Microbial Inoculants as Biofertilizer

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Abstract

Increasing application of chemical fertilizers in agriculture make country self dependent in food production but it depreciate environment and cause harmful impacts on living beings. The excess uses of these fertilizers in agriculture are costly and have various adverse effects on soil fertility. Further, soil microorganisms play an important role in the plant growth and development by various means *viz*. nitrogen fixation, phosphate solubilisation, phytohormone production etc. Therefore, bio-inoculants for agriculture purpose i.e. bio-fertilizers could be a better alternative to chemical fertilizers for agricultural as well as environmental sustainability.

Keywords

Microbial inoculants • Biofertilizers • PGPRs • Phytohormones • Nitrogen fixation

18.1 Introduction

Soil microorganisms are important component of integrated nutrient management and soil biodiversity system. They play a crucial role in the plant growth and development. In recent years, it is being noticed that excessive exposure to chemical fertilizers and pesticides which not only deteriorate soil health but also create several environmental impacts as global threat. Beneficial microorganisms offer the potential to meet our agricultural needs and thus, are better alternatives for sustainable agriculture practices. As compared to the chemical fertilizer, biofertilizers are

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safer with reduced environmental damage, has more targeted activity and effective in smaller quantities. Furthermore, they are able to multiply but simultaneously controlled by the plant and indigenous microbes. Moreover, microbial inoculants have quicker decomposition procedures and are less likely to induce resistance by the pathogens and pests.

Bio-inoculants for agriculture purpose are also known as bio-fertilizers. They can broadly defined as formulations of active or latent strains of microorganisms mainly bacteria either alone or in combination with algae or fungi components which, directly or indirectly, stimulate microbial activity and thereby increase mobilization of nutrients from soil. They are customized formulations employing functional attributes of the microorganisms to a range of soil systems and cropping patterns for attaining agricultural sustainability. PGPR includes many well known genera *Rhizobia*, *Azospirillum, Klebsiella*, *Bacillus*, *Burkholderia, Azotobacter, Enterobacter,* and *Pseudomonas* etc, but some of these genera include endophytic species as well. The best-characterized endophytic bacteria include *Azoarcus* spp, *Gluconacetobacter diazotrophicus*, and *Herbaspirillum seropedicae* etc. The practical use of biological fertilizers is well below its full potential, mainly due to non-availability of suitable inoculants. Therefore, further studies on bioinoculant formulations and their exploration will definitely help to understand the complexity and dynamism of microbial functioning and interactions in soils.

18.2 Plant Growth Promoting Rhizobacteria

The rhizosphere, the zone surrounding and influenced by plant roots, is a hot spot for several organisms and one of the most composite ecosystems on Earth (Mendes et al. 2013). The rhizosphere is the habitat for several bacteria, archaea, fungi, algae, viruses, oomycetes, nematodes, arthropods and protozoa. Mendes et al. (2013) described the rhizosphere microbiome in terms of "the good" (beneficial microorganisms), "the bad" (plant pathogens) and "the ugly" (human pathogens). Plant beneficial microorganisms not only promote their growth but also protect them from pathogen attack by a range of mechanisms.

PGPRs can induce plant's growth either directly or indirectly. Direct mechanisms comprise the production of substances like phytohormones, liberation of nutrients and stimulation of induced systemic resistance. For example, diazotrophs, Phosphate (P) solubilizing bacteria (PSB) *viz.* Rhizobia group, *Azospirillum*, *Agrobacterium*, *Pseodomonas & Dyadobacter*, etc (Singh et al. 2012; Rani et al. 2013; Kumar et al. 2014; Suyal et al. 2014). Furthermore, indirect mechanisms include stimulation of symbiotic relationships, stimulation for root growth and biocontrol ability. For example, bacterial genera like Azospirillum, Bacillus, and Pseudomonas can enhance plant growth by legume symbioses (Podile and Kishore 2006). Moreover, it is also important to know that in some cases, numerous mechanisms are involved when it comes to beneficial plant microbial interactions (Nihorimbere et al. 2011). Thus, the identification of the mechanisms accountable of plant growth represents a big challenge in present scenario.

18.2.1 Diazotrophs

Diazotrophs are able to reduce N_2 to NH_3, whereas others, including plants and animals must rely on a fixed form of nitrogen for survival *viz.* rhizobia, *Frankia*, *Azospirillum Pseudomonas, Dyadobacter* (Kumar et al. 2014; Suyal et al. 2014) etc. Though biologically fixed nitrogen has been found in a small number of non-legumes, this activity could have a great impact on the ecology of wild and cultivated ecosystems. Some of the well known diazotrophic genera are described below.

18.2.1.1 Rhizobia

Soil rhizobia are bacteria best known for their symbiosis with leguminous plants. Rhizobia include a range of genera, including *Rhizobium,* *Bradyrhizobium, Sinorhizobium, Mesorhizobium, Allorhizobium,* and *Azorhizobium*. Symbiotic nitrogen fixation is a major source of nitrogen, and the various legumes crops and pasture species have ability to fix as much as 200–300 kg nitrogen per hectare (Peoples et al. 1995). Inoculation of these rhizobial strains selected for high N_2-fixing capacity with legumes can improve N fixation in agriculture, mainly when local rhizobia are absent from soils or less effective.

18.2.1.2 Azotobacter

The genus *Azotobacter* belongs to the gama -subclass of the Proteobacteria. These are gram–negative, nitrogen–fixing soil bacteria that have extremely high respiration rates. The first species of the genus *Azotobacter*, named *Azotobacter chroococcum*, was isolated from the soil in Holland in 1901 and thereafter, six other species:, *A. vinelandii, A. beijerinckii, A. paspali, A. armeniacus, A. nigricans* and *A. salinestri* has been reported.

They benefits plants in multiple ways such as by producing ammonia, vitamins, growth substances, indole acetic acid, gibberllins, cytokinins etc. (DeLuca et al. 1996). The genus *Azotobacter* has a high respiratory rate, and its ability to fix atmospheric N_2 in O_2 stress at and above air saturation levels has intrigued researchers for many years (Verma et al. 2001).

18.2.1.3 Azospirillum

Azospirillum belong to the facultative endophytic diazotrophic group and has been reported to colonize the surface and/or the interior of roots of many grasses and cereals. It shows various plant growth promoting activities *viz.* N_2 fixation, production of plant growth-promoting substances etc.

18.2.1.4 Acetobacter

Presently, Acetobacteraceae family includes ten genera: *Acetobacter, Gluconacetobacter, Gluconobacter, Acidomonas, Asaia, Kozakia, Saccharibacter, Swaminathania, Neoasaia, and Granulibacter.* Among them, only three are N_2-fixing genera: *Gluconacetobacter, Swaminathania and Acetobacter. A. diazotrophicus*-sugarcane relationship, first observed in Brazil, was the first report of a beneficial symbiotic relationship between grasses and bacteria through nitrogen fixation (Cavalcante and Döbereiner 1988).

18.2.1.5 Pseudomonas

Several pseudomonas species have been studied for their plant growth promotion activities. Recently, plant growth promoting of Himalayan cold adapted diazotrophs *P. jesenii* MP1 (Kumar et al. 2014) and *P. migulae* S10724 (Suyal et al. 2014) has been revealed. These indigenous diazotrophs are particularly well adapted to the fluctuating temperatures of the hills and could be used effectively as a bioinoculant in high altitude agricultural lands.

18.2.2 Phosphate Solubilising Bacteria

Phosphorus is a plant macronutrient that has a vital role in plant metabolism, ultimately affects on crop yields. It is also important for the functioning of key enzymes that control the metabolic pathways. It is expected that about 98 % of Indian soils contain insufficient amounts of available phosphorus, which is essential to support plant growth (Vassilev and Vassileva 2003). P fertilizers are required for crop production, but only a small part of P is utilized by plants, rest is converted into insoluble fixed forms (Rodriguez and Fraga 1999). Solubilization of insoluble P by microorganisms was firstly reported by Pikovskaya (1948). Now days, many bacterial and fungal species are reported to have the potentials to solubilize inorganic phosphates and commonly known as phosphate solubilizing microorganisms (PSM). Among microbial populations present in soils, phosphate solubilizing bacteria (PSB) constitute P solubilization potential of between 1–50 %, while phosphorus solubilizing fungi (PSF) exhibit only 0.1–0.5 % solubilization (Chen et al. 2006). The commonly known P-solubilizers include *Pseudomonas,* *Bacillus*, *Arthrobacter, Rhodococcus, Serratia, Gordonia, Phyllobacterium, Delftia* sp. (Wani et al. 2005), *Azotobacter* (Kumar et al. 2001), *Xanthomonas*, *Chryseobacterium* (Singh et al. 2012), *Enterobacter, Pantoea*, Klebsiella (Chung et al. 2005), *Xanthobacter agilis, Vibrio proteolyticus* (Vazquez et al. 2000), *Rhizobium leguminosarum* bv. *Trifolii* (Abril et al. 2007), *Pseodomonas* sp. (Rani et al. 2013).

18.2.3 Mycorrhiza

Arbuscular mycorrhizal fungi (AMF), are the member of phylum Glomeromycota and can establish mutualistic symbiosis with several land plants. AMF are categorised into seven main groups: arbuscular (AM), ecto- (EcM), ectendo-, arbutoid, ericoid, monotropoid, and orchid mycorrhiza. AM and EcM are the most widespread and ecologically important mycorrhiza and the only ones commercially exploited in agriculture/forestry. The main benefit to use mycorrhiza is its greater soil exploration and increasing uptake and supply of N, P, K, Zn, Cu, S, Fe, Ca, Mg and Mn to the host roots (Mallik 2000).

18.3 PGPR Supporting Plant Growth under Abiotic Stress

It has been assumed that the rhizosphere microbial communities contributes to the ability of some plant species to survive under extreme environment (Jorquera et al. 2012; Mendes et al. 2013). For example, halotolerant bacteria thrive under salt-stress conditions and in association with the host plant are able to express qualities that promote plant growth (Jorquera et al. 2012). Upadhyay et al. (2009) isolated 24 halotrolerant bacteria from the rhizosphere of wheat plants grown in a saline zone, which showed the capability of producing indole-3-acetic acid, P solubilization, siderophores production and N_2 fixation. Similarly, regardless of the impact of low temperatures on nodule formation and nitrogen fixation, local legumes in the high arctic can nodulate and fix N at rates comparable to those reported for temperate climate legumes. There is great interest in agriculture and horticulture for bacterial and fungal inoculants that enhance growth of plants under low temperature (Mendes et al. 2013). For example, *Burkholderia phytofirmans* PsJN increased grapevine root growth and physiological activity at 4 °C (Barka et al. 2006; Mendes et al. 2013). When co-inoculated with *Bradyrhizobium japonicum*, *Serratia proteamaculans* stimulated soybean growth at 15 °C, the temperature at which soybean nodule infection and nitrogen fixation are normally repressed (Zhang et al. 1995, 1996). To identify mechanisms involved in plant growth promotion in cold environment, Katiyar and Goel (2003) selected cold-tolerant mutants of different *P. fluorescens* strains to solubilize phosphorus and to promote plant growth. They also identified two cold-tolerant mutants that were more efficient in P solubilization at 10 °C than their respective wild types (Katiyar and Goel 2003). Trivedi and Sa (2008) reported two phosphorus solubilizing mutants (of 115) that were more efficient than their wild-type strain within a temperature range from 4 to 28 °C (Mendes et al. 2013).

Other abiotic factors that may badly affect plant growth are pH and high concentrations of toxic compounds. Low pH soils or contaminated soils are main challenges in many production systems worldwide. Kawasaki et al. (2012), used a split-root model and a combination of T-RFLP, DGGE, and 16SrRNA gene pyrosequencing and showed that *Trifolium* and other legumes respond to polycyclic aromatic hydrocarbons contamination in a systemic manner. Similarly, Rani et al (2013) explored cadmium (Cd) resistant *P. putida* 710A for *Vigna radiata* (L.) Wilczek plant growth promotion and metal sequestering in Cd polluted soils. Also, fungi play an important role in rhizoremediation, for example, inoculation of the endophytic fungus *Lewia* sp. in the rhizosphere of *Festuca arundinacea* (Cruz-Hernandez et al. 2012).

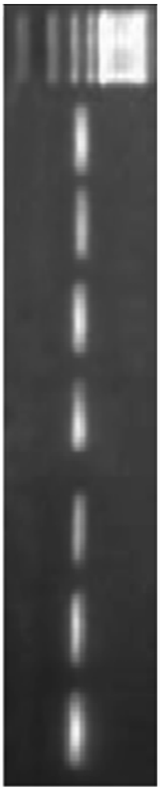
18.4 Himalayan Cold Adapted Diazotrophs for Sustainable Hill Agriculture

Isolation and characterization of the diazotrophs adapted to temperature is central to understanding the ecology of cold adaptive nitrogen fixers and their cold adaptive mechanisms. Previous reports highlighted the prevalence of *nif* and *csp* from the Indian Himalayas (Prema Latha et al. 2009; Singh et al. 2010). Predicted proteins look to be beneficiary in the agronomic practices at ice-cold heights of the Himalayas (Prema Latha et al. 2009). Recently, Suyal et al. (2014) isolated seven cold adapted bacteria from the rhizosphere of Red Kidney bean (*Phaseolus vulgaris* L.) from Western Indian Himalaya (Table 18.1). Furthermore, proteomics of S10724 strain revealed the up-regulation of stress proteins under cold diazotrophy, while most of the down regulated proteins were related to cell division (Suyal et al. 2014). In subsequent studies, net house studies were performed to determine the plant growth promoting ability of strain S10724 on native Green gram (*Vigna radiata* (L.) wilczek) (Suyal et al. 2014). The strain significantly ($p<0.05$) stimulated the growth of roots (45.3 %) and shoots (45.6 %) of Green gram plants (Table 18.2). Furthermore, other growth related parameters *viz*. fresh and dry weight was also found to be increased significantly. The total chlorophyll and nitrate reductase activity was also found to increase in S10724 inoculated plant as compared to their untreated control. Moreover, S10724 treatment increase the germination efficiency of the seeds by 22 % at 25 °C while 25 % at 12 °C unlikely to respective controls (Table 18.2). Similarly, Plant growth promoting properties of Himalayan psychrotroph *Pseudomonas jesenii* MP1 were tested against five native crops *viz. Cicer arietinum* L. (Chickpea), *Vigna mungo* (L.) Hepper. (Black gram), *Vigna radiata* (L.) Wilczek. (Green gram), *Cajanus cajan* (L.) Millsp. (Pigeon pea) and *Eleusine coracana* (L.) Gaertn. (Finger millet) (Kumar et al. 2014). The strain significantly ($p<0.05$) stimulated the growth of shoot length, root length, plant fresh weight and plant dry weight of each crop, over their respective untreated controls. Moreover, MP1 treatment significantly increases chlorophyll content, nitrate reductase activity and P content of the plants. MP1 inoculation showed better effect on Chickpea and Black gram in comparison to other crops. Further, total bacterial and diazotrophic count of MP1 treated soils along with their available Phosphorus (P) and Nitrogen (N) content were found to increase significantly, in comparison to their respective untreated controls (Kumar et al. 2014). These results suggest that *P. migulae* S10724 and *P. migulae* MP1 can be potential plant growth promoting diazotrophs under fluctuating temperature ranges and therefore, could be used effectively as a low cost bioinoculant in high altitudes agro-ecosystems successfully. The exploration of the psychrophilic diazotrophs for the agricultural purpose is in its infancy and therefore, further studies will definitely contribute to the understanding of low temperature diazotrophy mediated agriculture practices.

18.5 Bioinoculants as Biofertilizers

The majority of bio-inoculants used in last few years are mostly *Rhizobia*, constituting ~79 % of the global demand. Phosphate-mobilising bio-inoculants are ~15 %, with other bio-inoculants, such as mycorrhizal products, making up 7 % (Transparency Market Research 2014; Owen et al. 2014). *Azospirillum* species heads a long list of commercial free living PGPR products that are applied to crops in formulations. Some of them are good biocontrol agents and some improve plant growth as well. Additionally, one of the most important species of PGPR used for commercial products is *Bacillus subtilis* under the trade names Serenade, Kodiak, etc. The beneficiary crops are beans, cotton, legumes, pea, rice and soybean. Moreover, well known commercial product is *Agrobacterium radiobacter*, under the trade names Diegall, Nogall, etc. In this case, the beneficiary crops are: fruit, nuts, ornamentals and trees. Finally, *Pseudomonas fluorescens* has also been used to produce commercial inoculants under the trade names Conquer and Victus.

Table 18.1 Characterization of the N_2 fixing psychrophilic bacterial strains isolated from Himalaya (Suyal et al. 2014).

S. No.	Strain ID	Gram reaction and morphology	Accession no.	Nearest phylogenetic neighbour (NCBI-BLAST/EzTaxon) with % similarity	Temperature optima (°C)	*nif*H amplicon (Poly et al. 2001)
1.	S10103	Gram + ve, Rods	JX173281	*Bacillus megaterium* strain SVC4 (100 %)	15	
2.	S10105	Gram + ve, Rod/coccus	JX173282	*Arthrobacter* sp. BA51 (2011) (100 %)	15	
3.	S10107	Gram + ve, Rods	JX173283	*Rhodococcus qingshengii* (100 %)	15	
4.	S10501	Gram + ve, Rod/coccus	JX173284	*Arthrobacter nicotinovorans* strain KNUC2107 (100 %)	15	
5.	S10504	Gram + ve, Rods	JX173285	*Bacillus* sp. IPPBC p001 (100 %)	15	
6.	S10724	Gram −ve, Small rods	JX173286	*Pseudomonas migulae* (100 %)	12	
7.	S10725	Gram + ve, Rod/coccus	JX173287	*Arthrobacter* sp. bB6 (2011) (100 %)	15	

Despite their established economic and ecological benefits the application of such PGPR as biofertilizer must be carefully assessed because of their importance as opportunistic pathogens in nasocomial infections and in patients with diverse diseases (Mendes et al. 2013).

18.6 Conclusion

Besides promoting plant growth, bioinoculants can also alleviate biotic as well as abiotic stresses on crops, thus, providing an environmental friendly sound alternative for sustainable agriculture. However, successful implementation of microbial bioinoculants is dependent on shelf-life, variable efficacy across environments and different plants species other than soil forms. Moreover, the inconsistency of bio-inoculant performance and lack of independent validation does little to build confidence in their efficacy. Therefore, more elementary knowledge is required about microbial behavior and interactions along with dynamics of edaphic and biotic factors for sustainable agriculture. Nevertheless, targeted microbial inoculant for particular soil type is a better approach than uniform formulation.

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Table 18.2 *T*-test analysis depicting the effect of psychrophilic diazotroph *Pseudomonas migulae* strain S10724 on mung bean under net-house conditions after 60 days of germination (Suyal et al. 2014)

Treatment	*in vitro* seed germination assay		Pot trial data					
	% germination of the seeds							
	12 °C[a]	25 °C[a]	Shoot length (cm)[a]	Root length (cm)[a]	Plant fresh weight (g plant^{-1})[a]	Plant dry weight (g plant^{-1})[a]	Chlorophyll content (mg g^{-1} fr. wt)[a]	Nitrate reductase activity (mmol NO$_2$ g^{-1} fr. wt h^{-1})[a]
Control	30 ± 0.51 (6)[b]	70 ± 0.26 (3)[b]	7.22 ± 0.84	5.67 ± 0.65	0.44 ± 0.13	0.19 ± 0.43	0.40 ± 0.07	0.25 ± 0.16
P. migulae S10724	40 ± 0.32 (6)[b]	90 ± 0.12 (3)[b]	13.26 ± 0.55 (45.6)[c]	10.36 ± 1.02 (45.3)[c]	0.86 ± 0.21 (48.8)[c]	0.59 ± 0.61 (67.79)[c]	0.69 ± 0.16 (42)[c]	1.27 ± 0.16 (80.3)[c]
f-value	3.18	5.35	1.29	7.28	7.97	5.86	5.11	1.18
t-value	5.21	4.01	5.62	3.84	5.61	5.39	3.76	6.71

Note:

[a] Each value is mean of five replicates

[b] Values in parentheses indicate germination time in days

[c] Values in parentheses indicate percent increase over treatment

Data were analyzed statistically at the 5 % (p50.05) level of significance

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