

## Liquid Microbial Consortium- A Potential Tool for Sustainable Soil Health

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### Abstract

The green revolution brought amazing consequences in food grain production but with insufficient concern for agricultural sustainability. The availability and affordability of fossil fuel based chemical fertilizers at farm level in India have been ensured only through imports and subsidies which are largely dependent on GDP of the country. Dependence on chemicals for future agricultural needs would result in further loss in soil health, possibilities of water contamination and calculated burden on the fiscal system. Indiscriminate synthetic fertilizer usage has polluted the soil, water basins, destroyed micro-organisms and eco-friendly insects, made the crop more susceptible to diseases and depleted soil fertility at the primary levels as of today, which is the main concern of the write up. In this critical context Microorganisms have been emerged as the potential alternative for the productivity, reliability and sustainability of the global food chain. Carrier based biofertilizers has already proved to be the best over the agro chemicals and have been showing the tremendous effect on the global agriculture productivity since the past two decades. Rectifying the disadvantages of the carrier based biofertilizers, liquid biofertilizers have been developed which would be the only alternative for the cost effective sustainable agriculture. The article focuses on Liquid Biofertilizer Technology providing reliable reasons for their necessity, specificity and emphasizes that "Use of agriculturally important microorganisms in different combinations i.e. Liquid microbial consortium (LMC) is the only solution for restoration of soil health".

Even though biofertilizers are being produced and distributed constantly by private agencies, NGO's, State and Central Government production units for the last three decades, their corresponding usage is not in the satisfactory proportions. To cope with the rising demands for food commodities, serious efforts are being made by the State and Central Governments (under the National Projects) for the sufficient agricultural production by popularizing biofertilizers and making them available to the farmer community. In spite of these efforts, the rate of consumption of biofertilizers is not to the optimum level in comparison with the agrochemicals. The reason attributed is the "non-availability of good and suitable carrier materials" that raises contamination problems and shorter shelf life. To cope with this alarming situation, Liquid formulations (LFs) are being developed that ensure more quality over the conventional carrier based biofertilizers inaugurating a new era in the Biological input technology. These liquid formulations facilitate long shelf life (up to 2 years), minimum contamination, carrier free activity, handling comfort, storage and transport convenience, easy quality control, enhanced export potentials and are preferred by the farmer community as well as manufacturers.

**Keywords:** Alternative agriculture; Liquid microbial consortium; Plants nutrients; Viable microbial population

### Introduction

#### Why to explore bio-fertilizers?

India is an agricultural based country. In order to feed the ever growing populations, India has to increase the per unit area productivity. According to United Nations Food and Agriculture Organization (FAO) estimations, the average demand for the agricultural commodities will be 60% higher in 2030 than present time and more than 85% of this additional demand will be from developing countries [1]. For over half a century, the world has relied on the concept of increasing crop yields to supply an ever increasing demand of food. Therefore, vertical expansion of food production is necessary. In order to increase the unit area productivity of agricultural land, the role of different crop nutrients in contributing increased crop yield is vital. Among the crop nutrient, nitrogen as well as phosphorus play an important role in increasing the crop productivity. Further, the nitrogenous chemical fertilizers are manufactured industrially using nonrenewable petroleum products under high temperature and high pressure. Increase in petroleum cost day by day effects the cost of the chemical fertilizers. In addition, more than 50% of the applied N-fertilizers are somehow lost through different agricultural processes which not only lead to economical loss to the farmers and polluted environment consequently.

Feedstock/fossil fuels depletion and increasing fertilizer cost are making marginal farmer unaffordable. Growing concern about environmental hazards, increasing threat to sustainable agriculture are some of the other reliable reasons for the biofertilizer promotion. However, plant nutrients like N, P and K are highly essential for plant growth and Metabolism. It is also evident that plants utilizes nutrients in greater amounts from soil in modern intensive cultivation and needs replenishment. Under such conditions Micro organisms offer good alternative technology to replenish crop nutrients.

In agricultural eco-system, microorganisms have vital role in fixing/solubilizing/mobilizing/nutrient recycling. These microorganisms occur in soils naturally, but their populations are often scanty. In order to increase the crop yield, the desired microbes from rhizosphere are isolated and artificially cultured in adequate count and mixed with suitable carriers or as they are in suitable combinations (Microbial

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consortium) by artificial culturing. These are known as biofertilizers or microbial inoculants.

### Estimated demand and supply of biofertilizers

India is one of the important countries in biofertilizer production and consumption. In order to encourage the organic agriculture by biofertilizers, five biofertilizers namely *Rhizobium*, *Azotobacter*, *Azospirillum*, Phosphate solubilizing bacteria and *mycorrhiza* have been incorporated in the FCO, 1985 [2]. The average consumption in the country is about 45,000 tons per annum while the production being less than the half. At present in India there is a gap of about 10 million tones of plant nutrients between removal by crops and replenishment through fertilizers. It is evident that there is a tremendous gap between the annual demand and production of the biofertilizers globally especially in India. Hence, the judicious combination of chemical fertilizers and biofertilizers is also encouraged considering economical and ecological concerns. It is estimated that the present level of biofertilizer use is quite low and there is a substantial potential to increase it to 50,000-60,000 tons by 2020. The maximum production capacity is in Agro Industries Corporation followed by State Agriculture Departments, National Biofertilizers Development Centers, State Agricultural universities and private sectors.

### LBF Vs CBBF

Liquid biofertilizer formulation is the promising and updated technology of the conventional carrier based production technology which inspite of many advantages over the agrochemicals, left a considerable dispute among the farmer community in terms of several reasons major being the viability of the organism. Shelf life is the first and foremost problem of the carrier based biofertilizers which is up to 3 months and it does not retain throughout the crop cycle, LBF on the other hand facilitates the long survival of the organism by providing the suitable medium which is sufficient for the entire crop cycle. Carrier based bio fertilizers are not so tolerant to the temperature which is mostly unpredictable and uncertain in the crop fields while temperature tolerance is the other advantage of the liquid biofertilizers. The range of possible contamination is very high as bulk sterilization does not provide the desirable results in the case of CBBF, where as the contamination can be controlled constructively by means of proper sterilization techniques and maintenance of intensive hygiene conditions by appropriate quality control measures in the case of LBF. Moisture retaining capacity of the CBBF is very low which does not allow the organism viable for longer period and the LBF facilitates the enhanced viability of the organism [3].

However, the administration of LBF in the fields is comparatively easier than CBBF. The other disadvantages of CBBF like poor cell protection, labor intensity, and dosage controversy, limited scope of export, expensive package and transport, very slow adaptation by the farmer community are some of the strongest problems which are being solved by the Liquid biofertilizers very effectively. Therefore, LBF are believed to be the best alternative for the conventional carrier based biofertilizers in the modern agriculture research community witnessing the enhanced crop yields, regaining soil health and sustainable global food production.

### The concept of Liquid biofertilizers

“A preparation comprising requirements to preserve organisms and deliver them to the target regions to improve their biological activity (or) A consortium of microorganisms provided with suitable

medium to keep up their viability for certain period which aids in enhancing the biological activity of the target site”.

Liquid formulation is a budding technology in India and has very specific characteristics and uniqueness in its production methods. Liquid biofertilizers are the microbial preparations containing specific beneficial microorganisms which are capable of fixing or solubilizing or mobilizing plant nutrients by their biological activity [2].

They are broadly classified into three groups.

1. Nitrogen Fixing Microbes (NFM)
2. Phosphorus Solubilizing Microbes (PSM) and Phosphate Mobilizing Microbes (VAM) and
3. Potash Mobilizing Microbes (*Frateuria aurentia*)

Among the three groups Phosphorus solubilizing microbes' usage is to the larger extent in the current agro market. The commercial production of Potash mobilizing bacteria has been developed recently. The authors presently concentrate on solubilization or mobilization of Micronutrients by exploiting the biological activity of microbes like

1. Zinc and Sulphur Solubilizing Bacteria (*Thiobacillus* spp)
2. Manganese solubilizer (*Pencilium citrinum*)

The biggest challenge of the biofertilizer production units is the viability of organisms up to field application. Some of the beneficial organisms are very effective *in vitro*, but may fail at some stage of cropping, even at the stage of harvesting. The common and potential causes of this demise are poor stability of the biofertilizer (by means of the viability) which may not be constant from prior storage to field application, very low potential ingredients reaching the active site of the plant practically and rapid degradation of the fertilizer by means of acclimatization problems. Liquid formulations of the beneficial organisms (LBFs) or bioinoculants are the promising inputs which can effectively combat the present agro adverse Scenario, resolving the low viability of the organisms, maximizing the efficacy of the inputs and satisfying the concept of cost-effectiveness.

### Types of Inoculums

Basically Liquid biofertilizers have been classified into, 1) Dry products which include dusts, granules and briquettes and, 2) Suspensions containing oil or water-based and emulsions. A wider range of formulations with additives are also available in the global agri market.

### Dry inoculum products

These formulations comprise inoculums in semi dry condition. This includes dusts, granules and briquettes, based on particle or aggregate size. This group also comprises wettable powders formulated in dry powder. These powders require liquid or water as carrier just before the application to activate the inoculums. Dusts usually ranges from 5-20 mm in size and require low absorbance inert diluents as carriers. Low dusts particles, i.e. less than 10 mm size adhere best on the target site but are hazardous when inhaled. About 30% of the dry weight of dusts contains organisms as suspension. They are normally prepared by feeding the organism into an air stream for mixing with a blender. Particle size, bulk density and flow ability are extremely important.

### Granules, pellets, capsules and briquettes inoculum

These include masses of different sizes which are usually called as

Granules, pellets, capsules and briquettes. Granules are discrete masses 5-10 mm<sup>3</sup> size, pellets are of size >10mm<sup>3</sup>, and briquettes are large blocks up to several cubic centimeters; just like dusts, these products also contain inert carriers such as clay minerals, starch polymers and ground plant residues holding the organisms. Absorption (more important for formulating slurries of organisms), hardness, bulk density and product disburse rate in water etc., are the major factors taken into consideration in selecting a suitable carrier. Soft carriers e.g. Bentonite, disburse quickly to release the organism. Various materials are used to coat the product to slow down or control the rate of release, which also varies with the unit size. The usual concentration of organisms in granules is 20- 30%. There are three types of granular formulations in general: 1) the organisms attached to the outer surface of a granular carrier by a sticker in a rotating drum. 2) The organisms incorporated into a paste or powdered carrier which sets as a matrix size being controlled by passing the product through a sieve. 3) The organisms sprayed onto a rotating granular carrier without a sticker. When the carrier forms a protective coat around a core aggregate of organisms, the unit is termed as a capsule.

### Wettable powder inoculums

These formulations comprise charcoal, lignite, vermiculite powders which are predominated among all commercial products. These are blended with 3% gum to make them stable on the shelf during storage and stick readily to the seeds.

### The issue of formulations

Liquid formulations are sprayable propagules of the microbial agents suspended in suitable liquid medium (or) A consortium of microorganisms provided with suitable medium to keep up their viability for certain period which aids in enhancing the biological activity of the target site.

Basic characteristics of formulation

1. To stabilize the organism during production distribution and storage.
2. To deliver easily to the field in the most appropriate time.
3. To protect the microorganism from adverse environmental factors at the target site (field), thereby increasing persistence.
4. To enhance activity of the organism at the target site by increasing its viability, reproduction, contact and interaction with the target crops.

Although, India is considered to be the largest biofertilizers producer in the world, the creditability of biofertilizers is not up to the mark among farmer community. This is an alternative technology developed in the recent times resolving the adverse effects of agrochemicals and enhancing the activity of carrier based biofertilizers and biocontrol agents. This is not the usual broth culture from the fermenter or a water suspension of the carrier based biofertilizers.

Liquid biofertilizers are special liquid formulation containing not only the desired beneficial microorganisms and their biological secretions, but also special cell Protostans or substances that encourage the formation of dormant spores or cysts for longer shelf life and tolerance to adverse conditions.

### Dormant aqueous suspensions

Dormant technology is still being used by several current commercial producers. Generally, they are using growth suppressants,

contaminants suppressant like sodium azide, sodium benzoate, butanol, acetone, fungicides, and insecticides etc. for the long term viability. The *Rhizobium* dormant liquid formulations when used after 8 months reduce the size of nodules and effect nitrogen fixation process. This is due to the need of long duration of activation time. In case of *Azospirillum*, *Azotobacter*, PSM, it is observed that these bacteria crossed extreme dormant stage. Therefore, when applied in crops they take prolonged reactivation time. This long time is not desirable for short duration crops. Resolving this disadvantage a new technique was adopted in liquid formulations process involving the arrest of bacteria without preservatives.

### Dormant oil suspension

Oil serves as the perfect medium to supply the inoculants in viable condition. Microorganisms can be suspended in oil at high concentration in various degrees of dehydration and remain viable. This formulation delivers organisms in a physiologically dormant state and does not encourage the growth of contaminants during storage. The bacteria/fungi have been successfully dried by continuous aeration as a suspension in oil to provide inoculants with shelf lives of several years. Viscosity roughly equals the setting rate of the particles. This is achieved by the use of colloidal clays, polysaccharide gums, starch, cellulose or synthetic polymers.

### Factors Affecting LBF

#### Temperature

Temperature is important for the shelf life of microbial products and it can affect their activity pre or post microbial application. Temperature optima and limits vary with the microorganism. Colonization proceeds at field temperatures in the cropping season, but slows at lower temperature. Strains used in liquid formulation normally grow at 37°C [2] and able to tolerate temperature up to 45°C for two year or more. Whereas, solid base shelf life is hardly up to 3 months as rise in temperature beyond 35°C and start rapid decline of organisms.

#### Acclimatization

Effect of environment on bio fertilizers has been studied by many workers. It is reported that it has negative effect on different strains, but all the research was done on the carrier base biofertilizers. It is observed that the efficiency of liquid is almost same in all environments, but efficiency may reduce 20-25% in different climatic conditions in case of solid base. Normally in RCOF liquid formulation of an organism remain active for a long time after application, ideally throughout the period of the crop (2 years to the maximum), or in soil throughout the crop cycle. Temperature humidity, leaf surface exudates and competitors, etc are the critical factors which inactivates the microbes. They may be lost physically from the target location by the action of wind, rain or leaching depending on the fact why and where the product is used.

#### Effect of humectants

The effect of moisture content on the storage, stability and activity is up to the greater extent. Some organisms may need moisture for their activity. In case of Carrier based inoculums the organism gets stressed due to number of reasons when carrier become dry during transport and storage. Bacteria needs wet plant surface in order to establish them and show its activity. These conditions can be fulfilled only by liquid formulation as they contain humectants. The level of direct effect of humidity on spore forming bacteria in liquid forms is relatively very low.

### Sunlight intensity

Generally Microbes are sensitive to temperature. The most harmful rays UVB (280-320 nm) and UVA (320-400 nm) reaching the earth's surface have direct effect on microbes. Of course some are less damaging where as some have the maximum effects. However, microbes are sensitive to the wave-lengths outside this range. To counter the harmful effects of high temperature sunscreens are added to a formulation. Sunscreens act by reflecting and scattering physically or by absorbing radiation selectively, converting short wavelengths to harmless longer ones. However, no such types of sunscreens are available in solid base to resist the effect of sunlight.

### Effect of pH

The pH of a product plays a vital role in liquid inoculum preparation. It must be stabilized within certain ranges. The organisms are inactivated at the extreme high and low temperatures; therefore, a buffer is maintained by adding some additives which render the better shelf life in liquid. Maintenance of optimal pH improves shelf-life of some of microorganisms like *Azospirillum*, *Azotobacter*, Phosphorus Solubilizing bacteria (PSM), Potash Mobilizing Bacteria (KMB), *Frateuria aurentia* [2].

### Stabilization of liquid inoculums

The longest period in the life of a product elapses during storage, i.e. the time between manufacture and eventual use in the field. This period can range from several weeks to years. Organisms are required to remain viable during storage, with minimum loss of potency/ activity and without loss or breakdown of the desired formulation properties. The shelf life of liquid inoculum Vs. carrier based inoculum has been shown in Table 1. The liquid formulations are added with suitable additives for improving stability which promote growth throughout the plant lifecycle by maintaining proper storage conditions prior to application by appropriate processing after production. Stability of the product is improved by resolving various physical and contamination problems like adhesion to the target site, activation time and the purity of the mother culture used for the subsequent preparation of the product respectively.

### Handling and Application of Liquid Inoculums

Liquid inoculums ensure the facility of easy handling and application. This is exemplified by the addition of thickeners or suspenders to suspensions which aids in maintaining even distribution of the organism over the plant. Liquid prevents the clumping of the organism and ensure its ready resuspension after prolonged storage, where as uniformity is not maintained by dusts and other wetttable

powders. It is evident that the population count come down to 105 in the duration of six months at room temperature in the case of *Azospirillum* while it survives in liquid up to 2 years and maintains population up to 108/ml. Similarly the shelf life of *Azotobacter*, KMB and *Rhizobium* in solid state is up to 6 months and PSM can survive up to 8 months but liquid formulations of *Azotobacter*, PSM and KMB is up to 2 years followed by *Rhizobium* for 14 months.

### Typical characteristics of different Liquid biofertilizers

#### *Rhizobium*

*Rhizobium* is the classical example of symbiotic nitrogen fixing bacteria. The bacteria intact the legume root and form root nodules (nodular symbiosis) within which molecular nitrogen is reduced to ammonia that is readily utilized by the plant to produce valuable proteins, vitamins and other nitrogen containing compounds. It has been estimated that 40-250 kg N/ha/year could be fixed by the microbial activities of *Rhizobium* for various legume crops. When the plants die the fixed N<sub>2</sub> is released, making it available to other plants and this helps in fertilizing the soil. Majority of the legumes have this type of association, except a few genera (e.g., *Styphnolobium*) [1].The density of nodules occupied, dry weight of nodules, dry weight of plant and the grain yield per plant influenced by the multi strain inoculants was highly promising.

However, it is evident from the research that Liquid biofertilizers especially liquid *rhizobia* with 2% PVP were found to maintain higher CFU count and was very effective for enhancing the soya bean yield particularly in US (Hawaii Under NifTal), Brazil, Europe (e.g., Spain, Argentina) and Asia (China, Vietnam) [4].

#### Physical features of Liquid *Rhizobium*:

1. Dull white in colour
2. No bad smell
3. No foam formation, pH 6.8 to 7.5

**Use of fungicides/pesticides/herbicides with Liquid *Rhizobium* inoculum:** The effect of chemicals on nodulation and nitrogen fixing *Rhizobium* is negligible. Seed dressing fungicides such as Thiram and mercury have some adverse effect on the viability of *Rhizobium*, which can be overcome by doubling the doze of *Rhizobium* culture. Studies suggest that *Rhizobium* strain MRL3 may be exploited as a bioinoculant to augment the efficiency of lentil exposed to insecticide-stressed soils. [5]. On the other hand fungicides such as Dithane M-45 and Bevistin do not have any adverse effect on *Rhizobium*. The minimum tolerance doses of five different fungicides, Corbandazium, Dithane M-45,

Inoculum	Months											
	0	2	4	6	8	10	12	14	16	18	20	24
Azosp (L)	10.0	10.0	10.0	10.0	9.0	9.0	9.0	9.0	8.0	8.0	8.0	8.0
Azosp (S)	9.0	8.0	7.0	5.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Azoto (L)	9.0	9.0	9.0	9.0	9.0	8.0	8.0	8.0	8.0	8.0	7.0	7.0
Azoto (S)	8.0	8.0	7.0	6.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
P.S.M (L)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
P.S.M (S)	9.0	8.0	8.0	8.0	7.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil
K.M.B (L)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	8.0	8.0	8.0	8.0	8.0
K.M.B (S)	8.0	8.0	8.0	7.0	5.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Rhizo (L)	9.0	9.0	9.0	9.0	9.0	9.0	8.0	7.0	5.0	Nil	Nil	Nil
Rhizo (S)	9.0	8.0	8.0	7.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

**Table 1:** Liquid Inoculum Vs Carrier based Inoculum -Shelf life; Source: Chandra et. al. 2004, Biofertilizer vision 2004; L-Liquid (Liquid formulation without preservative was used); S-Solid (lignite carrier mesh size 150 micron, pre-sterilized in autoclave used).

Plant	N <sub>2</sub> fixed mg/g of substrate
<i>Sorghum bicolor</i> (Sorghum)	20
<i>Zea mays</i> (Maize)	20
<i>Panicum sp.</i>	24
<i>Cynodon dactylon</i>	36
<i>Setaria sp.</i>	12
<i>Amaranthus spinosa</i>	16
<i>Oryza sativa</i> (Paddy)	28

**Table 2:** Occurrence of *Azospirillum* and their nitrogen fixing capacity in different Plants.

Dithane Z-78, Topsin and Ceresin were tested for *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* locally isolated from *Glycine max* and *Pisum sativum*. Amongst these fungicides, ceresin was found to be more toxic to *Bradyrhizobium japonicum* and *Rhizobium leguminosarum*, since the minimum tolerance dilution is 107, whereas minimum dilution of Dithane Z-78 for soybean is 103; 104 for pea; Dithane M-45 for soybean is 104; pea is 105; Topsin for pea is 106; soybean is 107 and Corbandazium for pea and soybean being 106.

**Use of chemical fertilizer along with liquid *Rhizobium*:** Nitrogen is essential for the crops of the Fabaceae family. Pulses and legume oil seed crops require 30-40 kg of nitrogen and 40 kg phosphorus per hectare on an average for better growth and yield. Basal dose (5-10 kg) of nitrogen is not sufficient for the crop as nitrogen is essential to fulfill the initial requirement of crop up to 20-25 days, i.e. till the *Rhizobium* nodules start their functioning. Phosphorus is essential for better *Rhizobium* inoculation, so 30-40 kg of phosphorus (chemical) /ha is required as basal dose, if PSM is used the dose of chemical Phosphorus can be 15-20 Kg/ha. As potassium is also essential for better *Rhizobium* inoculation the amount of chemical fertilizer can be 10-15 Kg/ha instead of normal 30-40 kg/ha if KMB is used in appropriate combination.

**Use of sticky materials for seed treatment:** Generally sticky materials are used for the easy adhesion of the inoculums onto the target plant parts and subsequently increase bacterial population on the seeds for maximum nitrogen fixation. The germination and nodulation tests were performed on the pelleted seeds and results showed that although the sugar and molasses initially bound less inoculant and lime to the seed, the number of surviving *rhizobia* was similar to that obtained by the *Gum arabic* treatment after storage at 27.50°C for five days for fast growing *rhizobia*. The inoculated pelleted seeds of slow growing *rhizobia* showed promising results in germination and nodulation in acidic soil.

### ***Azospirillum***

*Azospirillum* belongs to bacteria and is known to fix considerable quantity of nitrogen in range of 20-40 kg N/ha in the rhizosphere in non-leguminous plants such as cereals, millets, oilseeds, cotton etc. *Azospirillum* is considered as the efficient biofertilizers because of its ability of inducing abundant roots in several plants like rice, millets and oilseeds even in upland conditions. An estimated amount of 25-30% chemical nitrogen fertilizer can be saved by the appropriate use of *Azospirillum* inoculants. The genus *Azospirillum* has three species viz. *A. lipoferum*, *A. brasilense* and *A. amazonense*. These species have been commercially exploited for the use of nitrogen supplying biofertilizer. One of the characteristics of *Azospirillum* is its ability to reduce nitrate and denitrify. Table 2 presents occurrence of *Azospirillum* and their nitrogen fixing capacity in different Plants. Both the species *A. lipoferum* and *A. brasilense* may comprise strains which may denitrify actively or weakly or reduce nitrate to nitrite. Therefore, for inoculation preparation, it would be necessary to select strains which

do not possess these characteristics. *Azospirillum lipoferum* present in the roots of some tropical forage grasses such as *Digitaria*, *Panicum*, *Brachiaria*, *Maize*, *Sorghum*, *Wheat* and *Rye*.

**Physical features of liquid *Azospirillum*:** The color of the liquid may be blue or dull white. Bad odors confirm improper liquid may be broth. Production of yellow gummy colour materials confirms the quality product. Acidic pH always confirms no *Azospirillum* bacteria present in liquid.

**Production of growth hormones:** *Azospirillum* cultures synthesize considerable amount of biologically active substances like vitamins, nicotinic acid, indole acetic acid, gibberellins. All these hormones/chemicals help plants in better germination, early emergence, and better root development.

### **Field applications of Liquid *Azospirillum*:**

- Stimulates growth and produces green color characteristics of a healthy plant.
- Aids utilization of potash, phosphorus and other nutrients
- Encourages plumpness and succulence of fruits and grains and increases protein percentage.

Inoculants of *Azospirillum* have been proved beneficial in a variety of crops including cereals, forage and other crops. The crop response however, varies greatly with types of crop and variety, location, season, soil fertility level; native microorganism interaction etc., the ability to fix atmospheric nitrogen and to produce photo hormones determines the crop response and yield. A series of experiments have been conducted on a range of crop varieties to evaluate the crop response to *Azospirillum* inoculants and a wide variation in production up to 40% have been observed. Nonfunctioning of *Azospirillum* in the fields can be identified by yellowish green color of leaves which indicates very poor or no nitrogen fixation which in turn results in arresting the growth promotion.

### ***Azotobacter***

*Azotobacter* belongs to the genus diazotrophic bacteria which is a free-living organism whose resting stage is a cyst. It is abundantly found in neutral to alkaline soils, in aquatic environments, and on some plants. *Azotobacter* is capable of performing several metabolic activities, including atmospheric nitrogen fixation by conversion to ammonia. *Azotobacter* spp. has the highest metabolic rate of any organisms. It serves as potential biofertilizer for all non-leguminous plants especially rice, cotton, vegetables etc. *Azotobacter* population is high in rhizosphere region and the rhizoplane does not contain *Azotobacter* cells. The lack of organic matter in the soil is a limiting factor in the proliferation of *Azotobacter* in the soil.

### **Liquid *Azotobacter* in tissue culture:**

1. The performance of *Azotobacter* liquid inoculants was comparatively better than all the treatments in 10% MS medium followed by *Azospirillum*.
2. The performance of *Azotobacter* liquid inoculants was comparatively better than all the treatments followed by *Azospirillum* for the growth of polybag sugarcane seedlings.

**Liquid *Azotobacter* as biocontrol agent:** *Azotobacter* have been found to produce some effective antifungal substance which inhibits the growth of some soil fungi like *Aspergillus*, *Fusarium*, *Curvularia*, *Alternaria*, *Helminthosporium*, *Fusarium* etc.

## Acetobacter

*Acetobacter*, a saccharophilic bacteria associated with sugarcane, sweet potato and sweet sorghum, coffee plants is acid-producing bacteria which fix atmospheric nitrogen. In fact, the *A. diazotrophicus*-sugarcane relationship is beneficial symbiotic relationship between grasses and bacteria which fixes about 30 kg/ha/year of nitrogen. This bacterium is being exploiting and commercialized extensively for sugarcane crop reducing the primary dependency in the chemical Nitrogen fertilizer. It is known to increase cane yield 10-20 ton/acre and sugar content by 10-15 percent.

**Liquid *Acetobacter diazotrophicus* in sugarcane:** Application of *Azospirillum* and Phosphobacteria for the cash crop sugarcane has been a regular practice for the past few years which is saving nearly 20% chemical nitrogen and phosphate applications in South India. It is evident from various studies that *Acetobacter diazotrophicus* present in sugarcane stem, leaves, soils have a capacity to fix up to 300 kg of nitrogen. This bacterium first reported in Brazil where sugarcane is cultivated in very poor sub-soil fields which are fertilized with phosphate, potassium and micro elements alone could produce a yield for three consecutive recorded harvests of 182 to 244 tons per hectare, without the use of any chemical nitrogen fertilizer. This manifests the assumption that active nitrogen fixing bacteria must be associated within the plant regularly. An extensive research work has been conducted by the scientists of Brazil and Australia on bacteria like *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae* and *Herbaspirillum rubisubalbicans* in leaves, stems and roots and revealed the fact that they are largely responsible for atmospheric nitrogen N-fixation.

***Acetobacter pasteurianus* for sulfur:** The physical structure of sulfur consists of several amino acids, including methionine and cystine, which are essential components of plant and animal proteins. Sulfur is important in synthesis of nitrogen in plants; Sulfur acts more like nitrogen than any other essential plant nutrients. Indeed, sulfur deficiency symptoms in plants closely resemble nitrogen deficiency symptoms. Moreover, leaching losses of sulfur from the soil are very much like those of nitrogen caused by percolation waters through light-textured sandy and salty soils. Thus, there is need for annual supplies of sulfur to meet the requirements of growing plants. Although, we are accustomed to use sulfur in the form of fertilizers but number of such farmers are very less, due to its cost factors etc. It was estimated that about 70-90% of total surface soil sulfur is found in organic matters. The remainder of the sulfur is present as sulfides and as soluble and insoluble sulfates. Usually, only 5-10% of the sulfates are found in the surface furrow slice of humid region, cultivated soils. In humid region soils, most of the sulfates are of 12-14 inches deep and are associated with oxides of iron and aluminum, especially in acid soils having Kalononitic type clay. These bacteria help in converting this non-usable form to usable form. It was experienced that use of 200 ml/acre bacteria influenced in level of sulfur in crops like vegetable, cabbage, turnip, onion, cotton, fruits, oilseeds, spices etc.

## Phosphorus solubilizing microorganisms

Microbial mineralization, solubilization and mobilization are the most important aspects of the phosphorus cycle, besides chemical fixation of phosphorus in soil. The enzymatic activity of microorganisms is responsible for the mineralization of organic phosphorus which is left over in the soil after harvesting, or added as plant or animal residues to soil [6,7].

Phosphorus solubilizing bacteria and fungi play a vital role in persuading the insoluble phosphatic compound such as rock phosphate, bone meal and basic slag and particularly the chemically fixed soil phosphorus into available form. These special types of microorganisms are known as Phosphate Solubilizing Microorganisms (PSM) which includes different groups of microorganisms such as bacteria and fungi that convert insoluble phosphatic compounds and fixed chemical fertilizers into soluble form. The species of *Pseudomonas*, *Micrococcus*, *Bacillus*, *Flavobacterium*, *Penicillium*, *Fusarium*, *Sclerotium*, *Aspergillus* and some other are considered as active in biophosphorus conversion.

These bacteria and fungi can be grown in the media containing  $\text{Ca}_3(\text{PO}_4)_2$ ,  $\text{FePO}_4$ ,  $\text{AlPO}_4$ , apatite, bone meal, rock phosphate or similar insoluble phosphate compounds are the sole source of phosphate. Such organisms not only assimilate phosphorus but also trigger the release of an excess amount of soluble phosphate than their actual requirements. Several rock phosphate dissolving bacteria, fungi, yeasts and *actinomycetes* were isolated from soil samples collected from rock phosphate deposits and rhizosphere soils of different legume crops. Some of the isolates solubilized and made available phosphorus to crop from rock phosphate and many isolates solubilized a very high quantity of tricalcium phosphate.

The most efficient bacterial isolates were identified as *Pseudomonas striata*, *Pseudomonas rathonis*, *Bacillus polymyxa*, *B. megatherium*. Apart from these, fungal isolates such as *Aspergillus awamori*, *Penicillium digitatum*, *Aspergillus niger*, *Schwanniomyces occidentalis* are identified as the potential phosphorous solubilizers. These efficient microorganisms have shown consistency in their capability to solubilize chemically fixed soil phosphorus and rock phosphate from different sources. PSM were found to mineralize organic phosphorus into soluble form by their enzymatic activity and save  $\text{P}_2\text{O}_5$  up to 30-50 kg/ha.

**Physical features of liquid PSM:** High turbidity is observed in the appearance of the solution. The color may be creamy mostly and it varies along with the strain. The odor may be pleasant but sometimes mild. The pH is 4.5 or buffer, but buffer with 6.5-7.0 ensures more than 2 years viability.

**Effect of liquid PSM under field conditions:** PSM encourages early root development, produce organic acids like malic, succinic, fumaric, citric, tartaric and alpha ketoglutaric acid which hastens the maturity and thereby increases the ratio of grain to straw as well as the total yield. Improves others beneficial microbial compatibility with the plants. Helps in rapid cell development in plants and consequently enhance diseases resistance towards pathogens. Increase micro nutrient content in soil like Mn, Mg, Fe, Mo, B, Zn, Cu etc., and make them available to the plant parts; stimulates formation of fats, convertible starches and healthy seeds.

Inoculants of phosphate solubilizing bacteria as fertilizer increases P uptake by the plant and enhance crop yield. There are many strains of different genera which promote the phosphate solubilization among which, strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* are the most powerful phosphate solubilizers. Phosphate solubilization by the organisms mainly involves the production of organic acids which is the principle mechanism solubilization, and the organic phosphorous in the soil is mineralized by acid phosphates which is a vital step. Cloning of several phosphatase encoding genes which are isolated is done and characterized for the better phosphate solubilization. Genetic manipulation of phosphate-solubilizing bacteria followed by their expression in selected rhizobacterial strains is the interesting

approach among the scientific community for the optimum phosphate solubilization [8]. The PSB has the greatest potential of utilization of India's abundant deposits of rock phosphates, much of which is not enriched [9].

### Phosphate mobilizing microorganisms

Compared with the other major nutrients, phosphorous is the least mobile and available to plants in most of the soils. In spite of its abundance in the most of the soils both in organic and inorganic forms, phosphorous is prime limiting factor for plant growth. Moreover plant species, nutritional status of the soil and ambient soil conditions determine the bioavailability of soil inorganic phosphorus. To circumvent the phosphorus deficiency in the soil phosphate solubilizing microorganisms (PSM) could play an important role in supplying phosphate to plants in a more suitable and eco friendly manner [10].

Several soil fungi are capable of mobilizing/making it available to the plant from the immobile form of phosphorous by its hyphal structures and hence called as phosphate mobilizing microorganisms. These soil microbes have mutualistic association with all crop plants except family *Brassicaceae*. Besides phosphorus this fungus also mobilizes zinc and sulphur. The literal meaning of *mycorrhizae* is "fungus root" which denote the non pathogenic association between certain soil fungi and plant roots. In general two typical types of mycorrhizal fungi are found in associations with plant roots namely *ectomycorrhiza*, *endomycorrhiza* and *ectendomycorrhiza*. Mycorrhizae increase the surface area of the plant root system for better absorption of nutrients from soil especially in phosphorus deficient soils. The *endomycorrhiza*, also known as *Arbuscular mycorrhiza*, aids in transfer of nutrients from soil into root system through specialized structures known as vesicles and arbuscules. The potential role of *mycorrhiza* in mobilizing & phosphate uptake and plant growth is being widely recognized in the recent times and *Arbuscular mycorrhizas* (AM) are the most common type which is being highly exploited. Advancement in biotechnology has promoted experimentation in artificial inoculation of plants with AM. Trials have mainly involved broadcasting and raking-in inocula over plant-growing substrates rather than application of inocula to seed before sowing. Since large-scale production of AM in axenic culture is not yet attained, inocula have been produced in pot cultures or in small field plots on plants grown under carefully controlled conditions, to avoid contamination by plant pathogens. Such inocula have comprised infected roots or spores and hyphae trapped in soil, peat and clay carriers. Introduction of AM into field on a large scale has tended to use inoculum at excessively high and impractical rates in order to guarantee rapid infection by the introduced fungi. Application of inoculum directly to seed may reduce the amounts of inoculums required. Experimental inoculation has involved coating spores, mycelium and infected root fragments, wet-sieved from soil, onto seeds using methyl cellulose as sticker.

According to the studies of root nodulation as well as N and P, uptake is improved by green gram plant on the application of favorably interacting rhizospheric microorganisms as the inoculants and hence, yield is increased in the phosphorous deficient soils [11]. *Bradyrhizobium* sp. (vigna) and phosphate solubilizing microorganisms (PSM), *Pseudomonas striata* or *Penicillium variable* have shown a significant effect in enhancing the nutrient uptake and plant yield. Combined inoculation treatment of AM fungus, *Glomus fasciculatum* the with *Bradyrhizobium* sp. (vigna) + *P. striata* further augmented the nutrient uptake and plant yield [10].

### Benefits and constrains

As the fungal hyphae facilitate increased surface area of absorption of nutrients like P, Zn, Cu etc., up to an extent of 8 cm the biochemical reactions in plant cycle are not interrupted promoting the plant growth.

Moreover, it is rather easy to inoculate on crops which are normally grown on nursery beds or root trainers or poly bags because of the difficulty in mass producing AM fungi. Nearly 25 to 50% of phosphatic fertilizers can be saved on a year with the inoculation of efficient AM fungi.

In spite of many advantages, AMF have some constraints which are causes for their limited usage. As it is an obligate symbiotic organism it need a host to survive, and it cannot be cultured in synthetic media in laboratory for large scale production. Variations and specifications in plant genotypes and soil nature make it difficult to use appropriate *mycorrhizal* strains for the phosphorous mobilization. Slow growth and development of *mycorrhizal* fungi association with the plant roots and poor resistance to the fungicides are some of the other potential constrains which are to be overcome by the rigorous field research with different approaches and optimizations.

### Potash Mobilizing Bacteria (KMB)

In addition to the regular biofertilizers like *Rhizobium*, *Azospirillum*, *Azotobacter*, *Acetobacter* and Phosphate solubilizing/mobilizing and microbes to meet N&P nutrition in plants, microbes responsible for Potassium mobilization has been isolated and developed from Banana rhizosphere which is having the ability to mobilize the elementary or mixture of potassium which can be easily absorbed by plants. It is estimated that 50 to 60% of potash chemical fertilizers usage can be reduced by using *Frateruria aurentia*, a new bacterial species (species conformation by IMTECH Chandigarh) as a bioinoculant. This new bacteria belonging to the family *Pseudomonaceae* have the extra ability to mobilize K in almost all types of soils especially, low K content soils, soils of pH 5-11 and it survives in the temperature up to 42°C. This potash mobilizing biofertilizers can be applied in combination with *Rhizobium*, *Azospirillum*, *Azotobacter*, *Acetobacter*, PSM etc. Potash mobilizing bacterial based product containing *Frateruria aurentia* producing plant growth promoting substances which offers plant a multifaceted benefits in terms of growth, by mobilizing potash and making it available to crops. It also enhances the efficiency of chemical fertilizer [12].

### Effect of Liquid Potassium mobilizer with AM

Optimization studies on the Liquid Potassium mobilizer revealed following facts. *Arbuscular mycorrhizae* (soil base) and Potassium mobilizing bacteria, individually, as well as in combination with organic compost and phosphocompost shown better results in capsicum, *Hungarian yellow* variety when compared to the controls (plain soil). Potassium mobilizing bacteria (*Frateruria aurentia*) enriched phosphocompost shows the highest results in growth parameters next to all the biofertilizers applied together. This may be because of significant increase in mobilization and uptake of K leading to release of growth hormones by the K-mobilizer.

### Constraints

Even though there is a considerable refinement in the field of biofertilizers for the past 3-4 decades the biofertilizer industry has been facing some technological constraints, some being resolvable the rest need strong research & development activities.

### Crisis of efficient strains

Unavailability of potential regional strains is one of the major reasons. The specificity and competitive ability of the strain is the key point on which the efficacies of the organism relay with respect to the hosting soil and plant variety. The ability to fix Nitrogen and survival capacity are the limiting factors of the Liquid as well as the carrier based biofertilizers [13].

### Possible genotypic changes

During the production of the fertilizers the organism may get interacted with other organisms which may leads to change in basic character of the organisms. Apart from this during fermentation the strains may undergo mutations which may alter the efficacy and viability leading to the economical loss.

### Lack of awareness

In spite of many ongoing projects on the development of biofertilizers, proper attention towards the technology is still needed in order to manifest the results at field level. Communication gap and miscommunication of the farmers by some commercial producers also is a considerable constraint. Lack of storage and marketing facilities, consistent awareness in the farmers by conducting community programs is still welcome [14]. As the production and distribution of biofertilizers is seasonal, the commercial production units may suffer from lack of demand. Moreover the establishment of microorganisms resisting the antagonistic activity of the native microbes is always challenging. Soil nature, temperature, humidity, ph, local insect population, etc., are some other acclimatization problems [15].

### Future Strategies

- Identification and characterization of potential organisms (unexplored) and their effective exploitation in the field of Agriculture is urgently needed.
- Selection and application of suitable bioinoculants with respect to soil nature, agro climatic condition, crop variety under proper agriculture practices is needed.
- Genotypic study of the strains and molecular characterization of the plant parts is necessary to understand the plant mechanisms.
- Study of soil texture and compatible studies with respect to microbial interventions.
- Exploring the novel soil bacteria and maintaining the genomic libraries for future exploitation.
- Identifying, studying the population genetics and preserving the useful endangered species and by advanced bio molecular techniques and bioinformatics tools.
- Suitable combinations of microbial formulations (liquid microbial consortium) with optimized field results are preferable for the sustainable production.
- Soil analysis, crop rotation, organic manure usage, maintenance of proper moisture content, regular sterilization practices are emphasized which are necessary to maximize the biofertilizer efficacy.
- Global standards in the research & development should be maintained during the production and storage of the formulations.

- Development of new strains with enhanced capabilities by genetic engineering techniques and rDNA technology is needed to maintain an eco friendly & sustainable agriculture.
- Constructive awareness and technical support by microbiologists and agricultural professionals must be provided to the Agrarians.

### Conclusion

Human has been relying on agriculture throughout the evolution and would be depending on it forever. The impact of globalization, transition in the technology is creating a negative shade on agriculture in the developing countries like India and declined the percentage of the farming community drastically resulting in raising demand for food commodities. Growing global population demanding the safe and sufficient food for the survival. Soil health has become the greatest assertion for the scientific community in this ever growing polluted globe. Uncertainty in the agro climatic conditions (edapho-climatic factors), monsoon failures by the priceless human activities, lack of proper awareness among the farming community are the direct causes for the agriculture failure in the developing countries like India. The rising demand for the fields like food processing, packing industries, ready to eat foods etc., witness the demand for raw materials in agriculture sector. Crises of agricultural land day by day, vertical increase in the cost of agriculture input technologies are leading to transitions in farming community. In such an agro critical scenario, a multifaced solution for different constraints in agro industry is necessary. It is evident that Biofertilizer technology has inaugurated a new era in biological input technology and recorded a tremendous raise in the annual agriculture production particularly in the past two decades. To combat the threat of global food crises the alternative technologies in the agriculture like liquid biofertilizers are obligatory. Liquid biofertilizer of course have the capacity to replace the traditional chemical fertilizers & carrier based biofertilizers and plays a major role in restoring the soil health, but a lot of measures in terms of technology, government support, subsidies, and constructive awareness by well trained technicians among the agrarians are emphasized.

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