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Role of Biofertilizers in Plant Growth and Soil Health

Murugaragavan Ramasamy, T. Geetha and M. Yuvaraj

Abstract

Biofertilizers nowadays have been realised for shifting fortunes in agriculture. It has been proven successful technology in many developed countries while in developing countries exploitation of bioinoculants is hampered by several factors. Scientific knowledge on bioinoculants and its usage will pave way for its effective usage. At the same time overlooking the significance of ensuring and maintaining a high quality standard of the product will have negative impact. Hence a proper knowledge of bioinoculants and its functioning will pave way to tape the resources in a better way. Thus the chapter provide overview knowledge about different bacterial, fungal and algal biofertilizers, its associations with plants and transformations of nutrients in soil. Adopting a rational approach to the use and management of microbial fertilizers in sustainable agriculture thrive vast potential for the future.

Keywords: biofertilizers, microorganisms, diazotrophics, bioinoculants, biological nitrogen fixation

1. Introduction

One of the present day challenges in agriculture is eco-friendly practices. Though the benefits of Green revolution has been reaped by us in terms of production, the other side of it, i.e., over usage of chemical fertilizers and its subsequent deterioration of soil health has been realised these days [1]. Hence awareness of practicing organic agriculture has been taken to various spheres and products of organic agriculture are fetching up huge market. One of the organic agriculture practices includes usage of biofertilizers in farming. Biofertilizers are likely called as bioinoculants as they are the preparations containing living or latent cells of microorganisms that facilitate crop plants uptake of nutrients by their interactions within the rhizosphere once applied through seed or soil. It accelerate bound microorganism processes within the soil that augment the extent of convenience of nutrients in a very type simply assimilated by plants [2]. Use of biofertilizers has several other advantages as well like they are cost effective, eco-friendly and renewable source of plant nutrients hence forms one of the important components of integrated nutrient management. As of now we could not claim bio-inoculants as a right alternative to chemical fertilizers but in near future the scientific understanding of the same will pave way for its right use and reap full benefits [3]. In addition to this in global scale, recent published works on biofertilizers states about the varied role of bioinoculants viz., other than nutrient transformations in different crops. To mention few, increase in root growth has been observed in wheat due to inoculation of bioinoculant consortia. Likewise *Rhizobium* inoculation increases deaminase

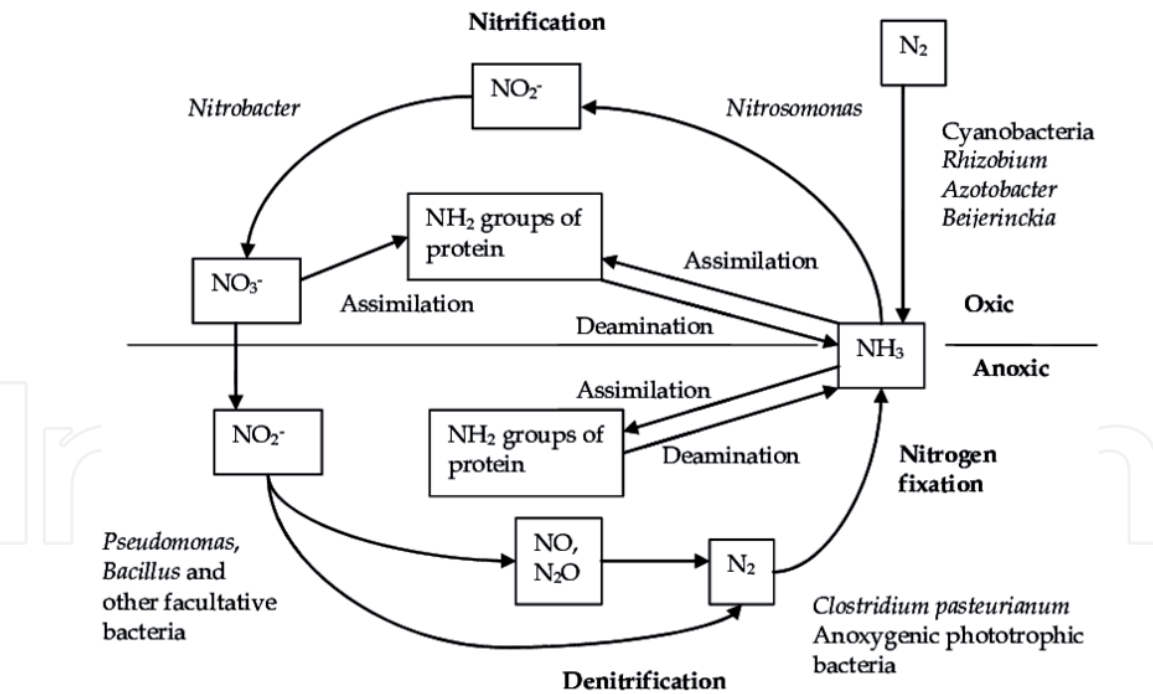
activity in pulses crops. Hence this chapter focuses on different bioinoculants and its uses in farming.

2. Importance of soil microbes in nutrient transformations

It is well established fact that soil microbes have versatile enzyme systems hence perform various nutrient transformations in soil which is very important for maintaining soil equilibrium and its health [4]. Among the nutrient transformations nitrogen and phosphors transformations forms significant importance, since they are the major plant nutrients derived from the soil.

3. Nitrogen transformations

Nitrogen cycle involves of transformations of nitrogen by particular group of soil microbes into organic, inorganic and volatile forms. In addition, a small part of the large reservoir of N₂ in the atmosphere is converted to organic compounds by certain free living microorganism or by plant microbe association that makes the element available to plant growth [5]. The atmospheric nitrogen constitutes about 78% in gaseous form which cannot be utilised by plant and other living organisms which is referred to as biological nitrogen fixation [6]. The details of nitrogen transformations occurring in soil with the role of microbes involved has been depicted below:



4. Biological nitrogen fixation

Biological nitrogen fixation is a component of nitrogen cycle which involves fixing up of atmospheric nitrogen by particular soil microorganisms. Nitrogen fixing ability has been restricted only to certain bacteria and few actinomycetes which belong to various groups and they are referred to as diazotrophs [7]. Diazotrophic microbes are ubiquitous to soil and are classified according to mode of nitrogen fixation to plants **Table 1**.

S. No.	Groups	Examples
1.	Free-living	<i>Azotobacter</i> , <i>Beijerinckia</i> , <i>Clostridium</i> , <i>Klebsiella</i> , <i>Anabaena</i> , <i>Nostoc</i>
2.	Symbiotic	<i>Rhizobium</i> , <i>Frankia</i> , <i>Anabaena azollae</i>
3.	Associative Symbiotic	<i>Azospirillum</i>

Table 1.
Groups of important diazotrophic organisms according to mode of nitrogen fixation.

The process of biological nitrogen fixation has been first documented in anaerobic bacterium *Clostridium pasteurianum* from which the enzyme nitrogenase has been isolated [8]. However today the organism has not been commercially used for the purpose. The nitrogen fixation is mediated by nitrogenase enzyme which reduces gaseous nitrogen to ammonia. All diazotrophs seemed to possess the enzyme and found to deliver quite similar mechanism of nitrogen fixation.

5. Important diazotrophs in commercial use

Rhizobium is the most studied bioinoculant which forms symbiotic association with legume plants. It was first shown by Boussingault that leguminous plant can fix atmosphere N₂ which Hellriegel and Wilfarth clarified that the process is done by bacteria residing in the roots of leguminous plants [9]. The purified bacterium was put into various examinations and now well-developed nitrogen fixing strains are available in various commercial production units.

This bioinoculant is specific for legume crops and forms nodules in the roots of the plants. It enriches the soil fertility also after harvesting of the crop. Hence it is the most preferred bioinoculant [10]. Other than root nodulating *Rhizobium* some of the strains found to nodulate stem known as *Azorhizobium* present in *Sesbania rostrata*. *Rhizobium* species are specific to legume crops because of nod factors they produce [11]. However some leguminous plants found to develop effective nodules on inoculation with the *Rhizobia* obtained from the nodules from other legume groups which is referred to as cross inoculation grouping

Table 2.

1.	<i>Rhizobium leguminosarum</i>	CIG	Host it can nodulate
	bv. <i>viceae</i>	Pea	Peas, lentils, vicia
	bv. <i>phaseoli</i>	Bean	<i>Phaseolus</i> spp
	bv. <i>trifoli</i>	Clover	<i>Trifolium</i> spp
2.	<i>R. meliloti</i>	Alfalfa	Alfalfa, clover, fenugreek
3.	<i>R. loti</i>	Lotus	Trifoli, lupine
4.	<i>R. fredii</i>	Soybean	Soybean
5.	<i>R. spp</i>	Cowpea group	<i>Vigna</i> , <i>Arachis</i> , <i>Cajanus</i> , <i>Dolichus</i> , <i>Sesbania</i> , <i>Acacia</i> , <i>Prosopis</i> , green gram and blackgram
6.	<i>R. spp</i>	Chickpea group	Chickpea

Table 2.
Cross inoculation grouping of *Rhizobium*.

5.1 *Azospirillum*

Azospirillum is considered as very important diazotrophs as it form associative symbiotic relationship with the roots of graminaceous plants. It is generally recommended for rice crop [12]. The organism is microaerophilic, some are aerobic motile and gram negative in nature hence suits well for rice field conditions. It was first isolated by Beijerinck and was named as *Sprillum lipoferum* later named as *Azospirillum*. In addition to nitrogen fixing ability, they also produce growth promoting substances such as IAA [13]. Some of the important species of *Azospirillum* has been listed below:

1. *A. brasilense*
2. *A. lipoferum*
3. *A. amazonense*
4. *A. halopraeferens*
5. *A. irkense*
6. *A. dobereineriae*
7. *A. largimobilis*

5.2 *Azotobacter*

Azotobacter are gram negative free living bacterium in the rhizosphere soil of many plant species, discovered by Beijerinck. The bacterium is very well recognised diazotroph and fixes atmospheric nitrogen in its habitat. Owing to its versatile adaptability and nitrogen fixing ability, they are commercially used in agriculture for many crops and are known with a brand name azotobacterin. Some species of *Azotobacter* known to produce alginic acid, a compound used in medical industry and in food industry it is used as additive in ice creams and cakes [14]. Apart from its nitrogen fixing ability, it also synthesise many phytohormones such as auxins and helps in promoting growth of the plants [15]. They are involved in mobilising heavy metals in the soil thus used for bioremediation purposes as well. Many species of *Azotobacter* are pigment producers and found to degrade aromatic compounds in the agriculture lands.

5.3 *Gluconoacetobacter diazotrophics*

They are endotrophic bacterium which resides inside the stem of sugarcane as it prefers high sucrose and acid content for its survival. They have the ability of capturing atmospheric nitrogen and converting into ammoniacal form [16]. Moreover they are known for stimulating plant growth by tolerant to acetic acid. The bacterium was first discovered in Brazil by scientists Vladimir A. Cavalcante and Johanna Dobereiner. They are originally known as *Acetobacter* belong to Acetobacteriaceae family and got the current name due to carbon source requirement. Besides nitrogen fixing ability they are known to synthesise indole-3-acetic acid which promotes the growth of the associated plant species [17]. Also reports suggest this bacterium controls pathogen especially *Xanthomonas albilineans* in sugarcane. Thus in recent years it is the most recommended bioinoculant for sugarcane.

Apart from these bacterial bioinoculants, cyanobacteria also fixes nitrogen which are referred as algal bioinoculants.

6. Algal bioinoculants

6.1 Algal biofertilizers

The potentiality of algal biofertilizers are realised long before by 1939, when WHO attributed the tropical rice natural fertility to green blue chlorophytic algae. Among algae, only blue green algae have biological nitrogen fixing ability due to the presence of heterocysts cells in them [18]. This bioinoculant is recommended only for rice crop and was proved to improve soil fertility by nitrogen fixation and organic matter enrichment after harvest. In some places, practice of culturing algae as dual crop along with rice has been done which found to inhibit small weed growth during cropping. Apart from this some of the algal species also promote growth by producing growth promoting substances [19].

The following list is some of the nitrogen fixing algal species:

- a. Examples of unicellular nitrogen fixing algae: *Gloeotheca*, *Gloeobacter*, *Synechococcus*, *Cyanothece*, *Gloeocapsa*, *Synechocystis*, *Chamaesiphon*, *Merismopedia*.
- b. Filamentous non heterocystous forms of *Cyanobacteria*, *Oscillatoria*, *Spirulina*, *Arthrospira*, *Lyngbya*, *Microcoleus*, *Pseudanabaena*.
- c. Filamentous heterocystous forms *Anabaena*, *Nostoc*, *Calothrix*, *Nodularia*, *Cylindrospermum*, *Scytonema*.

6.1.1 *Anabaena azollae*

Anabaena is a special type of algae which forms symbiotic association with free floating water fern *Azolla*. Water fern is bilobed in nature and algae resides in the roots of the fern. The common species of algae forming symbiotic association with *Azolla* are *A. microphylla*, *A. filiculoides*, *A. pinnata*, *A. caroliniana*, *A. nilotica*, *A. rubra* and *A. mexicana*. This algae takes shelter and carbon from the water fern and in turn fixes atmospheric nitrogen. They need sunlight and water for its multiplication and hence can be used for rice crop as dual crop. *Azolla* as dual crop in crop estimate to reduce nitrogen requirement by 20–25% [20].

6.2 Phosphate solubilizing and mobilizing bio inoculants

Next to nitrogen phosphorus is the key element for plant growth. Most of Indian soils contain significant amounts of inorganic form of phosphorus, but it is unavailable for plants as it is in the insoluble form. Hence it needs to be solubilised for plant use [21]. Moreover, phosphorus is also available in organic forms in soil which need to be mineralised for plant utilization. Thus mineralization and solubilisation of phosphorus in soil becomes important with respect to plant growth [22].

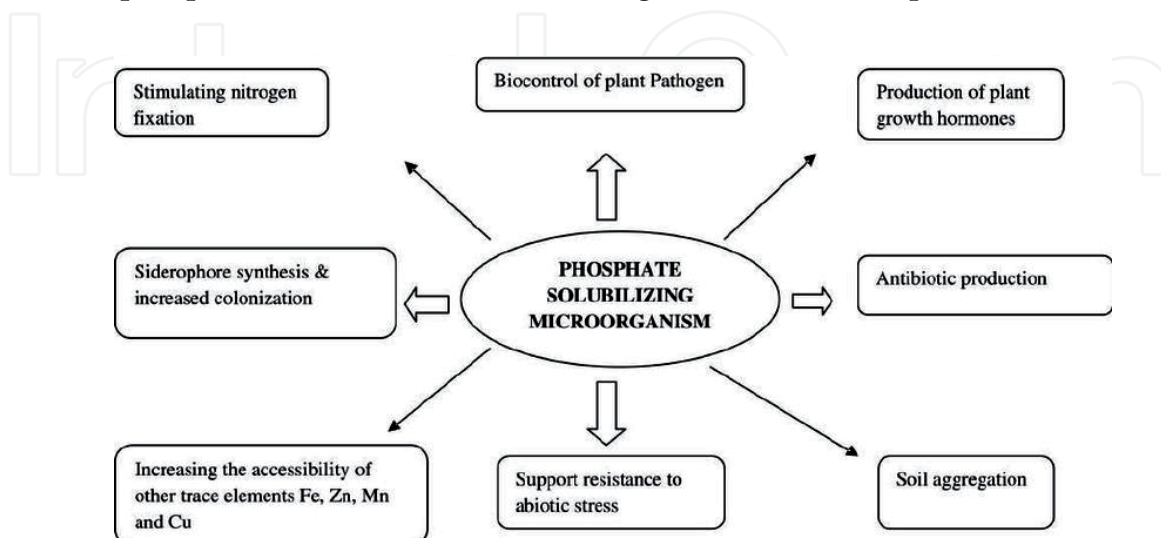
6.3 Phosphorus solubilising bioinoculants

The fate of phosphorus is that it forms apatite's with the salts present in the soil. In acid soil phosphorus will becomes Aluminium phosphates and Iron phosphates

while in alkaline soils it becomes calcium phosphates or sodium phosphates and becomes unavailable to plants [23]. In order to make these form of phosphorus to available form some of the bioinoculants produces organic acids which convert them to soluble form like hypophosphites which can be taken by plants [24].

Examples of phosphorus solubilising Bacteria: *Bacillus megatherium* var. *phosphaticum*, *Bacillus megatherium* var. *phosphaticum*, *Bacillus subtilis*, *Bacillus circulans*, *Pseudomonas striata* Fungi: *Penicillium* sp., *Aspergillus awamori*.

The phosphorus transformations occurring in soil has been depicted below:



6.4 Phosphorus mobilising bioinoculants

Mycorrhiza is the special type of relationship between fungi and plants. Existence of mycorrhizal fungi has been date back 450 million years ago [25]. The relationship is mutualistic in nature and the fungal members who enter into the relationship are members of Zygomycetes, Ascomycetes and Basidiomycetes [26]. Mycorrhizal fungi contribute According to the reports; roots of about 95% of all kinds of vascular plants are normally involved in symbiotic associations with mycorrhizae [27]. For angiosperms, gymnosperms, ferns and some mosses mycorrhizal association appears to be the norm. In the relationship fungus gets a supply of carbon from the associated plant and in turn plants gets lots of benefits from the fungus which is listed below:

- Fungi hyphae increases the root area hence produces more vigorous plants.
- Hyphae surrounding are thinner than roots, but longer than it hence absorb nutrients and water from deeper layers of soil. This helps the plants to tolerate drought.
- Mobilises phosphorus from distant places.
- Gives plants disease tolerance.
- Contribute to nutrient recycling due to production different enzymes.

Mycorrhizas are commonly divided into *ectomycorrhizas* and *endomycorrhizas* based on the mode of hyphal formation. The former do not penetrate the individual cells within the root, while the hyphae of later penetrate the cell wall and forms structures inside the cell membrane [28].

Ectomycorrhizas, are formed between the roots of around 10% of plant families, mostly woody plants including the birch, eucalyptus, oak, pine, and rose families, orchids, and fungi belonging to the *Basidiomycota*, *Ascomycota*, and *Zygomycota*. Thousands of ectomycorrhizal fungal species exist, hosted in over 200 genera [29]. In ectomycorrhizas the hyphae of the fungus do not penetrate the cells of plant roots. In ectomycorrhizae, the mycelium of the fungus forms a dense sheath over the surface of the root. These hyphae form a network in the apoplast, but do not penetrate the root cells. Ectomycorrhizae form a sheath and the fungus grows between the plant cells producing “Hartig net” [30].

One of the more important ectomycorrhizal fungi is *Pisolithus tinctorius*. Symbiosis begins when fungal spores germinate and emerging threadlike structures called hyphae, enter the epidermis of plant roots [31]. After colonization of the root, the fungus sends out a vast network of hyphae throughout the soil to form a greatly enhanced absorptive surface area. This results in improved nutrient acquisition and uptake by plant roots, particularly elemental phosphorus (P), zinc (Zn), manganese (Mn) and copper (Cu) and water. In return, the plant provides carbohydrates for the fungi [32].

6.5 Endomycorrhizae (AM fungi)

Endomycorrhizae form an association in which the hyphae penetrate and colonize epidermal and fleshy cortical cells of plant roots. The most common type of Endomycorrhizae is arbuscular endomycorrhizae (AM). Arbuscular mycorrhizae are characterized by the formation of unique structures such as arbuscles and vesicle by fungi within the plant root cortical cells [33]. Once the roots are colonized, individual hyphae extend from the root surface outward into the surrounding soil forming a vast hyphal network that absorbs nutrients and water that would otherwise be unavailable to the plant's root system. Endomycorrhizae can occur on most seed bearing plants, rain forest tree species, most agriculture crops and a vast variety of ornamental greenhouse crops.

Fungi forming AM associations include about 150 species belonging to genera *Gigaspora*, *Glomus*, *Sclerocystis*, *Acaulospora* and *Entrophospora*. Colonization of roots begins by the secretion of enzymes by arbuscular endomycorrhizae allowing hyphae to penetrate the epidermal and fleshy cortical cells of plant cells. Two to 3 days after colonizing the cell, the hyphae form structures within plant cells called arbuscules which resemble tiny trees and serve to facilitate the transfer of nutrients within the cortical cells.

Arbuscular endomycorrhizae provide the plant with certain mineral elements and water from the soil, and in turn, the plant provides sugars and other carbohydrates for the fungus. Between the cells, sac like structures, called vesicles may form midway or at the terminal ends of the hyphae. Vesicles contain lipids and serve primarily as storage organ for the fungus [34]. Vesicles can also serve as propagules that can colonize other parts of the plant root. Arbuscular endomycorrhizae hyphae also give rise to spores. Spores have very thick walls, which makes them very resistant to freezing and intense heat so they can survive for long periods of time. For this reason, spores are ideal for incorporating into growing media and for use as inoculants. It takes 2–6 weeks for arbuscular endomycorrhizae fungi to completely colonize plant roots and will remain with the plant throughout its life.

6.6 Plant growth promoting rhizobacteria (PGPR)

Other than these above mentioned bioinoculants some of the bacterium like *Pseudomonas*, *Bacillus thuringiensis* and fungi like *Trichoderma viride* are involved

in the control of pests and diseases of plants by colonising the rhizosphere of many plants. They are referred to as biocontrol agents. Among the biocontrol agents *Pseudomonas* is largely used in seed treatments and soil application in large number of crops. They are known for siderophore production which chelates iron in the rhizosphere region thus creating iron deficiency for the pathogenic microbes. Apart from these report suggests that they are also involved in nitrogen fixation and nutrient transformations in soil. Some bacteria living in rhizosphere affect plant growth positively and some are detrimental. Rhizosphere bacteria that favor plant growth are termed as plant growth promoting rhizobacteria (PGPR). They improve plant growth directly by producing plant growth regulators such as auxins, gibberellins and cytokinins; by eliciting root metabolic activities and/or by supplying biologically fixed nitrogen. Consequently, germination, root development, nutrient and water uptake are improved. Other PGPR affect plant growth by indirect mechanisms such as biocontrol activity by suppression of bacterial, fungal and nematode pathogens. The mechanism of biocontrol include competition for colonization space and for nutrients, antibiosis, excretion of hydrogen cyanide and other volatile compounds, synthesis and absorption of siderophores, excretion of lytic enzymes (chitinases, glucanases) and systemic resistance. The well-known PGPR include *Azotobacter*, *Azospirillum*, *Azoarcus*, *Klebsiella*, *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Enterobacter*, *Burkholderia*, *Serratia*, and *Rhizobium*.

7. Conclusion

In developing countries, the most important challenge is to produce sufficient food for the growing population from inelastic land area. Products of biological origin can be advantageously blended to replace a part of the energy-intensive inputs. It is in this context, biofertilizers can provide to the small and marginal farmers an economically viable lever for realizing the ultimate goal of increasing productivity. These microbes siphon out appreciable amounts of nitrogen from the atmospheric reservoir, solubilise phosphorus and enrich the soil with this important but scarce nutrient. The crop-microbial-soil ecosystem can, therefore, be energized in sustainable agriculture with considerable ecological stability and environmental quality.

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