Bacteriology & Biology of Eukaryotic Microbes

M.Sc. MICROBIOLOGY

SEMESTER-I,

PRACTICAL PAPER-II (106MB24)

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Bacteriology & Biology of Eukaryotic Microbes

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao
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M.SC. MICROBIOLOGY
SEMESTER-I

106MB24: Bacteriology and Biology of Eukaryotic Microbes Bacteriology

Bacteriology

1. Streak plat technique
2. Spread plate technique
3. Pour plate technique
4. Isolation of bacteria from soil
5. Contact slide technique
6. Simple staining
7. Gram's staining
8. Spore staining
9. Effect of temperature on bacterial growth
10. Effect of pH on bacterial growth
11. Effect of NaCl on bacterial growth
12. Effect of antibiotics on bacterial growth (disc method)

Biology of Eukaryotic Microbes

1. Observation of different cell organelles
2. Observation of different mitotic and meiotic stages
3. Identification of different algae from pond water
4. Isolation of soil algae
5. Isolation of fungi from soil, air and water
6. Isolation of Aspergillus from lemon
7. Observation of important fungal pathogens
8. Observation of important protozoan pathogens (slides)

CONTENTS

Bacteriology

Experiment No. 1

ISOLATION OF BACTERIA BY STREAK PLATE METHOD

Aim:

To perform the quadrant mode of streaking for the isolation of individual colonies.

Requirements:

Mixed culture broth, Nutrient agar plates, Spirit lamp, Inoculation chamber, Inoculating loop, Wax-marking pencil.

Principle:

Dilution of inoculum during the streaking and development of separate colonies from each isolated cells on agar plates during incubation.

Composition of Nutrient agar medium:

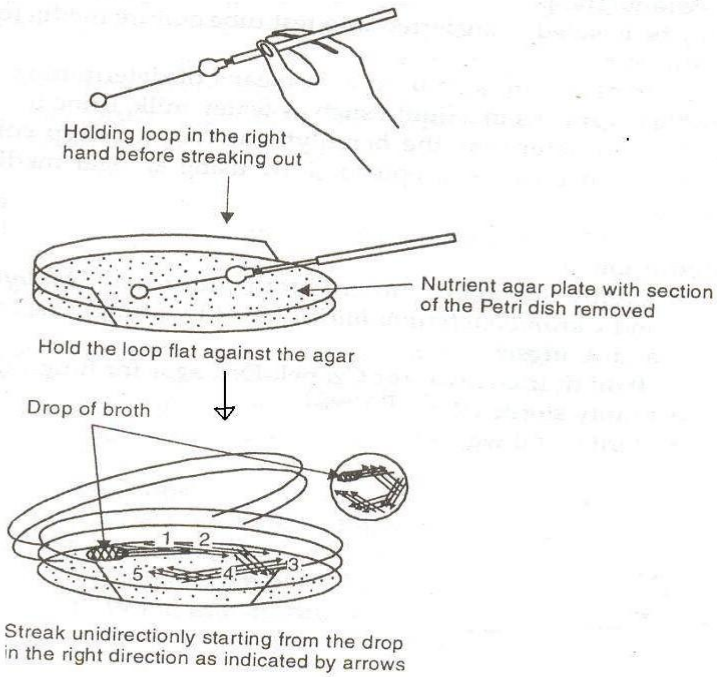
Peptone	– 5.0 g
Beef extract	– 3.0 g
NaCl	– 5.0 g
Agar agar	– 20.0 g
Distilled water	– 1000 ml

Procedure:

1. Prepare the nutrient agar medium as per the composition and sterilize the medium in autoclave at 15 lbs pressure and 121° C temperature for 20 minutes. Then cool the sterilized medium to molten state of temperature around 42°C.
2. Sterilize the clean petri plates in hot-air oven at 160°C temperature for 2 hrs. After sterilization transfer them to the inoculation chamber.
3. Pour about 20 ml aliquots of molten state sterilized agar medium into petri plates in inoculation chamber. Allow the plates for the solidification of the medium.
4. Sterilize the loop holding in the right hand, introduce the loop into the mixed culture broth and withdraw one loopful of culture.
5. Lift the upper lid of the agar petri plate with left hand and hold it at an angle of 60°.
6. Place the inoculum of the loop on the agar surface at the edge farthest from you and streak the inoculum from side to side in parallel lines across the surface of area.
7. Reflame and cool the loop and turn the petri plate to 90°. Touch the loop to a corner of the parallel lines in area 1 and streak the drawn inoculum across the agar in area 2 as in the previous step. The loop should never enter into area 1 again.
8. Repeat the same steps to streak the parallel lines in 3rd and 4th areas of the petri plate.
9. Replace the lid of the petri plate, after completing the streaking, and sterilize the loop by flaming.

10. Incubate the plates in an inverted position for 24 hrs. at room temperature.
11. Observe the plates for the presence of isolated and individual colonies on the streaked lines.

Result: During incubation, isolated colonies may develop on streaked lines of area 3 or area 4 of the petri plate, but with usual confluent growth in 1 and 2 areas.



Streak Plate Technique

Experiment No. 2

ISOLATION OF BACTERIA BY SPREAD PLATE METHOD

Aim:

To isolate pure cultures of bacteria in the form of discrete colonies on solid nutrient agar medium.

Requirements:

Nutrient agar medium, Petri plates, Mixed bacterial culture, Hot-air oven, Autoclave, Spreader or bent glass rod.

Principle:

During the spinning of petri plate meant for spreading of bacterial cells on solid medium surface, at some stage, single cells will be deposited by bent glass rod on the agar surface. These separated cells on agar surface develop into individual colonies on incubation.

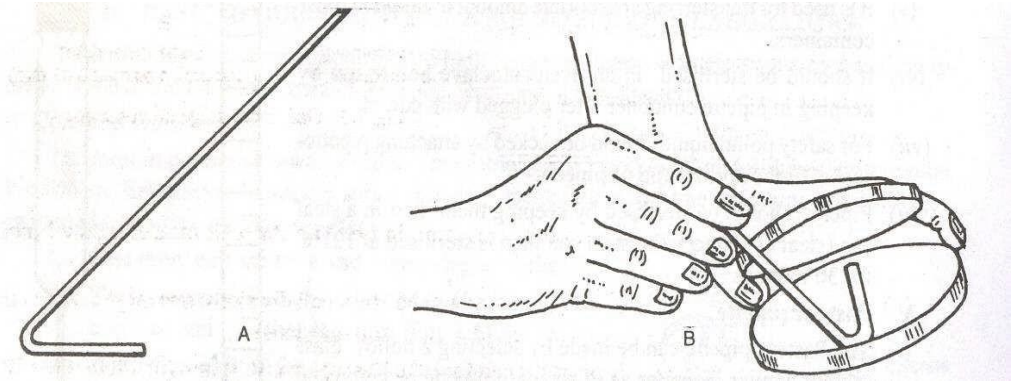
Composition of Nutrient agar medium:

Peptone	– 5.0 g
Beef extract	– 3.0 g
NaCl	– 5.0 g
Agar agar	– 20.0 g
Distilled water	– 1000 ml

Procedure:

1. Prepare nutrient agar medium as per the composition and sterilize in an autoclave at 121^{o}C and 15 lbs pressure for 20 minutes.
2. After sterilization, cool the medium to molten state, pour 20 ml aliquots into sterilized petri plates and allow to solidify to prepare nutrient agar plates.
3. Prepare culture suspensions of different dilutions by employing ten-fold serial dilution technique as follows –
   a) Take 1 ml of original broth culture into a test tube and add 9 ml of sterile distilled water to get 10^{-1} dilution.
   b) Tale 1 ml of suspension from 10^{-1} dilution and add it to 9 ml of sterile distilled water to prepare 10^{-2} dilution.
   c) In the same manner, prepare the suspensions of 10^{-3},10^{-4},10^{-5},10^{-6} and 10^{-7} dilutions.
4. Place 0.1 ml of culture suspension of 10^{-7} dilution on the nutrient agar surface and spread it with a bent glass rod through spinning the petri plate on turn table.
5. Repeat the step 4 for different dilutions.
6. Incubate the plates in inverted position at room temperature for 24 hrs.
7. Observe the plates after incubation, for the distributed colonies.

Result: Well isolated and discrete colonies will be seen on the surface of solid agar medium. The number of colonies in the plates progressively decreases with increasing dilution.



A. Glass Spreader B. Method of Spreading

Experiment No. 3

ISOLATION OF BACTERIA BY POUR PLATE METHOD

Aim:

To isolate pure bacterial species in the form of discrete colonies by using solid nutrient agar medium.

Requirements:

Nutrient agar medium, Mixed bacterial culture, Petri plates, Hot-air oven, Autoclave.

Principle:

The individual cells of bacterial inoculum in successive dilutions thoroughly mix with the molten agar medium during the rotation of petri plate and get fixed or seeded at different places in or on the medium on solidification of the medium. These fixed cells develop into individual colonies at those places on incubation.

Composition of Nutrient agar medium:

Peptone	– 5.0 g
Beef extract	– 3.0 g
NaCl	– 5.0 g
Agar agar	– 20.0 g
Distilled water	– 1000 ml

Procedure:

1. Prepare the culture suspensions of different dilutions by using 10-fold serial dilution method as follows –

a) Add 1 ml of original broth culture of bacteria to 9 ml of sterile distilled water in a test tube to prepare suspension of 10^{-1} dilution.

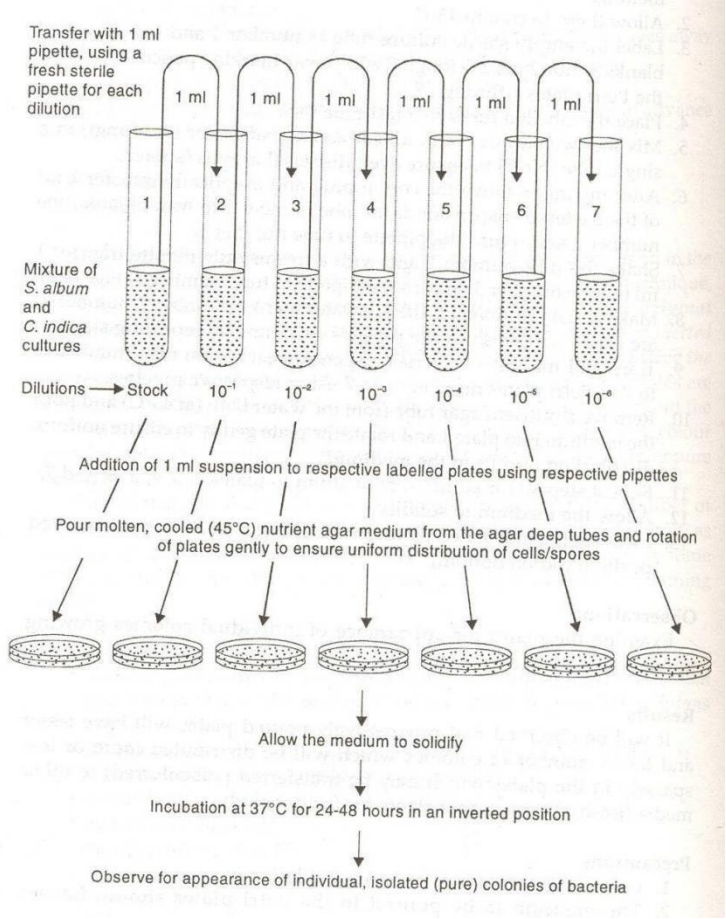
b) Take 1 ml of suspension from 10^{-1} dilution and add to 9 ml of sterile distilled water to prepare the suspension of 10^{-2} dilution.

c) Prepare the suspensions of other higher dilutions viz., 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions in the same manner as above.

2. Prepare nutrient agar medium as per the composition and sterilize the medium in an autoclave at 121°C temperature and 15 lbs pressure for 20 minutes.
3. Sterilize the clean glass petri plates in hot-air oven at 160°C temperature for 2 hrs.
4. Pour 1.0 ml of bacterial suspension of different dilutions into separate petri plates labeled with the corresponding dilution factor.
5. Into each petri plate, then, pour about 20 ml aliquot of molten state agar medium and rotate the plate gently to ensure uniform distribution of cells in the medium.
6. Allow the plates for the solidification of the medium.
7. Incubate the plates at room temperature in inverted position for 24 hrs.

8. Observe the plates after incubation for the distribution of bacterial colonies.

Result: Well isolated and distributed colonies of surface, sub-surface and bottom surface nature will be seen. The number of colonies per plate progressively becomes lesser and lesser with increasing dilution of the bacterial inoculum.



Serial Dilution and Pour Plate Method

Experiment No. 4

ISOLATION OF BACTERIA BY FROM SOIL

Aim:

To isolate bacteria from the given soil sample.

Principle:

Microorganisms are ubiquitous and soil is the one of the best sources to find all kinds of microorganisms. When a serially diluted soil sample is inoculated on Nutrient agar medium and incubated in suitable culture conditions, the representative bacteria grow on the medium in the form colonies.

Requirements

Nutrient agar medium, Soil sample, Glassware (test tubes, conical flasks, pipettes, Petri plates, etc.), Distilled water, Glass Spreader, Spirit lamp, Cotton and Laminar air flow.

Nutrient agar medium composition
Peptone -5g
Beef Extract -3g
Nacl -5g
Agar Agar -15g
Distilled H_2O -1000ml
pH -7.3 - 7.5
1N HCl and 1N NaOH

Procedure:

1. Collect the soil sample into a sterile container and bring it safely to the lab.
2. Prepare 10-fold serial dilutions of the sample in test tubes by using sterile distilled water.
3. Prepare Nutrient agar medium as per the composition, sterilize and pour the molten state agar medium into the sterile Petri plates and allow the medium to solidify in laminar air flow chamber.
4. After solidification, take 0.1 ml of the sample from each of 10^{-5}, 10^{-6}, and 10^{-7} dilutions of soil sample and spread separately on nutrient agar plates using glass spreader in aseptic conditions.
5. Invert the inoculated plates and incubate at 37°C temperature for 24hrs.
6. After incubation, observe and count the number of bacterial colonies grown on the medium.
7. Note the colony characters viz., size, shape, colour, margin, Elevation, etc.

Result: Basing on the characters of the colonies appeared, abundance of type of colonies present in soil can be understood.

Experiment No. 5

OBSERVATION OF SOIL MICROBES BY CONTACT SLIDE METHOD

Aim:

To study the relative preponderances of microorganisms in soil portions.

Requirements:

Soil sample, 250 ml beakers, Microscopic slides, Microscope, Erythrosine.

Principle:

The slides inserted into the soil serve as substratum for the growth of soil microbes in the same proportionality of their original distribution in the soil profile.

Procedure:

1. Weigh 200 g of soil sample and place in 250 ml glass beaker.
2. Add 20 ml of distilled water to the soil in beaker to maintain the moisture.
3. Insert two clean and dry microscopic slides in close contact up to ¾ of the slide length.
4. Incubate the set up for 2 weeks with intermittent wetting of the soil in beaker with few ml of distilled water.
5. After incubation, remove the slides carefully without disturbing the soil side of the slide.
6. Stain the slide on the side which is in contact with soil during incubation by using erythrosine.
7. Observe the stained slide for the occurrence and abundance of different microbes viz., bacteria, fungi and actinomycetes.

Result: Bacteria differ from fungi and actinomycetes by their unicelled nature. The filamentous fungi and actinomycetes differ by their diameter of the mycelium. The fungal mycelium diameter will be greater than that of actinomycetes. Abundance of these microbes will be assessed by relative proportions on the slide.

Experiment No. 6

SIMPLE STAINING OF BACTERIA

Aim:

To stain the bacterial cells with simple basic stain and observe the morphology.

Requirements:

24-hr. old bacterial culture of *Bacillus subtilis*, 1% methylene blue solution, Glass slides, Inoculating loop, Spirit lamp, Blotting paper, Microscope.

Procedure:

1. Take clean glass slides, wash and dry them.
2. Place a loopful of broth of bacterial culture on the clean glass slide at the center.
3. Spread the bacterial suspension thinly on the slide with inoculating loop to prepare the smear.
4. Fix the smear by heating gently over the spirit lamp flame.
5. Stain the smear by adding few drops of methylene blue solution to the smear and allow it to stand for 1-2 minutes.
6. Remove the stain and wash the smear gently with slowly running tap water.
7. Blot-dry the slide using blotting paper but do not wipe the slide.
8. Examine the preparation under oil-immersion objective of microscope.

Result: Rod shaped cells will be clear with a deep blue color of the stain.

Experiment No. 7

DIFFERENTIAL GRAM STAINING OF BACTERIA

Aim:

To differentiate Gram-positive and Gram-negative bacteria by differential Gram staining technique.

Requirements:

24 hr.-old cultures of *Bacillus subtilis* and *Escherichia coli*, Crystal violet stain, Gram's iodine solution, Ethyl alcohol, Safranin, Wash bottle, Inoculating loop, Glass slides, Blotting paper, Spirit lamp, Microscope.

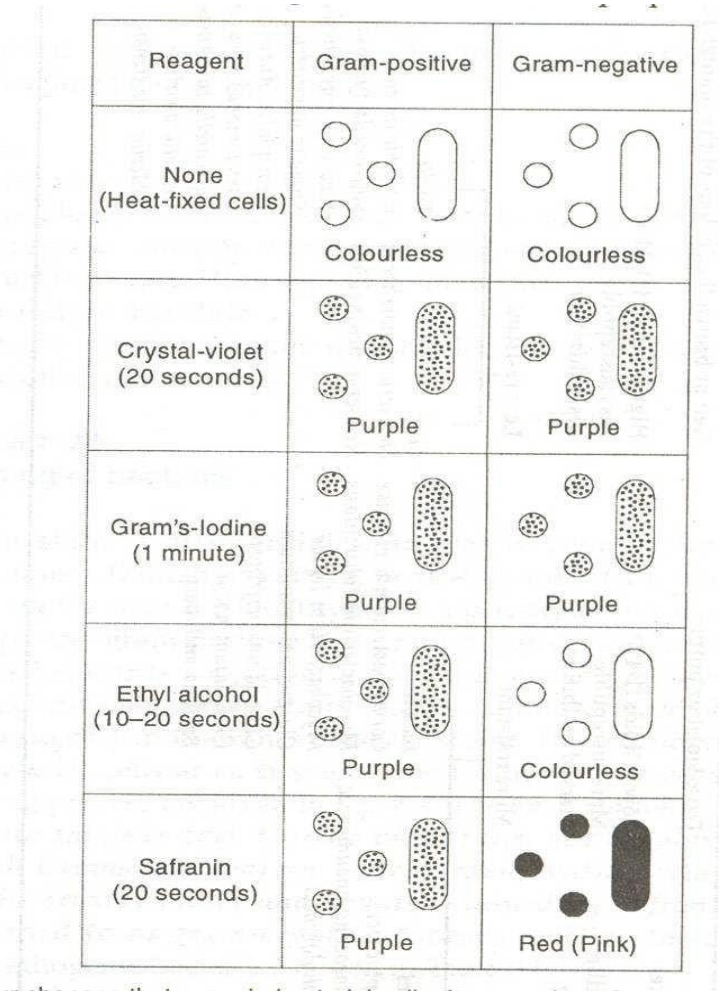
Principle:

The chemical and physical differences in cell walls of the bacteria respond differentially to the Gram stain in either retaining the primary stain (as in Gram-positive bacteria) or lose the primary stain and stained with counter stain (as in Gram-negative bacteria).

Procedure:

1. Make thin smears of *Bacillus subtilis* and *Escherichia coli* on separate glass slides.
2. Air-dry and heat fix the smears on the flame of spirit lamp.
3. Flood the smear with crystal violet stain for 30 seconds.
4. Wash the smear with distilled water for few seconds.
5. Flood the smear with Gram's iodine solution for 30 seconds.
6. Wash-off the iodine solution from the smear with 95% ethyl alcohol. During this step, add ethyl alcohol drop by drop keeping the slide in slant position until no more color of stain flows from the smear.
7. Wash the slides with distilled water and drain.
8. Apply the counter stain, safranin to the smear for 30 seconds.
9. Then, wash the smear with distilled water and blot-dry with absorbent paper.
10. Observe the preparation through microscope under oil-immersion objective.

Result: Bacteria that appear purple are referred to as Gram-positive and those appear pink are described as Gram-negative.



Gram's Staining Procedure

Experiment No. 8

STAINING OF BACTERIAL ENDOSPORES

Aim:

To observe the stained endospores of bacteria.

Requirements:

72 hr. old culture of *Bacillus cereus* or *Bacillus subtilis*, 5% malachite green, 0.5% safranin, Glass slides, Inoculating loop, Blotting paper, Spirit lamp, Microscope, Immersion oil.

Principle:

Special stains give the color to endospore by penetrating through the spore wall.

Procedure:

1. Prepare the smear of *Bacillus cereus* on clean, dry glass slide.
2. Air-dry and heat-fix the smear on spirit lamp flame.
3. Flood the smear with malachite green.
4. Heat the flooded smear intermittently on spirit lamp flame for 10 minutes by adding more stain to the smear from time to time.
5. Wash the slides slowly under running tap water, keeping the slide in slant position.
6. Counter-stain the smear with safranin for 30 seconds.
7. Wash the smear with distilled water and blot-dry with blotting paper.
8. Observe the slide under oil-immersion objective of microscope.

Result: The rod shaped vegetative cells stain red and elliptical or spherical or oval spores stain green and appear as green refractile bodies.

Experiment No. 9

EFFECT OF TEMPERATURE ON BACTERIAL GROWTH

Aim:

To study the effect of different temperatures on the growth of bacteria by measuring the turbidity of broth medium by using colorimeter.

Principle:

Every microorganism has cardinal temperatures viz., Minimum, Optimum and Maximum for its growth. Optimum temperature is that temperature at which the organism shows ideal and maximum growth. The cardinal temperatures vary from one organism to another organism. Basing on the range of cardinal temperatures, the microorganisms can be categorized into Psychrophiles, Mesophiles, and Thermophiles.

Requirements:

Bacterial culture, Nutrient broth medium, Boiling test tubes, Conical flasks, Measuring cylinder, Cotton plugs, Colorimeter etc.

Procedure:

1. Prepare the required amount of nutrient broth medium as per the composition (Beef extract – 3 gm, Peptone – 5 gm, NaCl – 5 gm, and Distilled water – 1000 ml) and dissolve the contents well.
2. Disperse about 25 ml aliquots of the prepared nutrient broth medium into separate boiling test tubes.
3. Sterilize the tubes containing the medium in an autoclave at 15lbs pressure and 121^{o} C temperature for 15 minutes. After sterilization, cool the tubes under running tap water.
4. Inoculate the tubes with a loopful of selected bacterial culture in an inoculating chamber.
5. Label the inoculated tubes as 10^{o} C, 37^{o} C and 50^{o} C in triplicates and incubate them at respective marked temperatures for 24 hours.
6. After incubation, read the optical density (O.D. values) of the medium of each test tube at 600 nm wavelength using colorimeter.
7. Tabulate the O.D. values of the triplicates of each temperature and take the average value.
8. Basing on the maximum growth (O.D. value) at any one the three temperatures tested, categorize the bacterium used in the study.

Result: Basing on the key given below, categorize your tested bacterium into the appropriate group.

Psychrophiles: Optimum growth between 0 – 15^{o} C temperature.
Mesophiles: Optimum growth between 15 – 45^{o} C temperature.
Thermophiles: Optimum growth between 40 – 80^{o} C temperature.

Experiment No. 10

EFFECT OF pH ON BACTERIAL GROWTH

Aim:

To study the effect of different pH concentrations on the growth of bacteria by measuring the turbidity of broth medium by using colorimeter.

Principle:

Generally, most of the bacteria prefer neutral pH (6.5 - 7.5) for their optimal growth. However, some bacteria may be acidophiles (below 6 pH) and some are alkaliphiles (above 9 pH) that can grow in acidic and alkaline pH ranges, respectively.

Requirements:

Bacterial culture, Nutrient broth medium, HCl (acid), NaOH (base), Boiling test tubes, Conical flasks, Measuring cylinder, Cotton plugs, Colorimeter etc.

Procedure:

1. Prepare the required amount of nutrient broth medium as per the composition (Beef extract – 3 gm., Peptone – 5 gm., NaCl – 5 gm., and Distilled water – 1000 ml) and dissolve the contents well.
2. Disperse about 150 ml aliquots of the prepared nutrient broth medium into 3 separate conical flasks.
3. Adjust the pH of the 1st flask to pH 5, 2nd flask to pH 7 and 3rd flask to pH 9 by adding diluted acid (HCl) or base (NaOH) as required.
4. Disperse 25 ml aliquots of the medium from different pH flasks to boiling test tubes separately and label them with respective pH values. Maintain 3 replicates for each pH.
5. Sterilize the tubes containing the medium in an autoclave at 15lbs pressure and 121^{o} C temperature for 15 minutes. After sterilization, cool the tubes under running tap water.
6. Inoculate the tubes with a loopful of selected bacterial culture in an inoculating chamber.
7. After incubation, read the optical density (O.D. values) of the medium of each test tube at 600 nm wavelength using colorimeter.
8. Tabulate the O.D. values of the triplicates of each pH and take the average value.
9. Basing on the maximum growth (O.D. value) at any one the three pH values tested; categorize the bacterium used in the study as acidophile or neutrophile or alkaliphile.

Result:

If the highest optical density value is observed at pH 5, then the given bacterium is acidophile.
If the highest optical density value is observed at pH 7, then the given bacterium is neutrophile.
If the highest optical density value is observed at pH 9, then the given bacterium is alkaliphile.

Experiment No. 11

EFFECT OF NaCl ON BACTERIAL GROWTH

Aim:

To study the effect of different NaCl concentrations on the growth of bacteria by measuring the turbidity of broth medium by using colorimeter.

Principle:

Bacteria are usually categorized based on the optimal NaCl concentration required for their growth or their tolerance towards the NaCl concentration into 'non-halophiles, slight halophiles, moderate halophiles, and extreme halophiles.

Requirements:

Bacterial culture, Nutrient broth medium, NaCl, Boiling test tubes, Conical flasks, Measuring cylinder, Cotton plugs, Colorimeter etc.

Procedure:

1. Prepare the required amount of nutrient broth medium as per the composition (Beef extract – 3 gm., Peptone – 5 gm., and Distilled water – 1000 ml) and dissolve the contents well.
2. Disperse 100 ml of the prepared nutrient broth medium into separate three 250 ml conical flasks.
3. To the first flask, add 1 gr of NaCl to provide 1% concentration. To the second flask, add 5 gr of NaCl to provide 5% concentration. To the third flask, add 10 gr of NaCl to provide 10% concentration.
4. Transfer 15 ml of medium from each conical flask into four separate boiling test tubes and label them with the respective NaCl concentration.
5. Sterilize the tubes containing the medium in an autoclave at 15lbs pressure and 121^{o} C temperature for 15 minutes. After sterilization, cool the tubes under running tap water.
6. Inoculate three tubes of each NaCl concentration with a loopful of selected bacterial culture in an inoculating chamber and leave the fourth tube without inoculation to serve as blank. Incubate the tubes for 24 hours at room temperature.
7. After incubation, read the optical density (O.D. values) of the medium of each test tube at 600 nm wavelength using colorimeter. Use the blank tube of each NaCl concentration to calibrate the colorimeter.
8. Tabulate the O.D. values of the triplicates of each NaCl concentration and take the average value.
9. Basing on the maximum growth (O.D. value) at any one the three concentrations of NaCl tested, categorize the bacterium used in the study.

Result: Basing on the key given below, categorize your tested bacterium into the suitable group.

Non-halophiles : grow best below 2% NaCl concentration
Slight halophiles : grow best between 2 to 5% NaCl concentration
Moderate halophiles : grow best 5 to 20% NaCl concentration
Extreme halophiles : grow best between 20 to 30% NaCl concentration

Experiment No. 12

ANTIBIOTIC SENSITIVITY TESTING BY DISC DIFFUSION METHOD

Aim:

To demonstrate the sensitivity or resistance of *Bacillus subtilis* against the selected antibiotics.

Requirements:

24 hr. old culture of *Bacillus subtilis*, Petri plates, Nutrient agar medium, Inoculation chamber, Forceps, selected antibiotic discs.

Composition of Nutrient agar medium:

Peptone	– 5.0 g
Beef extract	– 3.0 g
NaCl	– 5.0 g
Agar agar	– 20.0 g
Distilled water	– 1000 ml

Procedure:

1. Prepare the bacterial suspension of *Bacillus subtilis* by using sterile distilled water.
2. Prepare the nutrient agar medium as per the composition and autoclave at 121°C temperature and 15 lbs pressure for 20 minutes.
3. Cool the sterilized medium to molten state and add the bacterial inoculum to it.
4. Disperse 20 ml aliquots of the inoculated medium into petri plates to prepare nutrient agar plates seeded with inoculum.
5. Allow the plates for the solidification of the medium.
6. After solidification, place the selected antibiotic disc at the center of the plate with the help of sterilized forceps and gently press it towards the medium surface.
7. Incubate the plates in inverted position for 24 hours at 37°C temperature.
8. Observe the plates after incubation, for the formation of clear zone around the disc.
9. Calculate the area of clear zone by using the formula πr^2 for the comparison of antibiotic sensitivity against different antibiotics.

Result: Development of clear zone around the disc indicates the sensitivity of organism to that antibiotic and absence of any clear zone is an indicative of resistance of the organism.

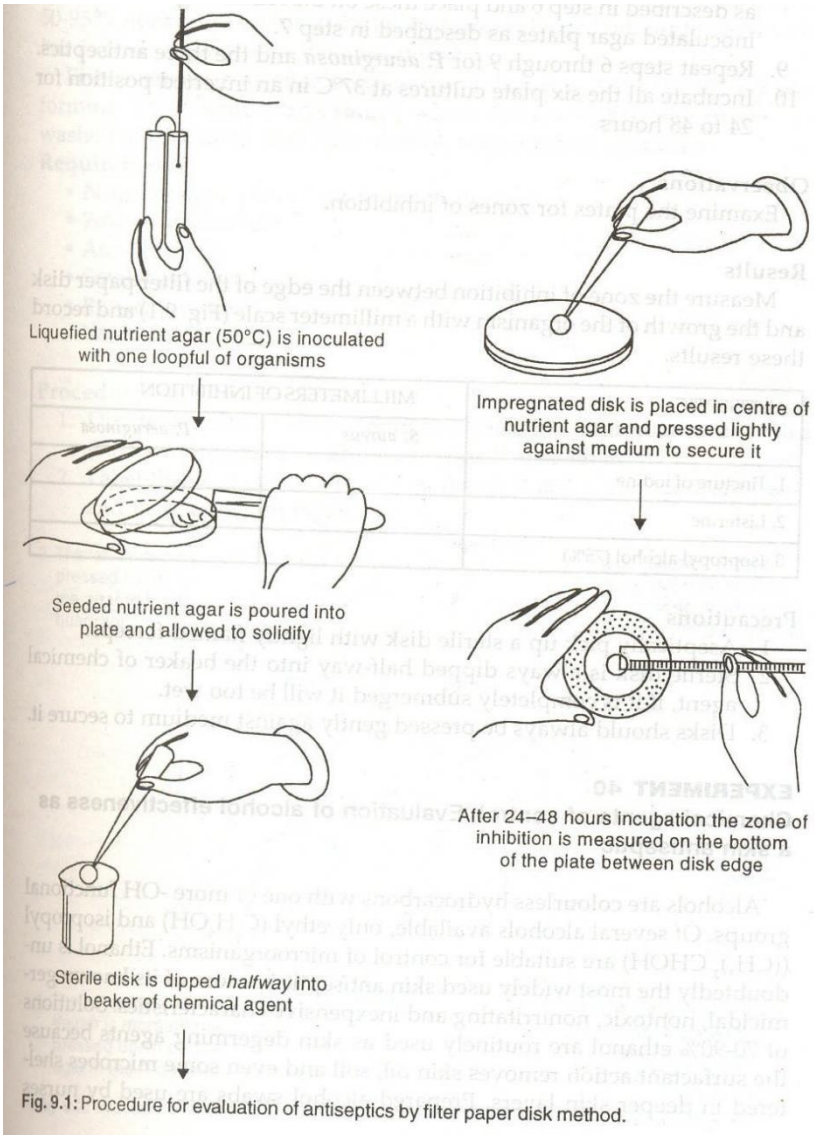


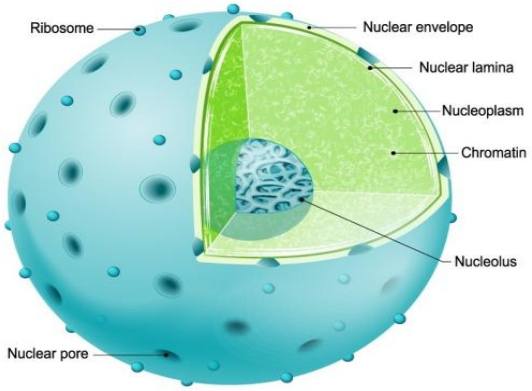
Fig. 9.1: Procedure for evaluation of antiseptics by filter paper disk method.

Antibiotic Sensitivity test by filter paper disk method

BIOLOGY OF EUKARYOTIC MICROBES

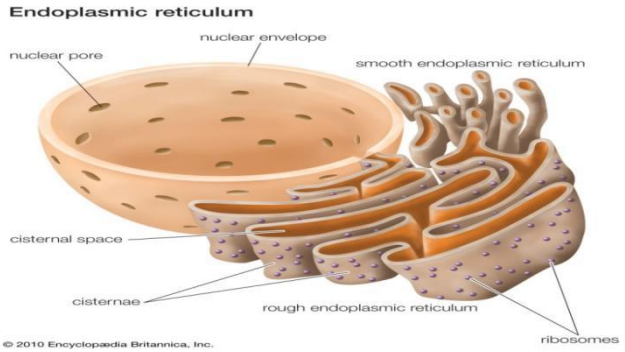
Experiment No. 1
OBSERVATION OF DIFFERENT CELL ORGANELLES

1. Nucleus:



1. The nucleus is a double-membrane organelle found in all eukaryotic cells and it is the largest organelle.
2. It functions as the control center of the cellular activities and is the storehouse of the cell's DNA.
3. Structurally, the nucleus is dark, round, surrounded by a nuclear membrane. It is a porous membrane and forms a wall between cytoplasm and nucleus.
4. Within the nucleus, there is tiny spherical body called nucleolus. It also carries essential structures called chromosomes.
5. Chromosomes are thin and thread-like structures which carry the genes.
6. Genes are the hereditary units in organisms and helps in the inheritance of traits from one generation (parents) to another (offspring). Hence, the nucleus controls the characters and functions of cells in our body.

2. Endoplasmic Reticulum



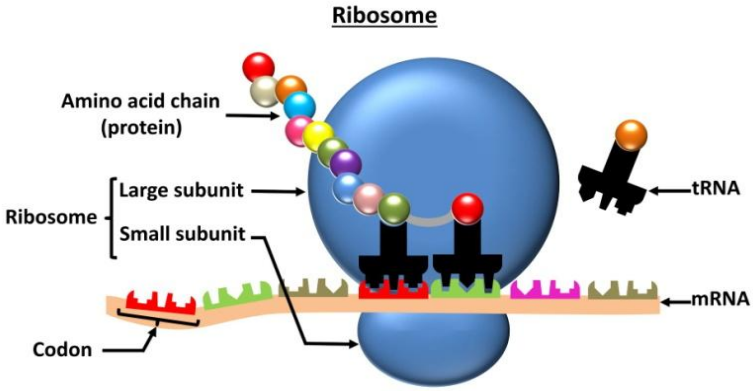
1. The Endoplasmic Reticulum is a network of membranous canals filled with fluid.

2. These membranes form continuous folds, eventually joining the outer layer of the nuclear membrane.
3. They are composed of cisternae, tubules, and vesicles, which are found throughout the cell.
4. They are the transport system of the cell, involved in transporting materials throughout the cell.
5. There are two different types of Endoplasmic Reticulum:

a) Rough Endoplasmic Reticulum – They are studded with ribosomes and are involved in protein manufacture.

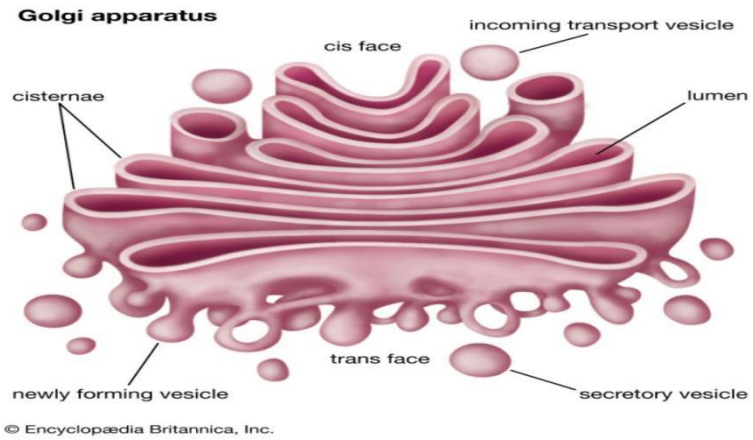
b) Smooth Endoplasmic Reticulum – They are the storage organelle, associated with the production of lipids, steroids, and also responsible for detoxifying the cell.

3. Ribosomes



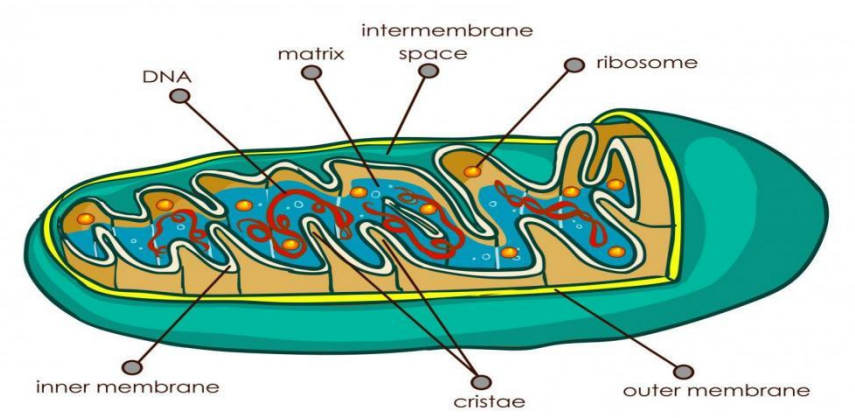
1. Ribosomes are no membrane-bound and important cytoplasmic organelles found in close association with the endoplasmic reticulum.
2. Ribosomes are tiny particles found in a large number in cells and are mainly composed of 2/3rd of RNA and 1/3rd of protein.
3. They are named as the 70s (found in prokaryotes) or 80s (found in eukaryotes). The letter 'S' refers to the density and the size, known as Svedberg's Unit. Both 70S and 80S ribosomes are composed of two subunits, smaller sub unit and larger sub unit.
4. Ribosomes are either encompassed within the endoplasmic reticulum or are freely traced in the cell's cytoplasm.
5. Ribosomal RNA and Ribosomal proteins are the two components that together constitute ribosomes.
6. The primary function of the ribosomes includes protein synthesis in all living cells that ensure the survival of the cell.

4. Golgi Apparatus



1. Golgi apparatus is also termed as Golgi complex. It is a membrane-bound organelle.
2. It is mainly composed of a series of flattened, stacked pouches called Cisternae, Tubules and Vesicles.
3. It is divided into three parts like Cis-golgi, Median-golgi and Trans-golgi.
4. This cell organelle is primarily responsible for transporting, modifying, and packaging proteins and lipids to targeted destinations.
5. Golgi apparatus is found within the cytoplasm of a cell and is present in both plant and animal cells.

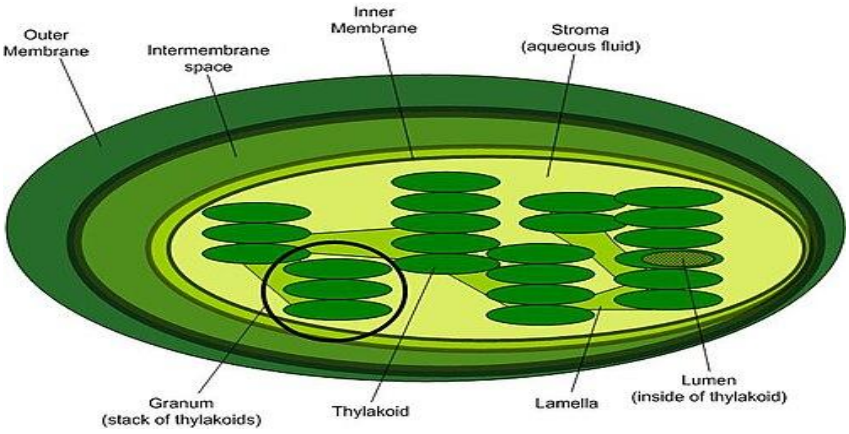
5. MITOCHONDRIA



1. Mitochondria are called the powerhouses of the cell as they produce energy-rich molecules for the cell.
2. The mitochondrial genome is inherited maternally in several organisms. It is a double membrane-bound, sausage-shaped organelle, found in almost all eukaryotic cells.
3. The double membranes divide its lumen into two distinct aqueous compartments. The inner compartment is called a ‘matrix’ which is folded into cristae, whereas the outer membrane forms a continuous boundary with the cytoplasm.
4. They usually vary in their size and are found either round or oval in shape.

5. Mitochondria are the sites of aerobic respiration in the cell, produce energy in the form of ATP and helps in the transformation of the molecules.
6. Mitochondria have their own circular DNA, RNA molecules, ribosomes (the 70s), and a few other molecules that help in protein synthesis

6. **Chloroplasts**



1. Chloroplasts are the double membrane-bound organelles, which usually vary in their shape – from a disc shape to spherical, discoid, oval and ribbon.
2. They are present in mesophyll cells of leaves, which store chloroplasts and other carotenoid pigments.
3. These pigments are responsible for trapping light energy for photosynthesis.
4. The inner membrane encloses a space called the Stroma. Flattened disc-like chlorophyll-containing structures known as Thylakoids are arranged in a stacked manner like a pile of coins.
5. Each pile is called a Granum (plural: grana) and the thylakoids of different grana are connected by flat membranous tubules known as Stromal lamella.
6. Like the mitochondrial matrix, the stroma of chloroplast also contains a double-stranded circular DNA, 70S ribosomes, and enzymes which are required for the synthesis of carbohydrates and proteins.

Experiment No. 2

OBSERVATION OF DIFFERENT MITOTIC STAGES OF ONION ROOT TIPS BY SQUASH TECHNIQUE

Aim:

To visualize and understand the different stages of mitotic cell division in onion root tips.

Principle:

Cell division occurs rapidly in growing root tips of sprouting seeds or bulbs. An onion root tip is a rapidly growing part of the onion, and thus many cells will be in different stages of mitosis. The onion root tips can be prepared and squashed in a way that allows them to be flattened on a microscopic slide, so that the chromosomes of individual cells can be observed easily. The supercoiled chromosomes during different stages of mitosis present in the onion root tip cells can be visualized by treating with DNA-specific stains like Feulgen stain and Aceto-carmine stain.

Materials Required:

Onion plant with root, Acetocarmine stain, 1N HCl, Scissors, Forceps, Razor blade, Microscope, slides and cover slips, Water bath, Light microscope.

Procedure:

1. Cut the freshly sprouted onion root tips to a size of 5-8 mm and discard the rest of the root.
2. Wash them in water on a clean microscope slide.
3. Place one drop of 1N HCl on the root tip and add 2-3 drops of acetocarmine stain to the slide.
4. Warm the slide gently over the spirit lamp for about one minute and do not allow the slide to get hot to touch.
5. Carefully blot the excess stain with blotting paper.
6. After 10-20 seconds, put a drop of water and blot carefully using blotting paper.
7. Again, put a drop of water on the root tip and mount a cover slip on it, avoiding air bubbles.
8. Squash the slide with your thumb using a firm and even pressure. (Avoid squashing with such force that the cover slip breaks or slides.)
9. Observe the slide under compound microscope in 10x objective. Scan the slide and focus to a region containing dividing cells and switch to 40x for a better view.

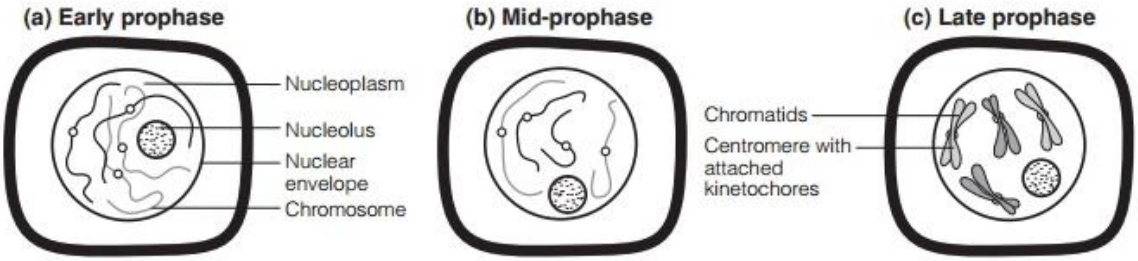
Result: Identify the different stages of mitosis and draw neat diagrams.

Different stages of Mitotic cell division

Prophase

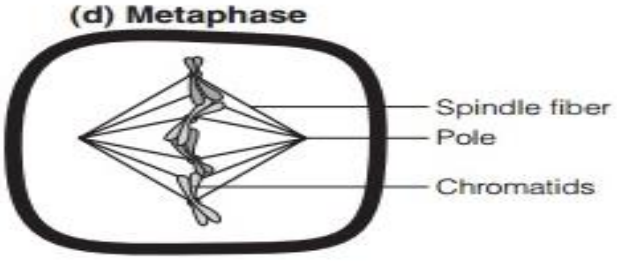
1. Prophase is the first stage of mitosis which is characterized by the appearance of thin-thread like condensing chromosomes.
2. During prophase, the cell becomes spheroid while the cytoplasm becomes more refractile and viscous and pale.

3. The chromosome in the prophase is composed of two coiled filaments, the chromatids.
4. As prophase progresses, the chromatids become shorter and thicker, and two sister chromatids of each chromosome are held together by a special DNA-containing region, called the centromere**.**
5. Similarly, the chromosomes approach the nuclear envelope, causing the central space of the nucleus to become empty.
6. In the meantime, two pairs of centrioles surrounded by microtubules radiating in all directions migrate to opposite poles of the cell.
7. Lastly, during prophase, the nucleolus gradually disintegrates, and this marks the end of prophase.



Prometaphase and Metaphase

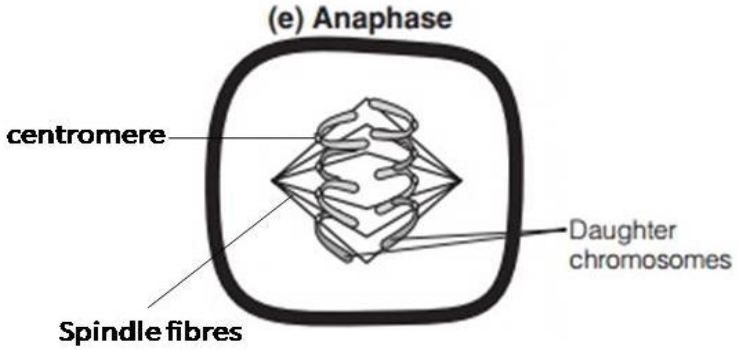
1. Prometaphase is initiated with the breakdown of the nuclear envelope, which enables the interaction of spindle fibers with the chromosomes.
2. At this stage, the chromosomes are violently rotated and oscillated back and forth between the spindle poles because their centromeres are capturing the ends of microtubules and are being pulled by the captured microtubules.
3. By the end of prometaphase, the sister chromatids are attached to the spindle fibers on the opposite ends and are held on the metaphase plate.
4. During metaphase, the chromosomes are shortest and thickest.
5. Their centromeres of the sister chromatids occupy the plane of the equator forming a metaphase plate, and the arms remain directed towards the poles.
6. Two chromatids of a chromosome repulse each other with the microtubules remaining stationary and under tension.



Anaphase

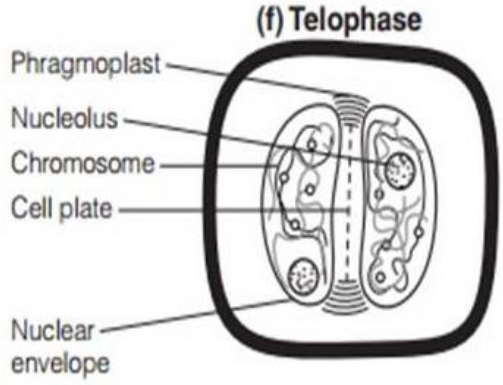
1. The anaphase begins abruptly with the synchronous splitting of each chromosome into its sister chromatids, called daughter chromosomes, separating for the centromere.
2. The splitting of each centromere during prophase is caused by an increase in cytosolic Ca^{2+}.

3. In anaphase, there is a movement of chromatids towards the pole due to the shortening of the microtubules.
4. During their pole ward migration, the centromeres remain forward so that the chromosomes characteristically appear U, V or J- shaped.
5. Inter zonal fibers expand and support the movement of chromosomes towards the pole.
6. A total of 30 ATPs are required to carry chromosomes to the poles.



Telophase and Cytokinesis

1. The end of the migration of the daughter chromosomes to the poles marks the beginning of the telophase.
2. During telophase, the events of prophase occur in reverse sequence.
3. A nuclear envelope reassembles around each group of chromosomes to form two daughter nuclei.
4. Events like the disappearance of mitotic apparatus, reduction in the viscosity of cytoplasm followed by synthesis of RNA take place during telophase.
5. The chromosomes resume their long, slender, extended form and the nucleolus reappears at the end of telophase.
6. Cytokinesis occurs by cell plate formation.
7. Golgi apparatus arrange themselves on the equator to form phragmoplast, which later forms the cell plate in plants.



Experiment No. 3

OBSERVATION OF DIFFERENT STAGES OF MEIOTIC CELL DIVISION IN FLOWER BUDS OF *ALLIUM CEPA*

Aim:

To visualize and understand the different stages of meiotic cell division in flower buds of *Allium cepa*.

Principle:

Reduction of the chromosome number by half, from a diploid (2n) to a haploid (n) state, while generating four genetically distinct daughter cells. This process is crucial for sexual reproduction, as it ensures that the resulting gametes (sperm and egg) have a single set of chromosomes, which, then combine during fertilization to restore the diploid number in the new offspring.

Materials Required:

Onion flowers, Carnoy's fixative, Acetocarmine stain, 70% ethanol, 2N HCl, 45% acetic acid, Glass slides, Cover slips, blotting paper.

Procedure:

1. Pluck the onion buds (preferably at morning) and transfer them to Carnoy's fixative (acetic alcohol 1:3 w/v) for 24hrs.
2. At the end of the fixation period, transfer the buds to a storage solution (70% ethanol) and keep these vials at 4-5°C until required for the experiment.
3. Remove the anthers from the onion buds stored in 70% ethanol.
4. Transfer the anthers to a watch glass and wash thoroughly with water (at least 3 times).
5. Drain off the water with a Pasteur pipette and add a few drops of 2N HCl. Leave it for 10-15 min at room temperature to allow softening of the tissue.
6. Remove HCl with a Pasteur pipette and wash the anthers again with water.
7. Transfer the anthers to 2% acetocarmine and stain for 10 minutes.
8. Destain the tissue by transferring it onto a clean slide and add few drops of 45% acetic acid with a dropping bottle.
9. Place cover slip carefully on the stained anthers and apply moderate pressure in a vertical direction with your thumb or with the flat bottom of a pencil. Tapping flattens the cells and spreads the chromosomes. A properly squashed tissue should be about three times its original size.
10. Examine the slides under the compound microscope at 10X and 40X.

Result: Identification of different stages of meiotic cell division.

Meiosis-I (heterolytic or Reductional division)

Meiosis-I has four different phases or stages:

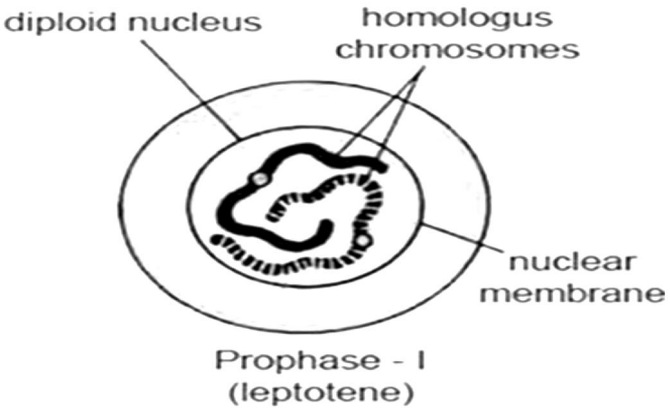
1. Prophase-I
2. Metaphase-I
3. Anaphase-I
4. Telophase-I

1. Prophase-I

1. It occupies the longest duration in Meiosis-I.
2. It is divided into five sub-stages or sub-phases.

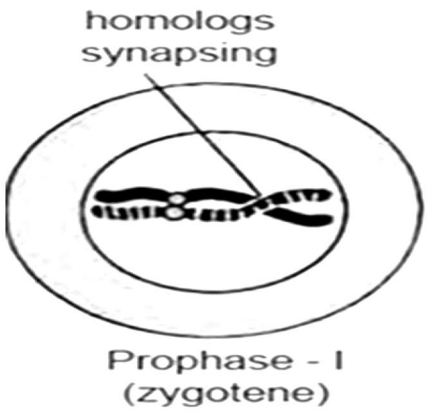
i. Leptotene

- This phase starts immediately after interphase.
- The size of cell and nucleus increases
- The chromosomes appear long, uncoiled thread-like in structure bearing many bead-like structures called chromomeres.
- The nuclear membrane and nucleolus remain as it is.



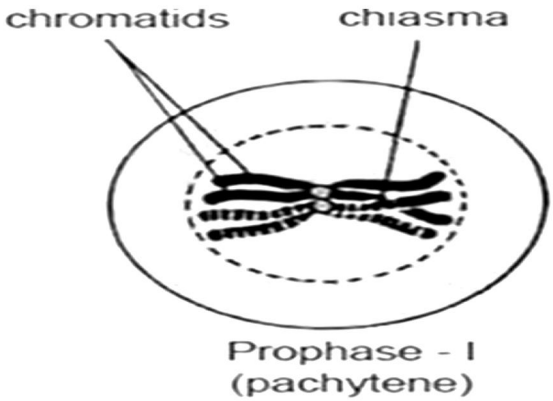
ii. Zygotene

- Homologous chromosomes come closer and starts to pair up along their length.
- The pairing of homologous chromosomes is called Synapsis and the paired homologous chromosomes are referred as bivalents.
- The homologous chromosomes are held together by ribonuclear protein between them.



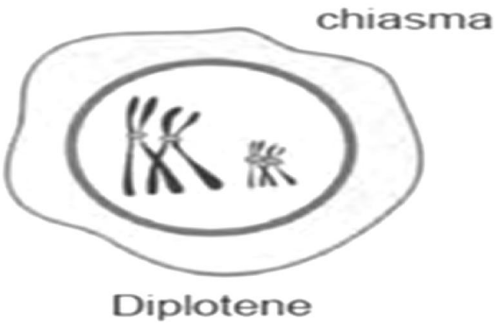
iii. Pachytene

- The chromosome becomes shorter and thicker.
- Each chromosome of the bivalents splits longitudinally to form two chromatids such that bivalents is composed of four strands and is known as a tetrad.
- The process of crossing over starts (crossing over; a small fragment of chromosome exchange between two non-sister chromatids of bivalent by breakage and rejoining).
- Crossing over is the most important genetic phenomenon of meiosis which causes variation in genetic characters in offspring.



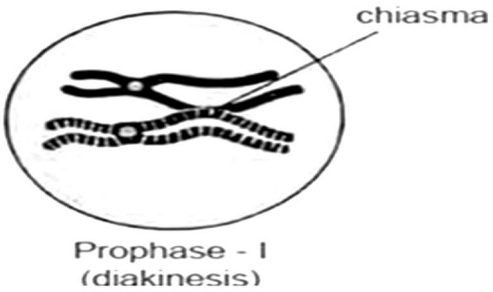
iv. Diplotene

- In this stage crossing over takes place.
- Bivalents (chromatids) repel each other.
- Homologous chromosome (two non-sister chromatids) begins to separates but separation is not complete, they remains attached to a point with a knot like structure called as chiasmata (singular – chiasma).
- The number of chiasmata varies. Depending upon the number of chiasmata, chromosome appears in different shapes.
  1 chiasmata: cross like
  2 chiasmata: ring like
  Many chiasmata: series of loop
- Nuclear membrane and nucleolus begins to disappear.



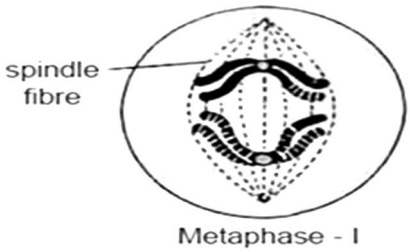
v. Diakinesis

- The chiasma moves towards the end of the chromosomes (tetrad) due to contraction of chromosome lastly slips over separating the homologous chromosome. This movement of the chiasmata towards the end of chromosome is called terminalization.
- By the end of diakinesis the nuclear membrane and nuleolus get completely disappeared and the chromosomes are free in the cytoplasm.
- Spindle fibers begin to form.



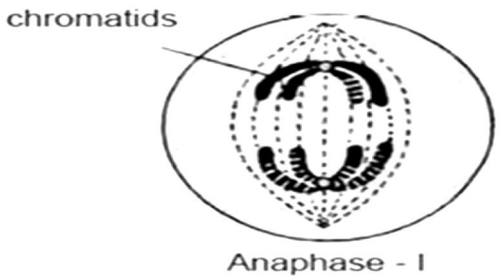
2. Metaphase-I

- The spindle fibers organized between two poles and get attached to the centromere of chromosomes.
- Chromosome moves to equator.
- The bivalent chromosomes are arranged in the equatorial plate in such a way that 2 metaphasic plates are formed.



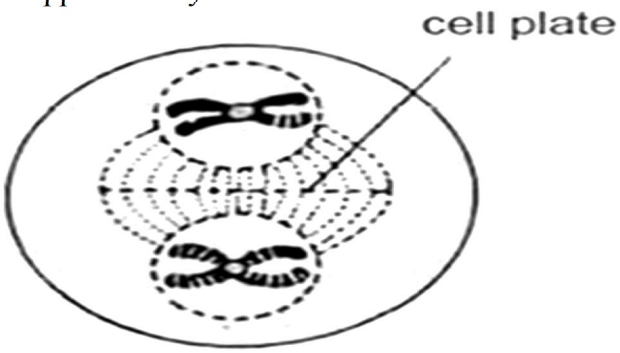
3. Anaphase-I

- Spindle fibers contracts and pulls the whole chromosomes to the polar region.
- The separated chromosome is known as dyads.
- No splitting of chromosomes occurs so the centromere of each homologous chromosome does not divide. Thus, the chromosome number of the daughter nuclei is reduced to half.
- Now the separated chromosome moves toward opposite poles.



4. Telophase-I

- Two groups of chromosome formed at each pole and organized into nuclei.
- The nuclear membrane and nucleolus reappears.
- The chromosomes get uncoiled into chromatin thread.
- The spindle fibers disappear totally.



Telophase - I

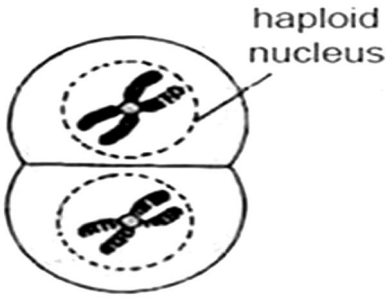
Meiosis-II (Homolytic or equational division)

- Meiosis-II is exactly similar to mitosis, so it is also known as meiotic mitosis.
- In this division, two haploid chromosomes splits longitudinally and distributed equally to form 4 haploid cells.
- It completes in 4 stages.

1. **Prophase-II**
2. **Metaphase-II**
3. **Anaphase-II**
4. **Telophase-II**

1. Prophase-II:

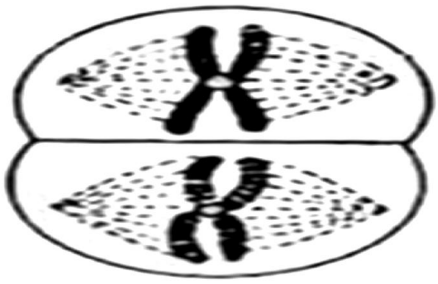
- The dyads chromosome becomes thicker and shorter.
- Nuclear membrane and nucleolus disappear.
- Spindle fiber starts to form.



Prophase - II

2. Metaphase-II:

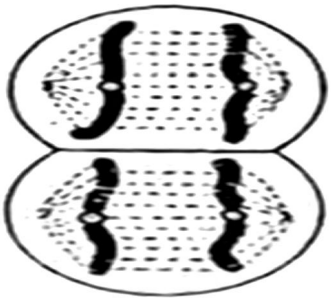
- The dyads chromosomes comes to equatorial plane.
- Spindle fibers organize between poles and attaches to centromere of chromosome.



Metaphase - II

3. Anaphase-II:

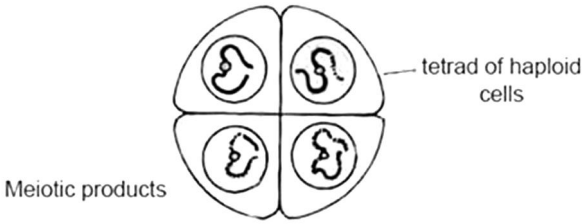
- Centromere of each chromosome divides and sister chromatids separates to form two daughter chromosome.
- Spindle fiber contracts and pull the daughter chromosome apart towards opposite pole.



Anaphase - II

4. Telophase-II:

- Chromosome become organizes at respective pole into nuclei.
- Chromosome elongates to form thin networks of chromatin.
- Nuclear membrane and nucleolus reappears.



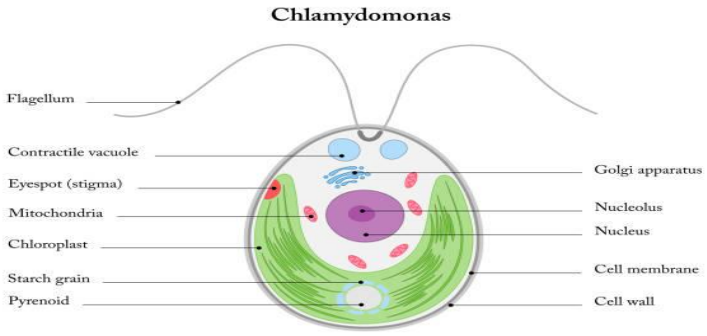
Cytokinesis

This separates each nucleus from the others. The cell wall is formed and 4 haploid cells are produced.

Experiment No. 4

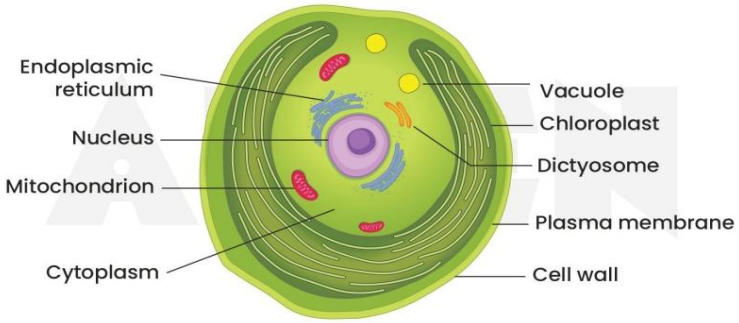
IDENTIFICATION OF DIFFERENT ALGAE FROM POND WATER

***Chlamydomonas*:**



- *Chlamydomonas* is mostly fresh water, but also grow in ditches, tanks, ponds, lakes marine water and moist terrestrial habitats.
- It is a motile, unicellular alga.
- It is a green algal member.
- Generally oval in shape and possess cellulosic cell wall.
- Possess two anterior whiplash type flagella of equal length.
- Contains a single haploid nucleus, large chloroplast, pyrenoid, and stigma or eyespot for phototactic response.
- At the base of the flagella, two small contractile vacuoles are present for osmoregulation.
- Reproduce asexually by zoospores formed through cell division.
- Sexual reproduction through gametic fusion.

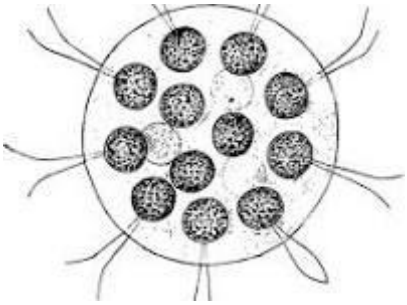
***Chlorella*:**



- Unicellular green alga found in freshwater, brackish water and terrestrial habitats.
- Non-motile, spherical, sub-spherical or ellipsoidal bound by a true cellulosic wall.
- Characterized by bell shaped or cup shaped parietal chloroplast with or without pyrenoid.
- Single small nucleus, mitochondria and golgi bodies are present.
- Flagella, eyespots and contractile vacuoles are absent.
- Only asexual reproduction is present and produces asexual autospores.

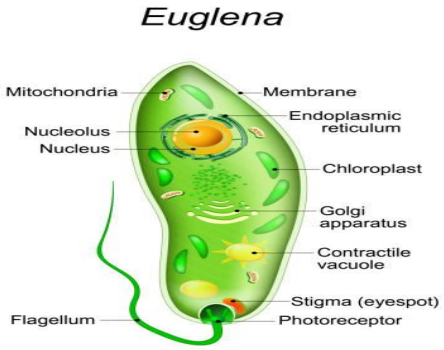
- Motile cells, zoospores or gametes are not produced.
- Few members may live as symbionts.

***Eudorina*:**



- Members are common inhabitants of freshwater ditches, pools, ponds and lakes.
- This alga forms motile coenobia which are ellipsoidal or spherical.
- Each coenobium has 32 or 64 globose cells embedded in mucilaginous matrix and arranged in definite tiers of 4,8,8,8,4 cells each.
- In cell structure it very much resembles with chlamydomonas.
- Vegetative reproduction is by fragmentation of coenobia.
- Asexual reproduction through the formation of gonidia, which produce daughter coenobia.
- Sexual reproduction is anisogamous or oogamous. Zygote formation is by gametic fusion. Zygote loose flagella, germinate and meiotically produce one or more zoospores that give rise to new individuals.

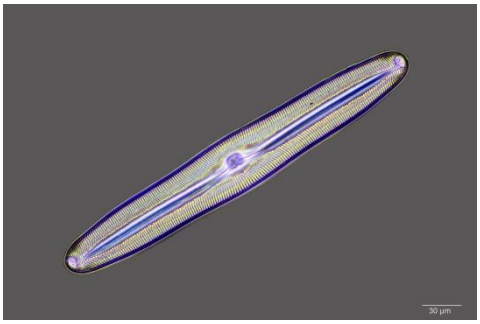
***Euglena*:**



- Unicellular and belongs to Euglenophyta.
- It is the representative genus.
- Typical ***Euglena*** cell is elongated and bounded by a plasma membrane.
- Inside the plasma membrane, an elastic pellicle which is composed of articulated proteinaceous strips lying side by side is present.
- Possess several chloroplasts that contain chlorophylls ‘a’ and ‘b’ together with carotenoids.
- Nucleus is large and contains a prominent nucleolus.
- Large contractile vacuole is present to regulate the osmotic pressure within the organism.

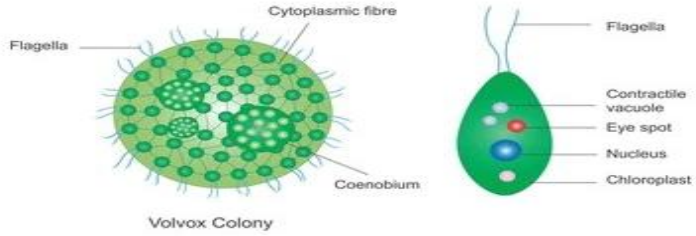
- Two flagella arise from the base of the reservoir.
- Near the reservoir, pigment spot called stigma is present.
- The primary storage product is paramylon.

***Pinnularia*:**



- Unicellular alga belonging to Chrysophyta.
- Possess a distinctive two-piece wall of silica called a frustule.
- The upper, older, overlapping bigger part or lid is called the epitheca.
- The lower, smaller, overlapped half is called the hypotheca.
- Each half consists of the main top and bottom surfaces called the valves.
- The incurved lateral sides at right angles containing the overlapping connecting bands are called the girdles.
- Contains smaller plastids.
- The single large nucleus of the vegetative cell is diploid.
- The cells lack the flagella.

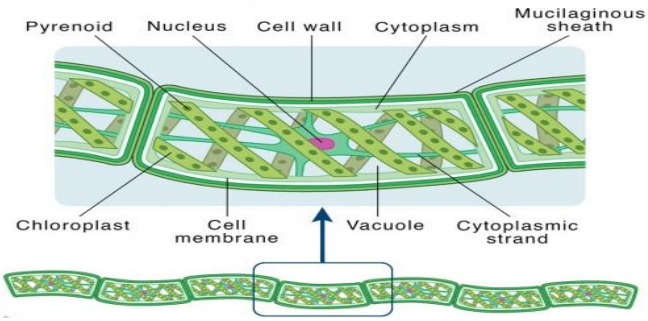
***Volvox*:**



- *Volvox* is a freshwater planktonic form occurring as green balls of pin head size.
- The colonies, usually called as coenobia, are generally spherical, oval or ellipsoidal in their shape.
- A definite number of cells form a globular outer hull which is held together by a highly viscous, gelatinous, glycoprotein sheath.
- The cell number per coenobium range from 500 to 50,000.
- The cells in the posterior region of the coenobium are usually larger than those in the anterior region.
- Coenobia are motile by the joint action of the flagella of individual cells.
- Each cell of V*olvox* resembles the *Chlamydomonas* cell with a pair of anteriorly inserted flagella.
- Chloroplast is cup shaped. Two or more contractile vacuoles and an eyespot are

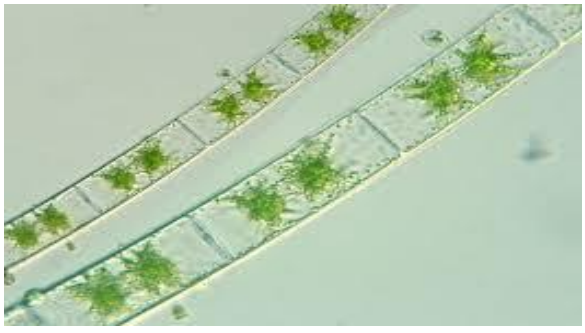
found in the anterior region.
- Both asexual and sexual reproductions are present.
- Pyrenoids are one or more in number.

***Spirogyra*:**



- *Spirogyra* is a freshwater, submerged or free-floating alga.
- It is unbranched and filamentous with uniseriate row of cylindrical cells.
- The basal cell frequently develops into a branched or highly lobed anchoring organ.
- The filaments are slimy to touch because of an outer mucilaginous wall layer.
- The cell wall consists of two layers, an inner cellulosic and an outer pectic layer.
- The septa between the cells may be plane or semireplicate or replicate.
- Contains one or a few spiral, ribbon-shaped chloroplasts, each with many pyrenoids.
- The cytoplasm forms a thin layer lining the cell wall.
- A single nucleus is situated in the central part of the cell and is connected with the peripheral cytoplasm through a number of radiating cytoplasmic strands.
- Vegetative reproduction is the common method and involves the fragmentation of filaments.
- The fusing gametes produced during sexual reproduction are morphologically isogamous and physiologically anisogamous.

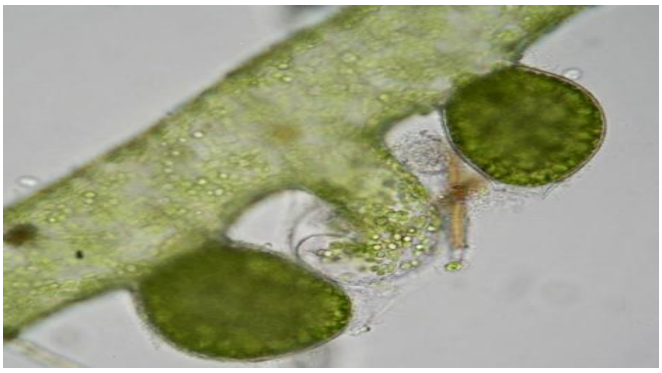
***Zygnema*:**



- *Zygnema* is a widely distributed free-floating freshwater alga.
- It is filamentous and unbranched .
- The cells of zygnema contain a pair of axile stellate chloroplasts.
- Each chloroplast harbours a single central pyrenoid with radiating starch grains.

- The single nucleus of the cell embedded in the middle of the cytoplasm that separates the two chloroplasts.
- Vegetative reproduction is by the fragmentation of the filament.
- Sexual reproduction mode is of isogamous type.

***Vaucheria*:**



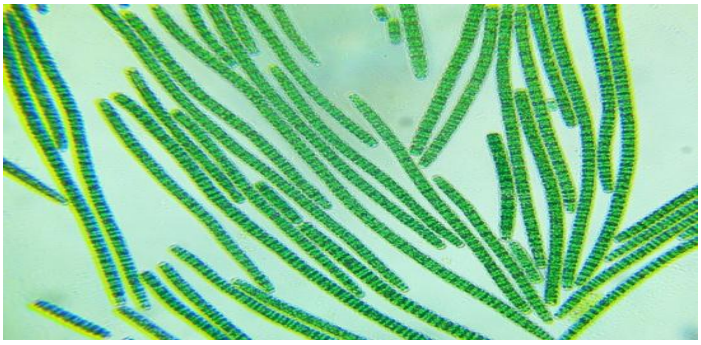
- Occurs widely in stagnant and flowing freshwaters, in shaded terrestrial habitats and on walls.
- The thallus is generally a sparingly branched, cylindrical tube lacking cross walls or septa.
- Rhizoid-like branches anchor the alga to the substratum, in terrestrial species.
- Thallus contains an outer cellulosic cell wall and a central vacuole that runs continuously from one end to the other end of the thallus.
- The protoplast is continuous and peripherally contains many discoid chromatophores devoid of pyrenoids.
- Numerous nuclei lie internal to the chromatophores.
- Asexual reproduction is through zoospore formation.
- Sexual reproduction is of oogamous type.

***Oedogonium*:**

- *Oedogonium* is a widespread freshwater alga that grows epiphytically in ponds, pools, and shallow tanks.
- It is unbranched and filamentous with cylindrical cells except the basal holdfast cell.
- The cell wall is thick and differentiated into an inner cellulosic, middle pectic and outer chitinous layers.

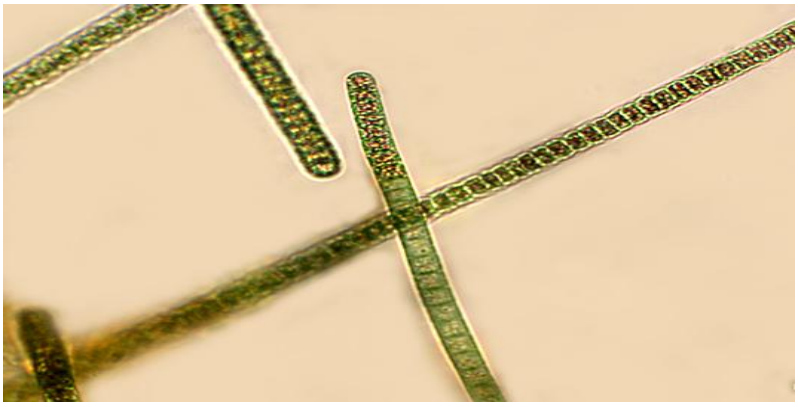
- The cells are uninucleate and have a central vacuole containing cell sap.
- The chloroplast is elaborate, reticulate with many pyrenoids.
- Fragmentation is the usual method of vegetative propagation.
- Asexual reproduction is by the formation of multiflagellate zoospores.
- Sexual reproduction is of oogamous type.

***Spirulina*:**



- It is a member of cyanobacterial order, Oscillatoriales.
- It occurs more commonly in brackish and salt water.
- It forms phytoplankton covering the surface of water.
- It is a protein-rich fresh water cyanobacterial form.
- The filaments are spirally twisted.
- Filament is an unbranched trichome with only vegetative cells.
- The mode of reproduction is binary fission in single plane.
- It is usually motile and does not have the heterocysts.

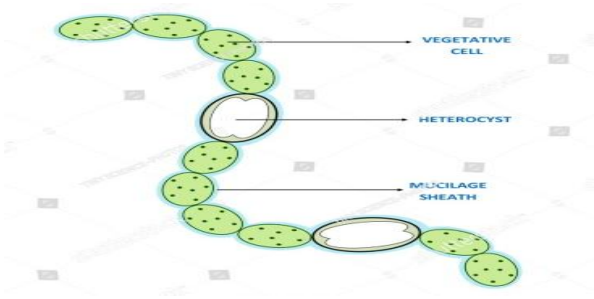
***Oscillatoria*:**



- It is a common filamentous form widely distributed in freshwater environments and damp soil and rocks.
- It is a member of cyanobacterial order, Oscillatoriales.
- The filaments are unbranched containing a single row of cells.
- The individual cells are much shorter than length.
- It exhibits oscillatorian movement.

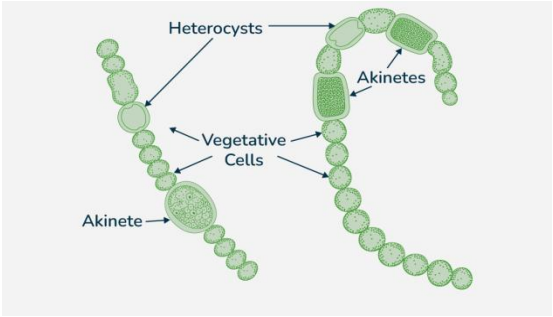
- Exhibits binary fission of reproduction in single plane and also fragmentation.
- Usually motile and does not have the heterocysts.

***Nostoc*:**



- Found in soil and freshwater habitats.
- Member of the cyanobacterial order, Nostocales.
- Occur in the form of minute balls of mucilage. Some occur in the intracellular spaces of bryophytes.
- The filaments are embedded in the mucilaginous mass and each individual filament is referred as trichome.
- Possess intercalary as well as terminal heterocysts in a trichome.
- Form hormogonia through fragmentation process.
- Reproduction is through single plane binary fission.
- Often motile and may produce akinetes.
- The heterocysts are prominently present.

***Anabaena*:**



- It occurs as free-floating forms in ponds, lakes, paddy fields and other freshwater habitats.
- Some species of *Anabaena* inhabits the coralloid roots of cycads.
- The gelatinous sheath is not as viscous as in *Nostoc.*
- *Anabaena* do not form large colonies like *Nostoc.*
- Often motile and may produce akinetes.
- The heterocysts are prominently present.
- Form hormogonia through fragmentation process.
- Reproduction is through single plane binary fission.

Experiment No. 5

ISOLATION OF SOIL ALGAE

Aim:

To isolate algae from soil sample.

Materials Required:

Conical flask, Beneck's medium agar medium, Petri plates, Hot air oven, Autoclave, Laminar air flow chamber, Incubator, Spirit lamp, pH strips, Soil sample.

Composition of Beneck's medium:

KNO_3	0.2 g
$MgSO_4.7H_2O$	0.2 g
K_2HPO_4or KH_2PO_4	0.2 g
$CaCO_3$	0.1 g
$FeCl_3$(1%)	2.0 drops
Agar	25 g
Distilled water to	1000 ml
pH	7.0 –7.5

Procedure:

1. Collect a representative soil sample and prepare slurry by mixing it with a sterile solution (like water or saline).
2. Prepare a series of progressively diluted samples, typically using a 10-fold serial dilution (1:10) in sterile liquid media. For example, take 1 mL of the slurry and mix it with 9 mL of sterile liquid, then take 1 mL of this diluted sample and mix it with 9 mL of sterile liquid, and so on.
3. Prepare the Beneck's medium as per the composition and steam sterilize at 121°C for 15 minutes. Phosphate should be autoclaved separately from the other components and mixed aseptically upon cooling.
4. Plate a known volume (e.g., 100 µL) of each dilution onto the agar plates containing a suitable growth medium for algae (Beneck's medium).
5. Incubate the plates under appropriate conditions (temperature, light, etc.) for a period that allows the algae to grow and form colonies.
6. After incubation, examine the plates for individual algal colonies that are distinct from other microorganisms.
7. Select the isolated colonies and further isolate them by transferring them to new plates or liquid media. The isolated algae can then be characterized using microscopy, DNA sequencing, or other methods.

Result: Observing the isolated colonies under microscope helps in identifying the algal species. The number of colonies on the plates can be used to estimate the initial algal concentration in the soil sample.

Experiment No. 6

ISOLATION OF FUNGI FROM SOIL

Aim:
To isolate fungi from soil.

Requirements:
Conical flask, Sabouraud's agar medium, Petri plates, Hot air oven, Autoclave, Laminar air flow chamber, Incubator, Spirit lamp, pH strips.

Composition of Sabouraud's Agar Medium:
Peptone – 10 gm
Dextrose – 40 gm
Agar – 20 gm
pH – 5.6 ± 0.2

Procedure:
1. Weigh the medium ingredients as per the composition and dissolve the components in distilled water.
2. Adjust the medium pH to 5.6 ± 0.2 by adding HCl or NaOH solution.
3. Sterilize the medium in an autoclave at 121°C and 15 lbs pressure for 15 minutes.
4. After sterilization, cool the medium under running tap water.
5. Pour 15-20 ml of aliquots of sterilized medium into sterilized petri plates and allow them to solidify.
6. Take 1 gm of soil sample and dissolve it in 100 ml of distilled water (This is 10^{-2} dilution).
7. Take 1 ml from 10^{-2} dilution and mix it with 9 ml of distilled water in a test tube using a pipette (10^{-3} dilution).
8. Repeat the process serially in test tubes up to 10^{-9} dilutions.
9. Using a micropipette or pipette, add 1-2 drops of soil suspensions from 10^{-3}, 10^{-4}, and 10^{-5} dilutions onto the separate solidified agar plates.
10. Spread the soil solution on the media using a spreader aseptically.
11. Mark the plates with the added dilutions.
12. Incubate the plates in an incubator at 28-30°C in an upright position for 3-4 days.

Observation:
After incubation, fungal colonies appear on the petri plates.

Result:
Morphologically distinct fungal colonies are observed on the Sabouraud Dextrose Agar medium.

Experiment No. 7

ISOLATION OF FUNGI FROM AIR

Aim:

To isolate fungi from air on fungal-specific media.

Requirements:

Petri plates, Sabouraud agar medium, Conical flask, Distilled water, pH strips, Laminar air flow chamber, Autoclave, Hot air oven, Spirit lamp.

Composition of Sabouraud Agar Medium:

Peptone – 10 gm
Dextrose – 40 gm
Agar – 20 gm
pH – 5.6 ± 0.2
Distilled water – 1000 ml

Procedure:

1. Dissolve the ingredients of the medium in a conical flask containing distilled water.
2. Adjust the medium pH to 5.6 ± 0.2 by adding HCl or NaOH.
3. Sterilize the medium in an autoclave at 121°C and 15 lbs pressure for 15–20 minutes.
4. After sterilization, cool the medium under running tap water.
5. Pour 15-20 ml aliquots of medium into sterilized Petri plates.
6. Allow the plates to solidify. After solidification, expose the plates to air.
3. Incubate the plates in an incubator at 25–30°C for 3–5 days.

Observation:

After incubation, the plates showed the growth of fungal colonies.

Result:

Different morphological fungal colonies were developed on the Sabouraud agar medium.

Discussion:

Aspergillus species are easily recognized by their conidiophores terminating in apical vesicles, and at the opposite end, the basal cell is inserted into the supporting hyphae. The identification of species depends primarily on colony and form of conical heads.

Experiment No. 8

ISOLATION OF FUNGI FROM WATER

Aim:

To isolate fungi from water.

Materials Required:

Conical flask, Sabouraud's agar medium, Petri plates, Hot air oven, Autoclave, Laminar air flow chamber, Incubator, Spirit lamp, Test tubes, pH strips.

Composition of Sabouraud's Agar Medium:

Peptone – 10 gm
Dextrose – 40 gm
Agar – 20 gm
pH – 5.6 ± 0.2

Procedure:

1. Weigh the medium ingredients as per the composition and dissolve them in distilled water.
2. Adjust the pH of the medium to 5.6 ± 0.2 by adding HCl or NaOH solution.
3. Sterilize the medium in an autoclave at 121°C and 15 lbs pressure for 15 minutes.
4. After sterilization, cool the medium under running tap water.
5. Pour 15-20 ml of aliquots of sterilized medium into sterilized petri plates.
6. Allow them to solidify.
7. Take 1 ml of water sample and dissolve it in 100 ml distilled water.
8. Add 1 ml of the prepared water sample to the solidified agar medium and spread the inoculum on the surface.
9. Incubate the inoculated plates in an incubator at 25–30°C for 3–5 days.

Observation

After completion of incubation period, fungal colonies were observed.

Result

Distinct fungal colonies were grown on the Sabouraud Dextrose Agar medium.

Discussion :

Aspergillus species are usually and easily recognized by their conidiophores, which terminate in an apical vesicle and at the opposite end in a basal cell inserted into the supporting hyphae. The identification of species primarily depends on colony colour.

Experiment No. 9

ISOLATION OF *ASPERGILLUS* FROM LEMON

Aim:

To isolate fungi from lemon.

Materials Required:

Czapek-Dox medium, Glass slides, Microscope, Inoculating loop, Inoculation chamber, Petri plates, Autoclave, Hot air oven, Laminar air flow chamber, lemon fruit spoiled with *Aspergillus* fungus.

Composition of Czapek-Dox Agar Medium:

Sucrose - 30 gm
Sodium nitrate ($NaNO_3$) - 2 gm
Dipotassium hydrogen phosphate (K_2HPO_4) - 1 gm
Magnesium sulfate ($MgSO_4$) - 0.5 gm
Potassium chloride (KCl) - 0.5 gm
$FeSO_4$ - 0.01 gm
Agar - 15-20 gm
pH - 7.3 ± 0.2

Procedure:

1. Weigh the medium ingredients as per the composition and dissolve them in distilled water.
2. Adjust the medium pH to 7.3 ± 0.2 by adding HCl or NaOH to the medium.
3. Sterilize the medium in an autoclave at 121°C for 15 lbs pressure for 15 minutes.
4. Pour the sterilized medium into petri plates and allow them to solidify.
5. Cut a small piece of spoiled lemon fruit and disinfect with 0.01 mercuric chloride solution followed by washing with sterile distilled water.
6. Then, blot-dry the cut piece and places it on the surface of the medium.
7. Incubate the Petri plates in an incubator at 25-30°C in an inverted position.

Observation:

After incubation, *Aspergillus* colonies are observed in Petri plates.

Result:

Black-colored *Aspergillus* colonies are developed on the Sabouraud's agar medium.

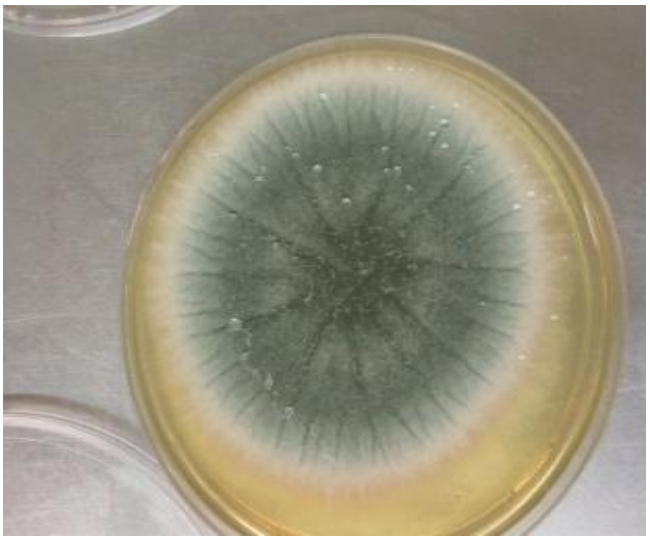
Discussion:

Aspergillus species are easily recognized by their conidiophores, which terminate in an apical vesicle, with a basal cell inserted into the supporting hyphae. The identification of species primarily depends on colony color and the form of conidial heads. *Aspergillus* is a cosmopolitan, saprobic fungus, and it is a common contaminant among fungi in the clinical laboratory.

Experiment No. 10
OBSERVATION OF IMPORTANT FUNGAL PATHOGENS

Aspergillus fumigatus

1. The most common pathogenic species are *Aspergillus fumigatus* and *Aspergillus flavus*.
2. *Aspergillus flavus* produces aflatoxin which is both a toxin and a carcinogen and which can potentially contaminate foods such as nuts. *Aspergillus fumigatus* and *Aspergillus clavatus* can cause allergic diseases.
3. Some *Aspergillus* species cause disease on grain crops, especially maize, and synthesize mycotoxins including aflatoxin.
4. Aspergillosis is the group of diseases caused by *Aspergillus*. The symptoms include fever, cough, chest pain or breathlessness. Usually, only patients with weakened immune systems or with other lung conditions are susceptible.
5. The spores of *Aspergillus fumigatus* are ubiquitous in the atmosphere. *A. fumigatus* is an opportunistic pathogen. It can cause potentially lethal invasive infection in immunocompromised individuals. *A. fumigatus* has a fully functional sexual cycle that produces cleistothecia and ascospores.



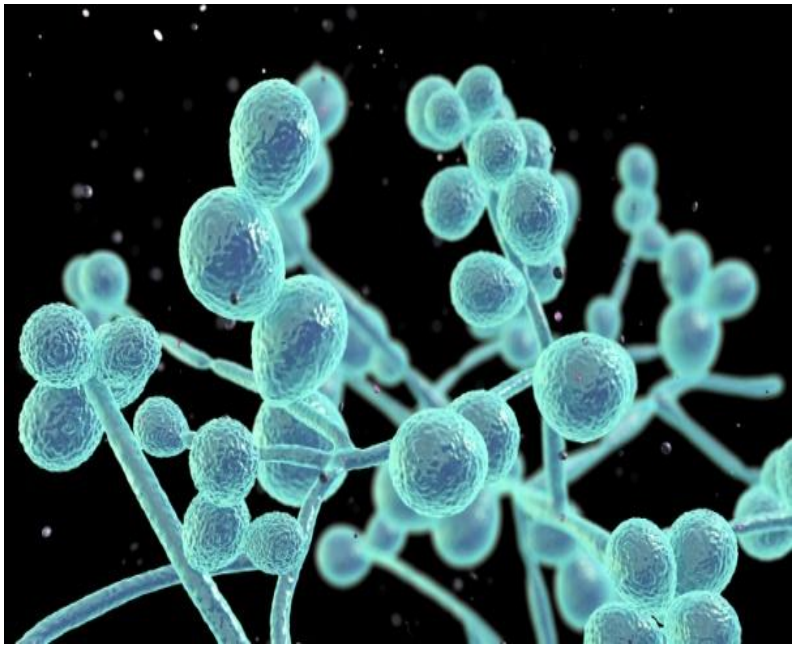
Aspergillus fumigatus colony in petridish

Candida

1. *Candida* is a genus of yeasts. It is the most common cause of fungal infections worldwide and the largest genus of medically important yeasts.
2. The genus *Candida* encompasses about 200 species. Many species are armless commensals or endosymbionts of hosts including humans.
3. When grown in a laboratory, *Candida* appears as large, round, white or cream (*albicans* means "whitish" in Latin) colonies, which emit a yeasty odor on agar plates at room temperature.
4. When it infects mucosal barriers are disrupted or the immune system is compromised, however, they can invade and cause disease, known as an opportunistic infection.

Candida is located on most mucosal surfaces and mainly the gastrointestinal tract, along with the skin.

5. Antibiotics promote yeast (fungal) infections, including gastrointestinal (GI) *Candida* over growth and penetration of the GI mucosa.
6. Women are more susceptible to genital yeast infections, men can also be infected. Certain factors, such as prolonged antibiotic use, increase the risk for both men and women. People with diabetes or the immunocompromised, such as those infected with HIV, are more susceptible to yeast infections.
7. *Candida antarctica* and *Candida rugosa* are a source of industrially important lipases. Lipases from *Candida rugosa* are also used to digest fats in laboratory assays because of their broad range of activity



Candida albicans

Fusarium graminearum

1. *Fusarium graminearum* is a plant pathogen that colonizes and infects cereal crops.
2. *Fusarium* head blight (FHB) caused by *F. graminearum* results in premature bleaching of cereal heads, particularly in wheat.
3. *F. graminearum* produces mycotoxins like deoxynivalenol (DON) and zearalenone in contaminated grains.
4. Mycotoxins can affect human health (e.g., food poisoning, abdominal pain) and livestock health (e.g., reduced feed intake, reproductive problems).
5. *F. graminearum* is a globally significant pathogen, particularly in regions where wheat and barley are major crops.
6. Managing FHB is challenging due to the emergence of fungicide-tolerant strains of *F. graminearum* and the need for more resistant crop varieties.

Fusarium culture on potato dextrose plate

Magnaporthe oryzae

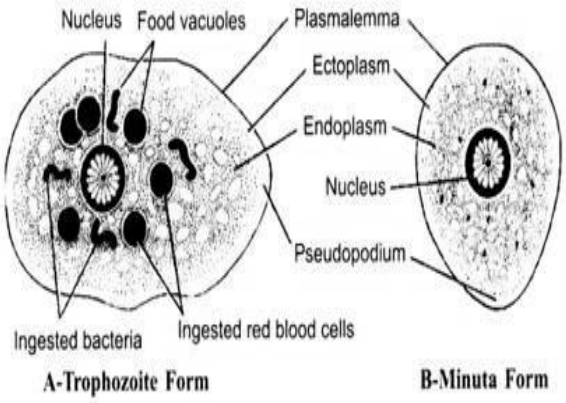
1. *Magnaporthe oryzae* is the primary pathogen responsible for rice blast disease.
2. Primarily known for rice blast, *M. oryzae* can also infect other crop species, including wheat and barley.
3. The fungus spreads through spores, which land on rice plants and germinate to produce appressoria, specialized structures that penetrate the plant tissue.
4. *M. oryzae* secretes effector proteins, which are crucial for invading the plant and suppressing its defense mechanisms.
5. Rice blast manifests as lesions on leaves, stems, and panicles, and can lead to significant yield losses.
6. Rice blast is a significant threat to global food security, as rice is a major staple crop for many populations.

Magnaporthe oryzae rice blast

Experiment No. 11

OBSERVATION OF IMPORTANT PROTOZOAN PATHOGENS

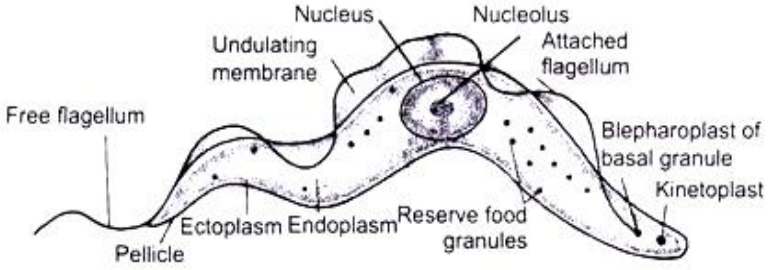
1. *Entamoeba histolytica*



Entamoeba histolytica

- *E. histolytica* is a typical unicellular protozoan. The cells are spherical to oval, 20-40 μm in diameter.
- They possess a central nucleus and cytoplasm is clearly seen as outer more dense ectoplasm and inner endoplasm. Cell membrane is thin and elastic.
- They show amoeboid movements with elongated pseudopodia which may be suddenly protruded and retracted.
- They feed on intestinal bacteria, mucins, RBCs etc. The cells transform into cysts under unfavorable condition. Cysts are thick walled, spherical resting structure of 10-20 μm in diameter. Immature cyst is uninucleate.
- As it matures, nucleus divides twice to become quadrinucleate. The pathogen may also invade and produce lesions in extra- intestinal foci, especially liver to cause hepatic amoebiasis. It occurs through hematogenous spread of the pathogen.
- The pathogen may also spread to lungs. However, all extra-intestinal amoebic lesions are secondary to ones established in the large intestine and cause Amoebiasis.

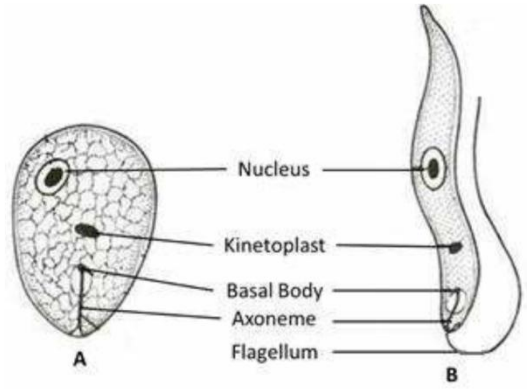
2. *Trypanosoma*



Trypanosoma

- African trypanosomiasis, also known as African sleeping sickness or simply sleeping sickness, is an insect-borne parasitic infection of humans and other animals.
- It is caused by the species *Trypanosoma brucei*. Humans are infected by two types, *Trypanosoma brucei gambiense* (TbG) and *Trypanosoma brucei rhodesiense* (TbR).
- TbG causes over 98% of reported cases. Both are usually transmitted by the bite of an infected tsetse fly and are most common in rural areas.
- Humans are infected by two types, *Trypanosoma brucei gambiense* (TbG) and *Trypanosoma brucei rhodesiense* (TbR).
- *T. brucei* is a typical unicellular eukaryotic cell, and measures 8 to 50μm in length. It has an elongated body having a streamlined and tapered shape.
- Its cell membrane (called pellicle) encloses the cell organelles, including the nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, and ribosomes.
- In addition, there is an unusual organelle called the kinetoplast, which is made up of numerous circular DNA (mitochondrial DNA) and functions as a single large mitochondrion.

3. *Leishmania*



Morphological forms of Leishmania. (A) Amastigote, (B) Promastigote

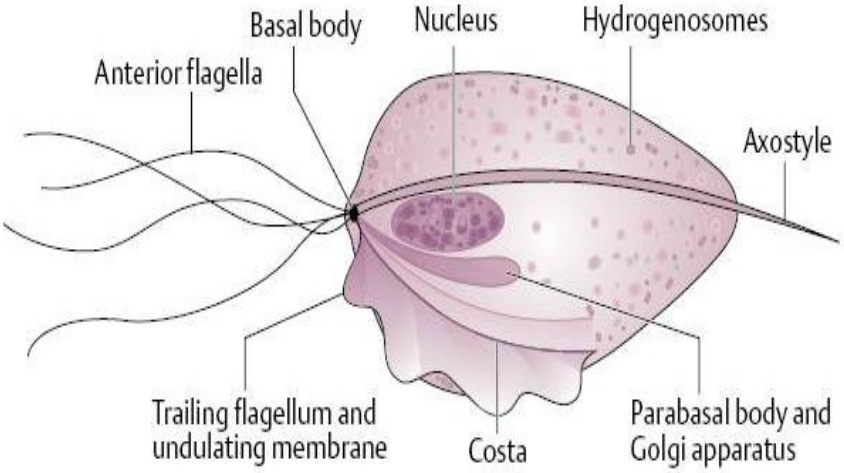
- *Leishmania donovani* is the causative agent of visceral leishmaniasis, also known as Kala-azar, Black Fever, Dum-Dum fever, Asian fever, Assam fever, or infantile splenomegaly in various regions.
- The parasite is named after the scientists who discovered it—Leishman and Donovan, both of whom reported the parasite in 1903.
- Leishman first identified the parasite in the spleen smear of a soldier in England who died from a fever contracted at Dum-Dum, Kolkata. Donovan found the same parasite in the spleen of a patient with kala-azar in India.
- The sand fly (*Phlebotomus argentipes*) was identified as the vector by the Indian Kala-azar Commission (1931-1934).
- The parasite has two forms: Amastigote and Promastigote.
- **Amastigote:** A flagellum-free stage of the parasite. Found inside macrophages, polymorphonuclear leukocytes, or endothelial cells in humans and other mammals. Amastigotes are small, round to oval, measuring 2-3 μm in length and known as LD bodies (Leishman-Donovan bodies).

  The cell membrane is delicate, visible mainly in fresh specimens. The nucleus is oval or round, less than 1 μm in diameter and centrally located. A rod-shaped kinetoplast lies at a right angle to the nucleus, containing both DNA and mitochondrial structures. The axoneme (rhizoplast) extends from the kinetoplast and reaches the body margin.

Vacuoles appear as clear, unstained areas adjacent to the axoneme.Giemsa or Wright stain shows a pale blue cytoplasm, a red nucleus, and a deep red kinetoplast.

- **Promastigote:** Found in the sand fly gut and in culture media. Promastigotes are slender, spindle-shaped, measuring 15-25 µm in length and 1.5-3.5 µm in width.A single nucleus is centrally located. The kinetoplast is located near the anterior end, and the single flagellum is delicate and measures 15-28 µm, often as long or longer than the body. The flagellum does not curve around the body, so there is no undulating membrane. Leishman stain shows a blue cytoplasm, pink or violet nucleus, and a bright red kinetoplast.

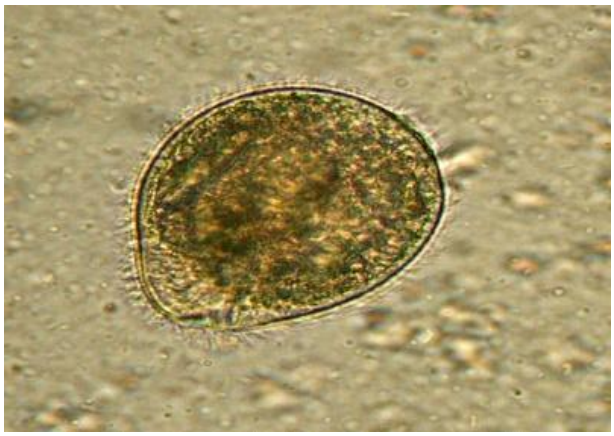
4. Trichomonas vaginalis



Trichomonas vaginalis

1. Trichomoniasis is a very common sexually transmitted disease (STD). It is caused by infection with a protozoan parasite called *Trichomonas vaginalis*.
2. *Trichomonas vaginalis* is one of the commonest sexually transmitted pathogens in the world, with an estimated 170 million cases occurring each year.
3. However, exact numbers are difficult to obtain because the infection is not nationally reportable and many infections are asymptomatic. It is more common in females than males.
4. *Trichomonas vaginalis* varies in size and shape, with the average length 10 µm and width 7µm. It exists in only one morphological stage, a trophozoite, and cannot encyst. The *T. vaginalis* trophozoite is oval or pear shaped.
5. Trichomonads have 4 flagella that project from the organism's anterior and 1 flagellum that extends backward across the middle of the organism, forming an undulating membrane. An axostyle, a rigid structure, extends from the organism's posterior.
6. The axostyle may be used for attachment to surfaces and may also cause the tissue damage seen in trichomoniasis infections.

5. *Balantidium coli*



Balantidium coli

1. *Balantidium coli* is the only ciliate known to parasitize humans. Ciliates represent a phylum of protozoa characterized, in at least one stage of development, by simple or compound ciliary organelles on the surface of their membranes that are used for locomotion.
2. Ciliates have 2 nuclei (one macronucleus and one micronucleus) and reproduce by transverse binary fission, conjugation, autogamy, and cytogamy. *Balantidium coli* has 2 contractile vacuoles.
3. Although contractile vacuoles are common to ciliates, they are rare in parasitic protozoa, which suggests that *Balantidium coli* has a unique osmoregulatory capacity.
4. *Balantidium coli* is a protozoan parasite responsible for the disease Balantidiasis.
5. *B. coli* is found worldwide, but disease occurs most commonly in parts of the developing world including Latin America, Southeast Asia, Papua New Guinea and parts of the Middle East.
6. Humans are usually resistant to infection; disease generally occurs in debilitated or poorly nourished patients.
7. Pigs are the primary reservoir for human infection and most cases occur in people in close proximity to pigs, although rats and other mammals may also transmit disease.