ANALYSIS OF MICROORGANISM INCUBATION TECHNIQUES FOR INCREASING AGRICULTURAL PRODUCTION

S. Anandhan [*1], M. Praveen[2], R. Manikandan[3], R. Janani[4]

[*1,2,3] *Student, B.Sc. (Microbiology), Bharathiar University Arts and Science College, Gudalur, The Nilgiris, Tamil Nadu-643212, India*
[4]*Assistant Professor, Department of Microbiology, Bharathiar University Arts and Science College, Gudalur, The Nilgiris, Tamil Nadu-643212, India*
[*1]Corresponding Author E-mail: anand281296@gmail.com

ABSTRACT

Microbes are an integral part of all earth particles and contribute to soil and plant health. Microorganisms have the ability to fix atmospheric nitrogen, solubilize and mobilize phosphorus, produce antibiotics and disease suppressing molecules. Owing to these properties, they are used in agriculture as biofertilizers and bio pesticides. They are also important in the treatment of solid waste and sewage. They clean up the environment by degradation of several pollutants like pesticides, hydrocarbons, dyes and paints. They also help in the enhanced recovery of oil and metals from low grade ores or aqueous streams. This article focuses on presenting a brief analysis of some of the best known microbes that are helpful to the soil and agriculture and describes methods for incubating them in laboratories. This work collects the aspects of different studies and compiles them to make a simple, general report on the beneficiary activities of the microbes specialized to the field of agriculture. The major goal of studying about these microbes is to help the farmers and other agricultural professionals to adapt themselves and increase the yield without degrading the soil nutrients.

Keyword: - *Microbes, nitrogen fixing bacteria, biofertlizers, bio-pesticides, blue green algae, rhizobium.*

1. INTRODUCTION

Microbes incorporate fungi, bacteria and virus. Agriculturists and farmers regularly consider microbes bugs that are damaging to their products or creatures (and additionally themselves), yet numerous microbes are valuable [1]. Soil microbes (microscopic organisms and parasites) are basic for breaking down natural matter and reusing old plant material. Some dirt microbes and parasites shape associations with plant roots that give essential supplements like nitrogen or phosphorus. Growths can colonize upper parts of plants and give many advantages, including dry spell resilience, warm resilience, imperviousness to bugs and imperviousness to plant illnesses [2]. Infections are quite often considered as specialists of sickness. This is on account of the ones that cause ailment is the ones that have been considered. About a large portion of the plants have infections, yet most don't appear to be wiped out by any means. The infections appear to live in the plants without doing any damage [3]. Microbes are important in human culture and health in many ways, serving to ferment foods, treat sewage, produce fuel, enzymes and other bioactive compounds. They are essential tools in biology as model organisms, and have been put to use in biological warfare and bioterrorism. They are a vital component of fertile soils.

Man is a host to assortment of pathogenic microorganisms, protozoa and infections. They can bring about different irresistible and non-irresistible ailments [4]. So as to control the ailment and its transmission, it is fundamental to segregate and recognize the causal specialist from blood, sputum, pee, stool or discharge. Different social and subatomic techniques can be utilized for ID of pathogen [5]. Sanitization procedures [6], utilization of disinfectants and immunization can help control transmission of illness.

2. BIOFERTILIZERS

Biofertilizers [7] are the items containing living cells of various sorts of microorganisms that enhance the supplement nature of soil. The fundamental wellsprings of biofertilizers are microscopic organisms, parasites and cyanobacteria (blue green growth). Most biofertilizers have a place with one of the accompanying classifications: nitrogen settling, phosphate solubilizing and activating, and plant development advancing rhizobacteria. A portion of the real biofertilizers and target harvests are given in table 1. Nitrogen settling biofertilizers settle barometrical nitrogen into structures which are promptly useable by plants. These incorporate Rhizobium, Azospirillum, Azotobacter, blue green growth and Azolla. While Rhizobium requires harmonious relationship with the root knobs of vegetables to fix nitrogen, others can settle nitrogen freely. Phosphate solubilizing microorganisms discharge natural acids that upgrade the take-up of phosphorus by plants by dissolving rock phosphate and tricalcium phosphate. Arbuscular mycorrhizal parasites are the most widely recognized phosphorus assembling sorts that are ubiquitous. A gathering of microscopic organisms that upgrade the development of plant through nitrogen obsession, phosphorus solubilization or creation of plant development advancing metabolites are known as Plant Growth Promoting Rhizobacteria (PGPR) [8]. Numerous PGPR strains can possibly be utilized as microbial inoculants to upgrade edit profitability.

Table-1: Major Biofertilizers and target crops [9**]**

BIOFERTILIZERS	TARGET CROP
Rhizobium	Leguminous crops
Azotobacter	Wheat, maize, cotton, mustard and vegetables (Potato, onion, tomato, brinjal and others)
Azospirillum	Cereal crops like wheat, maize, millets, sorghum, barley; and sugarcane
Blue green algae (BGA)	Rice
Azolla	Rice
Phosphate solubilizing microorganisms	All
Arbuscular mycorrhiza	Nursery raised crops and orchard trees
Plant growth promoting rhizobacteria	All

The development in rural creation over the most recent three decades has been joined by a sharp increment in the utilization of concoction manures, bringing about genuine concern. Principal among these worries is the impact of inordinate composts on the nature of soil and ground water. The utilization of ecological agreeable biofertilizers can chop down the utilization of substance composts. Biofertilizers have unequivocal preferred standpoint over concoction composts. It is sparing to utilize biofertilizers as they are a shabby wellspring of supplements when contrasted with substance composts. Biofertilizers notwithstanding nitrogen and phosphorus likewise gives certain development advancing substances like hormones, vitamins, and amino acids that enhances the plant wellbeing and power. Consistent utilization of concoction composts antagonistically influences the dirt structure while biofertilizers when connected to soil enhance the dirt structure. The concoction composts are poisonous at higher measurements while biofertilizers have no lethal impacts.

Nitrogen fixing bacteria

An air around us contains about 78% nitrogen that is in free shape and is not used by the plants. Plants take up nitrogen as smelling salts or nitrate [10]. Generally little measure of alkali is delivered by lightning. A few alkali likewise is delivered modernly by the Haber-Bosch prepare [11], utilizing an iron-based impetus, high weights and genuinely high temperature. Yet, the real transformation of N2 into smelling salts by the activity of compound nitrogenase, and thus into proteins, is accomplished by microorganisms in the process called nitrogen obsession (or dinitrogen obsession). Fig.1 demonstrates a portion of the gainful microbes.

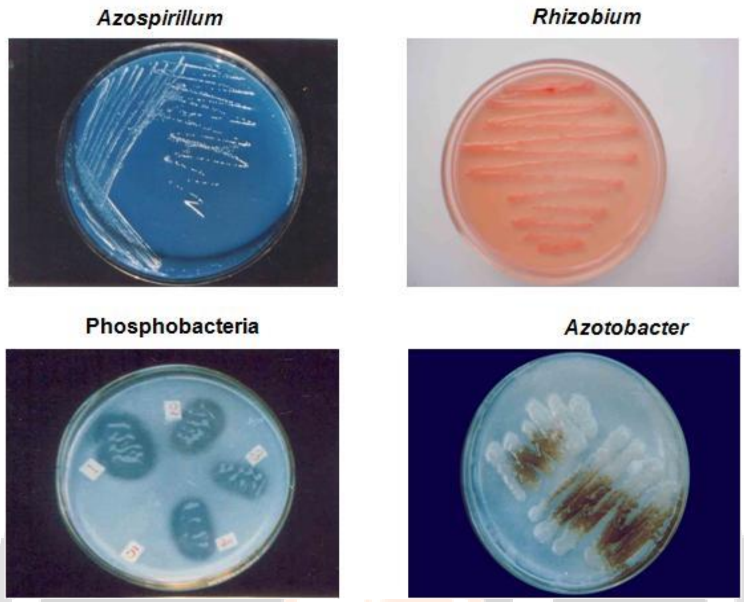

Fig -1: Beneficial Microbes

All the nitrogen-fixing organisms are prokaryotes. There are different groups of nitrogen fixing microorganisms (diazotrophs) present in the nature [12]. These are broadly divided into three categories, viz,

i. Symbiotic microorganism
ii. Asymbiotic or free living
iii. Associative Symbiosis

Examples of nitrogen fixing microorganisms for each category is given in [12] are summarized in table 2.

Table.2: Examples of nitrogen fixing bacteria belonging to different categories

CATEGORY	EXAMPLES
Symbiotic	*Rhizobium*- legume symbiosis *Rhizobium-Parasponia* (non-legume) Symbiosis *Frankia*- Trees (e.g *Alder, Casuarina*) *Azolla- Anabaena* *Azotobacter paspali - Paspalum notatum*
Free living 1. Aerobic	*Azotobacter* *Beijerinckia* Cyanobacteria (e.g *Nostoc, Anabaena,* *Tolypothrix, Aulosira*)
2. Facultative	*Klebsiella pneumonia* *Bacillus polymyxa*
3. Anaerobic	*Clostridium* *Desulfovibrio* *Rhodospirillum* *Rhodopseudomonas* *Desulfotomaculum* *Desulfovibrio* *Chromatium* *Chlorobium*
Associative	*Azospirillum* *Herbaspirillum* *Acetobacter diazotrophicus* *Azoarcus*

3. RHIZOBIUM INOCULANT

The bacteria of the genera Rhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium and Azorhizobium are collectively known as rhizobia, in advantageous relationship with leguminous plants decrease environmental nitrogen. The rhizobial states seem raised, wet, sparkling, and translucent or dark with smooth edge on yeast extricate mannitol agar (YEMA) medium. The vegetable rhizobia beneficial interaction finishes in the development nitrogen settling root or stem knobs. These one of a kind structures are agronomically critical as they give contrasting option to the utilization of vitality costly ammonium manure. Not all vegetables settle nitrogen. Of the three isolate groups of vegetables, the ability to frame knobs seem, by all accounts, to be missing from the greater part of types of Caesalpiniaceae. All individuals from family Mimosaceae and Fabaceae demonstrate arrangement of knobs with rhizobia. It is trusted that vegetable Rhizobium advantageous interaction contributes no less than 70 million metric tons N for every year. The measure of nitrogen settled shifts with the strain of Rhizobium, the plant species and ecological conditions. Fig.2 indicates run of the mill development of Rhizobium on yeast mannitol agar medium with congo red

Fig -2: Typical growth of *Rhizobium* on yeast

Rhizobium inoculation

Legume inoculation is a critical methodology for the control of rhizobial microflora and enhancing crop profitability and soil fruitfulness [13]. In any case, in tropical soils where there is nearness of sufficient local rhizobia and abnormal amounts of mineral N, vegetable inoculation frequently fizzles. Hence there is an earnestness to distinguish conditions where inoculation is required. Diverse analytic measures to choose about inoculation have been recommended by different laborers. Inoculation ought to be done if;

- population density of species-specific rhizobia is low,
- the same or symbiotically related legume is not grown in the area in the immediate past history
- waste-lands have to be reclaimed
- legume follows a non-leguminous crop in a rotation
- soil is poor in mineral N (nitrate)
- Soils are acidic, alkaline and saline.

Selection of rhizobial strains for inoculant production

An extensive scale screening ought to be done to distinguish perfect inoculant strain for various legume crops. The paradigm for choice may differ for specific soil sorts like acidic, sodic, saline, and nitrate-rich or overwhelming metal debased. Taking after are a portion of the attractive characters for a strain to be fit for use in business inoculants [14]:

1. Ability to form nodules and fix N on the target legume
2. Ability to compete in nodule formation with populations of native rhizobia present in the soil.
3. Ability to fix N across a range of environmental conditions;
4. Ability to grow well in artificial media, in inoculant carrier and in the soil
5. Ability to persist in soil, particularly for annually regenerating legumes
6. Ability to migrate from the initial site of inoculation
7. Ability to colonize the soil in the absence of a legume host
8. Ability to tolerate environmental stresses;

9. Ability to fix N with a wide range of host genotypes;
10. Genetic stability
11. Compatibility with agrochemicals

Inoculant production

i) Propagation

Rhizobia are not extremely specific in their wholesome prerequisites. Yeast- extract mannitol (YEM) medium is regularly utilized for refined of rhizobia. For business generation of cultures, less expensive sources like sucrose, molasses and corn soak alcohol can be utilized. Mass scale proliferation of rhizobia can be done utilizing arrangement of turning shaker or fermenter. In shake carafe culture, juices are brought up in cups with disturbance by round movement of revolving shaker. Fermenters are utilized for modern scale creation of bio-composts. Culture vessels going from 5 to 1000 L can be utilized relying on the necessity. The measure of inoculum culture to be included into the fermenter vessel relies on upon the span of the fermenters; however the proportion between the inoculum and the medium in the vessel ought to be kept up at 1:20 (5% inoculum rate). The stock is constantly circulated air through by constraining sterile air through permeable stainless steel sparger. Different aging necessities like air circulation, tumult and maturation time change from strain to strain. At the point when the quantity of rhizobia in the stock has achieved the required standard (108-109 cells ml-1) the soup ought to be added to the bearer for planning of transporter based inoculant. Ideal maturation conditions for mass augmentation of Rhizobium strains

ii) Carriers for rhizobial inoculants

The medium in which rhizobia are permitted to duplicate is an essential calculate rhizobial culture readiness. The expression "carrier" is for the most part utilized for a medium that conveys the live microorganisms. According to BIS particular, the carrier ought to be in powder shape and prepared to do going through 150-212 micron (72-100 work) IS strainer. A decent carrier material ought to

1. Have high water holding capacity
2. be non-toxic to rhizobia
3. be easy to sterilize by autoclaving or gamma irradiation
4. be readily and inexpensively available
5. Provide good adhesion to seed
6. Have pH buffering capacity
7. Have cation and/or anion exchange capacity.

A mixture of charcoal and soil in ratio of 3:1 is most commonly used as a carrier material. The preparation of charcoal based carrier is given below.

- The carrier material is sun dried up to a moisture level of 5%. The material is ground to a desired fineness preferably to pass 100-200 mesh sieves.
- The carriers are mixed with finely powdered calcium carbonate to neutralize if they are acidic. To make charcoal more suitable for the multiplication of rhizobia, $CaCO_3$ @ 1%, KH_2PO_4 @ 0.5% and soil @ 25% are mixed thoroughly with it. Finally the carrier is mixed with 10% water before sterilization.
- The pretreated carrier is sterilized in an autoclave at 15 lb psi for 3-4 hour continuously.
- Broth culture of *Rhizobium* containing 10^9 cells mL^{-1} is added to one-third of the water holding capacity of the carrier.

iii) Curing

In assembling inoculants, a time of "curing" (development) after expansion of broth culture to bearer enhances the nature of the item. In the wake of blending the bearer with the broth culture crude mixed transporter is kept for 24 hours for curing. Amid this time the rhizobia get acclimatized with the transporter.

iv) Packing

Subsequent to curing, the inoculant is gathered in polyethylene packs (high thickness; 0.075 - 0.09 mm) or polypropylene sacks. The pressing material ought to have the accompanying properties:

- Stable towards gamma irradiation
- Autoclavable
- High gas exchange capacity
- Should not allow high rates of moisture loss

v) Incubation and storage

Inoculants must be hatched for seven days in a room with an ambient temperature extending from 25-30^{o}C. Amid this period the bacterium increases and reaches to a required standard. The parcels may then be put away in a chilly room (4^o-15^{o}C) till its utilization

vi) Inoculant quality control

The quality of rhizobial inoculants is of awesome significance in guaranteeing field execution and additionally for the business prospects of inoculant industry. Essentially, quality means the nearness of the correct sort of smaller scale living being in dynamic frame and fancied numbers. Assessment of inoculant quality by specification of feasible rhizobia is a precise list of immunizing potential. Numerical contemplations are of such essentialness in deciding nature of inoculant items and their achievement in field that the need for quality control frameworks has been perceived in different nations. In India, Bureau of Indian Standards (BIS) (some time ago ISI) recorded the Indian standard particulars for Rhizobium inoculants in 1977 (IS: 8268-1976). This was reexamined in 1986 (ISI 1986).

Application of inoculants

The significant objective of legume inoculation is to present effective and focused strains in extensive numbers that can survive and build up in the legume rhizosphere and colonize the roots instantly [15]. Use of inoculant to the seed surface preceding sowing is the customary, most generally utilized and most easy to use methods for inoculation. There are various cements like gur, sugar, gum Arabic and methyl cellulose appropriate for joining inoculant to the seeds.

The strategy for seed inoculation incorporates readiness of 10% sugar or pharmaceutical review gum Arabic or 1% methyl cellulose arrangement. This arrangement is sprinkled on the seeds and the seeds are altogether blended to have a uniform covering. A check of 1000 suitable cells for each seed is to be achieved at the season of treating the seed and amount of culture utilized is as needs be balanced. The seeds are spread consistently to dry on a gunny sack or bond floor in shade dodging direct daylight.

Response of legumes to *Rhizobium* inoculation

Rhizobium inoculation enhances the efficiency of leguminous yield plants. The viability of Rhizobium inoculation has been built up in our nation without question by the consequences of facilitated trials directed by the Indian Council of Agricultural Research. The yield reaction differs with the inoculant strain, area and product assortment. Normal increment in yield of a portion of the beat crops because of Rhizobium inoculation [16] is shown in table 3.

Table- 3: Percent increase in yield of some leguminous crops due to *Rhizobium* inoculation

CROP	% INCREASE
Arhar	32
Mungbean	33
Chickpea	41
Groundnut	49
Lentil	50
Soybean	61

4. AZOTOBACTER

Azotobacter is a free living, heterotrophic nitrogen settling bacteria that happens in the hizosphere of assortment of plants [17]. The family Azotobacter has six species viz., A. chroococcum, A. vinelandii, A. beijerinckii, A. nigricans, A. armeniacus and A. paspali. But the last species, which is a rhizoplane bacterium, alternate individuals are to a great extent soil borne and rhizospheric. The capability of A. chroococcum and A. paspali as a biofertilizers for different non-legume crops is very much archived. Azotobacter is a high-impact, Gram negative, bar molded bacteria happens independently, in chains, or in bunches. It doesn't shape endospores yet do frame thick-walled cysts. These cysts are impervious to drying up and to some injurious synthetic and physic operators. They, nonetheless, can't withstand extraordinary temperatures. While in the cyst phase of their life cycle, they don't settle nitrogen and are optically refractile. It might be motile by peritrichous flagella or non-versatile. It can deliver a water solvent shade, yellow-green, fluorescent or red-violet/earthy dark. It develops well at an ideal temperature run in the vicinity of 20 and 30°C and at pH 7.0 - 7.5. They can develop on different sugars, alcohols, and natural acids. Fig.3 indicates Azobactor settlement and cyst.

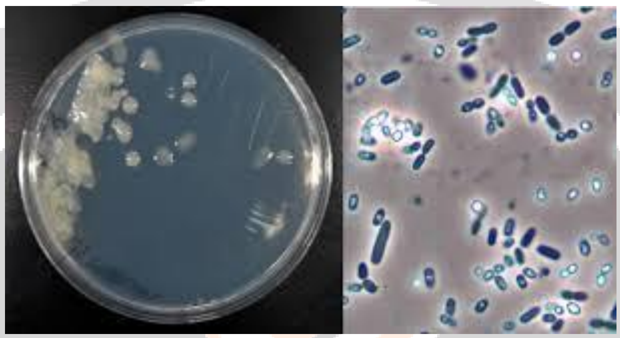

Fig -3:*Azobactor* colony and cyst

Azotobacter was first found utilizing a culture that was without a joined nitrogen source. Azotobacter is found on nonpartisan to antacid soils, in oceanic situations, in the plant rhizosphere and phyllosphere. A.chroococcum is the most widely recognized types of Azotobacter present in the dirt. Azotobacter sp. are known to impact plant development through their capacity to settle atomic nitrogen; generation of development advancing substances like IAA, gibberellin or gibberellin-like mixes and vitamins, discharge of smelling salts in the rhizosphere within the sight of root exudates; creation of against parasitic metabolites and phosphate solubilization.

Table-4: Effect of *Azotobacter* on crop yield

Crop	Increase in yield over yields obtained with chemical fertilizers (%)	Crop	Increase in yield over yields obtained with chemical fertilizers (%)
Food grains		Other	
Wheat	8-15	Potato	13
Rice	5	Carrot	16
Maize	15-20	Cauliflower	40
Sorghum	15-20	Tomato	2-24
		Cotton	7-27
		Sugarcane	9-24

The strategy taken after for mass increase of Azotobacter, readiness of bearer based inoculant and seed inoculation with transporter based Azotobacter is like that of rhizobial inoculation. Jensen's without n medium is routinely utilized for the mass augmentation of Azotobacter. Seed inoculation of A. chroococcum builds the yield of field crops by around 10 % and of oats by around 15-20%. The reaction to inoculation was expanded by manuring or by compost application. Coinoculations of Azotobacter with other bio-inoculants like Rhizobium, Azospirillum, P-solubilizers; vesicular-arbuscular mycorrhiza have been accounted for to upgrade the development and yield of legumes, oats and vegetable crops.

Useful impacts of Azotobacter chroococcum inoculation has been accounted for by different specialists on different grain, vegetables, oil seed, legume and money crops (Table 4). Inoculation explores different avenues regarding Azotobacter gave better yield execution just at lower levels of nitrogen (0 to 30 kg N ha-1). These diazotrophic bacteria require a lot of accessible carbon for their survival in soil. Expansion of barnyard fertilizer (FYM), compost and other natural corrections to horticultural soils enhances the proficiency of Azotobacter and henceforth the plant developed and yield.

5. AZOSPIRILLUM

Beijerinck in 1925 detailed a nitrogen-settling bacterium under the name Spirillum lipoferum. The capacity to fix nitrogen by specific spirilla was first recorded by him, who saw their nearness in improvement cultures of Azotobacter chroococcum [18]. Surface sanitization of roots by 70% liquor and production of miniaturized scale aerophilic (low oxygen prerequisites) conditions in the medium are the two fundamental strides for the seclusion of the living being. Azospirillum is perceived as an exceptionally universal soil creature fit for colonizing successfully not just the underlying foundations of a wide assortment of plants additionally their over the ground parcels shaping obviously a cooperative advantageous interaction. Fig.4 demonstrates the Azospirillum strain information.

Fig-4: *Azospirillum* strain data

The bacterium is Gram-negative, motile and large viroid fit as a fiddle and contains poly-β-hydroxy-butyrate granules. It is extremely motile and have a long, polar flagellum for swimming and sometimes, peritrichous flagella for swarming on surfaces. The cells change shape and size with culture age, and deliver cysts. They can become under anaerobic (NO^{3-} as acceptor of electrons, de-nitrification), small scale aerophilic (N_2 or NH_3 as nitrogen sources) and completely vigorous conditions with joined nitrogen just (NH_3, NO^{3-}, amino acids). Azospirillum species develop well on natural acids, for example, malate, succinate, lactate and pyruvate. On Rojo-Congo red medium, Azospirillum shapes unmistakable red, dry and wrinkled provinces.

Taxonomy

Azospirillum has a place with gathering 1 of the alpha subclass of the Proteobacteria. At present there are five known types of Azospirillum- A. brasilense, A. lipoferum, A. amazonense, A. halopraeferens and A. irakense.

Inoculant production

For mass augmentation of Azospirillum, the creature is permitted to develop in carafes containing NH_4Cl and malate medium and hatched at 35^o - 37^o C for 3 days. At the point when there is great development, the broth culture is blended with the bearer, and the transporter based culture is stuffed in polyethylene pockets [19]. The system utilized for arrangement of transporter based inoculant and for immunizing the seed or seedlings with Azospirlllum culture is same as that depicted before if there should be an occurrence of Rhizobium.

Crop response to *Azospirillum* inoculation

Azospirillum is broadly utilized as an inoculant for yield plants having a place with the family gramineae like wheat, sorghum, pearl millet, finger millet, grain and maize. Of the considerable number of crops tried, (Sorghum bicolor), pearl millet (Pennisetum americanum) and finger millet (Eleusine coracana) seemed, by all accounts, to be reliably receptive to Azosprillum at more than one area in India. Azospirillum species advance the yield of agronomically critical crops in a wide range of soils and climatic districts. By the utilization of this life form as a seed inoculant, funds of 20-30 kg N/ha counterparts can be accomplished in these crops. Nonetheless, the vital impacts of azospirilla go a long ways past outfitting nitrogen to host plants.

Once vaccinated onto plant roots, Azospirillum cells instigate noteworthy changes in the morphology and conduct of the whole root framework. For example, hairs near the root tip go up against a more particular appearance, and the general thickness and the length of the root framework increments). Root hairs comprise of extended root epidermal cells, which assume a part in water and supplement trades and furthermore help to stay attach to its environment. Vaccinating azospirilla onto plant roots likewise builds the breadth and length of both parallel and extrinsic roots and regularly prompts extra fanning of the sidelong roots. These advancements in the root framework are essential since they increment absorptive zone and volume of the dirt substrate accessible to the plant.

Strains of Azospirillum are known to deliver siderophores [20]. They are low atomic weight press restricting mixes integrated in extensive sums and discharged into culture medium by microorganisms under iron-inadequate conditions. Siderophores frame buildings with the metal particles in the culture medium taken after by translocation of the complex through bacterial envelope. The capacity of Azospirillum to combine siderophores may add to enhance the iron sustenance of plants and offer insurance from minor pathogens. Biosynthesis of development advancing substances like phytohormones, vitamins, antibacterial and hostile to contagious substances by Azospirillum is all around reported. The most widely revealed development promoters are IAA, gibberellins, cytokinin like-substances and vitamins.

The capacity of azospirilla to frame anti-microbial substances fluctuates from strain to strain. Fungistatic action of azospirilla against an extensive variety of phyto-pathogenic parasites has been accounted for e.g. certain azospirilla offer insurance to cotton plants against Thielaviopsis basicola and Fusarium oxysporum. These upgrading components of Azospirillum inoculation are likewise clear in field tests, with the bacteria expanding root numbers as well as enhancing yields of crops, for example, wheat, sorghum, pearl millet and maize. In field tries in Israel, Azospirillum vaccinated sorghum plants improved utilization of dampness put away in soils from winter precipitation than did uninoculated plants. In both green house and field tests, vaccinated plants are proficient at engrossing nitrogen, phosphorus, potassium and different microelements from soil than uninoculated plants.

6. ACETOBACTER DIAZOTROPHICUS

Acetobacter diazotrophicus [21] is a gram-negative, small scale oxygen consuming, nitrogen settling microorganism and was confined from washed roots and stems of sugarcane, utilizing semi-strong without n sugar medium fermented with acidic corrosive to pH 4.5. Cells of Acetobacter diazotrophicus are straight poles with adjusted closures, around 0.7 to 0.9 by 2 um, motile by sidelong or peritrichous flagella. Ideal development temperature is around 300C. Despite the fact that sucrose is the best C-hotspot for Acetobacter diazotrophicus however sugars like glucose, fructose, galactose, mannitol are likewise used. It develops well in the pH scope of 3.8 to 5.8 with great nitrogenase movement. Development and nitrogen obsession happen at sugar focus going from 10 to 30 %.

Acetobacter diazotrophicus, an endophytic diazotroph, has been discovered primarily connected with sugar-rich plants, for example, sugarcane, sweet potato, Cameroon grass, sweet sorghum and espresso. It colonizes roots, stems and leaves of host plants. Reports from Brazil shows that A. diazotrophicus contributes >50% of naturally settled nitrogen in sugarcane. Nitrogen settled by A. diazotrophicus is discharged as smelling salts into the medium. Strains of Acetobacter have been appeared to deliver significant measure of IAA. Synergistic consequences for plant development and yield taking after inoculation with Acetobacter diazotrophicus and AM growths have been accounted for sugarcane, sweet potato and sweet sorghum.

7. BLUE GREEN ALGAE

Blue-green algae (cyanobacteria) [22] are ubiquitous in distribution. They are either single celled or consist of branched or unbranched filaments. It is a group of free living organisms that has been demonstrated to be an ideal candidate as the biological nitrogen source in rice ecosystems. Some of them possess a peculiar structure known as 'heterocyst' and all heterocystous forms can fix nitrogen from air. Recently, some blue-green algae without heterocysts have also been found to fix nitrogen under special conditions like low oxygen tension. The algae that are generally used for field application are species of *Aulosira*, *Tolypothrix*, *Scytonema*, *Nostoc*, *Anabaena* and *Plectonema* as a mixture. Fig.5 shows a sample microscopic image of blue green algae.

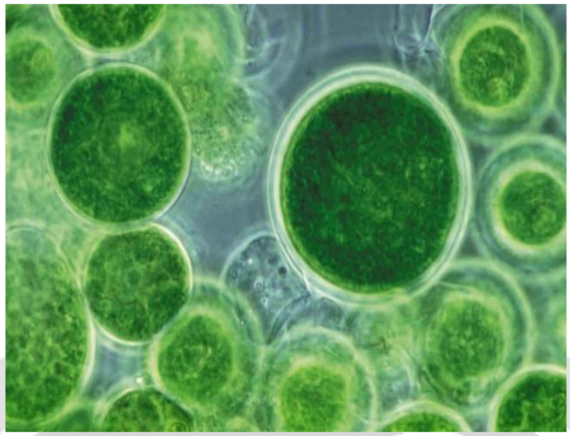

Fig -5:Blue green algae- Microscopic view

Cyanobacteria have capacity to do both photosynthesis and nitrogen obsession. Other than adding to the nitrogen economy of the dirt's these green growth have other useful impacts. Their extraordinarily great water holding limit, their capacity to think supplements, for example, nitrogen, phosphorus, settled carbon and follow components, their dirt restricting limit and their capacity to search sodium from salt influenced soils are extra biological favorable circumstances. The nearness of BGA in the quick region of rice seeds can diminish sulfide and iron damage to the plants. Cyanobacteria additionally create number of plant development substances like amino acids, little proteins and peptides, sugars, complex polysaccharides, vitamins and development hormones. Standing crops of nitrogen settling BGA run from 5-20 tons for every hectare new weight and contribute around 30kg nitrogen for every season per hectare of rice field. A main part of the natural matter delivered by algal development stays in the dirt and ends up noticeably accessible to the following yield as natural improvement.

Production of algae for field application

In view of the common biology of these algae, a straightforward country arranged outside technique for creating them in mass has been produced. The fundamental guideline is to develop them in regular daylight under conditions empowering these in the rice field. You can utilize a starter culture, comprising of soil-based blend of productive strains of BGA, provided by different rural colleges for mass augmentation.

Shallow stays (15cm x 7.5 cm x 22.5 cm) of electrifies iron sheet, or block and cement, or pits fixed with polythene sheets are readied. The size can be expanded if more material is to be delivered. Around 10kg soil is put and blended with 200 g super phosphate. The plate are then loaded with water (5-15 cm) contingent on the neighborhood conditions and rate of vanishing; the pH of the dirt ought to associate with unbiased. After soil settles down, saw-tidy and the starter culture are sprinkled on the surface of the standing water. The entire gathering is presented to daylight. In hot summer months, the development of the algae will be fast and in about seven days a thick algal tangle will be framed on the surface of the dirt and infrequently even buoys up. In the event that the day by day rate of dissipation is high, more water is included discontinuously. At the point when the algal development turns out to be adequately thick, expansion of water ought to be stopped and the water is permitted to go away in the sun. The dried algal drops are gathered from the surface and put away in packs for later use in the fields. The plates are again loaded with water and a little measure of the dry algal pieces is included, as further inoculum. The procedure is preceded as above. Once the soil in the plate is depleted (for the most part 3-4 harvests), new soil is placed, blend with super phosphate and the procedure is rehashed as some time recently. To keep the reproducing of bugs, utilization of Malathion (0.00075 ppm) or Carbofuran (3% granules) is suggested.

Algae are connected at the rate of 10kg/ha over the standing water in the field one week after transplantation. The field is kept waterlogged in any event for several days quickly after algal application.

8. PHOSPHATE SOLUBILIZING AND MOBILIZING MICROBES

Phosphorus (P) is a noteworthy supplement required for the development of plant. There are substantial stores of phosphorus in soils yet almost no sum is accessible to the plant. There are microorganisms in soil that can solubilize the inaccessible phosphorus and make it accessible to plant. They are called Phosphate solubilizing microorganisms (PSM). A gathering of parasites partners with the underlying foundations of higher plants and assemble the phosphorus from soil to the plant framework. Fig.6 indicates phosphate solubilizing and preparing microbes.

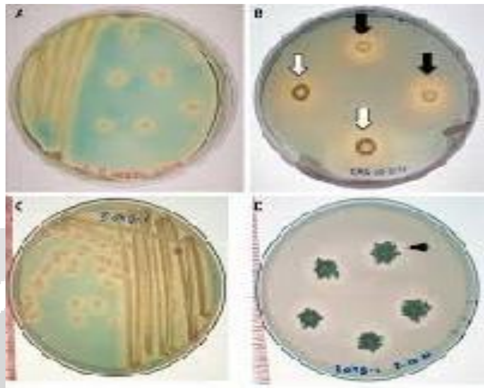

Fig-6: Phosphate solubilizing and mobilizing microbes

i) Phosphate solubilizing microorganism

The dominant parts of agricultural soils contain expansive stores of phosphorus of which an impressive part has aggregated as outcome of standard uses of P-compost. The marvel of obsession and precipitation of P in soil, which is very subject to pH, causes a low proficiency of dissolvable P manures. In acidic soils P is encouraged as Al and Fe phosphates though in calcareous soils high centralization of Calcium results in P precipitation. The dirt is an environment for differing gathering of life forms that utilize assortment of solubilization responses to discharge solvent phosphorus from insoluble phosphates. The capabilities of these phosphate solubilizing microorganisms has been acknowledged and are used as bioinoculants for harvest developed in soils poor in an ailable P and altered with shake phosphate or tricalcium phosphate.

Phosphorus solubilizing microorganisms [23] incorporate different bacterial, contagious and actinomycetes frames which help to change over insoluble inorganic phosphate into basic and solvent structures. Individuals from Pseudomonas, Micrococcus, Bacillus, Flavobacterium, Penicillum, Fusarium, Sclerotium and Aspergillus are a portion of the phosphate-solubilizing small scale life forms. They typically develop in a medium containing insoluble tri-calcium phosphate [$Ca_3(PO_4)_2$], apatite, shake phosphate, $FePO_4$ and $AIPO_4$ as sole wellspring of phosphate. The underlying disengagement of phosphate solubilizers is made by utilizing Pikovaskaya medium suspended with insoluble phosphates, for example, tri-calcium phosphate. The creation of clearing zones around the states of the creature means that the nearness of phosphate-solubilizing life forms. Such cultures are separated, distinguished and the degree of solubilization decided quantitatively. A few shake phosphate dissolving bacteria, parasites, yeast and actinomycetes were segregated from soil tests gathered from shake phosphate stores and rhizosphere soils of various leguminous crops. The most effective bacterial detaches were recognized as Pseudomonas striata, Pseudomonas rathonis and Bacillus polymyxa and contagious confines as Aspergillus awamori, Penicillium digitatum, Aspergillus niger and a yeast-Schwanniomyces occidentalis. These effective smaller scale life forms have indicated reliably their capacity to solubilize artificially settled soil phosphorus and shake phosphate from various sources. Moreover, these microorganisms were found to mineralize natural phosphorus to solvent frame because of enzymatic movement.

ii) Phosphate mobilizing microbes: Mycorrhizae

The term mycorrhizae was authored for advantageous affiliations framed by fungi with roots (Greek myces = fungus, rhizo = roots). Mycorrhizae are far reaching under characteristic conditions and happen almost in all soils from mine crown jewels to agricultural soils and in addition soil under plant or organic product crops. Over 95% of plant taxa shape mycorrhizal affiliations. The affiliation is by and large mutualistic in that the fungi acquire a carbon source from host, while the last advantages from upgraded supplement take-up through exchange from soil by means of the fungi. They are framed by most vascular plants aside from a couple of monocotyledons like cyperacea or juncacae and dicotyledons like chenopodiacea or brassicaceae. Mycorrhizea are generally separated into three morphologically unmistakable gatherings relying upon regardless of whether there is parasitic entrance of root cells: endomycorrhiza, ectomycorrhiza and ectoendomycorrhiza. Of the three gatherings, endomycorrhizae are essential as biofertilizer [24]. Fig.7 indicates Mycorrhizae fungi.

Fig-7: Mycorrhizae fungi

Endomycorrhizae are shaped by almost 90% of the land plants. In this affiliation the fungi shape outside hyphal organizes in the dirt and develop broadly inside the cells of the root cortex. This system of parasitic hyphae inside the root cortex is known as hartig net. Fungi having a place with basidiomycetes, ascomycetes or zygomycetes are included relying upon the kind of endomycorrhizal affiliation. Particular sorts of endomycorrhizae are shaped by individuals from the Ericaceae (Ericoid mycorrhizae) and orchidaceae (orchidaceous mycorrhizae), however the kind of mycorrhizae which is across the board is the arbuscular mycorrhizae (prior alluded as vesiculararbuscular mycorrhizae). It is shaped by 120 types of zygomycetes, all having a place with the request Glomales (Glomus, Acaulospora, Gigaspora, Sclerocystis, Entrophospora and Scutellospora). None of these fungi has yet been effectively cultured axenically.

iii) Plant Growth Promoting Rhizobacteria (PGPR)

The earth, or the volume of soil that is impacted organically and biochemically by living root, is known as rhizosphere. Root exudates and emissions make a rhizosphere impact that shows itself in the serious microbial movement that is related inside the prompt region of the root. Root related bacteria, additionally called rhizobacteria, can be advantageous, unbiased or injurious to the development of the plant. Plant Growth Promoting rhizobacteria (PGPR) are one class of useful bacteria possessing the dirt biological system [25]. The impacts of PGPR on plant development can be interceded by immediate or circuitous components [26]. The immediate impacts have been most generally credited to the creation of plant hormones, for example, auxins, gibberellins and cytokinins, or by providing organically settled nitrogen or solubilizing insoluble P. These PGPR additionally influence development by backhanded instruments, for example, concealment of bacterial, parasitic and nematode pathogens by the creation of siderophores, HCN, smelling salts, anti-toxins, unstable metabolites and so forth. By instigated systemic resistance and additionally by contending with the pathogen for supplements or for colonization space.

9. DISCUSSIONS

The proficient cultures have demonstrated ability to solubilize insoluble inorganic phosphate, for example, shake phosphate, tri-calcium phosphate, and iron and aluminum phosphates by generation of natural acids. They can likewise mineralize natural phosphatic mixes exhibit in natural compost and soils. Inoculation of PSM to seeds or seedlings builds the grain yield of crops. They are known to include 30-35kg P_2O_5 ha-1. The inorganic phosphate solubilization by microbes can be credited to fermentation, chelation, and trade response in development medium and additionally to the proton exchange amid ammonium osmosis.

The impact of mycorrhizae in expanding plant development has been very much recorded by various specialists for some plants. The valuable impact of mycorrhizae on plant development has for the most part been credited to an expansion in the take-up of supplements, particularly phosphorus. Mycorrhizal fungi enhance the dirt phosphorus accessibility by solubilizing inorganic types of phosphorus or by mineralization of natural phosphorus. Outer hyphae of mycorrhiza additionally has the ability to take up and convey different supplements to plants like NH^{4+}, NO^{3-},K, Ca, SO_4^{2-}, Cu, Zn and Fe. In trial chambers, the outside hyphae of AM can convey upto 80% of plant P, 25 % of plant N, 10% of plant K, 25% of plant Zn and 60% of plant Cu. Mycorrhiza additionally deliver ectoenzymes which furnish host plant with the possibility to get to natural N and P frames that are typically inaccessible to AM fungi or to non mycorrhizal roots.

The nitrogen settling and phosphate solubilising bacteria have been talked about independently. Other PGPR incorporate bacteria having a place with the genera Arthrobacter, Bacillus, Burkholderia, Enterobacter Klebsiella, Pseudomonas, Xanthomonas, Serratia and numerous all the more yet to be recognized. Impacts of PGPR on plant development have been assessed by numerous laborers on various crops. Increment in plant tallness and root and shoot biomass of wheat was accounted for taking after inoculation with 12 diverse segregates of PGPR having a place with Pseudomonas aeruginosa, P. cepacia, P. fluorescens and P. putida. Additionally treatment of wheat seeds with fluorescent pseudomonads (hostile to Gaeumannomyces graminis) brought about yield increments of 27% in field trials. PGPR are powerful inoculants yet are not marketed because of absence of consistency under field conditions. Late work proposes that blend of PGPR strains (at least two) which have separate method of plant development advancement or opposition against soil-borne pathogens are more powerful than single strain inoculum. Numerous bacterial genera have demonstrated their potential for bio-control both under in vivo and in vitro conditions. Agrobacterium, Arthrobacter, Alcaligenes, Bacillus, Escherichia coli, Enterobacter, Pseudomonas, Burkholderia, Rhizobium and Serratia were observed to be intense for concealment of soil-borne contagious pathogens. A large number of these bio-control operators displayed their viability under field conditions moreover.

10. CONCLUSION

This article aimed at providing a basic knowledge about the beneficial activities of microbes in agriculture and to a greater extend has succeeded in providing a clear understanding of beneficial microbes. The study of beneficial activities of microbes let the farmers and other agriculture related professionals in differentiating the microbes and employing them for the better yield in the commercial front and reducing the environmental hazards. Thus this article can be taken as a smaller step towards the research in microbial activities for the betterment of human resources.

11. REFERENCES

[1]. Pepper, I. L. (2000). Beneficial and pathogenic microbes in agriculture. Environmental Microbiology, 425-446.
[2]. Bisen, P. S., Debnath, M., & Prasad, G. B. (2012). Microbes in agriculture. Microbes: Concepts and Applications, 481-566.
[3]. Gallitelli, D. (2000). The ecology of Cucumber mosaic virus and sustainable agriculture. Virus Research, 71(1), 9-21.
[4]. Dethlefsen, L., McFall-Ngai, M., & Relman, D. A. (2007). An ecological and evolutionary perspective on human–microbe mutualism and disease. Nature, 449(7164), 811-818.
[5]. Schaad, N. W., Jones, J. B., & Chun, W. (2001). Laboratory guide for the identification of plant pathogenic bacteria (No. Ed. 3). American Phytopathological Society (APS Press).
[6]. Asselmeier, M. A., Caspari, R. B., & Bottenfield, S. (1993). A review of allograft processing and sterilization techniques and their role in transmission of the human immunodeficiency virus. The American journal of sports medicine, 21(2), 170-175.
[7]. Rao, N. S. S. (1982). Biofertilizers in agriculture. AA Balkema.
[8]. Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant and soil, 255(2), 571-586.
[9]. Mohammadi, K., & Sohrabi, Y. (2012). Bacterial biofertilizers for sustainable crop production: a review. ARPN J Agric Biol Sci, 7(5), 307-316.
[10]. Franche, C., Lindström, K., & Elmerich, C. (2009). Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant and soil, 321(1-2), 35-59.

[11]. Vojvodic, A., Medford, A. J., Studt, F., Abild-Pedersen, F., Khan, T. S., Bligaard, T., & Nørskov, J. K. (2014). Exploring the limits: A low-pressure, low-temperature Haber–Bosch process. Chemical Physics Letters, 598, 108-112.

[12]. Cannon, F. C. (1980). Genetic studies with diazotrophs. Genetic studies with diazotrophs., 367-413.

[13]. Duong, T. P., Diep, C. N., Khiem, N. T., Hiep, N. H., Toi, N., Lich, N., & Nhan, T. K. (1984). Rhizobium inoculant for soybean [Glycine max (L.) Merrill] in Mekong Delta. Plant and soil, 79(2), 235-240.

[14]. Mostasso, L., Mostasso, F. L., Dias, B. G., Vargas, M. A., & Hungria, M. (2002). Selection of bean (Phaseolus vulgaris L.) rhizobial strains for the Brazilian Cerrados. Field Crops Research, 73(2), 121-132.

[15]. Smith, R. S. (1992). Legume inoculant formulation and application. Canadian Journal of Microbiology, 38(6), 485-492.

[16]. Reddy, A. A. (2009). Pulses production technology: Status and way forward. Economic and Political weekly, 73-80.

[17]. Bagyaraj, D. J., & Menge, J. A. (1978). Interaction between a VA mycorrhiza and Azotobacter and their effects on rhizosphere microflora and plant growth. New Phytologist, 80(3), 567-573.

[18]. Okon, Y., & Labandera-Gonzalez, C. A. (1994). Agronomic applications of Azospirillum: an evaluation of 20 years worldwide field inoculation. Soil Biology and Biochemistry, 26(12), 1591-1601.

[19]. Okon, Y. (1985). Azospirillum as a potential inoculant for agriculture. Trends in Biotechnology, 3(9), 223-228.

[20]. Plazinski, J., & Rolfe, B. G. (1985). Influence of Azospirillum strains on the nodulation of clovers by Rhizobium strains. Applied and environmental microbiology, 49(4), 984-989.

[21]. James, E. K., Reis, V. M., Olivares, F. L., Baldani, J. I., & Döbereiner, J. (1994). Infection of sugar cane by the nitrogen-fixing bacterium Acetobacter diazotrophicus. Journal of experimental botany, 45(6), 757-766.

[22]. Krogmann, D. W. (1977). Blue-green algae. In Photosynthesis I (pp. 625-636). Springer Berlin Heidelberg.

[23]. Khan, M. S., Zaidi, A., & Wani, P. A. (2007). Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. Agronomy for Sustainable Development, 27(1), 29-43.

[24]. Richardson, A. E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Functional Plant Biology, 28(9), 897-906.

[25]. Kloepper, J. W., Lifshitz, R., & Zablotowicz, R. M. (1989). Free-living bacterial inocula for enhancing crop productivity. Trends in biotechnology, 7(2), 39-44.

[26]. Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology, 41(2), 109-117.