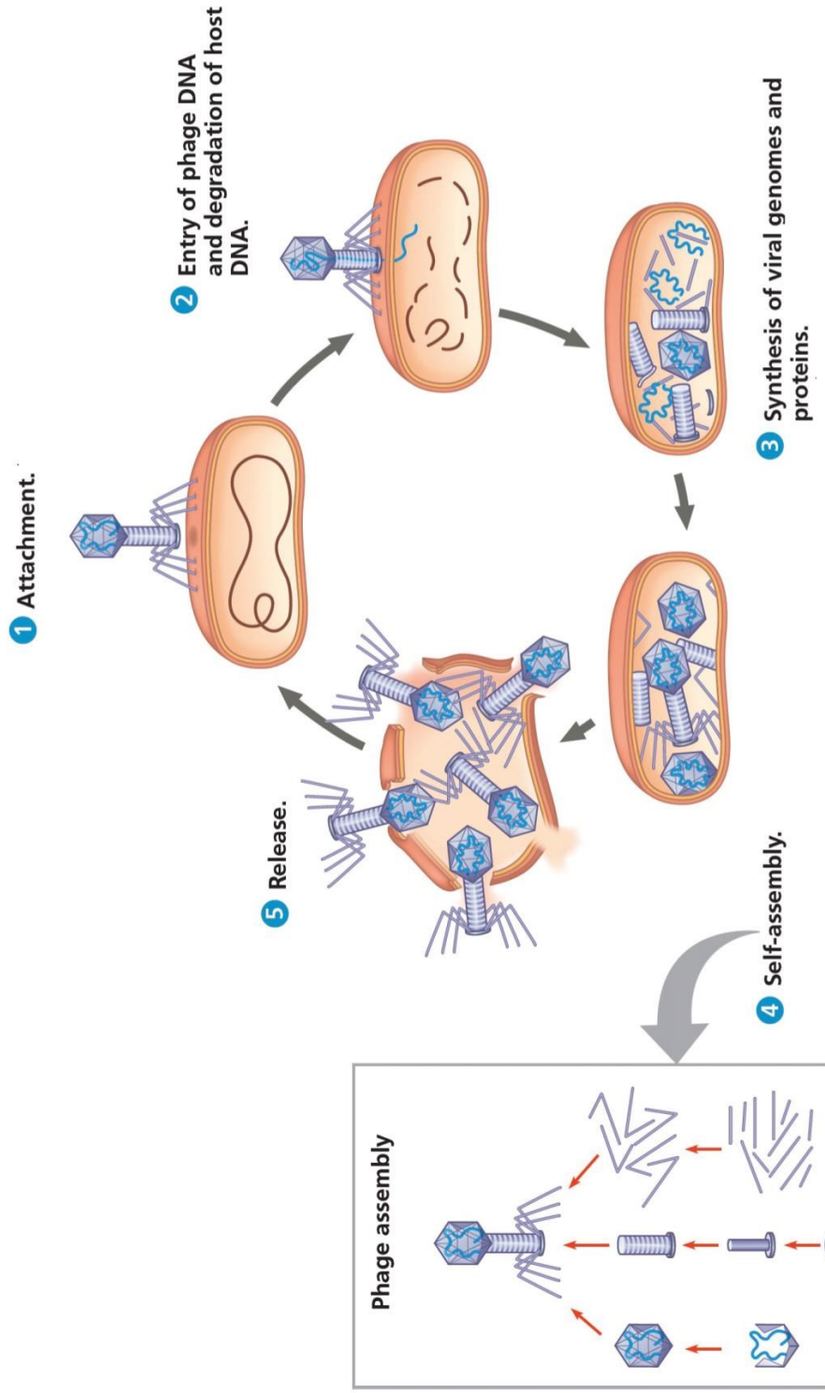
- Viroids consist only of …….. piece of naked ……... (Circular or linear) with ……... protective layer such as a protein coat. Viroids can only multiply within a living host cell using host cell resources. However, viroids do not contain any gene and only carry signals for their multiplication. Viroids infect plants, but no other life forms till to date.



Prions

- Prions are proteinaceous infectious particles. Their size is …….. than virus.
- Although prions ……... ……..... acid they can ……... with the help of a …….. gene that encodes the prion protein.
- They are found as disease causing agents in some birds and mammals. All these diseases are neurological diseases.
- Eg: Transmissible Spongiform Encephalopathies (TSEs), because large vacuoles develop in the brain giving sponge-like appearance. Mad cow disease was one of the serious disease emerged in cattle in 1987. Creutzfeldt-Jakob disease (CJD) is one of the human diseases caused by prions.
- Human to human disease transmission has been associated with transfusion of Infected blood and tissue and organ transplantation. Some TSE infections may be transmitted from cow to human.





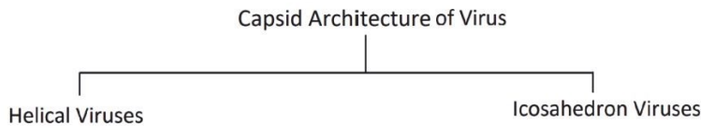
Virus

(a) Characteristic features

- Viruses are ……. prokaryotes nor eukaryotes and do not show any ……... organization. They do not possess any metabolic activity or …….. when they are out of living host cells. Thus, they are not considered as living organisms.
- However, once they get in to the host cells, they ……...and cause infection through various metabolic pathways, shows characteristics of living organisms.
- Since viruses can …….. multiply within a living host cell, they are ……..parasites.
- Viruses are very small can only be seen through an ……. ……....
- Viruses possess simple structures, usually are composed of a central core of a nucleic acid and surrounded by a protein ……..called the …….. which is made up of a fixed number of protein subunits called capsomeres.
- Viruses may have either ……... or …….. as their genetic material. They do not have protein synthesis machinery such as additional RNAs or enzymes for protein synthesis.
- Therefore, they depend on host cell's protein synthesis machinery.
- RNA viruses consist of reverse transcriptase enzymes for reverse transcribing RNA in to DNA.

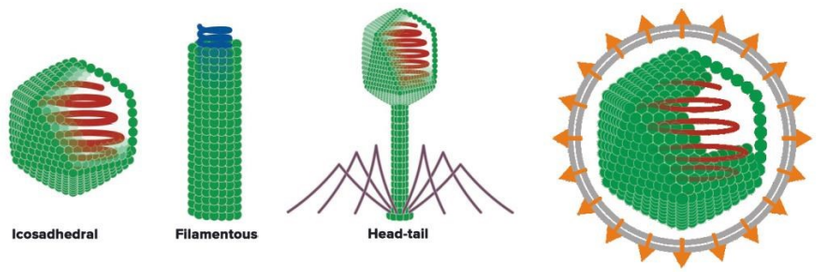
(b) Morphology and types of viruses

- On the basis of capsid architecture two basic morphological symmetries can be identified



- Based on the above symmetries viruses show four types of morphological forms; helical, polyhedron, complex and enveloped.

1. ……..	Long rigid or flexible rods. Eg: Rabies virus, Tobacco mosaic virus
2. ……..	Icosahedron symmetry present. Eg: Adeno virus
3. ……..	Exhibits more than one form of symmetry with additional structures. Eg: Bacteriophage
4. ……..	Eg: Roughly spherical. Capsid covered by envelopes. Eg: Herpes simplex virus. They can show either helical or icosahedral symmetry.



Multiplication of viruses

- A single virus can give rise to thousands of similar viruses in a single host cell. Therefore, viruses cause serious damages to their host leading to severe diseases in plants, animals and bacteria.
- Bacteriophages are typical group of viruses that are capable of Infecting bacteria. They multiply by two distinct mechanisms; lytic cycle or lysogenic cycle.
- Lytic cycle involves with the lysis of the host cell, whereas the lysogenic cycle allows viral DNA incorporating into host DNA and multiply without causing lysis of the host cell.

Lytic cycle of a Bacteriophage

- There are five distinct steps in the lytic cycle ; attachment, penetration, biosynthesis, maturation and release (Figure 9.9)

Attachment:

- The first step is the attachment of virus to a matching receptor site on the bacterial cell.

Penetration:

- After attachment, bacteriophage injects its DNA into the bacterial cell. This is facilitated by an enzyme which breaks down bacterial cell wall.

Biosynthesis:

- The next step is the biosynthesis of viral DNA and proteins in the host cytoplasm using host resources. This stage induces degradation of host cell DNA.

Maturation and assembly:

- Once bacteriophage DNA and proteins are synthesized, DNA and capsid are assembled to form complete virus particles. This is called maturation

Release:

- Finally, bacteriophage induce bacterial cell to break open (lyse). Newly produced bacteriophages are released from the host cell. These released bacteriophages can start another lytic cycle in cells in the vicinity.