Chapter 3
Microbes-Mediated Approaches for Improving Plant Productivity and Quality

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Abstract Most of the microbes present in soils are beneficial to the plant and the environment. Soil microbes assist plants in their development and growth and vice versa plants provide nutrition and shelter to the microbes for their development. Plant and microbe interaction enrich the soil in their texture and quality. Soil improvement reduces the dependency of plant on chemical fertilizers and provides many benefits to the plants. Microbes are natural organisms; their processes are slow. Genetic engineering and biotechnology tools may hasten the microbial process and could convert less utilized microbes into more utilization. In today's scenario, utilizing the microbial approaches in enhancing the productivity of plant is more progressive movement in the direction of sustainable agriculture and clean environment.

Keywords Soil improvement · Environment · Sustainable agriculture · Microbial approaches · PGPRs · Mycorrhiza

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3.1 Introduction

3.1.1 The Soil

Soil and the associated biodiversity harbors is a supplier to many ecosystem services which are of paramount significance to not only agriculture, but also the environment. The top layer of the earth's crust lithosphere which consists of soil is accountable for a myriad of functions such as shaping the local climate, relief features of the earth surface, water resources, the ecological circulation of the biogenic elements, and organic matter and their retention along with the creation of suitable conditions to sustain various life-forms like microbes, animals, and plants. Soil also protects and counteracts any changes in the environment through sorption properties besides providing an economic platform to the humans to work on the land as farmers. Thus, soil is a very dynamic entity entertaining over 30% of the species existing on earth. The soil organisms are a crucial aspect of soils and can be referred to as biological engine of the earth (Haygarth and Ritz 2009). Soil microbes are the chief part of the "working class" of the soil community profoundly affecting the functions of the soil in diverse ways. The soil microbial diversity is hugely responsible for the sustainable agricultural practices and in improving better usage of the natural resources (Bagde and Prasad 2016).

3.1.2 Soil Microbes

The soil microbes are invariably associated with the primary production of the organic matter and nutrient recycling (Basu et al. 2021). They promote the growth of the plants, either by suppressing the plant diseases or enhancing their root mass, water uptake, and retention capacities in rhizosphere, or help in the secretion of the plant hormones. They profusely contribute to climate changes through the synchronization of the C and N fluxes as well as modulating several greenhouse gases like CO_2, CH_4, and N_2O. They are also enlisted with the control of pest and diseases in humans, animals, and plants and the subsequent decontamination of the environment. The avalanche in the global food demand, scarcity of arable lands, and the concomitant environmental pressure call for a judicious and sustainable approach in modern agriculture. Soil microbial biodiversity is the linchpin in letting us achieve both our economic as well as ecological sustainable issues (Barea 2015). The amelioration in the soil texture and quality, plant nutrition, and health is the elemental role of the soil microorganism in agriculture.

3.1.3 Significance of Soil Microbial Consortium

The imperative complex natural processes occurring in the environment are largely controlled by the soil microbes. The soil microbiome in close relationship with plants is responsible for:

- Supplying essential nutrients (phosphorous and nitrogen etc.)
- Uptake of various nutrients
- Promoting plant protection
- Stimulating plant growth (through the production of plant hormones)
- Improving soil quality and texture
- Bioaccumulation or microbial leaching of inorganics
- Significant role in the bioremediation of contaminated soils (Brierley 1985; Ehrlich 1990; Middledrop et al. 1990).

The soil microbial biome consists of bacteria, fungi, actinomycetes, protozoa, and viruses. These exert positive and harmful effects depending on their positions. Organic farming hugely relies on the natural soil microbial flora. Microorganisms like *Pseudomonas, Flavobacterium, Bacillus, Micrococcus, Fusarium, Sclerotium, Aspergillus*, and *Penicillium* facilitate the solubilization of phosphorous for their own use which in turn is available to the plants. Sixty-five percent of the nitrogen requirement in agriculture is met through biological nitrogen fixation. Mutualist symbionts like the arbuscular mycorrhiza (AM) fungi and bacteria of the genera *Rhizobia* fix nitrogen in symbiosis with legume crops (Nihorimbere et al. 2011; De-Bruijn 2015a, b). Beneficial rhizospheric microbes boost plant growth via diverse regulatory pathways which can be intuited into direct and indirect mechanisms. These involve the manipulation of the plant hormonal signaling facilitating the bioavailability of the soil-borne nutrients and repelling the pathogenic microbial strains (Bargaz et al. 2018; Grover et al. 2021). Direct mechanism enables resource acquisition of macro- (N, P, K) and micronutrients. They articulate plant hormone biosynthesis and a varied other molecules either extracellularly in the proximity of the rhizosphere (i.e., siderophores) or intracellularly like aminocyclopropane-1-carboxylate deaminase which assists in the plant growth and development by lowering the ethylene concentrations and increasing the osmotic stress in plants (Nadeem et al. 2007; Zahir et al. 2008). Indirect mechanism employed by the soil microbes to enhance plant growth is by diminishing the inhibitory effects of the phytopathogens as they act as a biocontrol agent. They stimulate competition for nutrients, antimicrobial metabolite biosynthesis (such as HCN, hydrogen cyanate, phenazines, pyrrolnitrin 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide, tensin, etc.), and elicit induced systemic resistance to pathogen in the plant which can probably occur because of a beneficial interaction of the rhizobacteria with the plant root (Lugtenberg and Kamilova 2009; Planchamp et al. 2015).

3.1.4 Diversity of Microbial Interactions

The interactions of plants with the microbes are multifarious such as epiphytic (on plant surfaces), endophytic (within the plants), rhizospheric, and the soil microbes associated with the subsurface of the plant organs and soil interfaces. Plants achieve microbial interaction which can be competitive, exploitative, neutral, commensal, or mutualistic on an ecological scale. Although much of the research has focused around the pathogenic effects such as herbivory and infections, lately positive ecological microbial interactions enhancing the plant growth have taken precedence. A vast body of research has focused on the molecular mechanisms that elicit species-specific symbiotic collaboration of the legume plants with the soil rhizobia (Pinto et al. 2014). Flavonoids secreted in the root exudate are responsible for the legume host and the rhizobial interaction (Amit et al. 2021; Basile and Lepek 2021). A large group of soil microbes can trigger a systemic response in the plants, thereby activating the plant defense mechanisms. ISR or the induced systemic resistance can be activated by inoculating the plant with nonpathogenic root zone bacteria which elicits signaling pathways to provide a higher pathogen resistance to the host. Under abiotic stress conditions, species such as *Bacillus* induce ISR response. Endophytic bacterial species commonly employed as a biocontrol agent against various plant diseases might have a cutting advantage as they are protected from the relative competition in the soil environment besides usually growing in the same plant tissue where the plant pathogen usually resides (Heil 2001).

3.2 Guise of Beneficial Rhizospheric Microbes in Sustainable Agriculture

The main classes of the rhizospheric microbe which compliment plant growth, development and foster sustainable crop production can be discussed under the following categories:

1. **Decomposers/detrius:** The bacterial group actinomycetes decompose a wide array of substrates; they are predominantly important in degrading recalcitrant compounds such as chitin and cellulose and are active at high pH while fungi are prominent in degrading these compounds at low pH.
2. **Antagonists /biocontrol agent:** Most of the soil microfauna which act as biocontrol agents are competitive saprophytes, facultative plant symbionts, or facultative hyperparasites. Bacterial species such as *Streptomyces, Bacillus, Burkholderia, Lysobacter, Pantoea, Pseudomonas*, and fungal (*Ampelomyces, Coniothyrium, Dactylella, Gliocladium, Paecilomyces*, and *Trichoderma*) are some of the successful biocontrol agents. Other micro- and mesofauna predators like collembolan, mites, nematodes, annelids, and insect larvae activities reduce

pathogen biomass and often stimulate plant host defense by virtue of their herbivorous activities.

3. **PGPR**: Plant growth-promoting bacteria are profusely ascertained with a wide variety of ecosystem processes such as in biocontrol of plant pathogen, nutrient recycling etc. The N_2-fixing bacteria and the arbuscular mycorrhizal (AM) fungi are an example of beneficial mutualistic plant symbionts. Bacterial genera "rhizobia" have the capacity to fix atmospheric nitrogen in symbiosis with the legume plants. The nitrogen-fixing bacteria convert atmospheric nitrogen into ammonia and nitrate which is readily used by plants. The microbial consortium in agricultural soil interacts favorably to boost plant growth, which is often complex to predict (Prasad et al. 2020) (Table 3.1).

3.2.1 Plant Growth-Promoting Rhizobacteria

Plants have coevolved with soil microbes facilitating their growth and development in a symbiotic manner. PGPR are immensely exploited commercially and in scientific applications helping in making the soil ecosystem sustainable for crop production (Prasad et al. 2015). The PGPR associations have been investigated in oat, canola peas, tomato, lentil, barley, cucumber (Gray and Smith 2005). PGPR colonize plant root and enhance plant growth by diverse mechanisms involving various mechanisms such as: phosphate solubilization, nitrogen fixation, indole acetic acid (IAA), siderophore, 1-amino-cyclopropane-1-carboxylate (ACC) deaminase, and hydrogen cyanate production (Liu et al. 2016). PGPR are also involved in the degradation of environmental pollutants, heavy metal detoxification, salinity tolerance, and as an antagonist to plant pathogens and insects (Egamberdieva and Lugtenberg 2014).

3.2.2 Different Forms of PGPR

PGPR can be organized into two distinct classes: the extracellular plant growth-promoting rhizobacteria (ePGPR) and the intracellular plant growth-promoting rhizobacteria (iPGPR) (Martinez-Viveros et al. 2010). **ePGPR** colonize the rhizosphere (on the rhizoplane) or in the spaces between the cells of the root cortex and include the following genera: *Azotobacter*, *Serratia*, *Azospirillum, Bacillus*, *Caulobacter*, *Chromobacterium*, *Agrobacterium*, *Erwinia*, *Flavobacterium*, *Arthrobacter*, *Micrococcous*, *Pseudomonas*, and *Burkholderia*. Specialized nodules in the root cells are colonized by **iPGPR** which include the endophytic microbes such as *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, as well as *Frankia* species, which harbor the ability to fix atmospheric nitrogen specifically for higher plants (Bhattacharyya and Jha 2012).

Table 3.1 Relevant of some beneficial microbes

S. No.	Microbial species	Plant	Function	References
1	*Achromobacter xylosoxidans*	*Vigna radiata*	Affects plant homeostasis	Ma et al. (2009)
2	*Azospirillum brasilense*	*Zea mays*	Indole acetic acid synthesis induces plant growth	Orlandini et al. (2014)
3	*Bradyrhizobium japonicum*	*Glycine max*	Phosphate solubilization	Rathore (2015)
3	*Azotobacter aceae*	*Fagopyrum esculentum*	Fixation of nitrogen	Bhattacharyya and Jha (2012)
4	*Bacillus circulans, Cladosporium herbarum*	*Vigna radiata*	Phosphate solubilization	Oteino et al. (2015)
5	*Bacillus licheniformis*	*Piper nigrum*	Protection from *Myzus persicae*	Kumar et al. (2015)
6	*Bacillus megaterium*	*Zea mays*	Phosphate solubilization	Ibarra-Galeana et al. (2017)
7	*Bacillus mucilaginosus*	*Piper nigrum, Cucumis*	Enhanced potassium intake capacity	Liu et al. (2012)
8	*Bacillus cereus*	*Gossypium hirsutum*	Prevents from *Meloidogyne incognita* and *M. javanica*	Gao et al. (2016)
9	*Brevibacterium frigoritolerans* YSP40; *Bacillus paralicheniformis* YSP151	*Brassica juncia*	Uptake lead in metal-contaminated soil	Yahaghi et al. (2018)
10	*Burkholderia* spp.	Most of the fruit plants	Induces more ethylene production	Islam et al. (2016)
11	*Enterobacter agglomerans*	*Solanum lycopersicum*	Phosphate solubilization	Oteino et al. (2015)
12	*Flavimonas oryzihabitans INR*	*Cucumis sativus*	Protects from stripped cucumber beetle	Oteino et al. (2015), Bhattacharyya and Jha (2012)
13	*Paenibacillus polymyxa*	*Sesamum indicum*	Prevents fungal disease	Ngumbi and Kloepper (2016)
14	*Pseudomonas aeruginosa*	*Cicer arietinum*	Stimulates potassium and phosphorus uptake	Ahemad and Kibret (2014)
15	*Pseudomonas aeruginosa, Bacillus subtilis*	*Vigna radiata*	Prevents root knot formation	Ngumbi and Kloepper (2016), Ahemad and Kibret (2014)
16	*Pseudomonas fluorescens*	*Triticum aestivum*	Helps prevent *Fusarium culmorum*	Santoro et al. (2016)

(continued)

Table 3.1 (continued)

S. No.	Microbial species	Plant	Function	References
17	*Pseudomonas putida*	*Arabidopsis thaliana*	Improves utilization of plant secondary metabolites	Ahemad and Khan (2012)
18	*Pseudomonas* sp.	*Dianthus caryophyllus*	Prevents Fusarium wilt	Rathore (2015), Ahemad and Khan (2012)
19	*Rhizobium leguminosarum*	*Phaseolus vulgaris*	Phosphate solubilization	Ahemad and Kibret (2014)

3.2.3 *Role of PGPR in Enhancing Plant Growth*

Specialized traits enable the PGPR to enhance and stimulate plant growth and development through various direct and indirect mechanisms involving plant physiology and resistance to phytopathogens (Gupta et al. 2015). These includes nutrient fixation, neutralizing abiotic and biotic stress, and producing enzymes and other volatile compounds to prevent disease. The mode of operation depends upon:

- The type of host plant
- The biotic factors such as plant genotypes, development stage of the plant, and its subsequent defense mechanism and the other members of the soil microbe consortium
- Abiotic factors limiting the action of PGPR comprise of soil composition, soil management, and climatic conditions (Vacheron et al. 2013).

3.2.3.1 Nutrient Fixation by PGPR

PGPR have the propensity to increase the availability and concentration of nutrients by locking or fixing their supply for plant growth. Plants cannot utilize nitrogen directly; they quench their nitrogen requirement by absorbing nitrate (NO_3^-) and ammonium (NH_4^+) from the soil which are essential nutrients for the plant growth. In aerobic soils, nitrogen is predominantly available in the form of nitrates where the PGPR converts the atmospheric nitrogen into the nitrate. PGPR also possess the capacity to solubilize phosphate, resulting in an increased number of phosphate ions available in the soil and thus can be easily taken up by the plants. Species such as *Klebsiella pneumoniae* Fr1, *Bacillus pumilus* S1r1, *Acinetobacter* sp. S3r2, and *Bacillus subtilis* UPMB10 have been reported to fix atmospheric nitrogen and delay N remobilization. The microbe *Kocuria Turkanensis* 2 M4 isolated from the soil rhizosphere has been potent as a phosphate solubilizer, a siderophore producer, and IAA producer for many different plant species (Paredes and Lebeis 2016; Goswami et al. 2016).

3.2.3.2 Nitrogen Fixation

Symbiotic PGPR, documented to fix atmospheric N_2, include *Rhizobium* sp., *Azoarcus* sp., *Beijerinckia* sp., *Pantoea agglomerans,* and *K. pneumoniae* (Ahemad and Kibret 2014). Soil inoculation with a combination of rhizobacterial species improves soil quality and tremendously enhances nodule formation (Unkovich and Baldock 2010). Primarily *Nif* gene is responsible for the N_2 fixation, and other structural genes also involved in activating the iron protein, electron donation, biosynthesis of the iron molybdenum cofactor, and activity of the enzyme.

3.2.3.3 Phytohormone Production

PGPR have the capability to induce production of phytohormone like gibberellins, cytokinins, abscisic acid, ethylene, and auxin. PGPR help in root and shoot invigoration, such as *Rhizobium leguminosarum*, *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Paenibacillus polymyxa*, *Pseudomonas* sp., and *Azotobacter* sp. through the induction of phytohormones (Umesha et al. 2018). We can thus elucidate that PGPR manifest plant growth by invoking drastic changes in the soil microbial consortium in the rhizosphere. They assist in plant growth directly by either encouraging resource/nutrient procurement (nitrogen, phosphorus, potassium, and other essential minerals) or by altering plant hormone levels, or indirectly by diminishing the inhibitory effects of different phytopathogens in the forms of biocontrol agents. The general mechanisms of plant nutrient management by microorganisms include associative nitrogen fixation, lowering of ethylene levels, production of siderophores, production of growth regulators, VOCs, solubilization of nutrients, and promotion of mycorrhizal functioning (Fig. 3.1).

Phosphate Solubilizing Microbes (PSM): The second most essential macronutrient for plant growth is phosphorous. It plays cardinal role in all the metabolic processes such as energy transfer, signal transduction, respiration, macromolecular biosynthesis, and photosynthesis. Since most of the phosphorous in soil is immobilized and is either insoluble or in precipitated forms, plants cannot directly absorb it. Plants absorb phosphate only as monobasic $H_2PO_4^{+}$ and HPO_4^{++} dibasic ions. Many bacterial genera (i.e., *Azotobacter*, *Bradyrhizobium Arthrobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Microbacterium, Pseudomonas*, *Erwinia*, *Rhizobium*, *Mesorhizobium*, *Flavobacterium*, *Rhodococcus*, and *Serratia*), fungi (i.e., *Penicillium* and *Aspergillus*), actinomycetes (i.e., *Streptomyces*), and algae have the potency of solubilizing P-metal complex to release P in bioaccessible form such as orthophosphate through specific mechanisms generally involving organic acids, siderophore production, and phosphatase enzymes which efficiently hydrolyze organic P forms. Thus, PSM significantly contribute towards plant growth by enhancing the efficiency of P utilization through exudation of organic acids or by P-hydrolyzing phosphatase enzymes which in turn enhances the bioavailable P pool directly, or indirectly via the production of other high-value bioactive molecules like

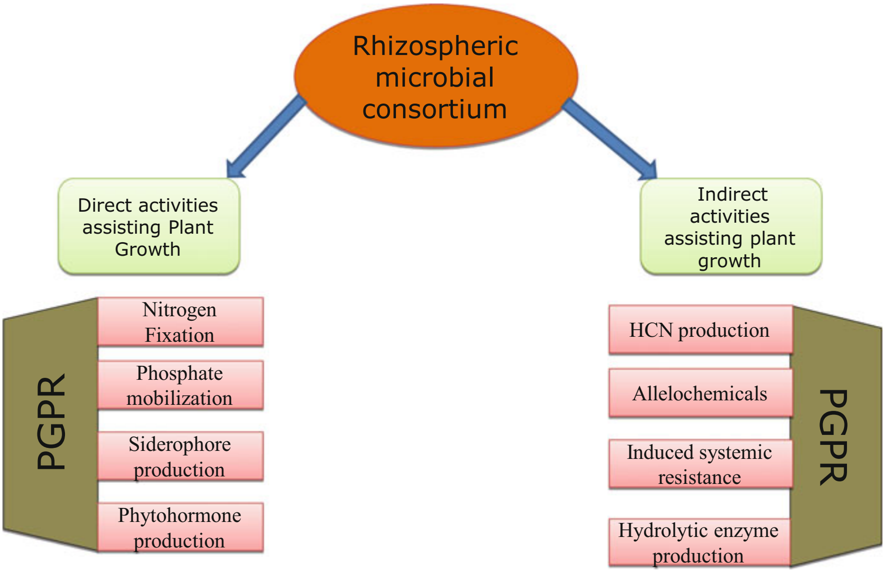

Fig. 3.1 Significance of PGPR

phytohormones, antifungal compounds, toxin-resistance compounds, which assist in building and strengthening robust shoot/rooting system, specially under biotic and abiotic constraints. The PSM have been implicated with the production of a number of organic acids such as acetic acid, gluconic acid, glucuronic acid, butyric acid, fumaric acid, citric acid, lactic acid, propionic acid, succinic acid, oxalic acid, and valeric acid out of which 2-keto gluconic acid and gluconic acid are the most common acids produced by gram-negative bacteria (Krishnaraj and Dahale 2014). The organic acids are efficacious in solubilizing P. The decrease in pH and the cation chelating properties have been attributed as the principal reason for the solubilization of P by organic acids. The concomitant acidification in the vicinity of microbial cell leads to the substitution of H^+ and Ca^{+2} (Zeroual et al. 2012; Behera et al. 2017).

3.2.3.4 Potassium Solubilizing Microbes

Potassium chiefly exist in the form of insoluble rocks and silicate minerals and thus are not available to plants in soluble form as their concentration is extremely low in soil. Low potassium concentrations results in poor seed production, slower growth rate, and stunted roots. PGPR are promising candidates in providing the required concentration of soluble potassium in soil and thus to plants as well. They solubilize potassium rocks by secreting organic acids; *Acidothiobacillus* sp., *Bacillus edaphicus*, *Ferrooxidans* sp., *Bacillus mucilaginosus*, *Pseudomonas* sp., *Burkholderia* sp., and *Paenibacillus* sp., have been reported to release potassium in accessible form from potassium-bearing minerals in soils (Liu et al. 2012). Thus, applying potassium-solubilizing PGP microbes as biofertilizer to improve agriculture can reduce the use of agrochemicals and support eco-friendly crop production.

3.2.3.5 Biological Nitrogen-Fixing Microbes

BNF implies a microbially mediated process where in the presence of an enzyme nitrogenase, atmospheric N_2 is reduced into ammonia (NH_3). Diazotrophs are the group of microbes which support such an enzymatic conversion. The process is carried out biologically either by symbiotic or nonsymbiotic interactions between microbes and plants. The legumes associate with certain soil rhizobial bacteria like *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*, and *Allorhizobium*. They utilize root nodules to sequester atmospheric nitrogen as ammonia, which can be easily utilized by the plants and further be incorporated into biomolecules including proteins and nucleic acids. In symbiotic nitrogen fixation, NF microbes transfer biologically fixed N directly from the bacteria to the host plant along with a significant transfer of photosynthetically fixed plant carbon to the NF bacteria. Some noteworthy illustrations of symbiosis between NF bacteria and eukaryotes include the associations of cyanobacteria with fungi that occur in lichens, cycads, and gunnera; the association of actinomycetes (i.e., Frankia) with a variety of angiosperms like Alnus and Casuarina are also significant (Varma et al. 2020). Nonlegume plants such as grasses have been extensively investigated for their propensity to fix N_2. Several nonsymbiotic NF bacteria of grass species, especially cereals, also exhibit PGP properties where they have been reported to significantly increase plant vegetative growth and grain yield. Species such as *Beijerinckia*, *Azotobacter*, *Azospirillum*, *Herbaspirillum*, *Gluconacetobacter*, *Burkholderia*, *Clostridium*, *Methanosarcina*, and *Paenibacillus* are well-known examples which help in promoting the plant growth. Unlike in the rhizobial association that lead to the formation of root nodules within their legume hosts, in nonsymbiotic NF bacteria reside either in the rhizosphere as free-living or live inside the living tissue (endophytic). They proliferate on account of the energy and nutrients derived from the plant roots. A cardinal feature of importance is that a direct controlled exchange of N and C between bacteria and plant hosts is not involved in associative N_2 fixation or the nonsymbiotic NF as in the symbiotic NF. Inoculation of biological N_2-fixing PGP microbes on crops and farm fields revitalizes growth-promoting activity, disease management, and maintains the nitrogen level in agricultural soil (Pankievicz et al. 2015).

3.3 Application of Soil Microbes as Inoculant to Facilitate Sustainable Agriculture

In the past decade, a comprehensive thrust has been given on formulating practical applications of high-quality microbial inoculants to sustain better crop yield production and improve soil health. Successful inoculation with rhizobia and other PGP microbes is globally recommended. However, the practical application still has many features to consider like (1) disseminating knowledge about different inoculant types and their proper applications on seed on soil or the plants etc., (2) standardizing quality control protocols, and (3) minimizing the fluctuations in field result.

3.3.1 Biofertilizers

The biofertilizers are progressive microbial inoculants containing live/dormant cells of efficient strains of nitrogen-fixing, phosphate-solubilizing, and cellulolytic microorganisms. They are not the source of nutrients but help plants in accessing nutrients in the soil. As compared to chemical fertilizers which are deleterious to the environment and soil, they help in improving the soil quality and texture and thus pave a way for sustainable production of the crops. The microorganisms which are generally used as biofertilizers include nitrogen-fixing soil bacteria (*Azotobacter, Rhizobium*), nitrogen-fixing cyanobacteria (*Anabaena*), phosphate-solubilizing bacteria (*Pseudomonas* sp.), and AM fungi. Similarly, microorganisms involved with the phytohormone (auxin) production and cellulolytic enzymes are also efficiently used as biofertilizer formulations. These organisms help in increasing the accessibility of nutrients to the plants by mediating certain biochemical processes.

Biofertilizers are one-stop shop for getting low-cost, renewable sources of plant nutrients. The efficient strains of the microbes are cultured and packed in suitable carrier (such as peat, lignite powder, vermiculite, clay, talc, rice bran, seed, charcoal, soil, rock phosphate pellet, paddy straw compost, wheat bran, or a mixture of such materials, etc. which provides better shelf life to biofertilizer formulation) in laboratory. The rapid momentum in the use of biofertilizer in recent times is because of its tremendous advantages: (1) it improves soil health, (2) increases crop yield and productivity, (3) controls soil-borne diseases, (4) diminishes the environmental pollutants by reducing the use of chemical fertilizers (Giri et al. 2019).

Currently, a variety of commercial biofertilizers formulations are available which ensure maximum viability of the microbes employed in such formulations. The above feat is achieved through various strategies which include: (1) optimization of the biofertilizer formulation, (2) application of the liquid biofertilizer, (3) application of biotic stress tolerant such as temperature and drought-tolerant genetically modified strains. The vast array of soil microbe association with the crop plants are exploited in the production of the biofertilizers. Table 3.2 enlists some of the microbial groups used as biofertilizers on the basis of their nature and function.

Table 3.2 Some common microbes utilized as biofertilizers

S. no.	Type of organisms	Function	Example	Reference
1	Free living	N2-fixing biofertilizers	*Azatobacter, Beijerinkia, Clostridium, Klebsiella, Anabaena,* and *Nostoc*	Choudhary and Kennedy (2004)
2	Associated symbiotic	N2-fixing biofertilizers	*Azospirillum*	Latef et al. (2020)
3	Symbiotic	N_2-fixing biofertilizers	*Rhizobium, Frankia, and Anabaena azollae*	Soumare et al. (2020)
4	Bacteria	P-solubilizing biofertilizers	*Bacillus megatherium var phosphaticum, Bacillus subtilis, Bacillus circulans, and Pseudomonas striata*	Khan et al. (2016), Igiehon et al. (2019)
	Bacteria	High $AlPO_4$ and $FePO_4$	*Burkholderiaceae*	
5	Fungi	P-solublizing biofertilizers	*Penicillium sp. and Aspergillus awamori*	Adhikari and Pandey (2019), Qiao et al. (2019)
6	Arbuscular mycorrhiza	P-mobilizing biofertilizers	*Glomus sp., Gigaspora sp., Acaulospora sp., Scutellospora sp., and Sclerocystis sp*	Etesami et al. (2021)
7	Orchid mycorrhiza	P-mobilizing biofertilizers	*Rhizoctonia solani*	Mosquera-Espinosa et al. (2013)
8	Pseudomonas	Plant-growth-promoting rhizobacteria	*Pseudomonas flurosence*	Nguyen et al. (2017)
9	Silicate and zinc solubilizers	Biofertilizers for micronutrients	*Bacillus* sp.	Maleva et al. (2017)

3.3.2 *Mycorrhiza*

Mycorrhiza are one of the most distinguished association of fungus with the roots of higher plant (Prasad et al. 2017). Although the system is complex to comprehend, it serves as basic model in understanding the mechanism behind stimulation of growth in the root cells because of the mycorrhizal intrusion an intricate signaling pathway ensures the formation of nodule-like structure and the penetration apparatus. The chief bioligands exuded by mycorrhiza and rhizobium are the Myc factors and the Nod factors which are seized by the host roots to incite an array of signal transduction pathways through unknown receptors (SYMRK and NORK) which activate the release of Ca^{+2} in the cytosol. The majority of the receptors implicated in this pathway are kinases-related proteins like DM1 and SYM71, which phosphorylate their substrate. Nuclear core complex and its associated proteins (NUP) incite calcium spiking. A DM1 protein helps in the frequent movement of calcium ions inside and outside the nucleus. Channel proteins like Ca^{++} along with certain transporters also corroborate in this process. The calcium calmodulin-dependent protein kinase CCAMK phosphorylates the product of CYCLOPS proteins which elicits the activation of multiple genes involved in the formation of penetration apparatus and nodule-like structure formation (Table 3.2) (Umesha et al. 2018).

3.3.3 Biopesticides

The United States Environmental Protection Agency (EPA) defines biopesticides as pesticides procured from natural materials (e.g., animals, plants, bacteria, etc.) and certain minerals (Kachhawa 2017). Biopesticides encompass a variety of different matter which may be living organisms (natural harmful pests), phytochemicals, microbial products, or other by-products, which can be used for pest management. The biopesticides are promising eco-friendly tool against the menace caused by phytopathogen in crop, alleviating the use of chemical pesticides which pose a serious threat to soil microbiome. Some common biopesticides include bioinsecticides (*Bacillus thuringiensis*), biofungicides (*Trichoderma* spp.), bioherbicides (*Phytopthora*), etc. (Table 3.3). These biopesticides are less harmful for agriculture as well as for animals and human beings.

Microbial biopesticides encompass a diverse group of organisms like bacteria, fungus, virus, protozoan, or alga as active agents (Pandey et al. 2010). One of the most notable examples of biopesticide is the bacterium *Bacillus thuringiensis* which possesses insecticidal properties. The *B. thuringiensis* produces a protein harmful to a specific insect pest (Dipteran). Besides the *B. thuringiensis*, other bacteria and fungus such as *Bacillus sphaericus*, *Trichoderma viride*, *T. harzianum* are also successful in controlling the phytopathogens. The efficacy of biopesticide bacteria such as *Bacillus circulans*, *Agrobacterium radiobacter*, *Bacillus pumilus*, and *Pseudomonas aureofaciens* and fungi such as *Ampelomyces quisqualis*, *Fusarium oxysporum*, *Gliocladium virens*, *Trichoderma harzianum*, and *Pythium oligandrum* has been utilized to support sustainable growth and development of agriculture in various countries (Hynes and Boyetchko 2006). *Pseudomonas fluoresens*, *Beauveria bassiana* have also been successfully employed for the pest management against different targets. Bioinsecticides are gaining widespread popularity (Table 3.3). They have shorter shelf lives, a low dose quantity results in higher efficacies, and are harmless towards animals and human beings in comparison to their synthetic counterparts (insecticides). They are target-specific with discrete mode of action. They mostly affect a single species of insect, and are often slow in action; however, the timing of their application is relatively crucial for their success. Besides bacteria and fungus, viruses have also been reported to possess bioinsecticidal potential (Fig. 3.2). Baculoviruses affect insect pests like corn borers, potato beetles, flea beetles, and aphids. A particular strain is being used as a control agent for Bertha army worms, which attack canola, flax, and vegetable crops (Kachhawa 2017). Conventional insecticides do not affect the worm until after it has reached this stage and by then much of the damage has been occurred. Now the scientists are

Table 3.3 Some commonly employed biopesticides

S. No	Type	Microbial species	Mode of action	Target organism/ pest	References
1.	Bactericide	*Agrobacterium radiobacter*	Antagonism and antibiosis	Crown gall (*Agrobacterium tumefaciens*)	Kawaguchi (2013)
		Bacillus velezensis	Antagonism and antibiosis	Crown gall	Gharsa et al. (2021)
		Bacillus subtilis	Colonization on plant root and competition	Bacterial pathogen	Hashem et al. (2019)
		Pseudomonas fluorescens	Overpopulates and controls the growth of plant pathogens	Several bacterial diseases such as frost-forming bacteria	Jain and Das (2016)
2	Fungicide	*Bacillus subtilis*	Colonization on plant root, competition, and antibiosis	Soil foliage, fungal pathogens such as *Rhizoctonia, Fusarium, Aspergillus,* and others	Hashem et al. (2019)
		Bacillus pumilus	Colonization on plant root, competition, and antibiosis	Seedling disease	Zhu et al. (2020)
		Burkholderia cepacia	Controls fungi via seed treatment	Fungal pathogens	Jung et al. (2018)
		Candida oleophila	Colonization of diseased tissues	Postharvest pathogens	Hernandez et al. (2019)
		Gliocladium catenulatum	Enzymatic mechanism	Seed-borne and soil-borne diseases	Pertit et al. (2019)
		Pseudomonas fluorescens	Seed and root exudates help in colonization and produce a diverse array of bioactive metabolites	Plant soil-borne diseases, fireblight	
		Pseudomonas syringae	Utilizes seed exudates, produces a wide spectrum of bioactive metabolites	Postharvest disease	
		Streptomyces	Mycoparasitism, antagonism, and antibiosis	Fungi-causing damping off, stem, and crown rots	
		Trichoderma viride/ Harzianum	Mycoparasitism, antagonism, and antibiosis	Soil-borne fungal disease	

(continued)

Table 3.3 (continued)

S. No	Type	Microbial species	Mode of action	Target organism/ pest	References
3	Insecticide	*Bacillus thuringiensis* (Bt)	Digestive system	Butterfly and moths (Lepidoptera)	Voirol et al. (2018)
		Metarhizium anisopliae	Penetration of the insect exoskeleton and grows directly through the cuticle to the inner body of their host	Coleoptera and lepidoptera, termites, mosquitoes, leafhoppers, beetles, and grubs	Sharma and Sharma (2021)
		Paecilomyces fumosoroseus	Parasitic	Whitefly and thrips	Gavira et al. (2020)
		Verticillium lecanii	Grows directly through the cuticle to the inner body of their host	Whitefly, coffee green bug, and homopteran pests	Sani et al. (2020)
4	Herbicide	*Alternaria destruens*		Dodder	Harding and Raizada (2015)
		Chondrostereum purpureum		Stump sprout inhibitor	Hamberg et al. (2020)
		Colletotrichum gloeosporioides		Northern jointvetch	Boyette et al. (2019)
		Phytophthora palmivora		Strangler vine	Harding and Raizada (2015)
5	Nematicide	*Bacillus firmus*	Competition, antibiosis	Nematodes	Huang et al. (2021)
		Paecilomyces lilacinus	Infection and destruction of nematode's eggs	Nematodes	Monjil and Ahmed (2020)

paying their attention to the development of sustainable agriculture in which the high productivities of plants are ensured using their natural adaptive potentials with a minimal environmental harm. The most promising strategy to reach this goal is to use alternative to the hazardous agrochemicals with environment-friendly preparations of symbiotic microbes, which could increase the nutrition of crops and livestock as well as their protection from biotic and abiotic stresses.

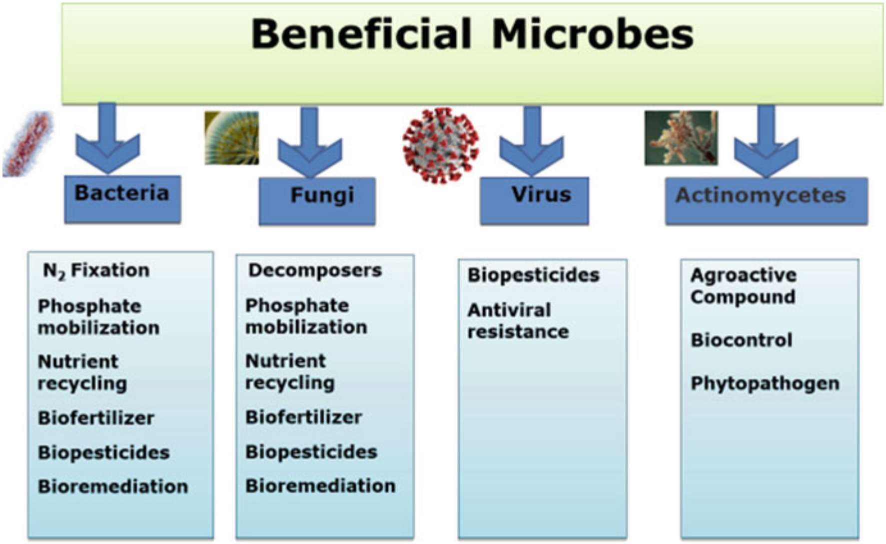

Fig. 3.2 Beneficial microbes and their uses

3.4 Role of Biotechnology in Microbes for Enhancing the Plant Productivity

Biotechnology has opened many fronts in agriculture for plant growth and development. Biotechnology approaches in the microbes assist in food security for increasing population. Productivity of crop depends not only on plants but also microbes present in soils. They are equivalent important for the crop yield and quality. Soil microbes participate in plant growth by many ways such as protection from the diseases, several biotic and abiotic stresses, assisting in nitrogen fixation, protecting from weed and from bioremediation (Lugtenberg 2015).

Plant rhizosphere have abundant amount of root exudates which consists of several chemical compounds for mediating the communication between the soil and plants through signaling (Verma and Verma 2021). Rhizosphere signaling is generally based on host patterns recognition receptors (PRR) and nod-like receptor (NLR) and microbial effector protein which alters the communication and affects the plant health and growth. In this contest, PRR on plant have the capacity to identify pathogen or beneficial microbes using the conserved pattern (Bukhat et al. 2020). Rhizosphere communication can be through quorum sensing molecules, volatile organic compounds, root exudates, flavonoids, rhizobia *nod* genes. PGPR inoculation also assist plants in their immunity and growth through signaling pathways. Plant receives the stimuli either from environment or PGPR which triggers local immune defense response at root zone and then translates into a systemic defensive response regulated by hormonal signaling pathways of salicylic acid, ethylene/jasmonic acid, etc. In this way, phytohormone plays important role in plant defense

(Denancé et al. 2013). Plants recognize microbes, pathogen, damage-associated molecular pattern (MAMPs, PAMPs, DAMPs) for the activation of signaling cascade for defense (Boller and Felix 2009). The mitogen-activated protein kinase and calcium-dependent protein kinases transduce primary signal PTI which is the (PAMPs triggered immunity) into several intracellular defensive responses. Activation of PTI followed the stimulation of ethylene signaling, stomatal closure, callose deposition, production of ROS, and secondary metabolite accumulation, particularly, antimicrobials (Zipfel and Oldroyd 2017; Li et al. 2016). Pathogens prevent the PTI signaling detection by producing the effector protein which leads to ETS (effector-triggered susceptibility) (Gimenez-Ibanez et al. 2016); in response plants have effector-triggered immunity (ETI) system which increases the resistance with the assistance of NB-LRR (nucleotide binding–leucine rich repeat receptor protein) (Pieterse et al. 2014). DNA methylation, histone acetylation, chromatin modification, translation inhibition, degradation and silencing phenomenon at the stage of transcription and posttranscription level also regulate the defense-related gene (Zhang et al. 2011a). The miRNAs and histone deacetylases also assist in plant immunity (Zhang et al. 2011b). After recognition of microbes-associated molecular patterns (MAMPs), plant activates SA, methyl jasmonate, brassinosteroid, abscisic acid, gibberellins, auxins, and cytokinin for defense signal (Pieterse et al. 2009; Shah and Zeier 2013). Abiotic stresses signal is initiated in plant by receptors and senses present on the cell membrane. These signals stimulate the intracellular chemicals such as ROS, inositol phosphate, calcium ion, nitric oxide, and sugars (Bhargava and Sawant 2013). Hormonal signaling, CDPKs, and MAPKs involved in abiotic stress signaling either repress or activate the transcription factor such as *bZIP, WRKY, NAC, MYB,* and *EREBP/AP2* (Danquah et al. 2014). There are different level of modifications at posttranscription stage apart from TFs in transcription stage such as sumoylation, ubiquitination which assist in the formation of complex regulatory signaling network for alteration in gene expression related to physiological and metabolic responses (Mizoi et al. 2013).

Rhizosphere engineering of PGPR microbes is another section in biotechnology which assists in the plant growth and development. PGPR assists in inducing the stress-responsive genes for tolerating the stress in plants (Tiwari et al. 2017). Rhizosphere zone comprises of plant root and soil microbes and modification of either or both the components changes the rhizosphere. Several studies have investigated the modification of plants and rhizosphere microbes but manipulating and engineering the microbiome is quite effective in terms of plant growth and development (Shrivastava et al. 2014; Bhatt et al. 2020). Genetic engineering technology in microbes assists plants in the development such as in genetically engineered bacteria *Pseudomonas syringae* which prevent frost damage in plants, genetically engineered *Rhizobium* which possess more nitrogen-fixation capacity from natural bacteria (De-Bruijn 2015a, b), another strain of bacteria *Pseudomonas fluorescens* genetically changed to produce more endotoxin for more insecticidal capability; the series of insecticidal *Bacillus thuringiensis* is also considered as biological insecticidal, genetic engineering in Baculoviruses infects only the insect (Kamita et al. 2017). *Bacillus* spp. could be engineered with NifH gene from *Paenibacillus* to

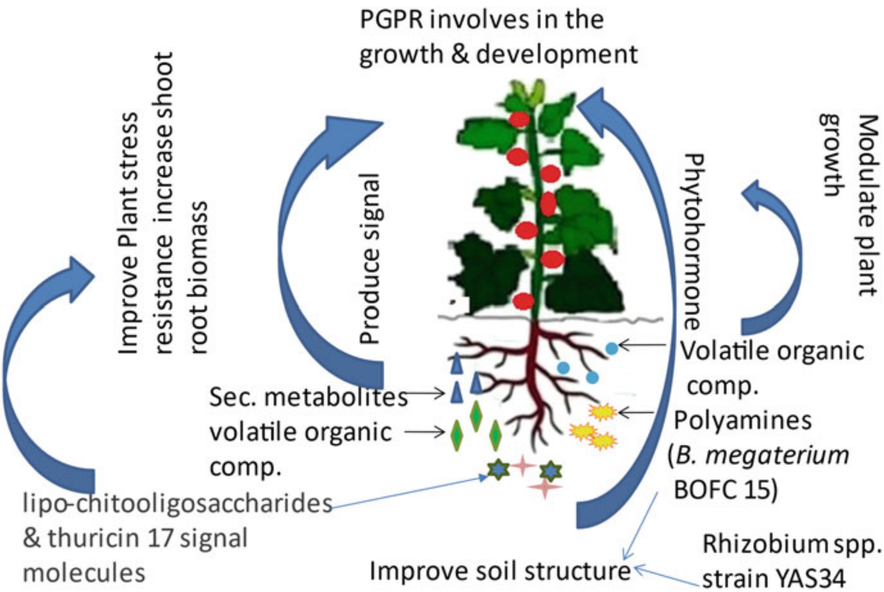

Fig. 3.3 Role of PGPR in enhancing the plants growth and productivity

contain a N_2-fixing machinery (Kim and Timmusk 2013). Many fungi could be utilized as insecticides such as *Metarhizium* and *Beauveria* belong to *Ascomycetes* genera (Lovett and Leger 2017). Plant phytohormones such as auxin, gibberellin, ethylene, cytokinin etc. are important in plant growth and development. So the modification in biosynthesis of plant hormone in microbes (Hedden and Phillips 2000). The field of microbes engineering paves the path for plant growth and development through rhizosphere (Fig. 3.3) (Table 3.4).

3.5 Conclusion

The emphasis on exploiting microbes to provide a holistic approach to sustain agriculture and improve yields has gained momentum during the past decade. The soil microbes open a plethora of opportunities to conserve our environment while catering to our nutritional demands and requirement in sustainable manner. The microbial rhizospheric activities such as BNF, P solubilization, dynamic nutrient recycling through the crops such as legume cereals foster a key role in making amicable approaches to meet the surplus nutritional demand which is all set to soar in the coming years whilst saving our environment and ensuring a better health for the living biome.

Table 3.4 Biotechnology approaches in rhizosphere

S. no.	Type of organisms	Approach	Effect	Reference
1.	Transgenic lotus	Engineering in root exudates which produces two opines (mannopine and nopaline) in rhizosphere to characterize different microbial community	Microbes present in transgenic lotus rhizosphere: Rhizobium and Duganella spp., Duganella, Afipia, Phyllobacterium, Arthrobacter, and Bosea spp., Proteobacteria, Rhizobiaceae family	Oger et al. (2004)
2.	Rhizosphere pseudomonas	Alteration in root exudates confirmed by RNA-seq profiling	Change in expression of genes encoding numerous catabolic and anabolic enzymes, transporter, transcriptional regulators, stress response	Mavrodi et al. (2021)
3.	*Populus trichocarpa*	Overexpression of *PtVP1.1* pyrophosphatase	Induces more acidic rhizosphere which upregulates the activity of the plasma membrane H^+-ATPase for auxin transport	Yang et al. (2015)
4.	Transgenic tobacco	Citrate synthase gene from *Pseudomonas aeruginosa in tobacco root*	Increased citrate efflux which results in improved aluminum tolerance	Delhaize et al. (2001)
5.	Soyabean	Engineered plant growth-promoting *Azospirillum brasilense* strains Ab-V5 used as biofertilizers	Impressive results of increases in root growth, biomass production, grain yield, uptake of nutrients and water, and increased tolerance to abiotic stresses	Santos et al. (2021)
6.	*Allium cepa* L.	Synthetic microbial community(*Azospirillum brasilense, Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae*, and *Burkholderia ambifaria)*	Increased crop productivity	Pellegrini et al. (2021)
7.	Rhizosphere *Klebsiella oxytoca*	Modification in nitrogen-fixating gene cluster	For more nitrogen in soil for plants	Temme et al. (2012)
8.	Grape vine	Plant engineering and rhizosphere engineering	For more sustainability, reduce the use of pesticide	Dries et al. (2021)
9.	Mosses	Bioprospecting of plant microbiomes	Enhanced richness in secondary metabolites, enzymes for the microbes	Muller et al. (2016)
10.	*P. fluorescens* SBW25	*merA* gene introduction	Mercury resistance	Hall et al. (2020)

3.6 Future Perspectives

As we face the global environmental issues affecting our biome, the incessant deterioration of forest, the constant rise in the pollutants, and global warming all endangering the nutritional demand of the global population, a direct need to shift our concerns towards innovative agri-input methodologies is required which can foster a healthy solution. We need to enable our agricultural system to adapt to the current environmental constraints while trying to find a remedial solution all the while. Exploiting microbial resources ensures to meet most of our current demands while offering us a promising approach to save our environment and help in sustainable agriculture. Biostimulants, a subcategory of bioinoculants, are among the beacons of hope which can become one of the major microbial inoculants involved in sustainable intensification of agriculture and ecosystem. They have shown profound result in fostering soil fertility and crop productivity in major cropping systems (Du Jardin 2015). Reproducibility of results is a major concern with the biostimulants as a lot of abiotic and biotic factors; the native soil microbiome all directly or indirectly affects its successful implementation. Rapid advancement in this area is dependent on broadening our understanding of all the associated factors to ensure successful manipulation of the beneficial microbes, their commercialization, and widespread use.

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