B.Sc. BOTANY LAB MANUAL

1st Semester

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Botany

MIDNAPORE CITY COLLEGE

B Sc (Honours) in Botany

[Choice Based Credit System]

CP1: Algae and Microbiology-Lab

1. **Electron micrographs/Models of viruses – T-Phage and TMV, Line drawings/ Photographs of Lytic and Lysogenic Cycle.**

Bacteriophages are bacteria infecting viruses. They are also called ‘phage’ or simply bacterial Viruses as any group of viruses that infect bacteria are referred to as Bacteriophage. A bacteriophage is a virus that parasitizes bacteria and reproduces inside it. They are of different shapes and show genetic variations. They may contain DNA or RNA as genetic material and may have gene count ranging from four to several thousand. The name bacteriophage describes an entity’s bactericidal ability and it translates to ‘’bacteria eater’’ in English. Not only do bacteriophages infect the bacteria but also archaea- the single-celled Prokaryotic organisms.

Characteristics of a Bacteriophage

- Several varieties of bacteriophages exist in the environment but one type can infect onlyone type or a few types of bacteria.
- They are classied in a number of Virus families. Examples include Inoviridae,Microviridae, Rudiviridae, and Tectiviridae.
- Like all other viruses, they are simple organisms consisting of a core of genetic materialsurrounded by a protein capsid.
- The genetic material can either be DNA or RNA in the bacteriophages.
- After infecting a cell, it completely takes control of the host cells and stops it from
- producing bacterial components and forces it to produce viral components.
- They eventually bring about the lysis of the host bacterial cell.

Characteristics of a Bacteriophage

A typical bacteriophage is composed of a polyhedral head, a short collar, and a helical tail.

The head of the phage consists of 2000 capsomeres with the genetic material- doublestranded DNA or Single-stranded RNA enclosed within the head.

The tail is composed of an inner hollow tube that is surrounded by a contractile sheath with 24 annular rings. The distal end of the tail consists of a basal plate that has tail bers at each corner.

Diagram of a Typical Bacteriophage

Life Cycle of a Bacteriophage

After the phage infects the host cell and inserts its genetic material into the host cell, it Follows either of the two life cycles, they are

1. Lytic Cycle (Virulent Cycle)

2. Lysogenic Cycle (Temperate Cycle)

Lytic Cycle

If they uptake the lytic cycle, bacteriophages infect the host cell and kill it to release progeny viruses. Steps involved in this cycle are as follows

Adsorption

This is the rst step of infection by phage in which the bacteriophage attaches itself to the surface of the host cell or bacteria. For attachment to take place, the tips of the tail bers attach to specic receptor sites on the surface of the bacterial cell.

Penetration

In the next step, the tail sheath of the phage contracts after adsorption has taken place. The base plate and the tail bers attach rmly to the bacterial cell surface. The phage lysozymes weaken a part of the host cell wall and the hollow core is pushed downwards through it. The phage DNA is then injected inside the bacterial cell.

Synthesis of Phage Components

The components of new virus particles are produced after the genetic material of the phage

is released into the host cell. The sub-units of phage then appear which includes the head, tail, and late protein. Early proteins and specic enzymes carry out the synthesis.

Release

The lysis of the bacterial cell takes place releasing the progeny phages. During the replication,

phage enzymes weaken the cell wall of bacteria.

Lysogenic Cycle

It is another pathway of viral reproduction in a host cell. In this phase the integration of phage nucleic acid into the host cell genome or the formation of a circular replicon in the cytoplasm of the host cell takes place. The host bacterial cell continues to live and reproduce normally in this phase. The genetic material of the phage also called prophage is transmitted to daughter cells at each subsequent cell division. The lysogenic cycle is dierent from the lytic cycle in the respect that the lysogenic cycle does not lyse the host cell straight away. The prophage may be converted into the lytic phase either naturally or articially by physical or chemical agents. The bacteria carrying prophage viruses without being lysed are known as "lysogenic bacteria".

In the event of multiplication of lysogenic bacteria, the prophage might be lost due to excision.

Gram Staining

Principle:

Different staining requires the use of at least three chemical reagents that are applied sequencetilly to a hit fixed smear. The first reagent is called primary stain. Its function is to impart its colour to all cells. In order to establish a colour contrast, the second reagent use is

the decolouring agent. Based on the chemical composition of cellular components, the decolouring agent may or may not remove the primary stain from the entire cell on only from certain cell structure. The final reagent, the counter stain has a contrasting colour than that of the primary stain. The following decolourization, if the primary stain is not washed out the counter stain cannot be observed and the cells on their components will retain the colour of the primary stain. If the primary stain is removed, the colourized cellular components will accept and assume the contrasting colour of counter stain. In this may, cell types or their structure can be distinguished from each other on the basis of the stain that the cells retained.

Purpose: to became familiar with;

1. The chemical & theoretical bases for differential staining procedure.

2. The chemical basis of gram stain.

3. Performance of the procedure for differentiating between the two principle groups of bacteria:

-Gram +ve bacteria

-Gram –ve bacteria

Materials: Cultures: 24 hours nutrient agar stain culture of sample

1. Reagents: Crystal violet (Primary stain) Gram's iodine (mordant) 70% Ethyl alcohol (Decolourising agent) Safranin (Counter stain) Equipments: Bunsen burner ,inoculating loop, staining tray, glass slide, bibulous, Paper, lens paper and microscope.

Procedure:

1. obtain 4 clean glass slides.

2. Using sterile technique prepared smears of each of the 2 sample organisms. The smear if prepared by placing a drop of water on the slide, then transferring each organism separately to the drop water with a sterile cooled inoculating loop.mix and spread he organism by the means of circular motion by inoculating loop.

3. Allow smears to air dry then heat fixed the smear by the Bunsen burner.

4. Gently flood the smear with crystal violet and let stand for 1 min.

5. Gently then wash it with tap water.

6. Gently flood smear with the gram's iodine mordant and let it stand for 1 min.

7. Gently wash it again with tap water.

8. Decolourised it with 90% ethyl alcohol. (Caution: do not over decolourise, add reagent drop by drop until alcohol runs almost clear, showing only a blue colouration.)

9. Gently wash it with tap water is when done, then the next step is to apply gently or counter stain gently with safranin for 45 sec.

10. Blot dry with bibulous paper and examine under oil immersion microscope.

Observation and result:

Draw a representative field. Cell morphology;

1) Shape — Round shaped

2) Arrangement — single

3) Cell colour — crystal violet (purple)

4) Gram Reaction — gram positive

Comment:

According to the above result, the sample contains gram positive stain, because it retains the crystal violet stain colour, hence it is a gram positive bacterium.

ENDOSPORE STAINING

Principle:

Some bacteria are capable of changing in to dormant structure that are metabolically inactivate and do not grow or reproduce since these structure are formed inside the cells hence are known as endospore . During sporutation a vegetative cell give rise to a new intracellular structure termed as endospore that is surrounds by a impermeable layer called aspore coat. Example:Bacillus, Clostridium, Coniella, Desulyoyomacula,

thermoactiinomycetes and sporo. These spore are differentially stained by using special procedure that help the dyes to penetrate the spore wall.An aqueous primary stain (malachite green) is applied and is steamed to enhance penetration of the impermeable spore coats ,once endospore are stained they do not readlydocolorize and appear green with red cells .

Requriements:

i) 48 hours nutrient agar cultures of Bacillicercusor Bacilli subtilisand Staphylococcus aureus.

ii) Malachite green(5%aqueous)

iii) Safranin(o.5%aqueous)

iv) Staning tray

v) Glass slides

vi) Inoculam loop

vii) Blotting paper

Viii) sprit lamp

ix) microscope.

Procedure:

i) Make smears of Bacillus sutilis and staphylococcus aureus on separate clean slide.

ii) Air dry and heat fixed the smnear

iii) Flood the smear with malachite green

iv) Heat the slide to steaming and stem for 5 minute adding move stain to yhe smear fron time to time.

v) Wash the slides under slowly running tap water.

vi) Counter stain with saffranin for 30 seconds.

Vii) Wash the smear with distilled water.

Viii) Bolt dry the slids that is wet with disttiled water

Observation:

Examine the slids microscopically under oil immersion objects from a reppresentitive microscopic field of each preparation warks drawing indicate the position amnd size of the endospore within individual cell as well as the size and also the exact shape of the free spore

Result:

In Bacillus subtilis the endospore staning and the vegetation cells stains red .The vegetation cells eae rod-shaped each containing an elliptical centrally located spores.

Comment:

From observation and result we can conclude that supplied sample contain endospore staning some cells have taken malachite green stain and from greenish colour and some cells have taken saffranin colour and from pink colour .So from above experiment we can concluded tha greenish cells area spore and pink cells are vegetation cells.

CP2: Biomolecule and Cell Biology-Lab

Staining procedure for algal specimen

- Put the species in clean slide and one drop cotton blue taken on it
- After preparation the slide heated by sprit lamp and waiting for 3-5 minutes.
- Added lacto phenol on the slide and spread well.
- After preparation the specimen is covered by cover slip and given pressure on the cover slip for clean the bubbles.
- At last seal the cover slip.

Phycology

Description and workout of algal specimen: A

Thallus Structure:

plant body is blue – green in colour,free floating colonical or attached to a substratum. Each colony contains a number of trichomes embedded within a matrix (common sheath) forming little Blass Nostoc balls. The trichoms consist of a single series of uniform, often torulose, bead- like ,ellipsoidal cells more or less depressed wich are often contorted and some times from densely interwoven masses. Cells of each trichomes are joined end to end to from moni liform (bead like)chains sheaths of individual trichomes are hetrocysts are bpresent . Heterocysts are distinguished from the vegetative cell by thick –walls, transparent contents,larger size and two polar nodules at two ends. Heterocysts separate the hormogonium

Reproductive structure:

Akinetes – Thy are different in appearance from vegetative cells. Akinetes are spherical or oblong and much larger than vegetative cells with dens Protoplasm.

IDENTIFICATION:

Thallus blue green in colour,cells devoid of conspicuous nucleus, absence of any sex organ

,presence of gelatinous sheath around the cells (in most case)absence of organised cell organelles

like plastids.

Thallus unbrunched, filamentous, presence of hormogonium, hetrocyst present some genera only.

The specimen belong the class: **CYANOPHYCEAE.**

Trichomes unbrunched ,interwoven and surrounded by sheath presence of heterocyst and akinets.

The specimen belong the order : **NOSTOCALES**.

Trichomes with single series of unifrome ,ellipsoidal(bead-like) cells presence of intercalary heterocysts and akinets.

The specie s belonging the family: **NOSTOCACEAE.**

Hence the specimen is under the Genus : **Nostoc**

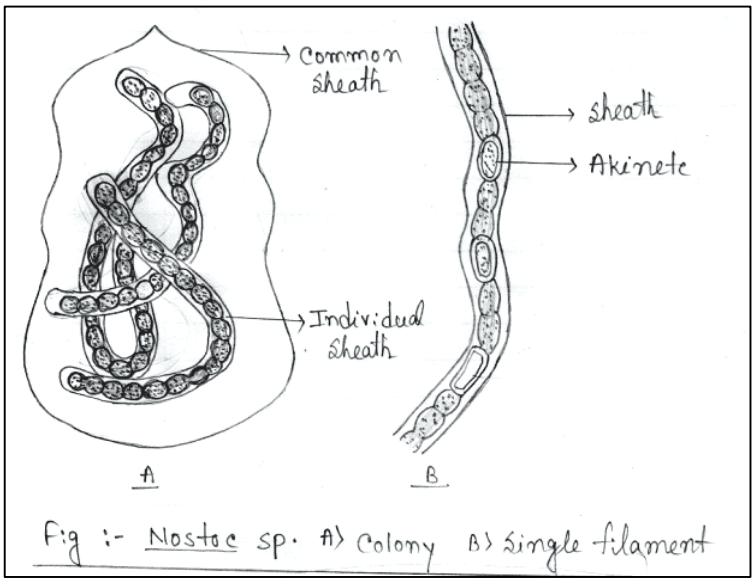

Fig :- Nostoc sp. A) Colony B) Single filament

WORK OUT OF SPECIMEN: B

Materials :-The specimen supplied from laboratory.

The plant body:-

I) Plant body of this specimen is filamentous, much brunched coenocytic and saponaceous thallus.

II) The coenocytic body contains many nuclei, septa may from during injury on the development of sex organ .

III) In terrestrial species the plant body remains attached to the soil surface with much branched thread like structure ,the rhizoid are either absent ort ill -developed.

IV) The filamentous body has a thin outer wall. which is less elastic .it is made up of outer pectin and inner cellulosic layers

IV) In the centre of the filament a continuous vacuole is present except at the apical region , which is filled with cell sap.

Sexual Reproduction :-

I) The sexual reproduction in specimen in specimen is of organous type.

ii) It takes place by antheridium ,the male sex organ and oogonium, the female sex organ

Development of the sex organ: Antheridium or male sex organ:

I) The nuclei of antheridium aggregate in the continue and divide Mibtically.

II) Each nucleus along with along with some cytoplasm metamorphoses into sivgta spindle shaped bi flagellate antherozoid.

III) The flagella are unequal in length ,dissimilar cone whiplash and othr tinsel and laterally in sorted.

Iv) The antherozoids are generally liberated though on opening developed at the apical region of antheridium

Oogonium or Femal sex organ :-

I) Identatically a small protuberance developes at on near the base of antheridial branch,due to occumulation of cytoplasm.

(II) The cytoplasm of this region is colourless which has many nucli and without any chromatophores .

III) The mature Oogonium contain a large nucleus at the centre with many chromatophores and all drof dispersed throughout the cytoplasm

IV) The protoplast along with nucleus round of and froms single ovum or egg.

V) It has an hyaline area towards the anterior known as receptive spot

IDENTIFICATION :-

I) Thallus yellowish green in colour filamentous cenocytic brunched.

II) Presence of oil as reserve food sexual reproduction complex Oogamous type.

Hence, the specimen is under the class – **XANTHOPHYCEAE.**

I) Plant body filamentous a sexuality reproduce by multiflagellate zoospore

Hence the supplied specimen is under the order – **HETRROSIPHOOONALES**

I) Filaments irregularly brunched .

II) Antheridia curved cylindrical and Oogonia.

Hence the specimen under the family –**VAUCHERTIACERAE.**

I) Filament long tubular spiraly brunched.

II) Antheridia hook like curved an d oogonium sessile on short stalked with a beak.

Hence the specimen is under the Genus – **VAUCHERIA**

So the supplied specimen is **Vaucheria sp**

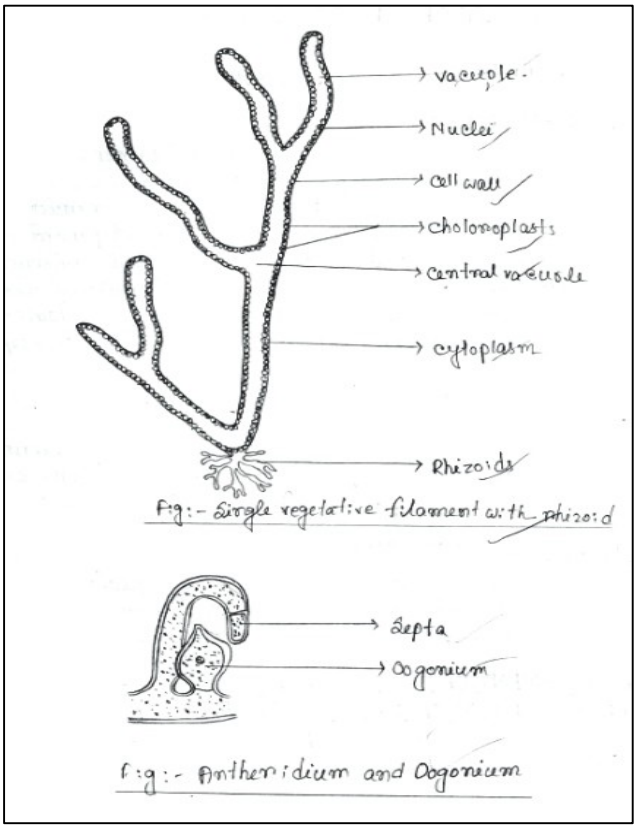

Fig:- Single vegetative filament with rhizoid

Fig:- Antheridium and Oogonium

WORK OUT OF SPECIMEN –C

Specimen: Supplied the specimen from the laboratory

Thallus structure:-

I) The plant body is promentous , brunched laterally or dichotomously and brownish red to purple red is colour.

II) The main axis and brunches process of polysiphonia appearance as the central axis cell is surrounded by pericentral cell of advisable number.

III) The cell is pronominent cell to cell organism connection and each cell has one nucleus and many discoid plastids embedded in dense cytoplasm. ultimate brunche are uniseriate structure and are known as trichoblast.

Reproductive structure : Spermatangium :

The lateral brunches of the male plant bear antheridium known as spermatangia in denseThe spermatangiaare short stalked colourless and spherical oval structure .it contain a singale.

Cystocarps :

It ius an unshaped structure formed cynoimoblast filament surrounded by sterile filaments. The terminal cells of the gonimoblast filament produce a carposporangium with one carpospores.

Tetrasporasngium :

It is brone on the central axis of the specialised filament called tetyrasporoohysis filamnent. Each sporangium contains four tetraspore.

Identification :

Presence of gelatinous material in the thallus cells contain chloroplast with pyrenoid. Presence of characteristic post lygotic structure called cystoicarp.

Hence the specimen belongs the class-**RODOPHYCEAE.**

Plant body hetrotrichous uniarial ios the growing and multi axial in the mature region carposporagium developing on filamentous gonimoblasts derived directly from the fertilized carposporangia.uni or multiaxias construction of thallus presence of tetrasporangia with tetraspores.

Hence the specimen belong the order –**SERAMIALES.**

Polysiphonous brunched thallus cystocarp urn shaped with a pore tetrasporangia with tetraspores

Hence the specimen belongs the family-**RHODOMELACEAE**

Cells of the central axis polysiphonus surrounded by peericentral cells presence of spermatangia on separate filaments

Hence specimen belong the genus **POLYSIPHONIA**

So the supplied specimen is **Polysiphonia sp.**

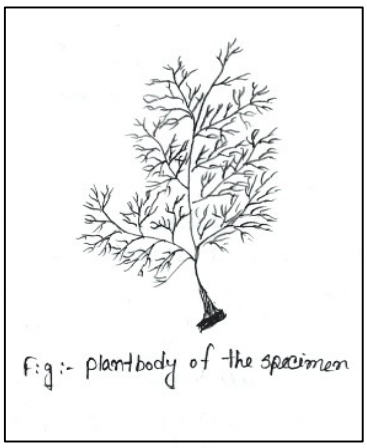

Fig:- plant body of the specimen

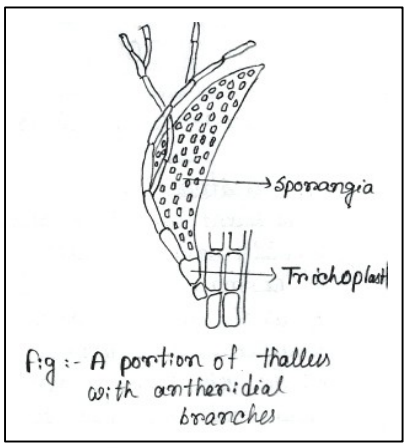

Fig:- A portion of thallus with antheridial branches

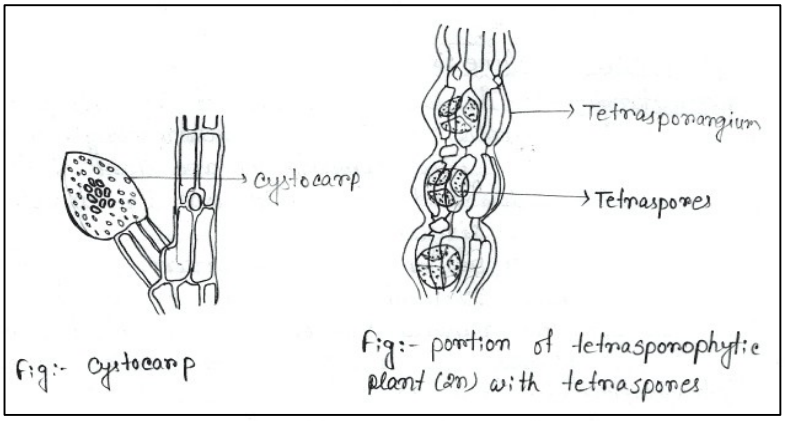

Fig:- Cystocarp

Fig:- portion of tetrasporophytic plant (enl) with tetraspores

WORK OUT OF SPECIMEN –D

Material:-

I) The plant body is filamentous and the filament are green, simple unbrunched consisting of row of cylindrical cells

II) The filamentous are usually free flating

III) The filamentous are silky hair like structures which are smooth to touch.

IV) The cells are generally more is length then in breath.

V) The protoplast is differentiated into structures such as plasma membrane chlololasts pyrenoids central vascular other cytoplast is nucleus.

VI) The cytoplast is surrounded by plasma membrane and it encloves a long vacuole fillec with tannin containing cell shape.

VII) The most prominante feature of this cells the presence of spinal or rtibbow shaped chloroplast which are partial in position and remain cubedded in the cytoplasm.

VIII) Chloroplast contain man pyrenoids.

Identifying character:-

I) Unbrunched filamentous type.

II) The filamentous forms are free floating

III) Filamentous forms are Silky hair like structure which are smooth touch.

IV) Cells are generally more in breath than in breadth.

V) Chloroplasts contain many pyrenoids

So, the supplied specimen is belongs the genus-***Spirogyra*** **sp.**

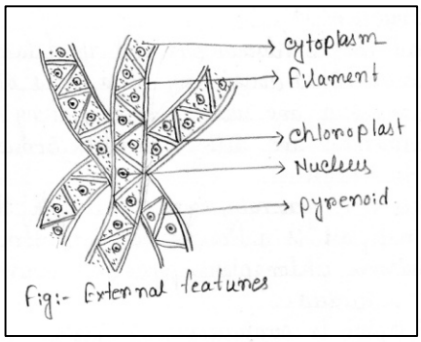

Fig:- External features

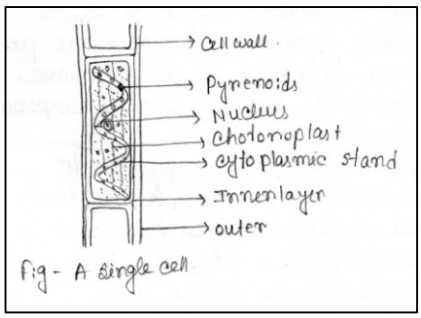

Fig- A single cell