M.Sc. Microbiology **2018-2019**

Semester - II

18MBP211 **ADVANCED PRACTICAL – III** **4H – 2C**

Instruction Hours / Week:L: 0 T: 0 P: 4 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 9 Hours

COURSE OBJECTIVES

To obtain outstanding practical skill in various techniques in Microbial Biotechnology and Agricultural Microbiology.

COURSE OUTCOME

This practical course renders a candidate the knowledge of advanced techniques involved in Microbial Biotechnology and Agricultural Microbiology. Candidates would be able to understand and perform molecular techniques which forms an integral part of core Microbiology.

EXPERIMENTS

1. Isolation of plasmid DNA from Bacteria
2. Isolation of chromosomal DNA from Bacteria
3. Determination of molecular weight by SDS Polyacrylamide gel electrophoresis
4. Isolation of microbes from soil
5. Isolation of free-living N_2 fixation from soil - Azotobacter
6. Isolation of symbiotic nitrogen fixers from root odule - Rhizobium
7. Isolation of phosphate solublisers, ammonifiers and denitrifers
8. Study of Mycorrhizae, Cynobacteria and Azolla
9. Determination of Dissolved oxygen of water
10. Determination of BOD (Biochemical Oxygen Demand) of water
11. Determination of COD (Chemical Oxygen Demand) of water

REFERENCES

1. Aneja K.R. (2001). *Experiments in Microbiology, Plant Pathology, Tissue Culture and Mushroom Production Technology*, (3rd ed.). New Age International (P) Limited Publishers, New Delhi
2. Cappucino, J.G., and Sherman, N., (2001). *Microbiology A Laboratory Manual*, (6th ed.). Benjamin Cummings, New York.
3. Chirikjan, J.G., Kisailus, E.C., King, B., Krasner, R., and Mortensen, H., (1995). *Biotechnology. Theory and Techniques,* Vol II, Jones and Bartlett Publishers, London.
4. Palanivelu, P. (2004). *Analytical Biochemistry and Separation Techniques*, (3rd ed.). Twenty First Century Publication, Madurai.

ADVANCED PRACTICAL III

EX.NO: 1

ISOLATION OF PLASMID DNA FROM BACTERIA

AIM

To isolate and analyse the purity of the plasmid DNA from bacteria by alkaline lysis method, from bacteria.

BACKGROUND

The term plasmid was coined by Joshua Lederberg. Plasmid is circular, double stranded extra chromosomal DNA present in bacteria. Naturally, they occurs to carry genes responsible for antibiotic resistance, to degrade certain substance such as hydrocarbon. It is also used in molecular biology/ recombinant techniques for cloning genes from various other sources and amplified them for farther steps. The size of plasmids range from 1-1000 kbps. Plasmid DNA miniprob is a fundamental technique in molecular biologyand efficient plasmid DNA isolation and purification from bacterial culture is a key to many molecular based experiments. They are important tools in genetic and biotechnology laboratories as well as industries where they are used to make large amount of protein.

Alkaline lysis method of plasmid isolation was originally developed by Birnboim and Doly in 1979. In this procedure bacteria containing the desired plasmid are harvested from culture medium by centrifugation and suspended in isotonic solution which is subsequently subjected to lysis by an alkaline solution containing a detergent (SDS) and (NaOH), while detergent serves to lyse cells and denature protein. Subsequent step is neutralized and results in renaturation of plasmid and genomic DNA. Since plasmid DNA covalently closed it reanneals properly and remains in solution insoluble, from white genomic DNA reanneals randomly resulting in the formation of precipitate. This precipitate is separated by high speed centrifugation, plasmid from the supernatant can be recovered by precipitation using isopropanol or ethanol.

1). Harvesting

Generally bacterial cells containing the plasmid are grown in a liquid media therefore it is essential to separate the bacterial cells from the culture medium. Almost all protocols use centrifugation to separate the bacterial cells. Centrifugation speed is optimized in such a way that it results in accumulation of all the bacterial cells in the form of pellet. It should be loose enough to be resuspended easily in resuspension buffer leads to incomplete lysis, resulting in low yield of plasmid separating bacterial cells from the rest of

culture medium is essential as bacterial culture mediummay contain inhibitors which can inhibit the enzyme activity (Eg: restriction enzymes)

2).Resuspension of pellet

Bacterial pellet isolated from first step is resuspended in resuspension buffer. Resuspension buffer contain glucose, EDTA, and trisbuffer. Glucose is required to make the solution isotonic. EDTA chelate the divalent cation (calcium and magnesium) which are released upon bacteria lysis. EDTA action results in inactivation of many enzymes which may harm plasmid DNA. Tris buffer acts as a buffering agent. Addition of Rnase A to remove RNA from the plasmid preparation.

3). Lysis of bacteria

In this step, bacterial suspension is treated with lysis solution which contains sodium dodecyl sulfate (SDS) and (NaOH). SDS is a detergent which solution the phospholipid and denature proteins. Components of the cell membranes leading to lysisand release of the cell contents. High alkaline condition due to NaOH denature the plasmid and genomic DNA.

4). Neutralization of lysate

Addition of neutralization solution acidic acetate brings the pH of the lysate back to neutral resulting in precipitation of protein and genomic DNA. Both plasmid and genomic DNA renatures upon addition of neutralization buffer, while plasmid DNA renatural in correct conformation due to its circular and covalent nature therefore remains in the solution. Genomic DNA precipitates due to its circular and covalent nature therefore remains in the solution. Genomic DNA precipitates due to random association of both strands. SDS reacts with potassium acetate and form insoluble potassium dodecyl sulfate.

5). Cleaning of lysate

Precipitate formed upon addition of neutralization solution is separated in aqueous solution by high speed centrifugation.

6). Recovery of plasmid from cleared lysate

Supernatant from the previous step contains plasmid DNA. Most protocol use precipitation to recover plasmid from solution. Plasmid DNA can be precipitatedby either addition of two volumes of absolute alcohol or 0.7 volume of isopropanol to the cleared lysate. Centrifugation at high speed results in collection of plasmid in the form of pellet.

7). Washing of plasmid pellet

Precipitation of plasmid DNA contains salts which need to be removed from the plasmid. For this purpose 70% ethanol wash is given to the pellet.

MATERIALS REQUIRED

- Solution I (Resuspension buffer)

50mm glucose

10mm EDTA

25mm Tris, pH 8.0

Autoclaved and stored at 4°C.

- Solution II (Lysis buffer II)

Freshly prepared 0.2NNaOH, 1% SDS stored at room temperature.

- Solution III (Neutralization buffer)

3m potassium acetate pH 6.0, 3m potassium acetate was prepared by mixing 29.45g in 60 ml of distilled water. To this 11.5 ml of glacial acetic acid was added and maked up to 100ml by adding 28ml of distilled water.

- Isopropanol – stored at 20°C
- 70% of ethanol
- TE Buffer(pH 8.0).

10mm Tris

20mm acetic acid

1mm EDTA.

PROTOCOL

- 5ml medium containing proper antibiotic was inoculated with a single bacterial colony and incubated at 37°C overnight with vigorous shaking (atleast 200 rpm).

- Bacterial cells were pelleted by centrifugation at 4000 rpm for 5 minutes at room temperature.
- The supernatant was discarded and bacterial pellet was resuspended in 150ml of ice-cold solution-I. The mixture was pipetted up and down or vortexed as necessary to fully resuspended bacterial cells. The content was stored for 5 minutes at room temperature.
- 300µl of solution II was added to the suspension and mixed thoroughly by repeated gentle inversion. Do not vortex. The mixture was incubated for 5 minutes on ice.
- 225µl of ice-cold solution III was added to the lysate and mixed thoroughly by repeated gentle inversion. Do not vortex. The tube was stored on ice for 5 minutes.
- The content was centrifuged at 1300 rpm for 15 minutes at 4°C and the supernatant was transferred to fresh tube.
- 0.7 volume of propanol was added to the supernatant to precipitate the plasmid DNA. The content was mixed thoroughly by repeated gentle inversion. Do not vortex and incubate for 5 minutes at room temperature.
- The tube was then centrifuged for 10 minutes at 1300 rpm at 4°C and supernatant was discarded.
- The pellet was then rinsed in 70% ethanol and air-dried for about 10 minutes to allow the ethanol to evaporate.
- 50ml of distilled water or TE was added to dissolve the pellet. After addition of 20.RNAase A (10mg/ml) the mixture was incubated for 20 minutes at room temperature to remove RNA.
- Agarose electrophoresis- Agarose gels were prepared at a final concentration of 0.8% in TAE buffer and the sample was loaded along with standard molecular weight DNA marker. Electrophoresis was carried out for 1 hour at 110V using TAE as the running buffer. The gel was then stained with (1ml/ml) Ethidium bromide and visualized under UV light in a transilluminator.

RESULT

The isolated plasmid DNA was loaded on to the agarose gel along with 1 Kbp DNA ladder. The plasmid DNA was spotted around 1 Kbp with reference to the marker, the plasmid DNA may be super coiled.

EX.NO:2

DETERMINATION OF MOLECULAR WEIGHT OF PROTEIN BY SDS POLYACRYLAMIDE GEL ELECTROPHORESIS

AIM:

To separate proteins of different molecular weight using sodium dodecyl sulphate – polyacrylamide gel electrophoresis.

BACKGROUND:

The separation of macromolecules in an electric field is called electrophoresis.Discontinous polyacrylamide gel electrophoresis(PAGE) is probably the most common analytical technique used to separate and characterize proteins.The most commonly used system is called Laemmli method named after U.K.Laemmli, who was the first to publish a paper employing SDS-PAGE in a scientific study.

- A solution of acrylamide and bisacrylamide is polymerized , acrylamide alone forms linear polymer, the bisacrylamide introduces crosslinks between polyacrylamide chains. The pore size is determined by the ratio of acrylamide. A high ratio of bisacrylamide to acrylamide and a high acrylamide concentration cause low electrophoretic mobility. Polymerization of acrylamide and bisacrylamide monomers is induced by ammonium persulfate(APS) which decomposes to form free radicals .TEMED, a free radical stabilizer is generally included to promote polymerization. Sodium dodecyl sulphate(SDS) also called as sodium lauryl sulphate(SLS) is an amphipathic detergent.it has an anionic head group and a lipophilic tail. It binds non covalently to proteins with stoichiometry o around 1SDS molecule per 2 aminoacids, SDS causesproteins to denature and dissociate from each other.it also confers negative charge,in the presence of SDS, the intrinsic change of a protein is masked. During SDS PAGE all protein is migrate towards the anode(the positively charged electrode). SDS treated proteins have very similar charge to mass ratios,and similar shapes. During PAGE the rate of migration o SDS treated protein is effectively determined by molecular weight.
- Polyacrylamide gels restrain larger molecules from migrating as fast as smaller molecules, because the charge to mass ratio is nearly the same among SDS dentured polypetides. The inal separation of proteins is dependent almost entirely on the differences in relative molecular mass of polypeptides. In a gel of uniform density the relative migration distance of a protein is

negatively proportional to the log of its mass. If proteins of known mass rate run simultaneously with the unknowns , the relationship between RF and mass can be plotted, and the masses of unknown proteins can be estimated.

- Regardless of the system preparation requires casting two different layers of acrylamide between glass plates, the lower layer is responsible for actually separating polypeptides by size, the upper layer(staching gel) includes the sample . it is designed to sweep up proteins in a sample between two moving boundaries so that they are compressed into micrometre, thin layers when they reach the separating gel.
- Protein separation by SDS PAGE can be used to estimate relative molecular mass, to determine the relative abundance of major proteins in a sample and to determine the distribution of proteins among fractions. The purity of protein samples can be assessed and the progress of a fractionation or purification procedure can be followed. Different staining methods can be used to detect proteins such as coomassie brilliant blue staining or silver staining.

Range of separation of protein in polyacrylamide gels:

Acrylamide concentration	Range of separation
5	60-350
10	20-300
15	10-100

The protein sample is mixed with the sample buffer and boiled for 3-5 minutes, then cooled to room temperature before it is loaded into the sample well of gels, protein samples , prepared for SDS PAGE analytes are denatured by heating in the presence of a sample buffer containing Tris-buffer to maintain the pH of SDS

to denature proteins. Reducing agent such as DTT or 2-mercapto ethanol to break disulphide bonds, glycerol to make the sample denser or heavier than water and sink neatly to the bottom of the well. A tracking dye (usually) Bromophenol blue is used to monitor the progress of the sample in the gel, bromophenol blue is a small molecule which travels just behind the ion moving down towards the bottom of the gel. few protein molecules travel ahead of this tracking dye, when the dye reaches the bottom of the running gel the current is turned off to make sure that proteins do not electrophoresis but of the gel into the buffer tank.

- To assess the relative molecular weight of proteins in a gel, a sample containing several proteins in a molecular mass run along the test sample in one or more lanes of the gel. such sets of known protein are called as protein molecular weight markers or protein loaders . several kinds of protein molecular weight markers are available that are labelled or pre stained for different modes of detection. These are pre reduced and therefore primarily suited or SDS PAGE rather than Native PAGE. Markers are detectable via their specialized labels(e g: fluoroscent tags) and by ordinary protein staining methods
- Gels are removed from the glass plates and stained with a dye coomassie brilliant blue dye.coomassie blue binds strongly to all proteins and unbound dye is removed by extensive washing of the gel. Protein bonds can there fore be located and quantified since the amount of bound dye is proportional to protein content. stained gas can be dried and preserved ,photographed or scanned with a recording densito meter to measure the intensity of the colour in each protein band.
- In general casting strands are used to prepare the mini slab gels,to clean plate with two teflon spares make a single carrete.The carretters are stacked upright in the stand with the bottom of the carretters filled tightly to the bottom of the stand.
- Using freshly prepared 10 percentage ammonium persulphate (APS) is always recommended.
- Once the catalysts are added polymerisation may occur quickly.Thus If it is necessary to have the casting stand and to have the overlay solution ready.
- Acrylamide polymerizes spontaneously in the absence of oxygen. so the polymerization process involves complete removal of oxygen from the solution. Polymerization is more uniform If the mix is degassed to remove much of the dissolved oxygen, by placing it under a vaccum for 5 minutes or so before polymerisation.
- Immediately after pouring the gel mix ,it must be overlayed with water saturated butanol to an additional height of 0.5 cm.the purpose of butanol is to produce a smooth, a completely level surface on top of the separating gel.so that bonds are straight and uniform.
- Combs are inserted, taking care not to catch bubbles under the teeth. And adjust to make them even if necessary.
- Acrylamide is a toxic substance and hence proper handling is required. Wearing gloves is mandatory while handling the solution.
- Any leftover should be discarded only after polymerization into gel.

MATERIALS REQUIRED:

1. 30% acrylamide stock solution:
   - ✓ Acrylamide = 30 g
   - ✓ Bis acrylamide =0.8 g
2. Separating buffer:
   - ✓ 1.5m Tris (pH 8.8)=100ml
3. Stacking gel buffer:
   - ✓ 0.5m Tris(pH6.8)
4. Running buffer:
   - ✓ 50mm Tris
   - ✓ 192mm glycine
   - ✓ 0.1% SDS
   - ✓ pH=8.3
5. 5X loading buffer:
   - ✓ 250mm Tris (pH 6.8)
   - ✓ 10% SDS
   - ✓ 4% sucrose
   - ✓ 5% beta- mercapto-ethanol
   - ✓ 10% Bromophenol blue(0-5% w/v solution in water)
6. Gel staining solution:
   - ✓ Coomassie brilliant blue R- 250 -0.1 g
   - ✓ Methanol – 40 ml
   - ✓ Glacial acetic acid – 10 ml
   - ✓ Distilled water- 50 ml
7. De staining solution:
   - ✓ Methanol – 40 ml
   - ✓ Glacial acetic acid – 10 ml
   - ✓ Distilled water – 50 ml
8. 10% SDS solution

PROTOCOL:

- The glass plates were thoroughly cleaned with soap, rinsed in distilled water and finally wiped with alcohol and air dried.
- It was then assembled properly using the spaces rubbed with petroleum jelly clipped and clamped in an upright position.
- 2% agar was then applied around the edges of the spaces to hold them in place and seal the chamber so as to be leak proof(water may be added to the seeded glass plates to check or any leakage)
- The following ingredients were mixed together for the preparation of 10% separating gel mixture in the order given.
    - ✓ 30% acrylamide stock solution-6.65 ml
    - ✓ Tris –Hcl (pH 8.8 , 4.0 ml)
    - ✓ Deionized or distilled water- 9.04 ml
    - ✓ 10%SDS -200 micro litre
    - ✓ Ammomium per sulphate-100 ml
    - ✓ TEMED -10 ml
- The contents were mixed gently and poured into the space between the plates to get the desired length or height of separating gel, butanol / 70% ethanol was layered on top of the mixture and left for about 30 to 60 minutes for polymerization.
- After making sure that the solution has been polymerized into gel , the layered butanol or 70% ethanol was removed and washed with stacking gel buffer.
- 4% stacking gel was prepared by mixing following solutions:
    - ✓ 30% acrylamide stock solution- 1.35 ml
    - ✓ Tris- Hcl (pH 6.8)- 1.0 ml
    - ✓ Deionized or distilled water-7.5 ml
    - ✓ 10% SDS -100ml
    - ✓ Ammonium per sulphate – 50 ml
    - ✓ TEMED – 10 ml
- The stacking gel mixture was poured , comb was placed properly and left for 30- 60 minutes for polymerization.
- After the gel has polymerized the comb was removed without de sorting the shape of well. this gel can be used immediately or can be stored in a leak-proof container

containing the running buffer.it was advisable to use the casted gel after overnight storage.

- After removing the clips and agar, the gel plate was carefully inserted in the electrophoretic apparatus.it was then filled with the running buffer and any trapped air bubbles at the bottom of the gel were removed.
- After determining the protein concentration ideal range 10 -50 micro gram in 50 micro litre. Sample were mixed with sample buffer and heated in a boiling water bath for 5 minutes.
- After the sample have cooled , it was carefully loaded into the wells using a micropipette standared marker proteins were loaded in the first/middle/ last well, according to the requirement.
- The current (25mA) was turned on until the samples reached the end of the separating gel
- After the run was completed the gel was carefully removed from the plates and soaked in staining solution placed over a rocking platform for about 2-3 hours.
- After staining the gel is immersed in de staining solution and shaked continuously over a rocking platform overnight or until the background of the gel is colourless. initially the de staining solution was changed frequently at least for 3 to 5 times.
- The protein bands may appear in blue colour and can be documented by gel documentation system. Alternatively it can also be scanned using scanner by placing the gel in the polythene bag.

RESULT:

- After de staining the thin, blue coloured bonds appear around 65k Da with reference to standared protein molecular weight markers. this is in the line with the size of sample used(Bovine serum albumin)

EX.NO: 3

ISOLATION OF MICROBES FROM SOIL

AIM

To isolate different microbial population from soil.

BACKGROUND

The soil is home to a wide range of plant and animal life. Roots are the largest form of plant life and certain rodents, insects, and earthworm and other largest animals through higher plants are the primary producers of chemical energy that sustains the terrestrial ecosystem. Plants are dependent on the invisible (other than certain fungal structures) community of soil microorganisms for continued supply of many essential nutrients. Nutrient elements bound in organic combination would be inaccessible to plants without microbial decomposition of matter and mineralization of these elements, not only would nutrient cycling step but also biological N-fixation.

Soil microorganisms are indispensable to life on earth. Soil microorganisms live in their film of water that surrounds soil particles. These organisms include microflora bacteria, fungi, and actinomycetes along with microfauna, protozoa and nematodes. In terms of numbers and biological activity, the microfloraare dominant. Bacteria are small (about 10µm) and occur in three general shapes. Bred "bacillus" spherical (coccus) and spiral bacilli and cocci are more common in soil. The branched hyphae exhibit cell division and fungal mycelia (hyphae mass) are often macroscopic. Actinomycetes are also filamentous and branched by smaller.

AGAR PLATE METHOD FOR MICROBIAL COUNT

In this method, soil is dispensed in an agar medium so that individual microbial cells, spores, or mycelial fragments develop into macroscopic colonies. The procedure involves successive dilution of soil. Depending upon the extent of dilution, plates may be filled with a huge enumeration of colony- forming units initially present in the soil is from plates, in between these extremes. This method requires sterile technique to avoid introduction of extraneous microbes, anyone of several different growth.

Medium can be used but no single growth medium is optimal for all microorganisms that inhibit soil. Thus, growth of certain organisms is favoured in the chosen medium and growth of organisms is studied. Antagonistic or antibiotic is added to the agar in the agar plate method.

MATERIALS REQUIRED

- Nutrient agar, CzapekDox agar, Starch Casein- nitrate agar.
- Flask containing 100ml of sterile saline.
- Test tubes with 9ml of sterile saline for serial dilution.

PROTOCOL

- Agar media plates such as nutrient agar, czapekdox agar, and starch casein nitrate agar were prepared for bacteria, fungi and actinomycetes respectively.
- 1g of the collected soil was suspended in 100ml of sterile saline and shaken vigorously.
- This suspension was serially diluted and until 10^{-7}, using spread plate technique. 1ml of this suspension was plated for each kind of microorganism as given below.
- For bacteria, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} dilutions were used to inoculate nutrient agar plates and incubated at 37°C for 24 hrs.
- For fungi 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilutions were used to inoculate czapekdox agar plates and incubated at room temperature for 3-5 days.
- For actinomycetes 10^{-3}, 10^{-4}, and 10^{-5} dilution were used to inoculate starch casein nitrate agar plates and incubated at room temperature for 3-7 days.
- Single colonies of bacteria, fungi, actinomycetes isolates were transferred to respective agar plates and maintained for further investigation.

RESULT

Nutrient agar

Colony morphology: most of the colonies were flat, wrinkled, irregularly margined with pear white colors that appeared after 84 hrs of incubation.

Czapekdox agar

Colony morphology: colonies with long branching filaments and fluffy/ cottony appearance. Different spore colors were seen at the back side of the plate after 5 days of incubation.

Starch casein nitrate agar:

Colony morphology: the colonies were flat with irregular margins. Fungi like filaments pick some colonies produce pigments that were seen at the back side of the plate after 5 days of incubation

EX.NO:4

ISOLATION OF FREE LIVING N2 FIXERS FROM SOIL AZOTOBACTER

AIM:

To isolate the free living nitrogen fixing bacteria, Azotobacter spp from rhizosphere soil.

BACKGROUND:

Azotobacter is free living – heterotrophic nitrogen fixing bacteria that occur in the rhizosphere of variety of plants. The genus Azotobacter has six species viz, A.chroococcum, A.vinelandi, A.beiferinkil, A.nigricans, A.arnelandi, A.armeniacus, A.paspali. Except the last species, which is a rhizoplane bacterium , the other members are largerly rhizosphere borne. The potential of A.chroococcum and A.paspali are used as biofertilizer for various non- leguminous crops.

Azotobacter is an aerobic gram negative rod shaped bacteria occur singly, in chains, or in clumps. It doesn't form endospores but do form thick walled cysts, these cysts are resistant to dessication and to some detrorious chemical and physical agent. They however, cannot withstand extreme temperatures while in the cyst stage of their life, they do not fix nitrogen and are especially refractile. It may be motile by peritrichous flagella or non- motile. It can produce a water soluble pigment- yellow- green, fluorescent or red/violet/brownish black. It grows well at or optimum temperature between 20^0C to 30^0C and at P^H 7.0- 7.5, they are able to grow on various carbohydrates, alcohols, and organic acids

Azotobacter was first discovered using culture medium that is devoid of combined nitrogen source. Azotobacter is found on natural alkaline soils, in aquatic environments in the plant rhizosphereband phycosphere. A.chroococcum is the most common species of azotobacter present in the soil. Azotobacter spp are known to influence plant growth through their ability to fix molecular nitrogen, production of growth promoting substances like IAA, gibberlins or gibberalline like compounds and vitamins, excretion of ammonia in the rhizoshpere in the presence of root exudates production of anti- fungal metabolites and phoshphate solubulization.

Jensen's nitrogen free medium and ashby's medium is routinely used for preparation and mass production of Azotobacter. Seed inoculation of A.chroococcum increase the yield of crops about 10% and cereals about 5-10%. The response to inoculation was increased by manuring or by fertilizer application , coinoculation of Azotobacter with other bioinoculant like Rhizobium,

Azospirillum , Phosphate solubulizers, Vesicular Arbuscular Mycorrizhae(VAM) have been reported to enhance the growth and yield of legumes, cereals, and vegetable crops.

Beneficial effects of Azotobacter chroococcum inoculation has been reported on various cereal, vegetables, oil seeds, legume and cash crops, Inoculation experiment with Azotobacter gave better yield performance only ate lower levels of nitrogen (0 to 30kg N ha^{-1}). These diazotropic bacteria require large amounts of available carbon for their survival in soil addition of farmyard manure(FYM) compost and other organic amendments to agricultural soils improves the efficiency of Azotobacter and hence the plant growth and yield.

MATERIALS REQUIRED:

- Jensens's medium, Ashby medium.
- Rhizosphere soil from sugarcane/paddy/cotton.
- Test tubes

PROTOCOL:

- Agar plates with Jensen's and ashby's medium were prepared.
- 1g of the collected rhizosphere soil was suspended in 100ml of sterile distilled water and gently mixed to form uniform suspension.
- This suspension was serially diluted until 10^{-5} and 0.1ml of sample was spreaded onto the plates by spread plate technique.
- The plates were incubated at room at temperature for 3-5 days.

EX.NO: 5

ISOLATION OF SYMBIOTIC NITROGEN FIXERS FROM ROOT NODULE – RHIZOBIUM

AIM:

To isolate Rhizobium spp. from root nodules of leguminous plants.

Backgrounds:

Nitrogen is an essential nutrient for plant growth and development planers usually depend upon combined or fixed forms of nitrogen such as ammonia and nitrate because it is unavailable in its most prevalent form as atmospheric nitrogen much of this nitrogen is provided to cropping systems, in the form of industrially produced nitrogen fertilizers, use of these fertilizers has to world wide ecological problems as well as affects the human health, Biological nitrogen fixation (BNF) is the cheapest and environment friendly procedure in which nitrogen fixing microorganisms.

interacting with leguminous plants, fix aerobic nitrogen into soil.

Soil contains many types of microorganisms such as bacteria, actinomycetes, fungi and algae which are important because they affect the physical, chemical and biological properties of soil amongst the soil bacteria a unique group called rhizobia has a beneficial effect on the growth of plants. It can live either in the soil or within the root nodules, where it converts atmospheric nitrogen to ammonia and provides organic nitrogenous compounds to the plants.

Legumes have been used in agriculture since concerns time and legume seeds or purses were among the first source of human feed and their domestication. In leguminous plant, the bacteria live in small outgrowths on the roots called nodules, within this nodules the bacteria do nitrogen fixation and the plants absorbs the ammonia, and the legume plants absorbs the ammonia, a unique ability to establish symbiosis with nitrogen fixing bacteria of the family Rhizobiacoas such group of Rhizobium, BradyrhizobiumAllorhizobium, Sinorhizobium and Mesorhizobium, these bacteria can fix atmospheric nitrogen only in root nodules of legumes and that too when it is in the bacteroid stage of its life cycle. It possess the entire complement of genes for nitrogen fixation,which are normally latent and become active only under species conditions.

Rhizobium are Gram negative motile rods, the size and morphology of the nodules formed by the Rhizobia vary with plant species, they can fix 150-200 kilograms of nitrogen contain the enzyme nitrogenase which catalyzes the following reaction,

$$N2 + 3H2 \longrightarrow 2N + H3$$

Yeast mannitol Agar with congo red is used for the cultivation of rhizobium species and for studying root nodulation. Yeast extract serves as a good source of readily available amino acids. Vitamin B complex and accessory growth factors for rhizobia, it also posses the oxidation reaction potential of medium in the range favorable for rhizobia and serves as hydrogen donor in respiratory process, mannitol is the fermentable sugar alcohol source. Magnesium provides cations, essential for the growth of rhizobia.

Congo red inhibits penicillin – susceptible strain colonies of rhizobia stand out as white, translucent and elevated with entire margins.

MATERIALS REQUIRED:

- ✓ Fresh pinkish root nodules,
- ✓ Yeast extract mannitol agar (YEMA) with congo red,
- ✓ 0.1% mercuric chloride (OR) 3% sodium hypochloride,
- ✓ 70% ethanol,
- ✓ Sterile – forceps, glass rods, blade and distilled water.

PROCEDURE:

1. A plant was carefully and root system was washed in running tap water to remove the adhering soil particles.
2. Healthy, unbroken, pink nodules were selected and washed in distilled water.
3. The nodules were then immersed in 70% ethanol for 5-10 seconds and followed by rinse in sterile distilled water.
4. The nodules were then surface sterilized by soaking in 0.1% acidified mercuric chloride for 1-2 minutes and following by rinse in sterile distilled water.
5. The process was repeated for three times and finally washed thoroughly in sterile distilled water and plated in sterile petridish.

METHOD-I:

1. By using a sterile blade, the nodule were cut into two harves and squeezed on the surface of the medium using sterile forceps.
2. Using this as a mother inoculum a quadrant streaking was performed with a inoculation loop.

METHOD-II:

1. The surfaced sterilized nodules were collected in a test tube and crushed using a sterile glass rod in the presence of few drops of sterile distilled water.
2. With an inoculation loop a loopful of the suspension was streaked on the medium.
3. Alternatively, a serial dilution of the suspension can be performed and plated either by spread or pour plate method.
4. All plates, used in either method were incubated at room temperature for 3-5 days.

OBSERVATION:

After incubation at room temperature for 4 days the YEMA plates showed white gristering colonies which may be Rhizobium spp.

EX.N0:6

ISOLATION OF PHOSPHATE SOLUBULIZERS, AMMONIFIERS, AND DENITRIFIERS

AIM:

To isolate phosphate solubulizing microorganism from soil.

BACKGROUND:

Phosphorous is one among the three important nutrients required for plants, animals, and microbial growth. It can occur in two forms.viz, Soluble phosphorous and Insoluble phosphorous. Soluble form can be easily assimilated by plant and microbes, It is not so in the case of organic and inorganic phosphorous that cannot be utilized by plants ,they are called as Phosphate solubulizing Microorganism(PSB).

Mechanism of mineral phosphate solubulization of PSB strains is associated with the release of low molecular weight organic/inorganic acids through with their hydroxyl and carboxyl groups chelate the cation bound to phosphate, there by converting it into soluble forms. Bacteria such as B.Subtilis, Polymyxa, Pseudomonas, Mycobacterium, Micrococcus flave bacteria and the fungi Aspergillus and Penicillium are some of the best Phosphate solubulizers.

PSB have been introduced to the agricultural community as biofertilizers.PSB have attracted the attention of Agriculturulist as soil inoculums to improve the plant growth and yield, wgen PSB used with rock phosphate, it can save about 50% of the crop requirement of Phosphate fertilizer , the use of PSB as inoculants increases uptake by plants by simple inoculation of seed with PSB gives crop yield responses equivalent to 30kg $P_2 0_5$/ha or 50%of the need for phosphate solubulizers.

Pikovaskaya's agar is used for detection of phosphate solubulizing bacteria from soil.Yeast extract in the medium provides nitrogen and other nutrients necessary to support bacterial growth. Dextrose acts as energy source, different salts and yeast extract supports the growth of organisms, phosphate Solubulizing bacteria will grow on the medium and form a clear zone around the colony, formed due to phosphate solubulization in the vianity of the colony.

MATERIALS REQUIRED:

- Soil Sample.

- Sterile Blanks for Dilution.
- Pikovskaya's Agar
- Sterile L- Rods

PROTOCOL:

- One gram of the collected soil sample was mixed in 100ml of sterile distilled water and it was further serially diluted upto 10^{-6} dilutions.
- From each dilution 0.1 ml of the sample was evenly spread over the agar medium by spread plate technique.
- The plates were incubated at room temperature for 3-5 days

EX.NO: 7

DETERMINATION OF DISSOLVED OXYGEN OF WATER

AIM:

To determine the dissolved oxygen content of water sample by winkler`s method.

BACKGROUND:

The test was originally developed by Ludwing winkler, while, working as a Doctoral student at Budapest university. In 1888 the DO determination measure the amount of dissolved (or free) oxygen present in water or waste water, to maintain healthy water, the amount of oxygen must be as high as possible when the maximum amount of oxygen possible is dissolved in water, it is said to be saturated, the saturation concentration decreases with increasing temperature and is smaller for salt water than for sea water. E.g.for 25°c the saturation concentration of oxygen in fresh water it is 6. 7mg L-1 the concentration dissolve oxygen it also temperature dependent at 10°c the saturated concentration of oxygen in fresh water is 11.3 mg L-1 while at 20°c it is 9.2 mg L-1, aerobic bacteria and aquatic life such as fish must have do to survive, aerobic waste water treatment processes are aerobic and facultative bacteria to break down the organic compounds found in waste water into more stable, products the will not harm the receiving waters, waste water treatment facilities such as lagoons or povels trickling filters and activated sludge plants depend on these aerobic bacteria to treat sewage. If sufficient oxygen is not naturally supplied through wind and turbulence to replace the depleted oxygen, the body of water will develop a low DO and became anaerobic.

Two methods are commonly used to determine DO. Concentration used to determine DO concentration is the method which is a titration-based method and depends on oxidizing property of DO and to the membrane electrode procedure which works based on the rate of diffusion of molecular oxygen across a membrane.

In the iodonetric method, divalent manganese solution is added to the solution following by adding of strong alkali in a glass-stopper bottle DO rapidly oxidise an equivalent amount of the dispersed divalent manganese hydroxide precipitates to hydroxides precipitates to hydroxides of higher valence states. In the presence of iodine ions in an acidic solution, the oxidized manganese reverts to the divalent state, with the liberation of iodine ions in an acidic solution, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent of the original DO content, the iodine is then titrated with a standard solution

of throsulfate the titration with a standard solution of thiosulfates. The titration and point can be detected visually with a starch indicator some oxidizing and reducing agents present in solution can interfere with the iodometric method agents liberate iodine from agents positive interference and some reducing agents reduce iodine to iodide (Negative interfered).

Also organic matter present in solution can be oxidized purifically in the presence of oxidized manganese precipitate, thus causing negative errors, thus some modification of procedure is required.

Steps in the Winkler method of oxygen-determination:

- Manganese (II) ions liberated from the manganese sulfate are loosely bound with excess hydroxide
  mn2++2oH---->Mn(oH2)
- Manganese (H) is oxidized to manganese (II) in the presence of a strong base and binds the dissolved oxygen.
  2Mn(OH2) +1/2 o2+H2O---->2Mn(OH3)
- Free iodine is produced upon oxidification of the sample at a rate of one l2 molecule for each atom of oxygen.
  2Mn(OH3) +2i+6+i---->2Mn2+12+6H2O
- Free iodine complexes with excess iodine ions.
  i2+i---->i3
- The iodine/iodide complex is reduced to iodide with thiosulfate.
  i3+2S2O2-3---->3+S4O62-

MATERIALS REQUIRED:

APPARATUS: -

300 ml BOD bottle, 500 ml beaker, BOD incubator, burette, pipetes.

REAGENTS: -

1. Nutrient solution:

A) Phosphate buffer:

Dissolve 8.5g kH2po4, 2i, 75g, k2Hpo4, 33, 4g Na2Hpo4, 7H2o and 1.7g NH4cl in approx 500ml reagent water. Dilute F2 II the pH should be 7.2 store in 4°c refrigerated check before each use for contamination (if there is any indication of microbial growth, discard remaining reagent and prepare fresh).

B) Magnesium sulfate solution:

Dissolve 22.5g mgso4, 7H2o in reagent water, dilute to 1L.

C) Calcium chloride solution:

Dissolve 27.5g cacl2 in reagent water, dilute to 1L.

D) Ferric chloride solution:

Dissolve 0.25g Fecl3, 6H2o in reagent water, dilute to 1L.

2. Manganese sulfate solution:

Dissolve 480g mnso4, H2o in reagent water, filter, dilute to 1L.

3. Alkali-iodide-axide reagent :

Dissolve 500g NaoH and 135g NaI in reagent water, dilute to 1L. Add 10g NaN3 dissolved in 40ml reagent water, this reagent should not give a color with starch solution when diluted and acidified.

4. Concentrated sulfuric acid:

5. Standard sodium thiosulfate 0.0250N/0.18M:

6. Starch solution:

Prepare an emulsion of 5g soluble starch in a martar or beaker with a small amount of distilled water, pour thus emulsion into 1L of boiling water, allow to boil for few minutes, and let settle overnight use the clear supernatant.

7. Dilution water:

If may be prepared immediately before use by adding 1ml of each nutrient solution per litre of dilution water, when volumes of sample used exceed 150ml, additional nutrients should be added to the sample bottke. Add an additional 0.1ml of nutrients for each 50ml of sample used in excess of 150ml.

8. Preparation of glucose-glutamic acid standard (GGA):

Dry reagent grade glucose and glutamic acid at 103°c for 1hrs and cool for one hrs in the desicattor. Dissolve 150mg (0.15g) of glucose and 150mg (0.15g) of glutamic acid in disttilrd water and bring up to 1/L note. Sear the bottles and sterilize them these sterilized portions can than be cooled and stored at 4°c. When a known standard is ren, 6ml of GGA standard from one of the sealed, sterilized container is added to each BODs bottles are filled 3/4 full with solution water (198+30.5mg oxygen/L is based on 9.27 dilution of GGA 6ml/300).

WINKLER TITRATION TECHNIQUE: -

PROCEDURE:

- ✓ Slowly drain off three portions of aerated dilution water into three separate BOD bottles, avoid adding atmospheric o2 to dilution water.
- ✓ To two of the three BOD bottles, add 1ml mnso4 solution, followed by 1ml alkali-iodine azide reagent-submerge pipette tips in sample when adding reagent rinse tips well between uses.
- ✓ Stepper carefully to exclude air bubbles. Mix by investing bottle several times.
- ✓ When precipitate has settle to about half the bottle volume, carefully remove the stopper and mix by gentle invasion until the iodine is uniformly distributed throughout the bottle.
- ✓ Transfer 203ml of sample into a white 500ml beaker and titration with 0.0250N sodium thiosulfate to a pare straw color. Add 1-2ml of starch solution and continue to titration of first disappearance of the blue colour (200ml of original dilution water is equal to 203ml of dilution water plus reagent).
- ✓ Titration two of the three sample results should be within 0.1ml.
- ✓ Completely fill two bottles with dilution water of be incubated as blanks.
- ✓ Label each bottle carefully as to sample and volume used.

RESULTS:

EX.NO: 8

DETERMINATION OF BOD (BIOCHEMICAL OXYGEN DEMAND OF WATER)

AIM

To determine the biochemical oxygen demand o various water samples including sewage.

BACKGROUND

The biochemical oxygen demand test measures the ability of naturally occurring microorganisms to digest organic matter usually in a 5 day incubation. BOD at 20°C by analysing the depletion of oxygen which measures biodegradable organic matter and are normally expressed as mg/L. The BOD gives an indication of the amount of oxygen needed to stabilize or biologically oxidise the waste. The advantage of BOD test is that it measures only the organic matter oxidised by the bacteria. The disadvantage is the 5 days time lag and the difficulty is in obtaining consistent repetitive values.

BOD samples are normally incubated in 300 ml BOD bottles and are diluted as necessary to allow atleast 1-0mg/L of DO(dissolved oxygen) to remain at the end of the 5 day period to be a varied test. Atleast 2mg/L of oxygen must be depleted during the incubation. If necessary samples must be pretreated to assure proper PH, temperature, and absence of toxic materials(chlorine) thus consuming a suitable environment for survival of the BOD consuming bacteria.

There is a limited amount of oxygen which will dissolve in the sample(about 9mg/L).These for dilution are prepared with a buffered mineral nutrient containing water.

The test does not determine the total amount of organic materials present, since many compounds are not decomposed by biological and biochemical reactions in the set conditions. In conducting the test several different dilutions are to be used to ensure that at least one will deplete the oxygen content by about 50%.Two differentnt dilutions of the same sample should generate valid results. The BOD values of the two dilutions are normally averaged. The BOD values for glucose/glutamic acid standared should be 60%-70% of the COD values for the same sample.

The method consists of filling with diluted and seeded sample in two overflowing airtight bottles of specified size and incubation.

MATERIALS REQUIRED

- Ferric chloride solution

Dissolve 0.025g Fecl3,6H2O in reagent water.Dilute to 1L.

- Manganese surface solution:

Dissolve 480g MnSO4 in reagent water, dilute to 1L.

- Alkali-iodide-Azide reagent:

Dissolve 500mg NaOH and 135g Nacl in reagent water. Dilute to 1L.Add 10g NaN3 dissolved in 240ml reagent water, this reagent should not give a colour with starch solution when diluted and acidified.

- Concentrated sulphuric acid
- Standared sodium thiosulfate
- Starch solution

Prepare an emulsion of 5g soluble starch in a mortar or beaker with a small amount of distilled water, pour this emulsion into1L1 of boiling water. allow to boil for few minutes,and let settle overnight. Use the supernatant.

- Dilution water

It may be prepared immediately before use by adding 1ml of each nutrient solution per litre of distilled water. When volume of samples used exceed 150ml additional nutrients should be added to the sample bottle. Add on additional 0.1ml of nutrient for each 50ml of sample used in excess of 150ml.

- Preparation of Glucose-Glutamic acid standared(GGA)

Dry reagent grade glucose and glutamic acid at 103 degree celcius or 1 hour in the desiccator .Dissolve 150mg(0.15g) of glucose and 150mg(0.15g)glutamic acid in distilled water and bring up to 1L

NOTE

Seal andSterilize the bottles. then they are cooled and stored at 4 degree Celsius. When the known standared is run 6ml,o GGA standared from one of the sealed/sterilized containers is added to each BOD bottles and filtered.

Winkler filtraton technique:

PROCEDURE

- Slowly siphon three portions of aerated dilution water into 3 separate BOD bottles. avoidadding atmospheric oxygen to dilution water.
- To two of the three BOD bottles add 1ml,MnSO4 solution followed by 1ml-alkali-iodide-Azide reagent submerge pipette tips in sample when adding reagent. Rinse tips well.
- Keep stopper carefully to exclude air bubbles, mix by inverting bottles several times.
- When precipitate has settled to above half the bottle volume carefully remove the stopper and 1ml concentrated sulphuric acid. restopper and mix by gentle inversion until the iodine is uniformly distributed throughout the bottle.
- Transfer 200ml of sample into a 500 ml beaker and titrate with 0.025N sodium thiosulphate to develop a pale straw colour.Add 1-2ml of starch solution and continue to titrate first disappearance of blue colour
- Titrate two of the three samples.
- Completely fill two bottles with dilution water to be incubated as blanks.
- Label each bottle carefully as to sample and volume used.

INOCULATION OF BOD IN SAMPLE

BOD5 is calculated by the following formulae

BOD5 = (DO-D5)/P

Where

DO is the dissolved oxygen of the diluted solution after preparation.

D_5 is the DO of the diluted solution after 5 day incubation.

P is the decimal dilution factor.

OBSERVATION

The biological oxygen demand of the given water sample after 5 days are found to be 4.8mg/L

Dissolved oxygen of water sample before incubation:

D_1 = 8x1000x0.25xg/d5= 8mg/l

Oxygen of water sample after incubation :

D_2 = 8x1000x0.025x0.8/D_5 =3.2mg/L

BOD of the water sample is D_2-D_1= 8-3.2=4.8Mg/l

EX.NO: 9

DETERMINATION OF COD CHEMICAL OXYGEN DEMAND OF WATER

AIM:

To detemine the chemical oxygen demand of various water sample.

BACKGROUND:

The chemical oxygen demand test most commonly used indirectly measure the amount of organic compounds in water, most applications of cod determine the amount of organic pollutants found in surface water (lakes and river)making COD a useful measure of water quantity. Its expressed in milligrams per liter(mg/l) which indicates the mass of oxyen consumed per liter of sollution. It is the measurement of the amount of oxygen in water consumed for chmical oxidation of pollutants,if detemines the quantity of oxygen required to oxidize the oragnic matter in water of waste water sample,under specific conditions of oxidizing agent, temperature and time,the applicable range is 3-900mg/l. The ratio of BOD to COD is useful toarror the amenability of waste for biological treatment.

Ratio of BOD to COD greater than or equal to 0-8 indicates that needs biological treatment BOD value is always industries waste water, COD value is about 2.5times than that of BOD values.

The organic matter present in the sample gets oxidized completely by pottassium dichromate k2(r207) in the presence of surpluric acid(H2S04) to produce C02 and H20,the sample is refused with a known amount of pottasium dichromate in the sulphuric acid medium and the excess pottasium dichromate determined by titration against ferrous ammonium sulphate,using ferrioin as an indicater,the dichromate consumed by the sample, is equivalent to the amount of oxygen required to oxidize the organic matter, the limitation of the test lies in its inability to differentiate between the biologically oxidizable and biologically inert material and to final out the system rate constant of aerobic biological stabilization.

MATERIALS REQUIRED:

Standard pottasium dichromate digestion solution (0.01667m)

- ✓ Sulphuric acid reagent
- ✓ Ferrion indicator solution
- ✓ Standard ferrous ammonium 0.10m

PROTOCOL:

- ✓ In a container (tubes or ampules) with 2.5ml of a sample ,1.5ml of pottasium dichromate solution were added.
- ✓ 3.5ml of sulphuric acid was added inside the vessels where an acid layer is formed under the sample digestion solution layer.
- ✓ After tightly capping the tubes,it was inverted several times to mix completely.
- ✓ The tubes were placed in blank digestor procheatoal to 150C and refluxed for 2 hours ,behind a a protective shield.
- ✓ It was then cooled to room temperature and placed in test tube rack
- ✓ One or two drops of ferrioin indicator was added to the tubes and stirred rapidly on magnetic stirrer,while titrating against standardized 0.10MEAS.
- ✓ The end point is a color change from blue –green to reddish brown although the blue-green may reappears within minutes.
- ✓ The procedure was repeatedely titrated with the blank containing.
- ✓ The reagent and a volume of distilled water equals to that of the sample.
- ✓ COD is given by,

$$COD(mg\ 0.1/l) = (AB)*m*8000)/\ V\ sample$$

Where;

A= Volume of FAS used for blank (ml)

B= Volume of FAS used for sample (ml)

C= Molarity of FAS

8000-mili equivalent weight of oxygen (8)*1000ml/l

EX.NO: 10

Study of mycorrhizae, Cyanobacteria and Azolla

Mycorrhizae

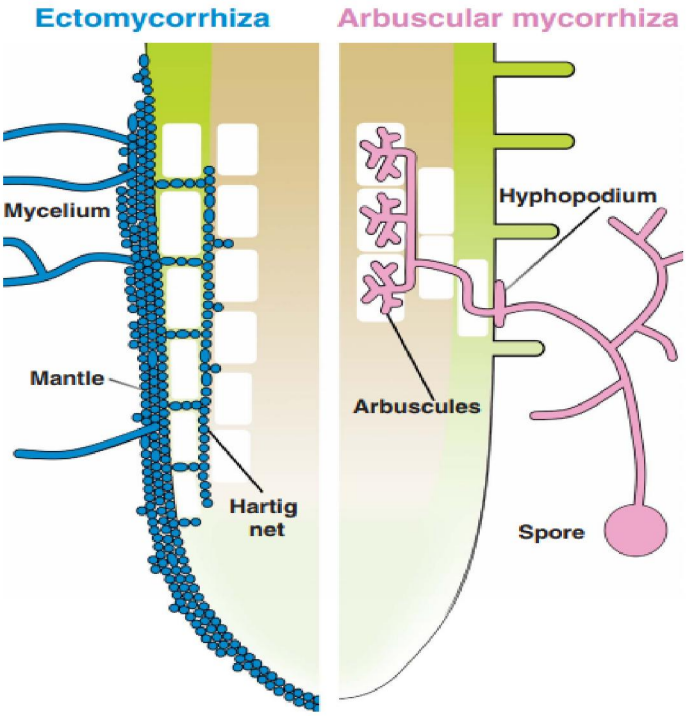
Owing to their filamentous organization, fungi exploit very diverse substrates on the basis of their nutritional strategy. Saprobes thrive in soil, water and on decaying animal and plant tissues. A smaller group of fungi, the parasitic and mutualistic symbionts, feed on living organisms. Such a classification cannot easily be applied to mycorrhizal fungi, a heterogeneous group of species spread over diverse fungal taxa. Although they can spend part of their life cycle as free-living organisms, mycorrhizal fungi always associate with the roots of higher plants, indeed over 90% of plant species, including forest trees, wild grasses and many crops. Both partners benefit from the relationship: mycorrhizal fungi improve the nutrient status of their host plants, influencing mineral nutrition, water absorption, growth and disease resistance, whereas in exchange, the host plant is necessary for fungal growth and reproduction.

Mycorrhizal fungi colonize environments such as alpine and boreal zones, tropical forests, grasslands and croplands. They have a major role in nutrient cycling through the specific activity of their mycelium in absorbing soil nutrients and supplying them to the plant, although their role in carbon flux is less well defined. The term mycorrhiza is derived from the Greek words for ‘fungus’ and ‘root’. Mycorrhizal fungi develop an extensive hyphal network in the soil, the aptly named wood-wide web, which can connect whole plant communities offering efficient horizontal transfer of nutrients. Mycorrhizas develop specialized areas, called symbiotic interfaces, to interact with the host plant.

There are two broad categories of mycorrhizal associations with plant roots, ectomycorrhiza and endomycorrhiza, which are differentiated by how they physically interface with the plant. The ectomycorrhizae (EM) occur mainly in the roots of woody plants (i.e. forest trees) and form a dense hyphal covering (fungal sheath or mantel) over the root tip from which hyphae grow into the intercellular spaces forming a net (Hartig net) of hyphae around the root cortex cells, but do not penetrate the cell walls. In contrast, the endomycorrhizae fungal hyphae grow into the root cortex and enter the cells forming fan-like, highly branched structure known as an arbuscule that remain separated from the cytoplasm by the plant plasma membrane. The endomycorrhiza can be further divided into the more widespread arbuscular mycorrhiza (AM) and the specialized orchid and ericoid mycorrhizas which, as the name implies, are colonizers of orchids and ericoid (e.g., cranberry) plant species. The AM fungal associations are the most abundant of all mycorrhizal associations. In both cases, the Hartig net and the arbuscules increase the contact area between the fungus and the plant through which the transfer of nutrients to the plant and carbon to the fungus occurs. Unlike the ectomycorrhiza, the endomycorrhiza are wholly dependent on the plant for

their carbon and when associations occur, both endomycorrhiza and ectomycorrhiza can demand up to 20-40% of the total photosynthetically fixed carbon the plant produces.

Schematic picture showing the difference between ectomycorrhizae and endomycorrhizae colonization of plant roots.



Cyanobacteria

Blue-green algae, also called cyanobacteria, any of a large, heterogeneous group of prokaryotic, principally photosynthetic organisms. Cyanobacteria resemble the eukaryotic algae in many ways, including morphological characteristics and ecological niches, and were at one time treated as algae, hence the common name of blue-green algae. Algae have since been reclassified as protists, and the prokaryotic nature of the blue-green algae has caused them to be classified with bacteria in the prokaryotic kingdom Monera.

Like all other prokaryotes, cyanobacteria lack a membrane-bound nucleus, mitochondria, Golgi apparatus, chloroplasts, and endoplasmic reticulum. All of the functions carried out in eukaryotes by these membrane-bound organelles are carried out in prokaryotes by the bacterial cell membrane. Some cyanobacteria, especially planktonic forms, have gas vesicles that contribute to their buoyancy. Chemical, genetic, and physiological characteristics are used to further classify the group within the kingdom. Cyanobacteria may be unicellular or filamentous. Many have sheaths to bind other cells or filaments into colonies.

Cyanobacteria contain only one form of chlorophyll, chlorophyll a, a green pigment. In addition, they contain various yellowish carotenoids, the blue pigment phycobilin, and, in some species, the red

pigment phycoerythrin. The combination of phycobilin and chlorophyll produces the characteristic blue-green colour from which these organisms derive their popular name. Because of the other pigments, however, many species are actually green, brown, yellow, black, or red.

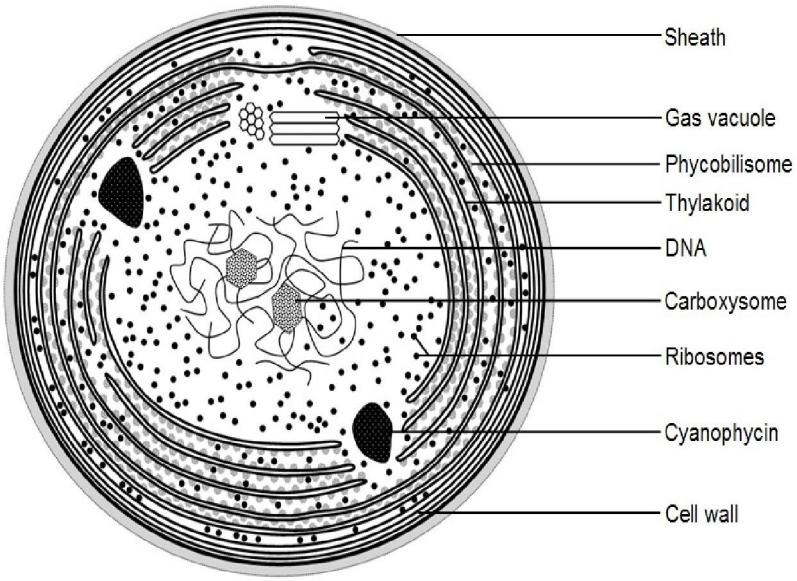
Most cyanobacteria do not grow in the absence of light (i.e., they are obligate phototrophs); however, some can grow in the dark if there is a sufficient supply of glucose to act as a carbon and energy source. In addition to being photosynthetic, many species of cyanobacteria can also "fix" atmospheric nitrogen—that is, they can transform the gaseous nitrogen of the air into compounds that can be used by living cells. Particularly efficient nitrogen fixers are found among the filamentous species that have specialized cells called heterocysts. The heterocysts are thick-walled cell inclusions that are impermeable to oxygen; they provide the anaerobic (oxygen-free) environment necessary for the operation of the nitrogen-fixing enzymes. In Southeast Asia, nitrogen-fixing cyanobacteria often are grown in rice paddies, thereby eliminating the need to apply nitrogen fertilizers.

Cyanobacteria range in size from 0.5 to 60 micrometres, which represents the largest prokaryotic organism. They are widely distributed and are extremely common in fresh water, where they occur as members of both the plankton and the benthos. They are also abundantly represented in such habitats as tide pools, coral reefs, and tidal spray zones; a few species also occur in the ocean plankton. On land, cyanobacteria are common in soil down to a depth of 1 m (39 inches) or more; they also grow on moist surfaces of rocks and trees, where they appear in the form of cushions or layers.

Cyanobacteria flourish in some of the most inhospitable environments known. They can be found in hot springs, in cold lakes underneath 5 m of ice pack, and on the lower surfaces of many rocks in deserts. Cyanobacteria are frequently among the first colonizers of bare rock and soil. Various types of associations take place between cyanobacteria and other organisms. Certain species, for example, grow in a mutualistic relationship with fungi, forming composite organisms known as lichens.

Cyanobacteria reproduce asexually, either by means of binary or multiple fission in unicellular and colonial forms or by fragmentation and spore formation in filamentous species. Under favourable conditions, cyanobacteria can reproduce at explosive rates, forming dense concentrations called blooms. Cyanobacteria blooms can colour a body of water. For example, many ponds take on an opaque shade of green as a result of overgrowths of cyanobacteria, and blooms of phycoerythrin-rich species cause the occasional red colour of the Red Sea. Cyanobacteria blooms are especially common in waters that have been polluted by nitrogen wastes; in such cases, the overgrowths of cyanobacteria can consume so much of the water's dissolved oxygen that fish and other aquatic organisms perish.

Cross section of Cyanobacterial cell



Azolla

Azolla commonly known as mosquito fern, duckweed fern, fairy moss, and water fern, is a small free floating aquatic fern native to Asia, Africa, and the America. It grows in swamps, ditches, and even in lakes and rivers where the water is not turbulent. The name Azolla is derived from the two Greek words, Azo (to dry) and Ollyo (to kill) thus reflecting that the fern is killed by drought. Due to their symbiosis with atmospheric nitrogen (N_2) fixing microorganisms (diazotrophs), the primary production of the plants is hardly ever N-limited under natural conditions. The diazotrophs live inside Azolla's leaf cavities and include the cyanobacteria Nostoc/Anabaena azollae that forms unbranched, multi-cellular chains that contain both photosynthetic, vegetative cells and N_2 fixing heterocysts. The endosymbiont, which is nitrogen-fixing, provides sufficient nitrogen for both itself and its host. The fern, on the other hand, provides a protected environment for the algae and also supplies it with a fixed carbon source. It has capability to fix atmospheric nitrogen as well as to produce biomass at a very high rate. Using nitrogenase enzymes, the diazotrophs reduce atmospheric N_2 to ammonium (NH_4^+), which is then excreted into the Azolla leaf cavity and taken up by the fern. In response to N-limitation, when there is no exogenous N available to Azolla, the heterocyst fraction increases in diazotroph chains. Azolla is commonly used as biofertilizer as well as green manure in the paddy field. Now a days Azolla (either fresh or in dried) is also used as a feed ingredient for ruminants and nonruminants type of livestock. Besides its utilization as biofertilizer and livestock feed, azolla, the 'green gold mine' of the nature is also used as medicine, water purifier, human food and for production of biogas.