Microbes in Crop Production: Formulation and Application

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Abstract

Agriculture depends upon expensive inputs of pesticides and chemical fertilizers to increase crop yields. This dependence on agrochemicals poses risks to human and environmental health such as disruption of nutrient cycling and demolition of beneficial microbial communities for higher crop production. Over the last decade, soil microbes have been widely exploited to enhance the crop production and plant and soil health management. The higher crop yields are reported after inoculation with plant growth-promoting microbes (PGPM). The PGPM signify as an effective and promising way to improve quality food production without environmental or human health hazard. This chapter will explore the current research and trends in microbial exploitation in growth promotion of different agricultural crops. We further discuss the key mechanisms underlying growth promotion and technological advances in bioformulation development to increase shelf life. Recent uses, development, and application of microbial formulation for managing a sustainable environmental system are also discussed.

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3.1 Introduction

The global human population is expected to increase approx. 9 billion from its current population of 7.3 billion by 2050 (Rodriguez and Sanders 2015). The increased population and global climate change have posed a serious threat to crop production and food security. The widespread use of mineral fertilizers and agrochemicals (like fungicides, insecticides, herbicides, etc.) in crop production for higher crop yields remains a common practice. The growing food and fiber demand has led to the expansion of conventional agricultural practices, which is neither economic nor environment friendly (Trivedi et al. 2017). These trends pose a series of unprecedented challenge to worldwide food and agriculture production leading to sustainably intensify food and agricultural crop production and find solutions to combat phytopathogens and abiotic stress.

The application of plant growth-promoting microbes (PGPM) in agriculture represents an economically attractive and environment friendly alternative to extensive chemical fertilization. The collective set of rhizospheric microbes is known as rhizosphere microbiome or rhizo-microbiome (Bulgarelli et al. 2013). A continued exploration and manipulation of rhizo-microbiome and their interactions with plant is a prerequisite for development of efficient microbial formulations (bioformulation). The application of bioformulations can enhance crop growth, vigor, and nutrient use efficiency and provide protection from phytopathogens and biotic and abiotic stress tolerance (Ahmad et al. 2018).

The widespread commercial use of PGPM requires a good screening and mass multiplication procedures that can promote quality, quantity, and product formulation with enhanced shelf life and bioactivity (Gopalakrishnan et al. 2016). In addition, new sustainable approaches will ensure competitive crop yields, crop protection, and soil health improvement. In this chapter, we discuss about soil microbes, role of PGPM in plant health management, and selection criteria of bioformulations.

3.2 Soil Microbes

Soil comprises a living and dynamic ecosystem containing approximately 90–-100 million bacteria along with around 0.2 million fungi (per gram soil). Most of the beneficial PGPM inhabit around the plant roots. The rhizo-microbiome depends on the plant root exudates like organic acids, amino acids, sugars, etc. that provide carbon as a food source (Glick 2018). The plant roots exude chemicals including signaling molecules and metabolites accessible to microbes. The plant-microbe interaction is considered beneficial, neutral, or detrimental for the plant growth. This interaction depends on the plant and specific microbes inhabiting the rhizosphere.

Soil microbial community consists of mixed populations that include bacteria, actinomycetes, fungi, algae, protozoa, and viruses. Nearly all soils contain a mixture of microbial populations. Among them, bacterial community is generally much higher than other groups. All microbial groups are important in bringing about numerous transformations and making up the soil environment. The microbial communities also contribute to various soil ecosystem functions including global biogeochemical cycling (C, N, P, Fe, etc.), organic matter cycling, soil aggregation, etc. Soil organisms influence the soil structure and aggregate formation, which are hotspots of microbial activity and diversity. Soil structure is thus both the cause and the product of soil biodiversity (Havlicek and Mitchell 2014).

The soil organic matter (SOM) decomposition is carried out by the activity of hydrolytic enzymes secreted by bacteria and fungi (primary decomposers). These primary decomposers determine both the magnitude of carbon (C) stored in soils and the rate at which nutrients become available to plants (Shelake et al. 2019). The high soil organic carbon (SOC) content improves the soil biological (microbial biomass), chemical, and physical properties, such as enhanced biological activity, improved soil structure, higher water-holding capacity, soil fertility, and sorption of organic and inorganic pollutants (Bhogal et al. 2018; Shelake et al. 2019). The growth and development of crops/plants is mainly affected by the soil microbial diversity, mineral nutrients, and physical properties of the soil.

3.3 Plant Growth-Promoting Microbes in Sustainable Agriculture

In recent times, agriculture faces numerous challenges like limited nutrient resources, extensive losses by phytopathogens, environmental deterioration through depletion of resources (air, water, and soil), and food security (Kroll et al. 2017). Sustainable agriculture involves a wide range of approaches to meet the growing food demand and fiber requirements without harming the environment (Barea 2015). This integrates three key objectives: healthy environment, economic profitability, and socioeconomic equity. The agricultural crop productivity is sturdily influenced by the activities of soil microbial communities. The microbial communities vary with soil type, soil pH and EC (electrical conductivity), availability of nutrients, and vegetation type (Wang et al. 2018). The exploitation of these beneficial microbial communities is of vital importance to agriculture for sustainable crop production and food safety. Soil microbes derive their energy and nutrients from decomposing organic substrate in the soil. They are involved in SOM transformation and nutrient immobilization and various soil processes ultimately improving soil fertility and productivity (Sharma et al. 2017a, b).

The PGPM are defined as the root-/rhizosphere-inhabiting microbes capable of colonizing root surface and can promote plant growth. The PGPM are divided into two distinctive groups: plant growth-promoting rhizobacteria (PGPR) and plant

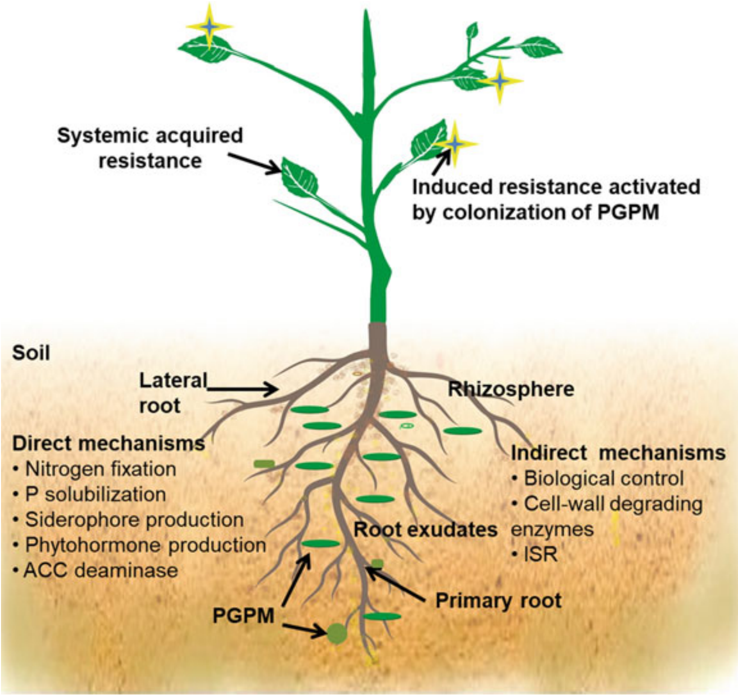

Fig. 3.1 Mechanisms used by PGPM for enhancing plant growth

growth-promoting fungi (PGPF) (Mishra et al. 2017). The term PGPR was coined by Kloepper and Schroth (1978) to beneficial soil bacteria inhabiting rhizosphere and able to colonize and promote plant growth. They are involved directly or indirectly in the growth and development of plant (Fig. 3.1). The mode of action by PGPR includes the nitrogen fixation, nutrient solubilization/mobilization, siderophore production, phytohormone production, and ACC-deaminase activity. Indirect effects include biological control through antibiotic production, cell-wall degrading enzyme activity, and induced systemic resistance (ISR) (Verma et al. 2016; Ahmad et al. 2018). The PGPF are nonpathogenic soilborne saprophytic filamentous fungi that facilitate plant growth. Several reported PGPF belong to fungal genera *Trichoderma*, *Aspergillus*, *Piriformospora*, *Fusarium*, *Penicillium*, *Phoma*, and arbuscular mycorrhizal (AM) fungi (Hossain et al. 2017). The PGPF colonize plant roots, stimulate growth, and suppress phytopathogens. They produce plant hormones, hydrolytic enzymes, antifungal metabolites, nutrient solubilization, organic matter degradation, and ISR in plants (Mishra et al. 2017).

The microbial use and application in crop production and soil health management is important for achieving sustainable agriculture. The use of PGPM largely excludes the use of chemically synthesized fertilizers, pesticides, and growth regulators and can increase crop productivity with environmental restoration. Understanding the rhizosphere structure and function will allow to harness plant-microbial interactions and improved crop productivity (Ahkami et al. 2017).

3.4 Plant-Soil-Microbe Interactions in the Rhizosphere

The term "rhizosphere" was coined by Lorenz Hiltner (1904), to describe the area around plant roots, inhabited and influenced by diverse microbial species and plant root exudates. This influence results from the release of organic compounds, also referred as rhizodeposition. The rhizodeposits include root exudates (sugars, amino acids, organic acids, etc.), insoluble materials (sloughed cells and root mucilage), dead fine roots, lysates, and gases, such as CO_2 (by root and microbial respiration) and ethylene (Cheng and Gershenson 2007). As a result, the rhizosphere soils are regarded as *mesotrophic*, favoring the microbial growth (bacteria, fungi, archaea, and viruses), and the bare soils are described to have *oligotrophic* environments (Dessaux et al. 2016). This chemically unique and complex environment supports the growth of remarkably diverse and unique microbial populations.

The rhizo-microbiome composition is complex and dynamic, controlled by several biotic and abiotic factors. The abiotic factors include the physicochemical properties of soil and environmental parameters, whereas biotic factors include the chemicals secreted by bacteria and plant together with their biological activities (Haldar and Sengupta 2015). The root exudate chemistry dictates the rhizosphere microbial communities (Ahmad et al. 2018). The rhizo-microbiome mediates interactions via the production and secretion of signaling molecules by both plants and microbes.

The signaling in the rhizosphere can be divided into three groups:

Microbe-microbe (via quorum-sensing molecules like N-acyl homoserine lactones (AHLs), diketopiperazines (DKPs), and diffusible signal factor (DSF).

The second group includes *plants to microbe* (via plant-secreted molecules, e.g., root exudates).

The third group contains *microbes to plants* (via microbially produced compounds like lipopolysaccharides, peptidoglycans, flagellin, and chitin).

This signaling between plants and rhizosphere microbes resulted in shaping the rhizo-microbiome, inducing systemic resistance (by priming) sustaining plant health, growth, nutrition, and stress tolerance (Venturi and Keel 2016).

3.5 PGPM Affect Root Growth and Development

Soil microbial communities are recognized to play crucial roles in agricultural and natural ecosystems. Their activities have a positive impact on chemical, biological, and physical soil properties (Levy et al. 2018). The rhizo-microbiome also depends upon the soil type and the composition of root exudates (like organic acids, sugars, amino acids, enzymes, fatty acids, phenolics, coumarins, anthocyanins, and flavonoids) secreted by the host plant (Chaparro et al. 2013; Badri and Vivanco 2009). The ability of rhizobacteria to colonize rhizosphere depends on their chemotactic response toward root exudates. This chemical communication among plants and rhizo-microbes results in altered microbial community structure, plant health, and growth. For example, plant roots exude rosmarinic acid which stimulates *quorum-sensing* response, influencing bacterial population in the rhizosphere (Corral-Lugo et al. 2016). Additionally, *salicylic acid* influences the colonization of specific bacterial families within the roots, thereby altering the microbial community structure (Lebeis et al. 2015). The beneficial rhizosphere microbes include PGPR, PGPF, and protozoa that have been reported for their positive effects on plant growth and development (Mendes et al. 2013, Weidner et al. 2017). The PGPR affect root system architecture (temporal and spatial distribution of roots in soil) by altering the cell division and differentiation (in primary root), thereby affecting root hair formation and lateral root development (Verbon and Liberman 2016).

Several PGPR species have been identified to increase lateral root formation and shoot growth and inhibit primary root growth (by decreasing the cell elongation) of plants. Some PGPR species are shown to induce cell division and differentiation at both the root apical meristem and lateral root emergence sites. The cell division is positively or negatively affected depending upon the type of species within the meristem. For example, *Pseudomonas simiae* WCS417 increases cell division, whereas *Bacillus megaterium* decreases cell division and growth conditions. The differentiation is induced close to the root tip in PGPR-inoculated plants, due to which root hairs emerge close to the root tip. As a result, root hair density and length increases upon colonization. Thus, rhizo-microbiome affects root growth and development by manipulating the host endogenous mechanisms by regulating postembryonic root development (Verbon and Liberman 2016).

3.6 Microbes in Crop Production

The plant health depends upon the interactions between living organisms and their environment. Both plants and microbes, the components of rhizosphere can be engineered, and the soil can also be amended to promote growth and development (Dessaux et al. 2016). Genetic engineering of crop plants has resulted in pathogen resistance, high metal concentration resistance, etc. In contrast, there are few reports of PGPR engineering to render it more effective, for example, a chitinase gene (isolated from *Bacillus subtilis*) was inserted into *Burkholderia vietnamiensis*, a PGPR, to suppress Fusarium wilt (cotton), sheath blight (wheat), and gray mold (tomato) (Zhang et al. 2012). A recent method involves engineering of set of microbial population rather than single strain. Alternate way consists of ecological engineering (plant-microbe interaction). In general, plants and their associated microbes are considered as a holobiont or superorganism rather than as "individual" (Dessaux et al. 2016). The microbes play a crucial role in plant adaptation to changing environments. The holobiont paradigm in plant world is transforming our understanding (Vandenkoornhuyse et al. 2015).

Plant and microbial engineering by modern techniques such as transgenic production involves several environmental and ethical issues. Emerging trend is the application of microbial formulations as an excellent alternative to agrochemicals. These microbial inoculants can substantially lessen the use of inorganic fertilizers and pesticides in agricultural crops, thereby enhancing the nutrient uptake and stimulating growth and protection against phytopathogens (Ahmad et al. 2018). The PGPM play a vital role in agricultural systems (Table 3.1). They increase the uptake of primary nutrients (*biofertilizers*), produce phytohormones (*phytostimulators*), and suppress diseases or phytopathogens (*biopesticide*) enhancing plant growth and development (Trabelsi and Mhamdi 2013). Different microbial inoculants are already commercialized and used for several crops (Table 3.2).

3.7 Microbial Formulations and Application

Many pot and field studies have shown that plants inoculated with PGPM stimulate growth and yield. The microbial formulations are defined as the preparations of single or consortia strains of known microbes in a user-friendly and organic or inorganic carrier material. The specific number of cells (differs among species, e.g., 10^6–10^7 cells/plant of *Azospirillum brasilense*) is needed to reach the threshold to obtain the anticipated response in plants (Bashan et al. 2014). Various kinds of bioformulations being used in agriculture include nitrogen fixers, potassium (K) and phosphorus (P) solubilizers and mobilizers, growth-promoting AM fungi and cyanobacteria, and other useful microbes (Table 3.3). The bioformulation thus includes the desired microbe, suitable carrier material, sticking agents, and osmoprotectant (Sahu and Brahmaprakash 2016). The development of PGPM-based formulations with multifarious PGP and biocontrol activity with improved shelf life could pave the way for its commercialization. They provide a suitable microenvironment, physical protection, and structure to the introduced microbes. The development of techniques for mass multiplication of pure inoculants would offer a potential solution for allowing extensive use of biofertilizers. The main advantage of PGPM-based formulations is the choice of desired microbial formulation, the carrier material selection, and delivery methods (Zayed 2016).

3.7.1 Selection of Appropriate Microbes

The development of successful PGPM formulation is a multistep process, which starts with the isolation of beneficial microbes from plants, in vitro screening, characterization of PGP, and antagonistic activities, followed by its testing in greenhouse and field. The development process varies depending on the microbial group (bacteria, fungi, yeast, viruses, and nematodes) used for bioformulation. For example, bacteria and yeast are produced by liquid fermentation, whereas fungi are

Table 3.1 Different microbes being reported as biocontrol agents, biofertilizers, and phytostimulators

Genus	Species
Bacteria	
Acinetobacter sp.	*A. lwoffii, A. baumannii, A. calcoaceticus*
Aneurinibacillus sp.	*A. aneurinilyticus, A. terranovensis, A. migulanus, A. danicus*
Arthrobacter sp.	*A. protophormiae, A. pokkalii, A. agilis*
Azospirillum sp.	*A. brasilense, A. lipoferum, A. amazonense*
Azotobacter sp.	*A. salinestris, A. chroococcum, A. beijerinckii, A. paspali, A. armeniacus, A. nigricans, A. salinestri*
Bacillus sp.	*B. subtilis, B. amyloliquefaciens, B. pumilus, B. mojavensis, B. velezensis, B. thuringiensis, B. licheniformis, B. cereus, B. safensis, B. methylotrophicus, B. megaterium, B weihenstephanensis, B. edaphicus, B. pantothenticus, B. subtilisformis, B. circulans, B. altitudinis, B. simplex, B. firmus, B. pasteurii, B. mycoides, B. sphaericus, B. brevis, B. coagulans, B. mucilaginosus*
Brevibacterium sp.	*B. halotolerans, B. iodinum, B. linens, B. frigoritolerans*
Burkholderia sp.	*B. pyrrocinia, B. cepacia, B. ambifaria, B. phytofirmans, B. phymatum*
Cellulosimicrobium sp.	*C. funkei, C. cellulans, C. terreum*
Chryseobacterium sp.	*C. indologenes, C. hispalense, C. cucumeris, C. elymi*
Enterobacter sp.	*E. aerogenes, E. cloacae, E. radicincitans, E. sakazakii, E. agglomerans*
Klebsiella sp.	*K. pneumonia, K. oxytoca*
Lysobacter sp.	*L. antibioticus, L. enzymogenes*
Novosphingobium sp.	*N. oryzae, N. pentaromativorans*
Ochrobactrum sp.	*O. anthropi, O. cytisi, O. intermedium*
Paenibacillus sp.	*P. polymyxa, P. mucilaginosus, P. illinoisensis, P. brasilensis, P. oenotherae, P. hemerocallicola, P. graminis, P. odorifer, P. expansum, P. azotofixans, P. macerans, P. peoriae*
Pantoea sp.	*P. agglomerans, P. dispersa, P. ananatis*
Paraburkholderia sp.	*P. phytofirmans, P. kururiensis, P. fungorum, P. tropica*
Pseudomonas sp.	*P. putida, P. fluorescens, P. aeruginosa, P. stutzeri, P. protegens, P. chlororaphis, P. brassicacearum, P. nitroreducens, P. geniculate, P. jesenii, P. migulae, P. tolaasii, P. picketti, P. savastanoi, P. cepacia, P. corrugate, P. striata, P. marginalis, P. oryzihabitans, P. gessardii, P. synxantha*
Sinorhizobium sp.	*S. meliloti, S. fredii, S. kostiense*
Serratia sp.	*S. marcescens, S. proteamaculans, S. nematodiphila, S. liquefaciens, S. plymuthica*
Sphingomonas sp.	*S. paucimobilis*
Stenotrophomonas sp.	*S. maltophilia, S. acidaminiphila*
Rhizobium sp.	*R. pusense, R. leguminosarum, R. tropici, R. etli, R. phaseoli, R. trifolii, R. japonicum, R. lupine, R. meliloti*

(continued)

Table 3.1 (continued)

Genus	Species
Fungus	
Acremonium sp.	*A. strictum, A. zeae*
Arbuscular mycorrhizal (AM) fungi	*Rhizophagus intraradices, Funneliformis mosseae, Glomus intraradices, Glomus mosseae, Gigaspora* sp., *Acaulospora* sp., *Scutellospora* sp., *Sclerocystis* sp.
Beauveria sp.	*B. bassiana, B. brongniartii, B. araneola*
Aspergillus sp.	*A. terreus, A. niger, A. aculeatus, A. oryzae, A. nidulans, A. fumigatus, A. flavus, A. versicolor, A. awamori*
Chaetomium sp.	*C. globosum*
Clonostachys sp.	*C. rosea, C. solani, C. rhizophaga*
Isaria sp.	*I. fumosorosea, I. javanica, I. poprawskii, I. farinosa*
Metarhizium sp.	*M. brunneum, M. robertsii, M. anisopliae*
Purpureocillium sp.	*P. lilacinum*
Penicillium sp.	*P. simplicissimum, P. bilaii, P. vermiculatum, P. expansum, P. citrinum*
Syncephalastrum sp.	*S. racemosum*
Talaromyces sp.	*T. flavus, T. wortmannii, T. pinophilus*
Trichoderma sp.	*T. viride, T. asperellum, T. harzianum, T. atroviride, T. polysporum, T. koningiopsis, T. gamsii, T. virens, T. longibrachiatum, T. hamatum, T. reesei, T. citrinoviride, T. brevicompactum, T. koningii, T. arundinaceum, T. ovalisporum*

produced by solid-state fermentation technology. The viruses and nematodes (possessing PGP traits) are scaled up by means of their alternate host or tissue culture method (Gopalakrishnan et al. 2016). It is important to select multiple compatible consortia forming beneficial associations with rhizo-microbiome, thus having a better chance to survive and provide multiple benefits to the host plant/crop, as compared to the single-strain bioformulations (Singh and Trivedi 2017; Wallenstein 2017). The PGPM formulation should possess:

1. High rhizosphere competency
2. Ability to enhance the plant growth
3. Highly competitive saprophytic ability and be more efficient
4. The ease of mass production or multiplication
5. The broad spectrum of action
6. Reliable control
7. Environmentally friendly and compatibility with other rhizobacteria
8. The ability to tolerate heat, desiccation, oxidizing agents, and UV radiations (Nakkeeran et al. 2005)

Table 3.2 Plant growth-promoting microorganisms, their application, and effect on different crops

Crops/plants	Microbe	Growth condition	Effect on plant	Application mode	References
Rice (*Oryza sativa*)	*Enterobacter* sp.	Growth room (under salt stress)	Seedling growth	Seed treatment (10^8 cfu/ml)	Sarkar et al. (2018)
	Bacillus spp.	Field	Enhanced yield and control blast diseases	Bacterial culture suspension (8×10^9 cfu/ml)	Rais et al. (2018)
	Bacillus amyloliquefaciens RWL-1	Growth chamber (under salinity stress)	Increased growth attributes, increased essential amino acids	Bacterial culture suspension (20 ml to root zone)	Shahzad et al. (2017)
	Bacillus amyloliquefaciens RWL-1	Growth chamber	Higher endogenous salicylic acid production	Bacterial culture suspension (2 ml to root zone)	Shahzad et al. (2016)
	Pseudomonas fluorescens	Field	Increase in seed germination, seedling vigor characters, yield	500 g/fed and 1000 g/fed (powder inoculation) for nursery and soil, respectively, and 1 liter fed^{-1} of liquid for foliar spray	Elekhtyar (2015)
Wheat (*Triticum aestivum*)	*Pseudomonas* sp. P34	In vitro	Promote root growth and dry matter accumulation	Bacterial culture suspension (10^9 cfu/ml)	Liu et al. (2018a, b)
	Aneurinibacillus aneurinilyticus, *Aeromonas* sp., and *Pseudomonas* sp.	Pot	Increased root and shoot length, increased germination and weight (fresh and dry) of plant	Seed treatment	Kumar et al. (2018)
	Bacillus sp. and *Brevibacterium halotolerans*	Pot	Increase plant growth-promoting traits and tolerate salt stress	Seed treatment (2.5×10^7 cells/seed)	Ansari and Ahmad (2018)
	Arthrobacter protophormiae, Dietzia natronolimnaea and *Bacillus subtilis*	Hydroponic (under salinity and draught)	Confer abiotic stress tolerance (salt and drought), improved growth	Seedling treatment	Barnawal et al. (2017)

	Bacillus megaterium, *Bacillus safensis*, *Enterobacter aerogenes*	Pot and field	Increased dry weight of root and shoot, increased length and seed weight	Seed treatment (10^7 cfu/ml, coated in carrier material)	Mukhtar et al. (2017)
	Bacillus megaterium, *Arthrobacter chlorophenolicus*, *Enterobacter* sp.	Pot and field	Increased grain and straw yield, plant height, nutrient acquisition, and micronutrient (Fe, Cu, Mn, and Zn) content in grain	Seed treatment (10^7 cfu/ml)	Kumar et al. (2014)
Maize	*Pseudomonas* sp., strain DSMZ 113134	Field	Increased Mg, K, and S contents	Soil treatment (pdx 22.7 kg/ha)	Holečková et al. (2018)
	Azospirillum spp.	Pot (under drought condition)	Enhanced drought tolerance and improved root and shoot gr	Seedling treatment (5×10^7 cfu/ml)	García et al. (2017)
Barley (*Hordeum vulgare* L.)	*Bacillus amyloliquefaciens*	Pot	Increased plant growth and tolerate salt stress	Seed treatment	Kasim et al. (2016)
	Curtobacterium flaccumfaciens	Pot (under salinity stress)	Increased plant growth	Seed treatment (10^7–10^8 cfu/ml)	Cardinale et al. (2015)
Chickpea (*Cicer arietinum* L.)	*Rhizobium pusense*, *Paraburkholderia*, *Stenotrophomonas maltophilia*	Greenhouse	Improved nodulation, N fixation, PGP, and yields	Seed treatment (10^8 cfu/ml) and soil drench (5 ml of 10^8 cfu/ml)	Gopalakrishnan et al. (2018)
	Streptomyces	Pot	Increased growth and host-plant resistance induction against *Botrytis cinerea* (when co-inoculated with *Mesorhizobium ciceri*)	Seed treatment (10^8 cfu/ml) and soil drench (5 ml of 10^8 cfu/ml)	Vijayabharathi et al. (2018)
	Pseudomonas geniculate, *Chryseobacterium indologenes*, *Stenotrophomonas*, *Pantoea dispersa*	Field	Improved nitrogen fixation, plant growth, and yield enhancements	Seed treatment (10^8 cfu/ml) and soil drench (5 ml of 10^8 cfu/ml)	Gopalakrishnan et al. (2017)

(continued)

Table 3.2 (continued)

Crops/plants	Microbe	Growth condition	Effect on plant	Application mode	References
Phaseolus vulgaris	*Cellulosimicrobium funkei* KM032184	Greenhouse (under heavy metal toxicity (chromium VI)	Enhanced seed germination, increased shoot and root length, total biomass, chlorophyll a, b, total chlorophyll, and carotenoid content	Seed treatment (10^8 cells/ml)	Karthik et al. (2016)
Oat *(Avena sativa)*	*Klebsiella* sp.	Hydroponic condition (under salt stress)	Improved shoot and root length, dry weight (root and shoot), and relative water content	Seed treatment (10^8 cfu/ml)	Sapre et al. (2018)
Soybean	Arbuscular mycorrhizal (AM) fungi	Pot (drought condition)	Increased growth	20 g of soybean root fragments, spores, and mycelia	Salloum et al. (2017)
	Pseudomonas putida H-2-3	Greenhouse (salt and drought stress)	Enhanced shoot length and fresh weight, chlorophyll content, abscisic acid, salicylic acid	Bacterial culture suspension (5 ml of 10^8 cfu/ml)	Kang et al. (2014)
Groundnut *(Arachis hypogaea L.)*	*Pseudomonas* sp.	Field	Increased in pod yield and reduces disease	Bacterial culture suspension	Le et al. (2018)
	Bacillus licheniformis A2	Pot (under saline soil condition)	Increased fresh biomass, shoot and root length	Soil treatment (talc-based bioformulation 0.5 g/kg)	Goswami et al. (2014)
Mung bean (*Vigna radiata*)	*Pseudomonas aeruginosa* and *Bacillus subtilis*	Pot	Enhanced root and shoot length, fresh and dry weight of root and shoot, leaf area, and chlorophyll content	Seed treatment	Kumari et al. (2018)
	Pseudomonas fluorescens MC46	Pot	Plant growth and health restoration, enhanced soil enzyme activities, rhizoremediation (triclocarban)	Formulated bacterial inoculant (saw dust) (4×10^9 cfu/g)	Sipahutar et al. (2018)

	Candida sp. AVGB4	In vitro	Increased growth, shoot and root biomass, rhizoremediation	Soil mixing (10^8 cfu/ml)	Silambarasan and Vangnai (2017)
Apple	*Pseudomonas putida*, *P. fluorescens*, *P. aeruginosa*	Field	Promoted plant growth, number of nodes, branches and chlorophyll content	Bacterial culture suspension (10^9 cfu/ml)	Sharma et al. (2017a, b)
Vigna radiata and *Glycine max* (L.)	*Ochrobactrum* sp. MC22	Growth chamber	Restored the damage to plant structure and root system, rhizoremediation (triclocarban)	Bacterial culture suspension ($2.9 \pm 0.3 \times 10^8$ cfu/ml)	Sipahutar and Vangnai (2017)
Alfalfa (*Medicago sativa*)	*Bacillus megaterium*	Growth chamber	Promoted plant growth	Bacterial culture suspension	Chinnaswamy et al. (2018)
Rapeseed (*Brassica napus*)	*Bacillus*, *Serratia*, *Arthrobacter*, and *Pantoea*	Field	Increased plant growth and yield	Bacterial culture suspension (10^9 cfu/ml)	Valetti et al. (2018)
Fenugreek (*Trigonella foenum-graecum*)	*Sinorhizobium meliloti*, *Pseudomonas fluorescens*	Pot	Improved leaf area, shoot fresh and dry weight, nitrogen, phosphorus and potassium content, and water use efficacy (WUE)	Seed treatment	Bolandnazar et al. (2018)
Amaranth (*Amaranthus hypochondriacus*)	*Bacillus* sp.	Field	Enhancement of essential amino acid (methionine, lysine, and tryptophan) contents and other nutritive chemical constituents' grains	Seed treatment (talc formulation 8.0 g/kg seeds)	Pandey et al. (2018)
Lentil (*Lens culinaris* L.)	*Bacillus cereus* and *Bacillus safensis*	Pot	Increased plant growth and suppresses *Alternaria* leaf spot and blight diseases	Bacterial culture suspension (5×10^6 cfu/ml)	Roy et al. (2018)
Ginger (*Zingiber officinale* Rosc.)	*Bacillus amyloliquefaciens* and *Serratia marcescens*	Green house and field	Enhanced sprouting, suppressed soft rot incidence and increased rhizome yield	Seed treatment (10^{10} cfu/ml) and soil drench (10^8 cfu/ml)	Dinesh et al. (2015)

(continued)

Table 3.2 (continued)

Crops/plants	Microbe	Growth condition	Effect on plant	Application mode	References
Pepper (*Capsicum annuum* L.)	*Bacillus velezensis*	Greenhouse	Promoted seedling growth and induced systemic resistance (ISR) pepper gray mold disease	Bacterial culture suspension (10^8 cfu/ml)	Jiang et al. (2018)
	Serratia nematodiphila PEJ1011	Growth chamber (under cold temperature stress)	Increased endogenous ABA levels	Bacterial culture suspension (40 ml of 10^8 cfu/ml twice)	Kang et al. (2015)
	Bacillus amyloliquefaciens	Greenhouse	Increased plant growth and suppresses anthracnose disease	Seed treatment (25 ml of 1×10^8 cfu/ml)	Gowtham et al. (2018)
Tomato (*Lycopersicon esculentum*)	*Pseudomonas stutzeri* E25 and *Stenotrophomonas maltophilia*	Pot	Higher shoot and root length, chlorophyll content, and total fresh weight of tomato	Bacterial culture suspension applied every week	Rojas-Solís and Santoyo (2018)
	Bacillus amyloliquefaciens subsp. *plantarum* strain 32a	Growth chamber	Promoted plant growth and reduces crown gall symptoms	Bacterial culture suspension (10 ml of 10^8 cfu/ml)	Abdallah et al. (2018)
	Bacillus altitudinis, Bacillus velezensis	Greenhouse	Increased plant growth and biocontrol against multiple plant diseases	Seed treatment (1 ml of 10^6 cfu/ml)	Liu et al. (2018a, b)
	Pseudomonas strains	Pot	Stimulate plant growth, increase yield, enhanced antioxidant enzyme activities and proline concentrations	Seed treatment (10^7–10^8 cells/ml)	Egamberdieva et al. (2017)
	Sphingomonas sp. LK11	Pot	Increased growth attributes	Bacterial culture suspension (50 ml of 10^8 cfu/ml)	Khan et al. (2017)
	Bacillus subtilis LK14	Pot	Increased shoot and root biomass and chlorophyll (a and b) contents	Bacterial culture suspension (5 ml of 10^8 cfu/ml)	Latif Khan et al. (2016)

Radicchio (*Cichorium intybus* L.)	*Pseudomonas* sp. and *Bacillus* sp.	Pot	Increased aerial parts and root dry biomass	Bacterial culture suspension (50 ml of 10^6 cfu/ml)	Stanojković-Sebić et al. (2018)
Lettuce	*Pseudomonas fluorescens*	Greenhouse	Increased shoot dry mater (SDM) and shoot N, P, Ca, Mg, Mn, and Na uptake rates	Seed treatment (2 ml of 10^7 cfu/ml)	Khosravi et al. (2018)
	Pseudomonas nitroreducens strain IHB B 13561	Growth chamber	Improved plant growth	Bacterial culture suspension (5 ml of 10^5 cfu/ml)	Trinh et al. (2018)
	AM fungi	Pot	Improved plant growth	Seed treatment (720 propagules/g)	Konieczny and Kowalska (2016)
Alemow (*Citrus macrophylla*)	*Pseudomonas putida* KT2440 or *Novosphingobium* sp. HR1a	Pot (under salinity stress)	Decreased the production of abscisic acid and salicylic acid, better plant performance	–	Vives-Peris et al. (2018)
Tobacco (*Nicotiana tabacum*)	*Pseudomonas fluorescens SS101*	In vitro and in planta	Enhanced plant growth	Bacterial culture suspension (10^7 cfu/ml)	Park et al. (2015)
Carrot (*Daucus carota*)	*Pseudomonas fluorescens* Pf	Field	Increased yield and suppress *Meloidogyne hapla* (root-knot nematode)	Seed treatment (100 ml/kg)	Seenivasan (2018)

3.7.2 Selection of Carrier Materials

In bioformulation development, carrier comprises the major portion of the inoculant (by volume or weight). It is used to deliver the PGPM (or active ingredient) in suitable physiological condition. The carriers include the following categories (Bashan et al. 2014): *soils* (coal, clays, peat, and inorganic soil), *plant waste materials* (composts, farmyard manure [FYM], wheat bran, press mud, spent mushroom compost, plant debris, etc.), *inert carrier materials* (ground rock phosphate, talc, vermiculite, perlite, etc.), *lyophilized microbial cultures and oil-dried bacteria* (these can be used as such or can be incorporated into a solid carrier), and *liquid inoculants* (like emulsions, oils, and broth). The carrier helps in protection and stabilization of cells during storage and transportation to the target site. These can be organic, inorganic, or synthesized from specific molecules. The desirable characteristics of an ideal carrier with organism (bioformulation) include (Bashan et al. 2014; Sahu and Brahmaprakash 2016):

1. Increased shelf life and stability (5–30 °C).
2. Deliver appropriate number of viable cells.
3. Cheaply and nearly sterilized to deliver the appropriate microbe.
4. It should be chemically and physically uniform.
5. It should be suitable for numerous microbes and must have high water-holding capacity.
6. It should be eco-friendly, i.e., nonpolluting, biodegradable, and nontoxic.
7. It should not be phytotoxic to the crop plants.
8. It should be well dissolved and release active component in water.
9. It should be able to tolerate adverse environmental conditions.
10. It should be able to work in diverse field conditions and soil types.
11. It should be cost-effective and compatible with agrochemicals.
12. It should be easily manufactured, and carrier material must be cheap and easily available.
13. It should be able to improve soil properties and resist pH changes during storage.
14. Its release in entrapped formulation should not be too fast or too slow.
15. It should complete the BIS norms for biofertilizers.

3.7.3 Application/Delivery Methods

The bioformulations come in various dispersal forms such as dry products (dusts, granules, and wettable powders), liquid products (oil, water, and emulsions), and slurry and microencapsulation (in polymeric matrix). The use of different bioformulations depends on the need of the type of crop, choice of farmers, market availability, and cost (Bashan et al. 2014). They can be readily delivered through *soil*, *seed*, *rhizomes*, *setts*, and *foliage* or through the combination of these methods (Nakkeeran et al. 2005). The seed inoculation/treatment uses the cell suspensions of specific microbe or the bacteria incorporated in dry products that can grow in

Table 3.3 Some of the commercially available PGPM products used in different countries

Product	Microbe	Crop	Company/country	Function	References
BioYield	*Bacillus amyloliquefaciens* IN937a and *B. subtilis* GB03	Tomato	Gustafson Inc. USA	Management of soilborne pathogens and suppression of *Meloidogyne incognita*	Xiang et al. (2018)
Serenade®	*Bacillus amyloliquefaciens* QST713	Maize and soybean	Bayer Crop Science, Thane, India	Increase in plant growth and protection against pathogens/ pests	Mendis et al. (2018)
VOTiVO®	*Bacillus firmus* I-1582	Maize and soybean	Bayer Crop Science, Thane, India	Promote plant growth and offer protection against pathogens/ pests	Mendis et al. (2018)
Organo,	*Bacillus* spp., *Pseudomonas, Enterobacter* spp., *Stenotrophomonas, Rhizobium*	Wide variety of crops	Amka Products (Pty) Ltd, South Africa	Solubilize P and K, produce phytohormones and siderophore	Raimi et al. (2017)
RhizoVital42	*Bacillus velezensis* FZB42	Beet, carrot, cucumber, pepper, potato, radish, squash, tomato, and turnip	ABiTEP GmbH Inc., Berlin, Germany	Promoting plant growth	Meng et al. (2016)
Onix®	*Bacillus methylotrophicus*	Carrot	Farroupilha's group Patos de Minas, Brazil	Increased plant growth and production	Clemente et al. (2016)
Rizos®	*Bacillus subtilis*	Carrot	Farroupilha's group Patos de Minas, Brazil	Increased plant growth and production	Clemente et al. (2016)
Quartz®	*Bacillus methylotrophicus*	Carrot	Farroupilha's group Patos de Minas, Brazil	Increased plant growth and production	Clemente et al. (2016)
Mazospirflo-2	*Azospirillum brasilense* AL	Soybean and maize	Soygro Ltd, South Africa	Enhanced N uptake in maize	Laditi et al. (2012)
PHC Biopak	*Bacillus* spp. and *Paenibacillus azotofixans*	Soybean and maize	Plant Health Product (Pty) Ltd. South Africa	Enhanced nodule mass	Laditi et al. (2012)
Ecomonas	*P. fluorescens* @ 10 g/l	Rice	PJ Margo Pvt. Ltd	Increase in yield and management of sheath blight (caused by *Rhizoctonia solani*)	Kumar et al. (2009)
Florezen P	*P. fluorescens* @ 2.5 g/l	Rice	Bab India Private Limited, Hyderabad, India	Increase in yield and management of sheath blight	Kumar et al. (2009)

association with plant roots. For example, the seed treatment with *Pseudomonas fluorescens* at the rate of 100 ml/kg of carrot (*Daucus carota* subsp. *sativus*) seeds led to increase in yield and suppress root-knot nematode (Seenivasan 2018). The soil inoculation with solid or liquid bioformulations is more convenient because of the less time required for application. In this regard, direct soil delivery of PGPM will elevate the population dynamics of augmented microbes in plant rhizosphere.

3.8 Conclusion

The conventional agriculture depends on the use of agrochemicals which is mainly exploited to increase the crop yield. It has a profound negative effect on the environment leading to pollution and degradation of natural habitats. The use of PGPM is a promising approach for sustainable and eco-friendly agriculture. The field application of bioformulation to crop plants is much less effective, mainly due to the varying climatic conditions and the type of carrier material. Therefore, the bioformulation efficacy needs to be enhanced through the usage of compatible mixture of PGPM rather than using a single agent. The development of bioformulation with more than one PGPM will ensure at least one of the mechanisms to function under field conditions. The bioformulation containing multiple strains will have the enhanced efficacy, reliability, and broad spectrum of action and can operate under variable environmental conditions. They are also involved in the remediation of pollutants and heavy metals from the soil and have a great potential to improve plant and soil health (Shelake et al. 2018).

The worldwide market for bioformulation has many products that have been commercialized for use in different crops. The development of new microbial bioformulations is a complex process. It requires competence and strong collaboration of experts in various fields. The product must be produced on a large scale, preserved, and formulated to ensure the biocompatibility. The production processes are patented before commercial use of the product. However, despite a huge number of patents, there are only a few products which have been registered for agricultural application (Timmusk et al. 2017). The future challenge is to produce more economic and improved mixed bioformulations at industrial scale with longer shelf life, increased effectiveness, and higher microbial count in varying field conditions.

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