Chapter 3
Plant Growth-Promoting Microbes (PGPM) as Potential Microbial Bio-Agents for Eco-Friendly Agriculture

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Abstract The use of chemical fertilizers generates environmental and public health problems. The exploitation of beneficial microbes as a biofertilizer has become of foremost importance in agriculture sector for their impending role in sustainable crop production. Eco-friendly approaches inspire a wide range of application of plant growth-promoting rhizobacteria (PGPRs), endo- and ectomycorrhizal fungi, cyanobacteria and many other useful microorganisms for improved nutrient uptake, plant growth and plant tolerance to abiotic and biotic stress. PGPR use different mechanisms like biofertilization including biological fixation of atmospheric nitrogen, phosphate solubilization, siderophore production and exopolysaccharides production; phytostimulation including production of indole acetic acid, gibberellins, cytokinins and ethylene; and biocontrol including stimulation of systemic resistance, competition for iron, nutrient and space, production of antibiotics, lytic enzymes, hydrogen cyanide and volatile compounds. In scrutiny of the recent advances in PGPR biotechnology, this chapter describes the rhizospheric PGPRs and the different mechanisms used by PGPR to promote the plant's growth and health.

Keywords Plant growth-promoting rhizobacteria (PGPRs) · Chemical fertilizer · Eco-friendly agriculture · Biofertilizer · Mycorrhizal fungi

3.1 Introduction

Organic farming has emerged globally as an important agricultural activity in view of the demand for safe and healthy food and long-term sustainability of the agroecosystem. Soil health and soil food web are being deteriorated by overapplication of agrochemicals particularly the inorganic nitrogenous fertilizers.

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The agricultural soils are facing problems like altered micro-ecological niches with depleted microbial diversity in terms of both species richness and species number and as such call for their replenishment. Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides. Such inputs to agriculture have contributed significantly to the spectacular improvements in crop productivity over the past 100 years (Junaid et al. 2013). In addition, additional chemicals are also being used in product security.

Keeping in view the phenomenon of sustainable agriculture or enhanced productivity using environmentally benign technology, inoculation with the free-living diazotroph, PSB or PGPR not only would be beneficial in terms of obtaining better yield but also would improve the soil microbial ecology and soil food web by establishment of inoculated biofertilizer for the benefit to the standing as well as subsequent crops. Besides, the long-term use of biofertilizers is economical, eco-friendly, more resourceful and available to small farmers. Biofertilizers being essential constituents of organic farming are the preparations containing live or latent cells of efficient strains of nitrogen-fixing, phosphate solubilizing or cellulolytic microorganisms, used for application to seed, soil or composting areas with the intention of increasing number of such microorganisms and to hasten those microbial processes which enhance the availability of nutrients to plants. Biofertilizers play a very significant role in improving soil fertility by fixing atmospheric nitrogen (N), solubilize insoluble soil phosphates and produce plant growth substances in the soil (Venkatashwarlu 2008).

3.2 Potential Characteristics of Biofertilizers and Bioagents

Rhizosphere, a narrow zone of soil adjoining plant roots, can comprise up to 10^{11} microbial cells per gramme of root and above 30,000 prokaryotic species that improve plant productivity (Venkatashwarlu 2008). Microbial composition of rhizosphere varies according to the plant species, stages of plant development and also soil type (Broeckling et al. 2008). *Proteobacteria* and *Actinobacteria* are the microorganisms most habitually found in the rhizosphere of numerous plant species. Collective rhizospheric microbial community enveloping plant roots is larger compared to that of the plant and is referred to as microbiome, whose interactions determine crop health by providing numerous services to plants, viz. organic matter decomposition, nutrient acquisition, water absorption, nutrient recycling and biocontrol. The agriculturally constructive microbial populations cover plant growth-promoting rhizobacteria (PGPRs), N_2-fixing microorganisms, mycorrhiza, plant disease suppressive beneficial bacteria and biodegrading microbes. Biofertilizers are a supplementary component to soil and crop management traditions, viz. crop rotation, organic adjustments, tillage maintenance, recycling of crop residue, soil fertility renewal and the biocontrol of pathogens and insect pests, which process can be significantly useful in maintaining the sustainability of various crop productions. Potential characteristics of biofertilizers and bioagents including nitrogen fixation, phosphate solubilization, zinc solubilization, plant growth hormone production, siderophore production, etc. are discussed later in the chapter.

3.2.1 Exploitation of Biofertilizer for the Betterment of the Nutrient Profile of Crops

Rhizosphere microbial communities have become a subject of great interest in sustainable agriculture and biosafety programme. A major focus in the coming decades would be the safe and eco-friendly methods to exploit the beneficial microorganisms in sustainable crop production. Soil microbes like *Pseudomonas*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Fusarium*, *Sclerotium*, *Aspergillus* and *Penicillium* have been reported to be active in the phosphorus solubilization process. Several fungi like *Aspergillus fumigates* and *A. niger* were isolated from decaying cassava peels that are found to convert cassava wastes to phosphate biofertilizers. *Burkholderia vietnamiensis* produces gluconic acid, which solubilizes insoluble phosphate. *Enterobacter* and *Burkholderia* isolated from the rhizosphere were found to produce siderophores and indole compounds. Potassium solubilizing microorganisms such as *Aspergillus*, *Bacillus* and *Clostridium* are found to be efficient in potassium solubilization and mobilization. Mycorrhizal mutualistic symbiosis with plant roots provides the nutrients to plants, which leads to enhance plant growth and development, and defends plants from pathogen attack. Mycorrhizal hyphae lead to absorption of phosphate from outside to internal cortical mycelia and thus transfer phosphate to cortical root cells. Nitrogen-fixing cyanobacteria such as *Aulosira*, *Tolypothrix*, *Scytonema*, *Nostoc*, *Anabaena* and *Plectonema* are commonly used as biofertilizers. Besides nitrogen fixation, growth-promoting substances and vitamins liberated by these algae increase the root growth and yield of plants. Bacterial genera such as *Acetobacter*, *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Herbaspirillum* and *Rhizobium* have also been reported as effective maize PGPR. Inoculation with *Azotobacter* and *Azospirillum* on field-grown maize significantly increased the plant biomass by 30.7%. Similarly, the co-inoculation of *Bacillus megaterium*, *Azotobacter chroococcum* and *Bacillus mucilaginous* significantly increased maize biomass and height equivalent to half of the chemical fertilizer inputs. Many similar effective N_2-fixing PGPR inoculation results have been reported from maize plants under low fertilizer-N (ca. 48 kg N ha^{-1}) condition, with strains such as *Bacillus* spp., *Klebsiella* spp., *Azospirillum* spp., *Azotobacter* spp. and *Pantoea* spp. Researchers attributed the increase in plant-N uptake and dry biomass of inoculated plants to PGP abilities such as BNF, phosphate solubilization and root promoting phytohormone production, namely, indole-3-acetic acid (IAA), cytokinin and gibberellins (Saini et al. 2015).

3.3 Plant Growth-Promoting Bacteria

3.3.1 Nitrogen-Fixing Bacteria: Symbiotic, Associative, Free-Living

Biological nitrogen fixation (BNF) can contribute to the replenishment of soil N and reduce the need for industrial N fertilizers (Saikia and Jain, 2007; Lanier et al. 2005). The interaction of rhizobia with roots of leguminous plants results in the establishment of effective N_2-fixing symbiosis. In this process, rhizobia reduce atmospheric N to ammonia using enzyme nitrogenase and supply this essential nutrient to the host plant cells. It is an energetically unfavourable reaction, carried out by prokaryotic microorganisms including bacteria, cyanobacteria and actinomycetes, in symbiotic or non-symbiotic association with plants (Giller 2001). Although most *Rhizobium* isolates can nodulate more than one host plant species, several different bacterial species are often isolated from a single legume (Young and Haukka 1996). The exchange of chemical signals between compatible strains of *Rhizobium* and legumes has been named as molecular dialogue (Cooper 2007), which serves to initiate nodule development. In the legume rhizosphere, the rhizobia become affected by the chemotactic and growth-promoting compounds.

Azotobacter spp. is an obligate aerobe although it can grow under low O_2 concentration. The ecological distribution of this bacterium is a complex subject and is related to diverse factors which determine the presence or absence of this organism in a specific soil. Bacteria of the genus *Azospirillum* are a well-known example of so-called associative N fixers which are widespread in the soils of tropical, subtropical and temperate regions. These bacteria develop close relationships with the roots of various wild and agricultural plants (Shridhar 2012).

Cyanobacteria or blue-green algae are a diverse group of prokaryotes that often form complex associations with bacteria and green algae in structures known as cyanobacterial mats. They are the major N_2 fixers in freshwater and marine systems. In large areas of the world's oceans, cyanobacteria provide an important source of nitrogen to the marine ecosystem. Much of our understanding of the ecology and biogeochemistry of oceanic diazotrophy has been derived from studies on the filamentous cyanobacterium *Trichodesmium* spp., a cosmopolitan cyanobacterium in tropical marine systems, including oligotrophic regions throughout the Atlantic and Pacific Oceans (Matthew et al. 2008). Cyanobacteria also grow and fix nitrogen in many terrestrial environments, from rainforests to deserts and are able to survive in extreme environments because of unique adaptations like resistance to desiccation. Because of the ability to fix atmospheric nitrogen, cyanobacterial mats have been used as biofertilizer in modern agriculture. One of the commonest examples includes *Nostoc* and *Anabaena* which have the ability to fix atmospheric nitrogen through symbiosis with fern named *Azolla*. Therefore, it is considered an important potential source of nitrogen for wetland rice. The contribution of nitrogen from *Azolla* spp. to wetland rice plants has been found to be maximum when incorporated into the soil as green manure.

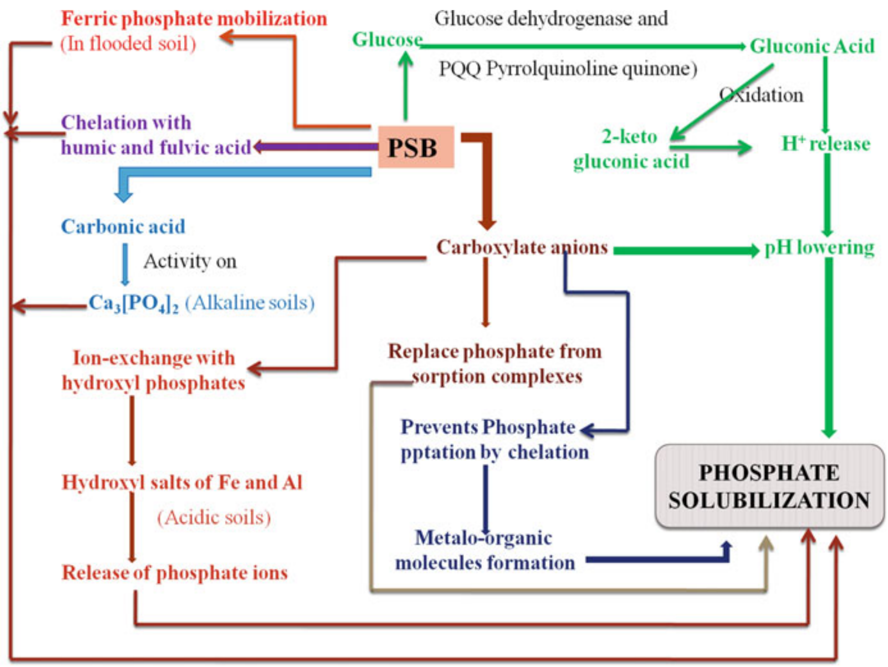

Fig. 3.1 Various organic/inorganic substances produced by PSB responsible for phosphate solubilization in soils

3.3.2 Phosphate Solubilizing Bacteria

Phosphorus (P) is an essential plant nutrient with low availability in many agricultural soils. Thus application of phosphatic fertilizers is a must to make up for the P lost due to the fixation of P by the soil constituents and P run-off from P-loaded soils (Vikram and Hamzehzarghani 2008). Phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{2+}, depending on particular properties of a soil. On the other hand, much of this P is in mineral form and is only slowly available to plants. The role of microorganisms solubilizing inorganic phosphates in soil by acidification, chelation, exchange reactions and production of gluconic acid (Chen et al. 2006) and making them available to plants is well known (Fig. 3.1). Phosphate solubilizing bacteria (PSB) act principally by production of organic acids, acid phosphatases and proton transfer. Another mechanism of phosphate solubilization is by phytase production, since organic P can constitute between 30 and 50% of the total P of the soil, a high proportion of which corresponds to phytate. Phytase-producing rhizobacteria have been reported to belong to genera *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Serratia* and *Staphylococcus*. Further, Jorquera et al. (2008) isolated PSB from the rhizosphere of five cultivated plants (*Lolium perenne*, *Trifolium repens*, *Triticum aestivum*, *Avena sativa* and *Lupinus luteus*), which presented more than one mechanism for utilizing insoluble form of P. Recent reports revealed that optimum P solubilization takes place in the presence of NaCl concentration from 0 to 1.25%, but higher concentrations increase time of P solubilization from 48 to 72 h (Deshwal and Kumar 2013). The major limitation today, for use of these organisms, is the lack of consistency in mobilizing P under field conditions. This is likely due to competition with the native microflora and environmental factors that either limit the population size or activity of the PSB. Microbial biomass assimilates soluble P and prevents it from absorption or fixation.

3.3.3 Zinc Solubilizing Bacteria

Zinc is an essential micronutrient that plays a vital role in various metabolic processes in plants, and its deficiency adversely affects the growth and development of crop plants. The crop and soil management practices mine large amounts of zinc from the native pool of the soil. Additionally, the total zinc content is substantially high although it exists in fixed forms such as smithsonite ($ZnCO_3$), sphalerite (ZnS), zincite (ZnO), franklinite ($ZnFe_2O_4$), willemite (Zn_2SiO_4) and hopeite ($Zn_3(PO_4)_2{\cdot}4H_2O$), which are sparingly soluble. Consequently, large inputs of zinc fertilizers are required to be added to the soil to meet the zinc needs of crops. However, exogenous application of zinc sulphate also gets transformed into different unavailable forms like Zn(OH) and $Zn(OH_2)$ at pH of 7.7 and 9.1, $ZnCO_3$ in calcium-rich alkali soils and $Zn(PO_3)_4$ in near neutral to alkali soils of high P application and gets accumulated in the soil (Gandhi et al. 2014).

Most of the zinc-deficient plants exhibit low levels of auxin such as indole-3-acetic acid (IAA) because Zn plays an essential role in the biosynthesis of IAA. In the absence of IAA, plant growth is stunted. Bacteria such as *Stenotrophomonas maltophilia*, *Mycobacterium brisbanense*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Xanthomonas retroflexus* isolated from the rhizosphere have the capability to synthesize IAA in vitro in the presence or absence of physiological precursors, mainly tryptophan (Sunithakumari et al. 2016). This necessitates a system that releases essential quantity of zinc from the unavailable state for good growth.

Organic acids secreted by the microflora increase soil Zn availability by sequestering cations and by reducing rhizospheric pH. 5-ketogluconic acid was the major organic acid produced in the intermediary of solubilization by *Gluconobacter diazotrophicus*, while in *Pseudomonas* it was 2-ketogluconic acid that mediated solubilization process. Gluconic acid and ketogluconates are sugar acids having multiple conformations which chelate the metal cations apart from solubilization suggesting that the solubilization process might be a direct consequence of increased hydrogen ion activity in the solution. The solubilization process was not accompanied by a reduction in the pH of the medium when ZnO or $ZnCO_3$ was supplemented, and this might be partly because of their intrinsic buffering potential. Also, ZnO may act as an excellent buffer consuming 2 mol of protons per mole for solubilization. Thus gluconic acid production appears to be related to phosphorylative and direct oxidative pathways of glucose metabolism involving enzymes pyrroloquinoline quinone (PQQ)-dependent glucose and gluconate dehydrogenases. Hence isolation and identification of such bacteria are an eco-friendly approach to eradicate zinc deficiency in plants (Saravanan et al. 2007).

3.3.4 Plant Growth-Enhancing/Hormone-Producing Bacteria

3.3.4.1 Auxins

Although plants are able to synthesize IAA themselves, the microorganisms that are the inhabitants of the rhizosphere also contribute to plant's auxin pool. Auxins synthesized by the plant and the microorganisms differ only in the biosynthetic pathway (Fig. 3.2), depending on the plant and/or microorganism. Major IAA-producing bacteria belong to *Aeromonas*, *Bacillus*, *Azotobacter*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Microbacterium*, *Sphingomonas*, *Mycobacterium*, *Kocuria varians* and *Rhizobium*, with some exceptions. Auxin regulates the expression of different genes in *Rhizobium*-legume interactions that are involved in plant signal processing and attachment to plant roots.

3.3.4.2 Gibberellins

Gibberellins are tetracyclic diterpenoid acids that are involved in a number of developmental and physiological processes in plants (Crozier et al. 2000). Apart from *Gibberella fujikuroi*, *Azospirillum* sp. and *Rhizobium* sp., production of gibberellin-like substances has also been claimed in numerous bacterial genera (Bottini et al. 2004) like *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae* (Bastián et al. 1998) and *Bacillus* sp. (Gutiérrez-Mañero et al. 2001), *Pseudomonas monteilii* (Pandya and Desai 2014) as well as *Actinomycetes* (Patil and Patil 2012).

3.3.4.3 Cytokinins

Cytokinins are adenine derivatives. Studies with the slime mould *Dictyostelium discoideum* revealed that 5′-AMP is a direct precursor of isopentenyl adenosine 5′-phosphate ([9R-5′P]iP). Zeatin is the major representative of the group cytokinins.

Many rhizosphere bacteria can produce cytokinins in pure culture, e.g. *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Erwinia*, *Pantoea agglomerans*, *Pseudomonas*, *Rhodospirillum rubrum*, *Serratia* and *Xanthomonas*.

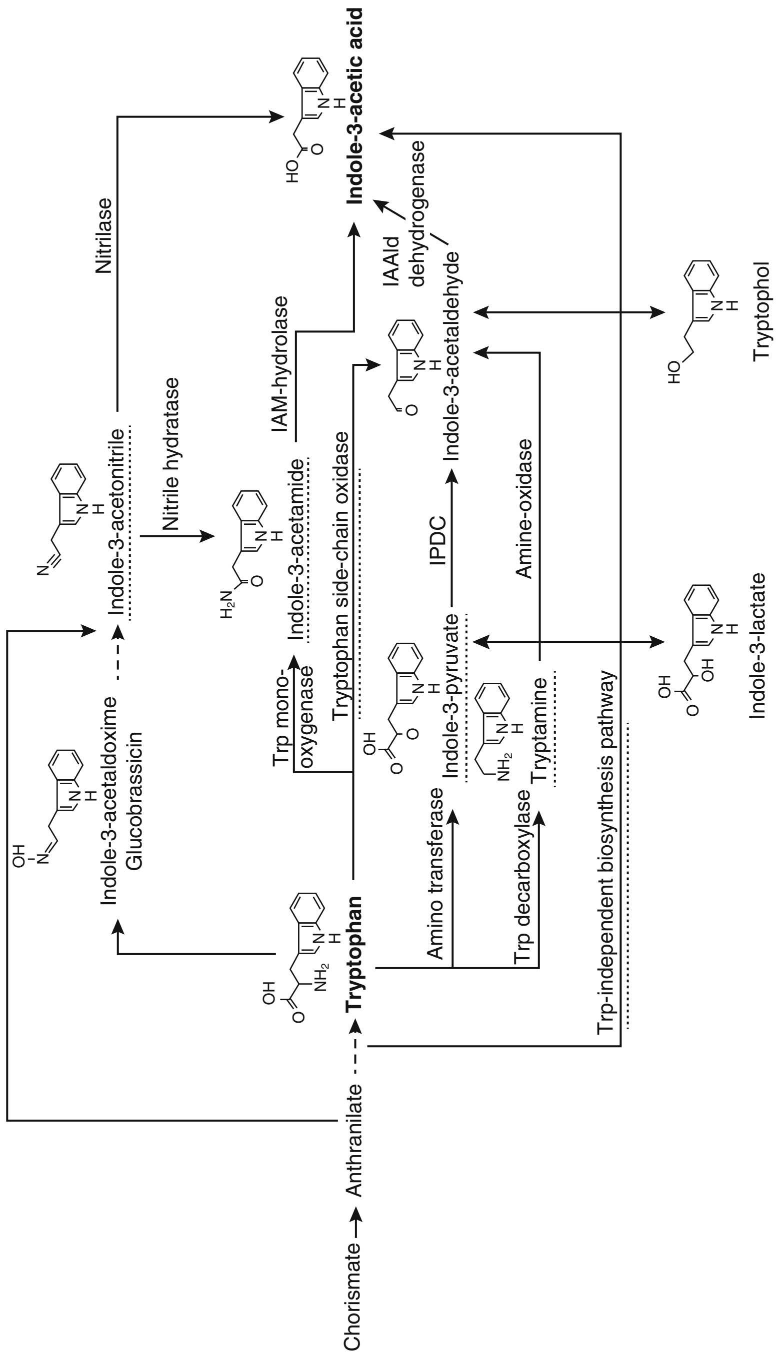

Fig. 3.2 IAA production strategies adopted by different organisms

The cytokinins produced by rhizospheric bacteria becomes part of the plant cytokinin pool and thus influencing plant growth and development.

Concerning the mechanism of action of cytokinins, one speculates that cytokinin produced by rhizosphere bacteria becomes part of the plant cytokinin pool and thus influences plant growth and development. Cytokinins, by affecting cell division, growth, nutrient translocation, retardation of senescence and plant defence, undoubtedly play an important role in the growth-defence trade-off. Großkinsky et al. (2016) has indicated towards the ability of *Pseudomonas fluorescens* G20–18 to efficiently control *Pseudomonas syringae* infection in *Arabidopsis*, allowing maintenance of tissue integrity and ultimately biomass yield using mutant analysis. While cytokinin-deficient loss-of-function mutants of G20–18 exhibited impaired biocontrol, functional complementation with cytokinin-mediated biocontrol was correlated with differential cytokinin levels.

The ability to produce auxins and cytokinins is a virulence factor for the pathogen *Agrobacterium tumefaciens* which produces crown galls. This bacterium can transfer the genes for production of auxins and cytokinins to the plant and incorporate these genes in the plant's DNA. Another bacterium from this genus, *A. rhizogenes*, modifies cytokinin metabolism, resulting in the appearance of masses of roots—instead of callus—from the infection site (Lugtenberg et al. 2013).

3.3.4.4 ACC-Deaminase Activity

Under different types of environmental stress, such as cold, drought, flooding, infections with pathogens and presence of heavy metals, plants respond by synthesizing 1-aminocyclopropane-1-carboxylate (ACC), which is a precursor for ethylene (Glick et al. 2007), a growth hormone. It has been reported that certain PGPRs also have ACC-deaminase activity that changes ACC into alpha-keto-butyrate and ammonia and thereby lower the level of ethylene in the plant. Rhizobacteria with ACC-deaminase activity belong to genera *Achromobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Pseudomonas* and *Rhizobium*. More specifically, the soilborne fluorescent pseudomonads have gained particular attention throughout the global scene because of their catabolic versatility, excellent root colonizing ability and their capacity to produce a wide variety of enzymes and metabolites that favour the plant to withstand varied biotic and abiotic stress conditions. Glick et al. (1999) suggested a model explaining how ACC deaminase containing PGPR can lower plant ethylene levels and in turn stimulate plant growth. According to this model, PGPRs attach either to seed surface or roots of developing plant, in response to tryptophan and other amino acids produced by the seeds, and thus synthesize the auxin (IAA). Together with the plant produced IAA, the bacterial IAA stimulates synthesis of ACC synthase, which is responsible for the rapid transformation of S-adenosyl-L-methionine into ACC. Besides, plants inoculated with PGPR having ACC deaminase are more resistant to the injurious effects of the stress ethylene that is produced as a result of stressed environments (Saini et al. 2015).

3.4 Fungi and Their Potential as Biofertilizers

3.4.1 Mycorrhizal Fungi

The term "mycorrhiza" was coined by Albert Bernhard Frank (TAC 1989) to describe the symbiotic association of plant roots and fungi. Mycorrhiza literally meaning "fungus root" results from mutualism between roots of higher plants and certain fungi. Though the word "mycorrhiza" was coined in 1885, mycorrhizal fungi appear to have coevolved with plants for over 400 million years to become part of the root system as evidenced by fossil mycorrhiza found in carbonaceous deposits. These fungi in soil are ubiquitous throughout the world and form symbiotic relationships with the roots of most terrestrial plants. In natural ecosystems, it is exceptional for a plant not to possess a mycorrhizal root system. Therefore, it could be said that mycorrhizal association is very common or almost universal phenomenon in plant kingdom (Bagyaraj 2014).

3.4.2 Types of Mycorrhizae

3.4.2.1 Ectomycorrhiza

Ectomycorrhiza are most common among temperate forest tree species in the families Pinaceae, Salicaceae, Betulaceae, Fagaceae and Tiliaceae, as well as in some members of Rosaceae, Leguminosae, Myrtaceae and Juglandaceae. Numerous fungi have been identified as forming ectomycorrhiza. Most of them are basidiomycetous fungi belonging to the genera *Boletus*, *Suillus*, *Russula*, *Hebeloma*, *Tricholoma*, *Laccaria*, *Rhizopogon*, *Scleroderma*, *Alpova*, *Pisolithus*, etc. Some ascomycetous fungi also form ectomycorrhiza such as *Tuber* and *Cenococcum*. Thus, they are mostly fungi-forming mushrooms, puffballs or truffles. The mycorrhizal association helps in the uptake of nutrients from soil, protects roots against invasion by pathogens and also decomposes organic matter. These fungi can be cultured in the laboratory on suitable media and used for inoculating forest nurseries.

3.4.2.2 Ericoid Mycorrhiza

Ericoid mycorrhizal fungi usually colonize plants belonging to the families Ericaceae, Empetraceae and Epacridaceae, which are commonly referred to as heath plants, e.g. azalea, rhododendron, blueberry, cranberry, etc. These plants occur in temperate regions of the world. The fungus that forms mycorrhizal association is *Hymenoscyphus ericae*, earlier called as *Pezizella ericeae*, which is an apothecium-forming ascomycete. Most ericaceous species characteristically grow on nutrient-poor, acidic soil where ammonium predominates over nitrate. Ammonium ions are relatively immobile in soil. Thus, ericoid mycorrhizal fungi help in the uptake of both N and P. In India, azaleas and rhododendrons are grown in Himachal Pradesh and North Eastern regions. The possibility of using the ericoid mycorrhizal fungus for enhancing the productivity of these plants can be an area for future research (Smith and Read 2008).

3.4.2.3 Orchid Mycorrhiza

Orchids belong to the family Orchidaceae. This family has nearly 30,000 species. Orchid seeds contain very limited reserves in the form of starch or lipid. At the time of germination, seeds absorb water and swell slightly, and the seed coat breaks exposing the epidermal hair, and this structure is referred to as the "protocorm". The protocorm has to be infected by the mycorrhizal fungus to develop into a plant. Protocorms wait up to 6 months to be infected by the mycorrhizal fungus. If not infected by the fungus, it dies. Orchids are thus obligatorily dependent on mycorrhizal fungi. The fungi involved were initially identified as *Rhizoctonia solani*, *R. repens* and other *Rhizoctonia* spp. Later, their perfect stage was discovered, and they belonged to the genera *Thanatephorus*, *Ceratobasidium*, *Sebacina* and *Tulasnella*. Extensive studies have been made in the orchid mycorrhizal fungus *Tulasnella calospora*. This fungus can be cultured in the laboratory. If the host is not available, it can survive as a saprophyte (Krishnamurthy and Senthilkumar 2005).

3.4.2.4 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal (AM) fungi are ubiquitous in soil habitats and form beneficial symbiosis with the roots of angiosperms and other plants. The AM fungi belong to the family *Endogonaceae*, of the order *Muccorales*, class *Zygomycetes*. The AM-forming genera of the family include *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*. A wide range of AM fungi is found in India. Bakshi (1974) was the first to give an account of 14 spore types: *Glomus macrocarpum* Tul and Tul var., *G.geosporum*, *G.mosseae*, *Glomus* sp., *Sclerocystis coremioides*, *Sclerocystis* sp., *Gigaspora*, *Calospora*, *Acaulospora* sp., *Endogone gigantea*, *E. microcarpum*, *Endogone 1*, *Endogone 2* and *Endogone 3*. Gerdemann and Bakshi (1976) reported two new species, viz. *Glomus multicauli* and *Sclerocystis sinuosa*. In general, the distribution of AM spores in rhizosphere soil is governed by edaphic and certain climatic factors. pH is the only edaphic factor which determines the abundance of AM fungi. However, pH did not influence the mycorrhizal spore density and frequency. High soil P and N content caused a reduction in infection and number of AM spores as well as decreasing the dependency of the plant on the fungal association.

Vesicular arbuscular mycorrhizas (VAM) are produced by aseptate mycelial fungi and are so-called because of the two characteristic structures, vesicles and arbuscules, found in roots with the type of infection. They are by far the commonest of all mycorrhizas and are found in Bryophytes, Pteridophytes, Gymnosperms, excluding the Pinaceae, and in virtually all families of Angiosperms. They are of general occurrence in the Gramineae, Palmae, Rosaceae and Leguminosae, which all include many crop plants. Indeed most crop plants, including herbs, shrubs and some trees, possess this type of mycorrhiza. Associations resembling modern-day VAM were present very early in the evolution of land plants. VAM may colonize the roots of a host plant either intracellularly or extracellularly; this filamentous network promotes bidirectional movement of nutrients where carbon flows to the fungus and inorganic nutrients move to the plant, thereby providing a critical linkage between the plant root and rhizosphere. VAM fungi help plants to capture nutrients such as phosphorus and micronutrients from the soil. It is believed that the development of the VAM symbiosis played a crucial role in the initial colonization of the land by plants and in evolution of the vascular plants. It has been said that it is quicker to list the plants that do not form mycorrhizae than those that do.

3.5 Biofertilizers in Relevance to Environmental Stress Tolerance by Plants

3.5.1 Genetics

It is important to combine genome manipulation techniques with microbiological interference, as it will help in improving potential of microbial assets. The role of microorganisms in inducing genes related to plant defence response has been well documented. *Medicago trunculata* showed induction of various defence-related genes with mycorrhizal colonization (Liu et al. 2007). Expression of ENOD11 and many defence-related genes and root remodelling genes gets upregulated during entry. Subsequently, this allows the formation of a pre-penetration apparatus or PPA (Bucher et al. 2009). Many disease resistance genes that work via jasmonate/ethylene signalling as well as osmotic regulation via proline synthesis genes were differentially expressed with UFLA285 induction (Baharlouei et al. 2011). Various differentially expressed genes were identified which include metallothionein-like protein type 1, a NOD26-like membrane integral protein, ZmNIP2–1, a thionin family protein, an oryzain gamma chain precursor, stress-associated protein 1 (OsISAP1), probenazole-inducible protein PBZ1 and auxin- and ethylene-responsive genes (Brusamarello-Santos et al. 2012). The expression of the defence-related proteins PBZ1 and thionins was found to get repressed in the rice with *H. seropedicae* association, suggesting the modulation of plant defence responses during colonization (Brusamarello-Santos et al. 2012) (Table 3.1).

Table 3.1 List of plant growth-promoting microbes of interest

Bio-inoculants	Plant sp.	Conditions	Effects produced	References
Bacillus sp.	*Wedelia Trilobata*	Aseptic	The phenotypic growth (root RCR, shoot diameter and shoot length), clonal growth and biomass (above ground mass and total mass) increased greatly	Dai et al. (2016)
Twenty-three strains (11 rhizospheric and 12 root endophytes) isolated from tomato root region	*Arabidopsis Thaliana*	Plate	A total of 73% isolates were able to produce organic acids, 89% IAA, 83% ACC deaminase and 87% siderophores. The most striking result was remarkable increases in the formation of root hairs for most of the inoculated plants	Abbamondi et al. (2016)
Rhizobium daejeonense, *Acinetobacter calcoaceticus* and *Pseudomonas mosselii*	*Agave americana* L.	Plant inoculation assay	All of the strains were able to synthesize IAA and solubilize phosphate and had nitrogenase activity. Significant effect on plant growth and the sugar content was also witnessed	De La Torre-Ruiz et al. (2016)
Endophytic bacteria from leaf, stem, root and rhizosphere of sugarcane	*Zea mays* L.	Greenhouse	Isolates were observed to solubilize phosphate; fix nitrogen; produce IAA, HCN, chitinase, ammonia, cellulase and pectinase; and promote plant growth	Rodrigues et al. (2016)
Streptomyces sp.	*Triticum Aestivum*	Pot	Production of IAA, siderophore, ACC deaminase, ammonia and hydrogen cyanide, solubilization of mineral phosphate, significant increases in shoot and root length, plant fresh weight, plant dry weight, number of leaves and number of roots	Anwar et al. (2016)

(continued)

Table 3.1 (continued)

Bio-inoculants	Plant sp.	Conditions	Effects produced	References
Streptomyces sp., SAI and VAI	*Cicer arietinum* L.	Field	Production of siderophore, cellulase, lipase, protease, chitinase, hydrocyanic acid, indole acetic acid and 1,3-glucanase. An increase in nodule number, shoot weight and yield, enhanced total N, available P and organic C was also observed under field conditions	Sreevidyaa et al. (2016)
Trichoderma longibrachiatum	*Triticum aestivum*	Laboratory	The relative water content in the leaves and roots, chlorophyll content and root activity were significantly increased, and the accumulation of proline content in leaves was markedly accelerated with the plant growth parameters. The antioxidant enzymes, superoxide dismutase, peroxidase and catalase, were increased by 29, 39 and 19%, respectively, under salt stress	Zhang et al. (2016)
Eighty-one isolates associated with *Firmicutes* and *Proteobacteria* phyla	*Langsdorffia hypogaea* Mart.	Laboratory	Of the total isolates, 62, 86 and 93% produced, respectively, siderophores and IAA and were able to fix N_2. In addition, 27 and 20% of isolates inhibited the growth of enteropathogens and phytopathogens, respectively	Felestrino et al. (2017)
Bacillus aquimaris DY-3	*Zea mays* L.	Pot	Tolerance to salinity was achieved. Chlorophyll content, leaf relative water content, accumulation of proline, soluble sugar and	Li and Zhang (2017)

(continued)

Table 3.1 (continued)

Bio-inoculants	Plant sp.	Conditions	Effects produced	References
			total phenolic compound and activities of superoxide dismutase, catalase, peroxidase and ascorbate peroxidase were enhanced, while lipid peroxidation levels and Na^+ content decreased	
Azotobacter vinelandii, *Pantoea agglomerans*, *P. putida*	*Onobrychis sativa* L.	Pot	Increased root and shoot lengths, shoot dry weight, nutrient uptake and mitigation of drought stress were observed	Delshadi et al. (2017)

3.5.2 Multiple Inoculations

Application of PGPR for improvement of crops has been investigated for many years, with recent attention focused on co-inoculation of PGPR with different growth attributes for growth promotion and stress tolerance. Populations of bacteria have functional roles within communities that permit their survival. Distinct microbial populations in rhizosphere frequently interact with each other. Syntrophic relationships between different organisms have been demonstrated in several microbial ecosystems. Bacteria live in consortia bound to surfaces as in biofilms, flocs or granules. Under these conditions the bacteria are positioned in a heterogeneous environment. It is increasingly apparent that in nature, bacteria function less as individuals and more as coherent groups that are able to inhabit multiple ecological niches. When these strains are made into an inoculum consortium, each of the constituent strains of the consortium not only outcompete others for rhizospheric establishments but complement functionally for plant growth promotion. Pandey and Maheswari (2007) described the relationship between two distantly related isolates, *Burkholderia* sp. MSSP and *Sinorhizobium meliloti* PP3. They discovered that in combination both the strains promote growth of host plants because of increased indole-3-acetic acid (IAA) production and phosphate solubilization than single inoculation under laboratory conditions. About 25% increase in mean growth rate was recorded for *S. meliloti* PP3 when grown in mixed-species, two-species culture with respect to single-species culture. This interaction also indicates that in soil, association with *Burkholderia* sp. MSSP favours *S. meliloti* PP3 as an adaptation of high rate of reproduction—a well-known strategy that enables organisms to successfully survive and maintain themselves in communities. Seneviratne (2003) has mentioned that co-inoculation and coculture of microbes have been observed to perform the tasks better than the individual microbes.

3.6 Constraints in Biofertilizer Technology

In spite of a significant growth of biofertilizer industry over the last 50 years, they are still far from their actual potential. Restricted nutrient mobilization potential compared to their chemical counterparts and slow impact on crop growth are the major constraints. Incoherent responses in the field under varied agroecological niches and cropping systems have also contributed to their low recognition by farmers. Besides these, there are some technological, biological, field and marketing constraints, which restrict the fast growth of biofertilizer industry. Some of the major constraints and limitations of the industry are as follows:

1. Crop-specific local strain development.
2. Vulnerability of strains to high chemical fertilizer use.
3. Declining interest in scientific community on development of biofertilizer technologies.
4. Deficiency in technology in respect to carrier suitability and product formulations.
5. Lack of automation in product handling.
6. Liquid inoculants are coming up as solution, but the technology is still immature and not available in public domain.
7. Distribution channels through government agencies are not effective which are leading to cut throat competition among bidders, resulting in low-cost poor-quality inoculant production.

3.7 Current Scenario and Future Prospects

Biofertilizers being important components of organic farming play imperative role in maintaining extended term soil fertility and sustainability by fixing atmospheric nitrogen, mobilizing fixed macro- and micronutrients or converting insoluble P and Zn in the soil into forms available to plants, thereby increasing their efficiency and accessibility (Mishra et al. 2013). In the context of both the cost and environmental impact of chemical fertilizers, excessive dependence on chemical fertilizers is not so viable a strategy in the long run. In this context, organic manures would be the practical option for farmers to increase productivity per unit area.

Ecological stresses are becoming a foremost problem and productivity is declining at an unprecedented rate. Excessive dependence on chemical fertilizers has not only adversely affected the quality of human consumption and environment but also disturb the ecological balance. Biofertilizers can help solve the problem of feeding an increasing global population at a time when agriculture is facing various environmental stresses. It is important to realize the useful aspects of biofertilizers and implement its application to modern agricultural practices. New technology developed using the powerful tool of molecular biotechnology can enhance the biological pathways of production of phytohormones and other growth-promoting secondary metabolites. Identified and transferred to the useful PGPRs, these technologies can help provide liberation from environmental stresses. However, the lack of attentiveness regarding enhanced protocols of biofertilizer application to the field is one of the few reasons why many useful PGPRs are still beyond the knowledge of ecologists and agriculturists. Nonetheless, the recent progresses in technologies related to microbial science, plant-pathogen interactions and genomics will help to standardize the requisite protocols. The success of the science related to biofertilizers depends on inventions of pioneering strategies related to the functions of PGPRs and their proper application to the field of agriculture. The major challenge in this area of research lies in the fact that along with the identification of various strains of PGPRs and their properties, it is essential to scrutinize the actual mechanism of implementation of PGPRs for their efficacy towards exploitation in sustainable agriculture.

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