Semester – I

18MBU111 BASIC MICROBIOLOGY - PRACTICAL (3H – 2C)

Instruction Hours / week: L: 0 T: 0 P: 3 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

COURSE OBJECTIVES

To develop skills related to

- Isolation and culture techniques of bacteria
- The external feature of bacteria and colony characteristics.
- Various staining techniques

COURSE OUTCOME

This practical paper will give those hands on experience in handling of various important instruments. They also will develop knowledge on preparing permanent temporary mounts for fungi, protozoans and algae.

EXPERIMENTS

1. Microbiology Good Laboratory Practices and Biosafety.
2. To study the principle and applications of important instruments (biological safety cabinets, autoclave, incubator, BOD incubator, hot air oven, microscope, pH meter) used in the microbiology laboratory.
3. Preparation of culture media for bacterial cultivation.
4. Sterilization of heat sensitive material by membrane filtration and assessment for sterility.
5. Demonstration of the presence of micro flora in the environment by exposing nutrient agar plates to air.
6. Temporary mounts Lacto phenol cotton blue mount – *Rhizopus, Penicillium, Aspergillus*.
7. Study of *Spirogyra* and *Chlamydomonas*, *Volvox* using temporary mounts.
8. Study of the following protozoans using permanent mounts/photographs: *Amoeba*, *Entamoeba*, *Paramecium* and *Plasmodium*

SUGGESTED READINGS

1. Tortora, G.J., Funke, B.R, and Case, C.L. (2008). Microbiology: An Introduction. 9th edition. Pearson Education.
2. Madigan, M.T., Martinko, J,M., Dunlap, P.V., and Clark,D.P. (2014). Brock Biology of Microorganisms. 14th edition. Pearson International edition.
3. Cappucino, J., and Sherman,N. (2010). Microbiology: A Laboratory Manual. 9th edition. Pearson Education Limited.
4. Wiley, J.M., Sherwood, L.M., and Woolverton, C.J. (2013) Prescott's Microbiology. 9th edition. McGraw Hill International.
5. Atlas, R.M. (1997). Principles of Microbiology. 2nd edition. WM.T.Brown Publishers.
6. Pelczar, M.J., Chan, ECS., and Krieg, N.R. (1993). Microbiology. 5th edition. McGraw Hill Book Company.
7. Stanier, R.Y., Ingraham, J.L., Wheelis, M.L., and Painter, P.R., (2005). General Microbiology. 5th edition. McMillan.

EXPERIMENT NO: 1

MICROBIOLOGY GOOD LAB PRACTICES AND BIO SAFETY

A rewarding lab experience demands strict to prescribe the rules for personal safety in terms of laboratory accidents. The later requires maintaining clean lab settings which prevent contamination of experimental procedures by microorganism from exogenous sources. Most of the microbiological laboratory procedures requires the use of living organism.

Thus the microbiologist must develop an aseptic technique for the preparation and handling of various pathogens. The following are the basic steps for the laboratory and to reduce the microbial flora in the environment.

- Upon entering the laborartory place the coats,masks and other materials at its specified locations on the bench tops.
- Doors and windows are kept closed during laboratory session to prevent contamination from air currents.
- The bench tops should be wiped with disinfectant solution at the start and end of the practicals.
- Do not place contaminated instruments such as inoculation loops, needles and pipettes on bench tops.
- Place the cultures and materials in the disposed ares in the laboratory.
- The fungal cultures are processed rapidly to prevent the spread of reproductive spore in the laborartory environment.

The following are regulations to prevent accidental injury and infection.

- Wash the hands with liquid detergent before and after any lab procedures.
- Wear a lab coat or an apron, a paper cap to tie back long hair (minimize exposure, working in the laboratory to protect clothing from contamination or accidental discoloration while staining solution)
- Never apply cosmetics or insert contact lenses in the lab.
- Eating, drinking and smoking is strictly prohibited.
- Carry cultures in a test tube rack or in a tray when moving around in the lab.
- Spilled cultures or broken cultured tubes must be washed or cleaned with disinfectant solutions.
- Broth cultures or chemical reagents should not be pippeted by mouth. The use of mechanic pipetting device is required.
- Self stick labels are used for identification of experimental cultures.

The following are procedures while handling body fluids.

- Disposable glasses should be worn while handling the materials.
- Hands must be washed immediately when accidentalcontact from dust materials happen.
- The dust material and the other materials used must be treated with a disinfectant prior to autoclaving.

EXPERIMENT NO:2

To study the principles and application of important instruments

Biological safety cabinet:

Laminar air flow can maintain area devoid of contaminants many medical and research laboratory requires sterile working area devoid of in order to very out specialized work. Laminar flow cabinet's glade article free working environments by projecting air through a filtration system and exhausting it across work surface in a laminar air flow uni-directional air steam. They provide an excellent clean air environment in a number of laboratory requirements.

The process of laminar air flow can be described is airflow where an entire body of air flows with steady uniform velocity. Laminar air flow cabinet work by the use of inflow laminar air flow through one or more HELA, filters and designed a create a franticly free working environment and Froude product production. Air is taken through filtration system and then exhausted across the work surface as part of laminar flows process commonly the filtration system comprises of a prefilter and a HEPA filter. The laminar flow cabinet is enclosed at the end constant positive air pressure is maintained to prevent the intression of contaminated room air.

Types of laminar flow cabinets:

Laminar air cabinets can be produced as both horizontal vs vertical. There are many different types with a variety of airflow patters of different purposes.

Vertical laminar flow cabinet

Horizontal laminar flow cabinet

Laminar flow cabinet and hoods

Laminar flow benches and cloths

All ensure a work space during devoid of contaminants and may be tailored in the lab required.

Horizontal laminar flow cabinet:

Horizontal laminar flow cabinet receive their name due to the direction of the flow which comes from above but then changes direction and is across the work in horizontal direction. The constant flow of filtered air provides material and product production.

Vertical laminar flow cabinet:

Vertical laminar flow cabinet functions equally wells horizontal flow cabinet with a laminar air directed vertically downwards on to the working area. The air can the working area air holes in the base vertical flow cabinets can provide greater protection.

Autoclave:

Autoclave is an instrument used for the sterilization works under the principle of moist heat sterilization was first discovered by charleschamberland pressure is used to produce high temperature steam. It is based on the principle that when water is boiled at an increased pressure the temperature at which boils and of the

steam it forms rises. Hot durated steam rapidly it condenses cooler objects it ensures the destruction of bacterial endospore as well getative cells by coagulation and denaturing mmicrobial protein and enzymes.

Method of use:

Add correct volume of water to the autoclave place all materials to be sterilized in the inner amber of autoclave.

Do not over load the autoclave.

Secure the lid as directed by the manufacturer when the air lock (air outlet) and close off knob apply heat electrically as the water boils air and steam will emerge through the airlock.

When all the water emerge droplets have been expelled and only steam is emerging wait for 1 minutes and lose the air lock. This will cause the pressure to use.

When the required pressure has been reached and the excess steam begins to be released from the safety value reduce the heat and begin thining.

Hold all materials at 121*c for 15min with 15lbs pressure (holding time will vary depends on the material to be sterilized).

At the end of sterilization time turn off the heat and allow the autoclave to cool naturally. This usually takes few hours.

Check that the pressure gauge is showing zero. When at open airlock and them wait fpr few min before opening the lid to allow time for autoclave fully

vented.

Remove all sterilized material from the autoclave due to coal naturally. This tighter the bottle caps.

Control of autoclave:

A biological method is available to mcontrol the performance of an autoclave. It is called bioindicator sterilization. Its spare will be destroyed at 121*c in 10 min. so people set autoclaving temperature at 121*c for 10 min.

Incubator:

In microbiology an incubator is a device for autoclaving the temperature humidity and other indications in which a microbiology culture is being grown. The simplest incubator are incubated upon 0-65*c. more elaborate incubators include a timer ability to lower temperature (via refrigeration) or the ability to control humidity or co_2 levels. Most incubators include a timer some can also be programmed to cycle through different temperature humidity levels etc., incubator can vary in size from table top to the size of small rooms. Incubator also contains certain features such as shaken, measured by evaluation for minutes. Common incubation material temperature or bacteria are approximately 86-87*c and for the fungus are 25-30*c. incubatory condition may vary with the type of microorganisms.

Biological oxygen demand incubator:

All the aquatic animals rely on the oxygen present in the atmosphere (dissolved oxygen) to live. Aquatic microorganisms use the organic matter discharge into the water as food sources of organic matter include plant decay and leaf fall. Bacteria

will break down the organic matter using the dissolved oxygen in the water and there by product less complex organic substances with increased disposal of waste material (include organic compounds) the ability of dissolved oxygen by the microorganisms will also be increased. So the water becomes depleted in oxygen. In this anaerobic condition, microorganisms will produce offensive products and may not results in undesirable effects like fish as phyxications. So the mount of dissolved oxygen in the water is an indicator of the quality of the water.

Biological oxygen demand is a widely used technique to express the concentration of organic matter is sewage which containing high BOD value. Digestion of these organic compounds in neutral ecosystem such as lakes etc., can deplete available oxygen and result in fish asphyxication.

Some microbes are to be grown at all lower temperature for specific purposes. The BOD low temperature incubator which can maintain temperature from 50*c and as low as 2-3*c is used for incubation is obtained by rotating the knob finely trail and error and notching the temperature fixed on incubator most of the modern BOD incubator are programmed which do not need trail and error temperature setting. Here the operator gets the desired temperature and reduced period of time. The incubator automatically maintain it accordingly.

The BOD of water sample is generally measured by incubator. The sample is 20*c for 15 days in a work room under aerobic condition (in BOD incubator) the water samples where more than 70% of initial oxygen is consumed it is necessary to aerate or oxygenate and dilute the sample with BOD free water (de ionized glass distilled water) pass through a column of activated carbon and redistilled the avoid

o_2 stress.

Working principle:

Under alkaline condition (by adding alkaline iodine-oxide) the manganese sulphate produces a white precipitate of manganese this reacts with the dissolved oxygen present in the same to form a brown precipitate. On acidic condition manganese diverts to its divalent state and released iodine. This released is treated against sodium thiosulphate using starch as in indicator.

An own is heated on the principle where dry heat or hot air accomplishes sterilization. The sterilization process in an oven is longer than autoclaving dry heat removes water from microorganisms while moist heat adds water to them. In addition moist heat gas greater penetrating forces than dry heat.

It is used for sterilization glass water like petriplates tubes pipettes metal instruments oils powders axes etc., commonly used temperature for hot air when sterilized is 10 minutes at 180*c for 40 minutes at 170*c, 60 minutes at 160*c, 150 minutes at 150*c 180 minutes at 140*c, 480 minutes at 480*c. an own consist of an incubated cabinet, which is held at the constant temperature by means of an electric heating mechanisms and ethermostant. It is filtered with a fan of keep the hot air circulating at a constant temperature.

Microscope:

A microscope is an optical device having arranged in such a manner to enlarge or magnify a minute object.

Principle:

There are 2 principles involved in development of microscope

a) To magnify the object as the extreme capacity.
b) The magnify should be such that all the details should be seen clearly and closely.

these are two types of microscope:

1) Simple microscope
2) Compound microscope

The early microscope has at least two lenses of which eye piece and the other is objective.

Resolving power:

Resolving power of microscope is its ability to distinguish two objective close to one another generate and distinct objects. The reading power of microscope is general by the wavelength of light used to illuminate object and also the numerical aperture of objective lenses.

Numerical aperture:

The numerical aperture of a lens is a measure of its light gathering capacity so the numerical aperture can be defined as

$$NA = n \sin \varphi$$

Where, n = refractive index of the medium between the objective and object.

φ = half of the angle come of light entering into the objective from center of object. Subsequently oil immersion technique can give high resolution of the image. So the limit of the resolution of the image can expressed as,

$$d = \lambda / 2NA$$

where, λ = wavelength of light used,

NA = Numerical aperture

Working parts of microscope:

The microscope consist of two parts,

Mechanical part

Optical part

Mechanical part:

Mechanical parts are necessary for the operation of microscope. There is the faze which is mostly horse shoe shaped from the base the filter arises at the top of which inclination. A nab is attached at the top of the similar. The stage is with a central opening shifts to hold the slides. The body tube mousse up and down by two adjustments. This adjustment coarse and fine system moves the body tube over a greater vertical distance and bring the specimen with the body tube slowly for

five focusing. There is a substage below the stage carrying a condenser which focus the light rays upon the object attached to the regulation of light passing through the mirror into the condenser. The mirror has two surfaces:

Flat and concave

Optical part:

Optical part grown the magnification of the image it includes mirror objectives eye piece. All of them should be reception of optical axis for the results objectives are there type lower power high power oil immersion.

The total magnification of system is determined by multiply magnification power of eye pieces.

If the microscope has only one eye piece it is called monocular microscope and binocular microscope has two eye pieces.

Ways of handling:

Place the slide on the stage with the specimen slide up the central part of the specimen to be remained is placed on the for in the center of the stage .

Adjust the mirror until it a reflect maximum amount of the light through the specimen and place the light through in a lowest power position.

More the body tube with the help of the course adjust time at unitill the objective time is a 1-2cm slide, look through the eye piece and slowely raise and the object time until the specimen is in appropriate focus never more the course

adjustment downward by looking through the eye piece. Brimg the specimen for sharp focus with the fine adjustment.

After examining the specimen in a lower power lens turn to high power object time. Turn the nose piece till the object time click to position use only fine knob for sharp focusing.

Raise the body tube and rotate the position and place one or two drops of the immersion out on the lower side and the lower body tube carefully till the oil immersion objective touches the oil drops and then focus with fine adjustments.

Each time after the use of oil immersion objectives then the oil drop from the lens with help of xylene and lens paper .

While caring the microscope, the right should hold the arm, the left hand should hold the base of the microscope.

Stretching of a objective lenses while focusing the specimen should be avoided immersion oil should be used only oil immersion objective (100x).

Microscope should be kept in dust free cabinet after use.

pH METER:

A pH meter is an electronic instrument to mean the ph of a liquid. A typical ph meter consist of a special measuring probes connected to an a electronic meter that measures and displace the ph reading. The first commercial ph meter where build around 1936/radiometer in a Denmark and by doctor Arnold radiometer Backman in the untitled status Danish biochemist sorensarensen incented the ph scale in

1999.

A standard ph meter as two electrodes, one glass and the other is a reference electrode. The voltage produced in one ph unit (say ph = 7.00 to 8.00) is a typically about 60mb mille volte. Present is a contain micro processor that make the necessary connection for the temperature and calibration the reference

electrode with traditionally silver chloride (Agcl) and has been suppressed by calomel mercuric chloride ($Hgcl_{2)}$ this electrode used mercuric chloride ($Hgcl_2$) in a potassium chloride (Kcl) solution. A ph meter measures essentially the electro chemical potential between a known liquid inside the glass electrode (membrane) and an unknown liquid outside. The calomel reference electrode consist of a glass the with a potassium chloride (Kcl) electrolyte which is in intimate contact with a mercuric chloride element at the end of the solution. it is a fragile construction, joined by a liquid junction top made of focus ceramic or similar material. This kind of electrode is not easily poisoned by heavy metals. The glass electrode consist of a study glass tube with a this glass half welded to it. Inside is a known solution of potassium chloride preferred at a ph of 7.0.a solution silver electrode with the inside solution. To minimize electronic interference, the probe is shielded by a foil shielded often found inside the glass electrodes.

EXPERIMENT NO:3

PREPERATION OF CULTURE MEDIA FOR BACTERIAL CULTIVATION

INTRODUCTION

For studying the characteristics of different organisms it is essential to consider them. For this, the culture media is required and the primary role is to provide a balancing media of required nutrient concentration that will permit too.

NUTRITIONAL NEEDS:

Nutritional needs of microbial cells are supplied in the diet through a variety of medium. The following tests illustrate the nutritional diversity that exist among the microbes.

CARBON:

This is the most essential and central atom common to all cellular structure and functions. Among microbial cell two carbon different type are known;

i)heterotrophs:

These organisms cannot be grown in a dominant medium consisting of inorganic compound. They must be applied with organic nutrients, primarily glucose.

ii) autotrophs :

These organism can be cultivated in a ,medium consisting of organic compound especially they use inorganic carbon in th form of carbon dioxide.

PHOSPHOROUS:

This is necessary for the nucleic acids, DNA, RNA and also for the synthesis of high energy organic compound. Adenosine triphosphate phosphorous is supplied in the form of photosynthetic salt inside the microbial cell.

METALLIC ELEMENTS

Ca^{2+}, Zn^{2+}, Na^{2+} ,K^{+} ,Cu^{2+} ,Mg^{2+} ,Mn^{2} ,Fe^{2+} are some of the metallic ions necessary for obtaining efficient performance of various cellular activity, osmoregulation of enzyme activity and electron transportation during oxidation. These enzymes are micronutrients and are prepared to trace concentration only as organic salts and are supplied moderately.

VITAMINS

The organic substance contribute to cellular growth and essential in minor concentration for all activities. They are also sources of co enzymes which are required for the formation of active enzyme system. Some microbes require vitamins to be supplied in a performed range for normal metabolic activities. Some posses vitamins synthesis pathway where as some can synthesise only limited numbers from each component present in the medium.

NITROGEN

These organisms can be cultivated in a medium consisting inorganic compound especially they use inorganic carbon in the form of carbon dioxide.

This is also an essential atom in many cellular macromolecules particularly protein and nucleic acid protein and serve as structural molecules forming the so called fabric of the cell and as functional molecules enzyme dat responsible for the metabolic activity of the cell. Nucleic acid will lose DNA genetic bases of the cell. RNA which plays an active role in protein synthesis within the cell. Some microbes are autotrophic nitrogen others use nitrate salts and still others require nitrogen containing organic compound such as amino acids.

NON METALLIC SALTS

The major non metallic ions used for cellular nutrients are;

i) sulphur : this is integrated to some amino acid and therefore a compound of protein sources include organic compounds such as sulphur containing amino acids, inorganic compounds such as sulphates and elemental sulphur.

ii) water : all needs require water as the medium so the two molecular weight

nutrients can cross the cell.

iii)energy : active transport, biosynthesis and biodegradation of macromolecules are the metabolic activities of cellular organism. These activities can be sustained only if there is a constsnt availability of energy between the. Two big enegetic types of the microorganism present in air are,

a)phototrophs:

these depend on the radiant energy as solar energy source.

b)chemotrophs:

these depends on the oxidation of chemical compound as their energy sources. Some microbes use molecules such as glucose. Others utilize inorganic compound such as H_2S or $NaNO_2$.

ION CONCENTRATION

Growth of an organism in a medium may completely inhibited if the pH is not under limit. The enzyme of microorganism is greatly affected by this type. Therefore the pH of the medium should be adjusted to an organism level to face the minimum growth of organism under study.

STERILISING AGENTS:

Liquid media are frequently termed broth. They may be small to solid material by adding whole , egg white or blood serum and heating a coagulation poiny. When gelatin is added to the nutrient broth medium is called nutrient gelatin or nutrient agar.

NUTRITIONAL BROTH:

To 100ml of distilled water in 250ml flask beef extract and NaCl were added. Then it was stirred to dissolve the contents and pH was adjusted to 7. Broth was pipetted into test tube and bottom is plugged. It was then autoclaved. After autoclaving the test tubes were allowed to cool at room temperature. This can be used for inoculation.

NUTRIENT AGAR

To 100ml of distilled water in 250ml flask peptone, beef extract and NaCl were added. Then it was stirred to dissolve the contents and pH was adjusted to 7 and the agar was added. The contents were autoclaved. The flask with the medium was cooled to about 45C.

Four petriplates were taken and sterilized.

The flask was held at an angle and the stopper was removed with the 4^{th} and 5^{th} figures of the other hand. The mouth of the flask was heated by pasiing it briefly through the flame

The cover from the 1^{st} plate was removed with the hand holding the plug quickly and neatly the agar medium was dispensed in petridishes without air bubbles.

RESULTS

The media , nutrient broth and nutrient agar were prepared by the above described technique. It was then dispensed in sterile petriplates and test tube.

EXPERIMENT NO. 4

STERILISATION OF HEAT SENSITIVE MATERIAL BY MEMBRANE FILTRATION AND ASSESSMENT FOR STERILITY

INTRODUCTION

Microbiology membrane filters provide a useful----of studying material such as vaccines antibiotic solutions and other solutions that may be damaged or denatured by high temperature or chemical agents .Filtration process does not destroy but removes the microorganisms .Membrane filters were used for sterility testing of water and air. The filter contain pores small enough to allow the organism free fluid to pass through. The liquid is then collected in a sterile flask. Filters with a pore diameter from 2.5nm to0.45nm are usually used in this procedure

.Application of filtration for sterilisation of gases : HEPA(high efficiency particular air) can remove ways to 99.99% of particles greater than 0.3micrometer in diameter .Air is first passed through profilters to remove larger particles and then passed through HEPA filter .The performance of HEPA filter is monitored by pressure differential and air flow rate measurements. There are two ways of filters used in filtration utilisation.

DEPTH FILTERS :

It consist of fibrous or granular materials to packed as to form twisted channals of minute dimensions .They are made of diatomatious earth analysedporclain filter ,sintered glass or asbestors. These are porous membrane about 0.1mm thick made

of cellulose acetate ,cellulose nitrate polycarbonate and polyanilidine fluoride or some other synthetic material .The membrane are supported on a frame and held in special holders. Fluides are made to transverse membrane by negative pressure or by centrifugation.

Materials required

Media used in sterility testing :Nutrient agar medium generally used for tests for sterility.

PROCEDURE:

The filter should be a membrane filter disc of cellulose ester or other suitable plastics ,having normal average spore diameter not exceeding of o.45nm.The membrane should be held firmly the filtrate in unit which consist of a supporting base for the membrane ,a receptacle from the field to be tested ,a collecting reservoir for the filtered fluid, and the necessary tubes or connections the apparatus is so designed that the solution to be filtered can be introduced and filtered under aseptic condition it permits the aseptic removal of the membrane or transfer to medium or it is suitable for carrying out incubation after adding the medium to the apparatus itself .Cellulose nitrate filters are recommended for aqueous ,oily and weakly alcoholic solutions and the entire unit should be sterilized by appropriate means with the membrane filter and sterile airways in place. The method of sterilization should not be deleterious to the membrane filter and sterile airways in place.eg: weaker it or change the nomial average -------- diameter The sterile be free should provide pure access to the sterilizing agent . After sterilization ,the apparatus should be free of leaks to the atmosphere except through the sterile

airways .The solution to be the filter sterilized was filtered through cellulose nitrate membrane filter---------- .The passed out fluid was checked for sterility spread (0.2ml)by plating on to nutrient agar medium. The plates were circulated at 37°c.

RESULTS

No growth was observed showing the material was filter sterilized properly

EXPERIMENT NO:5

DEMONSTRATION OF THE PRESENCE OF MICROFLORE IN THE ENVIRONMENT BY EXPOSING NUTRIENT AGAR PLATE INTO AIR

INTRODUCTION

Air is the simplest one. The relative qualities of air gases in air , by volume percentage are nitrogen 78%, oxygen 21%, argon 0.9%, carbon dioxide 0.03%, hydrogen 0.01% and other gases in trace amounts. Air is mainly a transport or dispersal medium for microorganisms. They occur in relatively small numbers in air when compared to soil and water. The air in the atmosphere, which is found outside the buildings is reffered to as outside air. The dominant flora of outside air is fungus. The common fungi are Deuteromycetes, Cladosporium, and the Basidiomycetes, Sspoerebotomycetes. Widely occurring bacterial forms are spore of Bacillus, Clostridium resistant non spore formers such as Sarcinalutea and Micrococcus luteus, non pathogenic species of cornybacterium and few gram negative rods such as coliforms and chromobacteriacea. About 2-500 cells per m^3 were detached upto the height of 4 km from earth surface. Number of microorganism may vary from place to place. For eg; about several hundreds of bacteria found per cubic meter in cities. The air found inside the building is referred an indoor air. The commonest flora are penicilium, mucor, and aspergillus. The frequent bacterial species are Staphylococcus, Bacillus and Clostridium perfringes. Sources of air microbes are man made activities like digging

angploughing of soil, aerosols and air currents bring microbes from plant or animal sources. The optimum rate of relative humidity for the survival of microbes is between 40-80%, viruses survive at 17-25%

AIM

To demonstrate the ubiquitious nature of microbes experimentally.

MATERAILS REQUIRED

1) Sample chosen for the analysis in air

2) Other material required are nutrient agar, petriplates, cotton swab ,Bunsen burner, L-rod.

PROCEDURE

a) Inoculation

- Prepare and sterilize nutrient agar
- Pour the medium into sterilized petriplates.
- Allow all plates to solidify
- Expose plate to air for 5 minutes to understand the air microbial nature.
- Incubate all nutrient agar plates ate 37C for 24hours and rosebengal plates at 30C for 48 hours.
- Observe plates for growth and record the results.

RESULTS

Bacteria or fungi were observed in the medium from air samples. This indicate microorganism are ubiquitious in nature

EXPERIMENT NO:6

TEMPORARY MOUNT- LACTOPHENOL COTTON BLUE MOUNT- *RHIZOPUS* SPS, *PENICILLIUM* SPS, *ASPERGILLUS* SPS

OBJECTIVE

To stain fungal species by using lactophenol cotton blue.

BACKGROUND

The branch of science that deals with study of fungal species is called as mycology. Fungi are eukaryotic organism and they are classified into the main group as yeast and mould. The cell wall of mold is made upof chitin. Fungal cells have both macroscopic as well as microscopic structure. It is both beneficial and haemful for human beings as it produce many antibiotics, natural products as well as used in industrial fermentation process. It causes human, plant and animal disease as well as produce basic substances hence it is very important to study fungal species for the purpose of their identification, staining of these fungal cells is an important step. Some examples of common fungi are *Rhizopus, Aspergillus, Penicillium, Fusarium, Mucor.*

The lactophenol cotton blue wet mount preparation is the most widely used method of staining and observing fungi, the preparation has three components: phenol, which kills any other organism and cotton blue which preserves fungal

structures and cotton blue which is an acid dye that stain the chitin present in the cell wall of fungi.

MATERIALS REQUIRED

Fungal cultures: *Aspergillussps, Penicilliumsps, Rhizopussps*

PROCEDURE

- Lactophenol cotton blue stained clean grease freeglass slide and cover slips were taken.
- Sterile needles and tearing needles .
- A drop of lactophenol cotton blue stain was placed at the centre of clean slide.
- A fragment of the fungal colony was removed using an inoculation loop and placed in the drop of the stain.
- The fungal filaments were placed gently so as to separate them individually along with their sporulating structures.
- A coverslip was placed over the stain, care was taken not to introduce air bubbles. The slide was then first examined under low power objective to locate a well separated and healthy stained fungal hyphae with its fruiting body. Once located then it was finally viewed under high dry objective for the presence of characteristic mycelia and fruiting structues.

MORPHOLOGY OF FUNGI

1) *RHIZOPUS* SPECIES:

Rhizopus are rapidly growing white coloured fungus species over entire planet. It has the aerial mycelium which gives rise to straight sporangiophores that terminate with black sporangium containing a columella; root like hyphae (rhizoids) penetrate the medium.

2) ***ASPERGILLUS* SPECIES**:

The colonies are white in colour when young, become greenish, blue, black or brown as the culture ages. Conidia in chains develop at the end of the stigma arising from the terminal.

3) *PENICILLIUM* SPECIES:

Matured colonies are usually greenish or blue green. Conidia in chains develop at the end of the sterigma from the medulla of the conidiophores arise from a septate mycelium.

EXPERIMENT NO:7

STUDY OF *SPIROGYRA, CHLAMYDOMONAS* AND *VOLVOX* USING TEMPORORY MOUNT

SPIROGYRA

Spirogyra is a member of the algae. There are simple plants ranging from single celled organisms *(Chlamydomonas*, *Euglena*) to complex sea weeds.

They contain chlorophyll and make their food by photosynthesis. Spirogyra is a filamentous algae. Its cells form long thin strands that in large numbers contribute to the familiar green, slimy, blanket weed in ponds.

Seen under in the microscope, each filament consist of an extensic chain of identical cell.

Each cell contain a helical chloroplast, a number of cytoplasm and a vacuole enclosed in a cell wall.

Each cell can divide transversely and grow to full size thus increasing the length of the filament. When the filament breaks, this is a form or a sexual reproduction but there is a sexual process called conjugation.

In certain times of the year, tubular structures grows out from each cell of a pair of filament lying parallel to each other. The tubules join upto make a passage between each cell and its partner. The chloroplast and other structures become less distinct and the cytoplasm pulls free from the cell wall to form a round

structure. The cytoplasmic contents of the (conjugation tube) and use with the cytoplasam of the cell of the adjacent filament.

CHLAMYDOMONAS:

Chlamydomonas is the name given to a genus of microscopic, unicellular green plants which lives in fresh water.

Typically they are single celled body and these approximately spherical, about .02mm across with in the cell world surrounding the cytoplasam and central nucleus.

To filaments of cytoplasam, flagella, extend from one cell of the *Chlamydomonas* through the water and rotate it at the same time.

A single cape size chloroplast expels the greater thrust of the cell. In the chloroplast is a protein region called a peprenoid, which is involved starch production and maybe surrounded by storage granules.

Chlamydomonas make its in the same way as the green plants but without the elaborate system of roots, stem and leaves of the the higher plants. It is surrounded by water containing dissolved carbon dioxide and salt so that the light, with the aid of its chloroplast, it can build up starch by photosynthesis. No special breathing system organs are present, the oxygen needed of respiration diffuses in from the water through the entire surface of the cell. Similarly, carbon dioxide diffuses during photosynthesis.

In favorable condition the *Chlamydomonas* individuals will continue to grow

and then attain a certain size, reproduce by cell division.

***VOLVOX* :**

VOLVOX is a polyphyletic genus of chlorophite green alage in the family volvocaeceae.

Its form spherical colonies of up to 50,000 cells they live in verity of fresh water habitats and where first reported by Anton Von Leuwenhoek in 1700.

Each mature *VOLVOX* is composed of numerous flagellate cell similar to the *Chlamydomonas*, up to 10,000 in total and embedded in the surface of a hollow sphere or coenobium containing an extra cellular matrix made up of gelatinous glycoprotein.

The cells have eye spot, more developed near the anterior, which enables the colonies to swim towards light. The individual algae in some species are interconnected by thon strands of cytoplasm called protoplasmates.

Volvox can reproduce both sexually and asexually

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In asexual reproduction, gonidia develop into new organism that break out of the parent (when they dies).

In sexual reproduction two types of gametes are produced. *VOLVOX* species can be monoecious or dieceous. Male colonies release numerous microgamete or sperm while in female colonies single cell enlarge to become oogametes or egg.

EXPERIMENT 8

STUDY OF THE FOLLOWING PROTOZOANS USING PERMENANT MOUNT PHOTOGRAPHS; AMOEBA, ENTAMOEBA, PARAMEACIUM, PLASMODIUM

AMOEBA;

*Amoeba proteus*is a microscopic living organism which consists of a single cell.

Like most plant and animal cells it has cytoplasm, nucleus cell membrane and verity of inclusion in the cytoplasm.

It is about .3mm across and inhibit the mud at the bottom of the fresh water ponds.

Although it's just a single cell it exhibit all the essential functions of any living organisms.

Pseudopodium -A protuberance from the surface of amoeba into which cytoplasm flows and in this way the amoeba moves about over at the bottom of the pond.

Amoeba feeds on microscopic organism seen as single celled algae and bacteria, when amoeba encounters a suitable organism, the cytoplasm flows around the prey and enclaves it, with a drop of water in the food vacuole.

The cytoplasm secretes enzymes into the food vacuole. The enzymes digests the soft part of the prey and the soluble products are absorb back into the cytoplasm. Any undissolved residue is left behind as the amoeba flows on.

Amoeba for changing its direction of moment where this cytoplasm extend to the endoplasm flows into form pseudopodium.

Phylum: Protozoa

Class: Rhizopoda

Order: Lobosa

Genus*:* *Entamoeba*

Species: *histolytica*

The amoeba are parasitic in the intestine and transmission from man occurs through cyst.

The cyst are round and oval in outline, refractile, pearly in color having a definite wall. cyst may contain 1-4 nucleii and about 12mm in diameter.

The uncyst amoeba consist of a small mass of cytoplasm and inner dense mass called endoplasm.

If the amoeba have been isolated from feces of human being the ingested wet cell, which also are present in the feces.

The multiplication invade mucous membrane of large intestine and multiply in it.

The uncysted amoeba fail to infect due to destruction by enzymes of gastric origin.

Entamoeba histolytica causes amoebiasis or amoebic dysentery and result constipation. In chronic cases, it causes diarrhea leading to blood and mucous. It

also causes amoebic hepatitis. The infection caused can be cured by treating the patients with emotine hydrochloride.

PARAMECIUM:

It is a ciliate protozoan. Ciliate bodies are covered with fine cytoplasmic hair like structure called cilia. Flickering moments of cilia propel the organism through the water and also create feeding currents.

Paramecium has distinct and permanent shape and certain areas of cytoplasm specialized to carry out specific function.

During locomotion individuals cilia bend and straightened rapidly in such a way that the recovery stoke, offers minimal resistance and the bending stroke helps propel paramecium through the water.

The whole compliment of cilia beat in rhythmic pattern do that they are the waves of contraction pass over the cell body like wind blowing through ripe corn.

Paramecium on the other hand, can take in food only at the cytostome. The cilia in the oral groove create a current of water which drafts the food organism up to the cytosome where they are ingested in a food vacuole. This food vacuole then follows a specific root through the cytoplasm on its travel, enzymes are then absorbed into the cytoplasm.

Any undigested matter is expel through the oval pore. This contracts with amoeba which can expel the undigested remains from almost any point.

Paramecium reproduces like amoeba, by binary fission. The ciliate stops moving and both mega and micronucleus divide and move to opposite end of the organism. The cytoplasm then divides at right angle to the long axis and the daughter paramecia separate binary fission may take place two three times each day.

This is also a complex sexual process in which two paramecia join by their oval surfaces.

PLASMODIUM

They belong to

Phylum: Apicopelxa

Order: Sporozoa

Genus: *Plasmodium*

There are few species as a)*Plasmodium vivax*, b)*Plasmodium falciparum* c) *Plasmodium ovale*, d) *Plasmodium malariae.*

The malarial parasite, *Plasmodium* lives in the blood stream of human but unlike the trypanosomes, the parasite enter the red cells and feed on their cytoplasm.

The parasite are transmitted from person to person by female mosquitoes of the genus they seek from the superficial skin capillaries.

Malaria is characterized by intermitted fever associated with chills and rigors in the patient. There may be enlargement of the liver and spleen in the patient.

The life cycle of *Plasmodium* species involves several different stages both in insect and the vertebrate host

These stages include sporozoite which are injected by the insect vector into the vertebrate hosts blood. Sporozoites infect the host liver, giving rise to merozoites and hypnozoites. These moves into the blood where they infect red blood cells. Other forms more merozoites to infect more red blood cells or produce gametocytes which are taken by insects which fed on the vertebrate host. In the insect host, gametocyte emerge to sexually reproduce. After sexual reproduction, parasite move to the insect salivary glands, from which they can infect a vertebrate host bitten by the insect.