

Microbe-based Inoculants: Role in Next Green Revolution

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Abstract

Increasing food demand, with growing population, has been a major concern throughout the globe. The aim can only be achieved with the onset of next green revolution being much defined by sustainable approaches. The past green revolution had its negative impact due to excessive use of agrochemicals contaminating the environment and further challenging the food security. Henceforth, designing the blueprint of next green revolution requires the application of effective and sustainable approaches which enhance the yield of plants while still maintaining the decorum of sustainability. In this regard, microbes have been concluded as the best players finding their roles in plant growth promotion and also stress management. Currently, there are several bacterial-, fungal-based inoculants available in the market along with genetically modified organisms, forming the base of upcoming green revolution. Thus, the future of sustainable agriculture is related to the efficiency and action of these microbes.

Keywords

 $\label{eq:microbial} \begin{array}{l} \mbox{Microbial inoculants} \cdot \mbox{Green revolution} \cdot \mbox{Environmental sustainability} \cdot \mbox{Plant} \\ \mbox{growth-promoting rhizobacteria} (PGPR) \cdot \mbox{Stress} \end{array}$

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9.1 Introduction

Agricultural productivity has always been a worldwide concern since centuries due to ever-growing population. The pressure to meet the demands of population and to lessen the problem of hunger led to the evolution of new scientific technologies through which advancement in agronomic practices was made possible in order to increase yield of crops (Pinstrup-Andersen and Hazell 1985). These improved mechanizations in scientific field contributed to the emergence of "green revolution" (GR) (Rena 2004). This revolution was also named as modern agriculture technology or seed-fertilizer-water technology (Das 2017). The extraordinary era of crop productivity evolved in 1966 and lasted till 1985, and the later two decades are known as post-GR period (Pingali 2012). New technologies that enabled an increase in agricultural productivity included better quality seeds, improved water supplies, and the use of fertilizers and pesticides for plant protection (García-Fraile et al. 2017; Arora 2018a). Professor Norman Borlaug, a plant pathologist and a wheat breeder, with the help of his associates developed a high-yielding variety of wheat, and later, for his contribution, he was awarded the Nobel Prize in 1970 (Dalrymple et al. 1974; Barker et al. 1985). This led to an increase of 5000-6000 kg production of wheat in Mexico in 1965. In other developed nations, the yield of products also increased manyfold between 1975 and 1995, e.g., there was an increment of 78% in cereals, 113% in fishing, 127% in beef, 331% in eggs, and 280% in poultry products (FAO 1998). This strategy was later adopted by many other developing countries. The establishment of over-expanding list of hybrid technologies in the form of genetically modified (GM) crops and discovery of Ti-plasmid in Agrobacterium tumefaciens by J Schell in 1974 was also noticeable which also set a milestone in advancement of GR (Zanden 1991). GM crops whose genetic code has been with desirable characteristics by introducing foreign genes have been very close to green revolution, and this technique emerged as a gene revolution (Pingali and Raney 2005; Jain 2010). In modern farming, agricultural biotechnology based on genetically engineered crops represents one of the major advanced technological innovations (Chern 2006). Some of the well-known examples of genetically engineered crops include Bt cotton, insect resistance varieties of eggplant, maize, and herbicide-tolerant crops of soybean, canola, and alfalfa (Brookes and Barfoot 2015).

In India, the advent of green revolution began in the mid of 1960s in the kharif season under the name 'High Yielding Varieties Program' (HYVP) with the main focus to introduce such variety of crops which could withstand the expanded use of fertilizers and pesticide (Sebby 2010). Kalyansona wheat (1967) and Jaya rice (1968) were first two modern varieties that kicked off the onset of green revolution in India (Janaih et al. 2005). With the implementation of transgenic crops and other improved varieties of crops, there was an undisputed increment in the production but at the cost of losing the sustainability of the ecosystem (Evenson and Gollin 2003). Excessive use of GM crops, inorganic fertilizers and pesticides, and plant protection chemicals in the past decades has led to a number of long-term environmental problems (Arora et al. 2012) including ecosystem degradation on a large scale and

health of cultivated land including degradation of soil, deforestation, greenhouse gas emissions, accumulation of chemical pesticides and fertilizers, groundwater pollution, and decrease in water table due to excessive irrigation (Tilman et al. 2002; Foley et al. 2011). The strategy of HYVP worked well in increasing the yield but failed to retain the genetic base resulting in loss of indigenous varieties as well as useful conventional agricultural practices (Shiva 1993). On the other hand, shift of traditional methods to monoculture technique is known to degrade quality of soil making it prone to soil erosion and hence rendering it unproductive (LaSalle and Hepperly 2008). The unprecedented use of fertilizers also increased drastically during this period which led to imbalance in the threshold of nitrogen (N), phosphorous (P), and potassium (K) contents and loss of other micronutrients in soil (Das and Mandal 2015). Estimates reveal that production of global N and P fertilizer increased by 8 and 3 times, respectively, during 1961 and increment of N/P ratio was observed by 0.8 g N g⁻¹ P per decade during 1961–2013. Global pesticide production was reported to increase at a rate of about 11% per year from 0.2 million tons in 1950 to 5 million tons by 2000 (FAO 2017; Carvalho 2017). These fertilizers and pesticides are also one of the greatest sources of greenhouse gases (GHGs) like nitrous oxide (N_2O) , methane (CH_4) , and carbon dioxide (CO_2) of which N_2O is 310 times more potent than CO₂ (IPCC 1996). Nitrogenous fertilizers like ammonium nitrate, calcium ammonium nitrate, urea, and urea ammonium nitrate are its major sources (Wood and Cowie 2004). On the other hand, phosphate fertilizers such as mono- and diammonium phosphates, single superphosphates, and tri-superphosphates result in CO₂ emissions (Davis and Haglund 1999), whereas NPK fertilizers were found to release CO₂ and N₂O, and most of the CO₂ was liberated during production of ammonia, and 100% N₂O was produced during nitric acid production (Kongshaug 1998). GHGs thus produced add to the adversities like global warming, air pollution, and climate change (Pardis and Devakumar 2014). Climate change gives rise to several other abiotic stresses like drought and flood and also contribute to salinization of the agricultural soils that intercepts growth and development of plants along with effects like nutrient imbalance (Tewari and Arora 2013), osmotic stress, toxicity of Na^+ and Cl^- , production of ethylene, adverse result on plasmolysis, and disturbance in soil fertility (Drew et al. 1990; Arora et al. 2018). In case of pesticides which are recalcitrant in nature and persist in the environment for centuries causing biomagnifications at trophic levels, such compounds are highly carcinogenic, and their toxicity gets accumulated in the soils deteriorating its biology. The crops planted in such soils absorb these toxic compounds adversely affecting human health leading to generation of diseases such as cancer, neurological imbalance, and infertility in humans (Zahn and Ward 1998; Adesemoye et al. 2017).

The increase in fertilizers and pesticides significantly decreased the biodiversity of different ecosystems (terrestrial and aquatic) and has rapidly increased rate of extinction since the last 50 years (Singh et al. 2016). Birds, fishes, and many other nontargeted species are often reported to be affected by pesticides contamination leading to their high mortality. In this way, it has resulted in the genetic erosion of the keystone species especially in case of microbial biodiversity (Aktar et al. 2009;

Arora 2018b). Microbes are sensitive to slight changes in the environment; hence, indiscriminate use of plant protectants deteriorates structure and functioning of microbes in soil leading to loss of important ecological processes such as biogeochemical cycling of nutrients (Hartman and Richardson 2013). It was reported by de Vries et al. (2006) that organic fertilization technique increases the bacterial/fungal biomass ratio in soil, and a vice versa effect was observed when inorganic fertilizers were used establishing the fact that sustainable agriculture practices favor microbial biomass. Nitrogen-fixing ability by free-living bacteria is known to decrease in presence of glyphosates in soil (Santos and Flores 1995). Similarly 2,4-Dichlorophenoxyacetic acid (2,4 D) reduces nitrogen fixation in cyanobacteria and is also known to affect their growth (Calgan and Sivaci-Güner 1993). Mycorrhizal fungi are also affected in similar ways and were reported to reduce in number in presence of triclopyr and oxadiazon (organic pesticides) (Chakravarty and Sidhu 1987: Moorman 1989). Many European countries have banned the use of such synthetic plant protectants, but still on large part of agricultural lands, intensive chemical inputs are being applied on crops causing several environmental issues (Baez-Rogelio et al. 2017).

To halt this loss, major efforts should be adopted which are organic in origin and, most importantly, which promise to ensure the sustainability of the environment. This could be achieved when farming practices would incline toward the use of biological agents in order to protect plants from pests and diseases, to enhance their growth, and, above all, to maintain an eco-friendly environment. In this regard, microbial inoculants are seen as a substantial example and as an excellent substitutes having capability to reduce the use of synthetic fertilizers and pesticides (Trabelsi and Mhamdi 2013). Soil is a complex biota and harbors dynamic varieties of microorganisms. Approximately 10¹⁰ bacterial species reside in a gram of soil along with approximately 4×10^3 to 5×10^4 species of other organisms (Torsvik et al. 1990; Roesch et al. 2007). Hence, soil is considered as a black box of microbial diversity (Prashar and Shah 2016). Among this huge diversity of microbiota reside certain microbial communities which are known to promote growth of plants by several mechanisms and are known as "plant growth-promoting microorganisms" (PGPM). They promote growth by acting as biofertilizers for the plants in order to provide nutrients such as growth regulators or by providing resistance to the plants from phytopathogens thus acting as biocontrol agents (Abhilash et al. 2016). In this way, microbial inoculants are the best alternatives to their chemical counterparts. Scientists from different parts of the world are already exploiting the use of these inoculants in different ways such as a single inoculum of bacteria, in combination with two or more different bacterial inocula or consortia of fungal and bacterial species (Malusá et al. 2012). Therefore use of such beneficial microorganisms as biocontrol agents and as biofertilizers could be a step toward "second green revolution" or more aptly the "era of evergreen revolution." In this chapter, the role of these bioinoculants in next green revolution as an approach to sustainable agricultural practice has been explained. In further sections, various types of bioinoculants such as bacterial, fungal, and other types of inoculants have also been discussed.

9.2 Beneficial Soil Microorganisms and Their Role in Next Green Revolution

A plant microbiome encompassing a lot of microbes in the soil is much similar to human microbiome and functions by lending a hand to nutrient absorption and protection from vicious and harmful microbes. Consequences and harmful side effects of using chemical fertilizers and pesticides in excessive amounts are already known. This also comprises of degradation in soil fertility, environmental pollution, disruption in the environment, and harmful effect on human health (Ayala and Rao 2002). Microorganisms are very well expected to pave way for the next agricultural green revolution and can be the great option for better and improved sustainable organic farming practices. A number of microbial bioformulations are commercially available throughout the globe providing effective way to eradicate chemical fertilizer and pesticides. Microbe-based bioinoculants gained attention mainly because of their eco-friendly nature and threats affiliated with chemical fertilizers. Considering the good impact of microbial inoculants in terms of nutrient supplementation, biofortification, biocontrol, and bioremediation, they need to be encouraged in the future for implementation in agriculture for a stable and productive agroecosystem. The mechanisms involving role of microorganisms in growth promotion in addition to microbial inoculants and their types have been discussed below in detail.

9.2.1 Nutrient Assimilation and Biofortification

Mineral elements including phosphorous (P), nitrogen (N), calcium (Ca), iron (Fe), zinc (Zn), and manganese (Mn) have the most pivotal role in all living organisms, and an appropriate balance is required to achieve proper growth and development of plants. Among these P and N are the critical and important macronutrients responsible for building of nucleotides, amino acids, and proteins. Micronutrients such as Ca, Mg, Zn, Mn, boron (B), molybdenum (Mb), and sulfur (S) are required in small quantities and play a significant role in plant metabolism by acting as cofactors in enzymatic reactions (López-Arredondo 2013). Microbial inoculants have emerged as the most reliable approach to assimilate nutrients and increase their bioavailability to plants and improve the fertility of soil (Rashid et al. 2016). Nutrient assimilation in plants is a complex cascade involving bacteria, fungi, protists, and animals (Müller et al. 2016). Diverse microbes are known to play noteworthy role in the acquisition of nutrients and favoring the plant health (Vessey 2003). PGPR thriving in the vicinity of roots increase the plant growth by enhancing the nutrient uptake through nitrogen fixation, phosphate solubilization and iron and zinc chelation through various mechanisms (Stamenković et al. 2018).

Nitrogen is an essential macronutrient needed in significant amount by plants for their growth, physiological and developmental processes, and metabolism, but often its limited availability renders the soil unhealthy for cultivation (Bouguyon et al. 2015). Although there is huge abundance of nitrogen in atmosphere, yet the availability of N₂ to plants is only around 50%, while 25% is exuded to atmosphere, 20%

runs off into water bodies, and only 5% persists in the soil (Postgate 1982; Galloway et al. 2008). The capability of microbes to fix nitrogen to ammonia has been used to overcome the excessive use of chemical N fertilizers. Both free-living and symbiotic nitrogen fixers are known to thrive in the rhizosphere. This capability to fix the nitrogen by converting elemental nitrogen into plant utilizable form (Gothwal et al. 2009; Kuan et al. 2016) is confined to some of the bacteria and few of methanogenic archaea (Bae et al. 2018), and the process is known as biological nitrogen fixation (BNF). Approximately 200 million tonnes of nitrogen is fixed annually by BNF (that is two thirds of nitrogen fixed globally) (Peoples et al. 2009; Gouda et al. 2018) which is a very cost-effective solution allowing the farmers to save millions of dollars which otherwise would be spent on chemical fertilizers. Symbiotic nitrogen fixation is an essential biological process which governs the sustainability of agriculture and maintains the nitrogen content of soil. Inside de novo organs called as nodules formed on leguminous roots, bacteria fix nitrogen with the aid of enzyme nitrogenase (Sulieman and Tran 2014). The nitrogen-fixing symbiosis initially involves molecular dialogue between the plants and microbes, involving signals predominantly flavonoids and isoflavonoids attracting the rhizospheric microbes toward plants. Endosymbionts like rhizobia (including Rhizobium, Bradyrhizobium, Sinorhizobium, and Mesorhizobium) take up these signals and bind to the transcriptional regulator NodD of conserved nod genes, inducing the cascade of releasing nod factors which later aid in infection stages and nodule formation in leguminous plants (Long 1996; Mus et al. 2016). Rhizobia possesses large genomes (about 10.5 Mbp), with multiple replicons for symbiosis (MacLean et al. 2007). Sinorhizobium meliloti and Medicago species endosymbiont has 3.65 Mbp chromosome and two megaplasmids, pSymA and pSymB (1.35 and 1.68 Mbp), majority of which carry symbiotic genes. With the starvation of nitrogen, Rhizobium-legume symbiosis is triggered selecting the specific *Rhizobium* partner from the population of bacteria in the rhizosphere (Maróti and Kondorosi 2014). The genera of Frankia member of actinomycete family are symbionts of non-leguminous plants often referred to as actinorhizal plants. Their successful symbiosis forms root nodules where the microbe provides nitrogen to plant in exchange of carbon. A tripartite symbiosis of actinorhizal plant-Frankia-mycorrhiza has also been reported which forms a much stable association and has the efficiency even in poor marginal soils (Dawson 2008). Cyanobionts including Nostoc are characterized by mechanism of nitrogen fixation including heterocysts (nitrogen-fixing cells), akinetes (spore-forming resting spores), and hormogonia (motile filaments), all of which lead to infection of plants for symbiotic nitrogen-fixing associations (Adams and Duggan 2008; Santi et al. 2013).

Phosphate is another limiting nutrient found in soil (Shi et al. 2013) as only the anionic form can be obtained and consumed by plants. In average soil, the normal content of phosphorous available to the plants is about 0.05% (w/w), out of which only 0.1% is procurable by the plants (Achal et al. 2007; Zhu et al. 2011). It has been reported that plants are unable to avail 80% of phosphorous applied to the soil and nearly 5.7 billion hectares land globally are deficient of phosphate naturally (Vassilev et al. 2006). In the last few years, attention has been given to the biological processes using phosphate-solubilizing microorganisms for making phosphorous

available to plants and soils (Fasim et al. 2002). These microbes regulate the organic transformation of the insoluble form to an assessable form of phosphate, i.e., orthophosphate, thus controlling immobilization and mineralization of phosphate occurring in the soil. The mechanism behind phosphate chelation involves synthesis of assimilating compounds including organic acids, hydroxyl ions, siderophores, protons, and CO_2 (Sharma et al. 2013). Organic acids like gluconic acid, aspartic acid, malonic acid, glycolic acid, glutamic acid, malic acid, oxalic acid, etc. along with hydroxyl ions or protons lower the pH and chelate cations releasing P by substitution of H⁺ for Ca⁺² (Goldstein 2000; Alori et al. 2017). Another mechanism involves direct exchange of H⁺ for cation uptake or through H⁺ translocation ATPase (Rodríguez and Fraga 1999). Enzyme-based mobilization includes via phytases and phosphatases (Calvo et al. 2014; Owen et al. 2015). Both fungi and bacteria are known to solubilize P in the soil. Arbuscular mycorrhizal fungi (AMF) through their extended radical hyphae are able to fix P from allochthonous sites (up to 8 cm) (Millner and Wright 2002; Smith and Read 2008). Gram-negative bacteria directly solubilize phosphorous through direct oxidation of glucose to gluconic acid (Goldstein 2000; Alori et al. 2017). These are important attributes found in phosphate-solubilizing bacteria (PSB) and AMF being used predominantly in inoculations (Fankem et al. 2006; Khan et al. 2007; Sharma et al. 2013).

Another vital nutrient needed for stabilized growth of the plant is potassium (K) on which variety of indispensable mechanisms are dependent directly or indirectly. It is involved in several key functions including energy transfer and activation of enzymes in many plant physiological reactions such as photosynthesis, protein synthesis, and starch synthesis, and it also helps in combating phytopathogens and disease resistance (Rehm and Schmitt 2002). The concentration of K in the soil differs extensively ranging from 0.04% to 3%, although 2.5% of lithosphere is depleted of K (Sparks and Huang 1985). Beneficial soil microbes including saprophytic bacteria, fungi, and actinomycetes enhance the fertility of soil and growth of plants through chelation of soluble K forms from insoluble complexes by various mechanisms such as synthesis of organic and inorganic acids and polysaccharides, acidolysis, complex reactions, chelations, exchange reactions, etc. (Meena et al. 2015; Saha et al. 2016a; Etesami et al. 2017).

Iron, the fourth most abundant element found in the Earth's crust, is needed by all the life forms. The availability of both forms, i.e., ferrous (II) and ferric (III), rely upon the oxygen level and pH in the soil. The concentration of iron in the soil ranges from 7000 to 500,000 mg kg⁻¹. The ferric form of iron has been found to preponderate in the soil, while plants can consume and uptake the ferrous ion form (Kobayashi and Nishizawa 2012). The activity of some microorganisms increases the concentration of ferrous ions in the soil including the rhizosphere. The mechanism behind Fe chelation involves lowering of the redox potential (Nikolic and Römheld 1999) commended by chelators known as siderophore, has been reported to show affinity toward Fe³⁺ and is known to be secreted by fluorescent pseudomonads (Schalk and Guillon 2013; Chen et al. 2016). Many studies favor that microbe-mediated uptake of Fe even at very

low concentration consequently enhances the agricultural productivity (Fageria et al. 2009; Saha et al. 2016b).

Micronutrient deficiency often termed as hidden hunger along with decrement in crop yields is a matter of serious concern nowadays, gaining attention worldwide and accounts for increased human mortality rate in conjunction with reduced socioeconomic development. The phenomenon of adding essential mineral elements to improve the nutritional quality of staple crops is known as biofortification which renders a sustainable eco-friendly solution to this issue of malnutrition. Biofortification technologies still need to go beyond these programs in the coming years for excelled and upgraded nutritional quality of food crops. Microorganisms are well known to be engaged in one or more direct or indirect mechanisms, and their function to improve the quality of food crops has gained attention worldwide (de Souza et al. 2015). There are many reports available which support the use of microorganisms as key biofortification agents resulting in improvement of bioavailability and concentration of micro and macronutrients content in various crops (Rana et al. 2012).

Zn is an influential micronutrient for all the living forms and is found in a range of 10–300 ppm in soil (Lindsay 1972). It is important for plants helping in photosynthesis, phytohormone activity, disease resistance, carbohydrate metabolism, gene expression, enzyme activity, seed production, protein synthesis, etc. (Jaivel et al. 2017). Approximately 50% of the soils are known to be deficient in zinc (Review 2008), and this scarcity may lead to inappropriate development of the plants (Alloway 2004). Rhizospheric microbes have shown mechanisms of zinc solubilization involving production of organic and inorganic acids and acidification, whereby they lower the surrounding pH and chelate Zn and also the anions trap Zn and enhance their solubility (Alexander 1977; Jones and Darrah 1994). Production of siderophores, protons and oxido-reductive reactions on cell membranes and trapped ligands also solubilize Zn (Wakatsuki 1995; Chang et al. 2005).

Manganese is another essential micronutrient that participates in the photosynthetic proteins and enzymatic structure. It influences the water splitting PS II complex that is known to supply electrons for photosynthesis, and thereby its deficiency can be detrimental for chloroplasts (Millaleo et al. 2010). The oxidized form of Mn (Mn^{4+}) is unavailable to plants, while the reduced form (Mn^{2+}) is utilized for plant processes (Rengel 2014). The reduction of Mn is chiefly based upon occurrence of electron-carrying reducing agents and presence of protons. Arbuscular mycorrhizal fungi (AMF) are known to facilitate Mn reduction making it available to the plants (Cely et al. 2016; Chen et al. 2017).

9.2.2 Combating Phytopathogens

Phytopathogens are known to be present with plants in the rhizosphere from the very genesis of agriculture. FAO (2015) reported 20–40% annual reduction of the global crop yield caused by pests and plant diseases. Application of chemical pesticides during green revolution had exacerbated the scenario by addition of xenobiotics such

as DDT and methyl parathion, which are still persisting in the soil as nondegradable moieties (Singh and Arora 2016). Thus, the concept of next green revolution cannot be enacted until this issue is mitigated through sustainable approaches. Microbial inoculants have shown influential results in sublimating phytopathogenic biotic stress and reclaiming the quality and quantity of agricultural production (Arora et al. 2007; Mishra et al. 2015). The offensive biocontrol strategies enabled by microbes include synthesis of allelochemicals, niche exclusion, and competition for nutrients. Antibiosis is the major biocontrol mechanism gaining popularity over the last two decades. Antibiosis operating cascade involves GacA/GacS or GrrA/GrrS, RpoD, and RpoS, N-acyl homoserine lactone derivatives for quorum sensing (Bloemberg and Lugtenberg 2001; Haas and Keel 2003). Antibiotics produced include pvoluteorin. pvrrolnitrin. tensin. tropolone. 2,4-diacetylphloroglucinol (DAPG), zwittermicin А, kanosamine, cvclic lipopeptides, oomycin A, DDR, viscosinamide, butyrolactones, N-BBS, pantocin A and B (Bhattacharyya and Jha 2012), etc. Microbes also cause biocontrol through production of cell wall-hydrolyzing enzymes such as chitinase, laminarinase, cellulase, protease or proteinase, and glucanase (Jadhav and Sayyed 2016). These enzymes show inhibitory activity against oomycetes and fungal sporulation and mycelial extension (Saraf et al. 2014). Similar to the regulatory mechanism of antibiotics, lytic enzymes also are controlled by GacA/GacS or GrrA/GrrS (Sacherer et al. 1994; Corbell and Loper 1995). Apart from these, microbes suppress the pathogens by producing toxic secondary metabolites such as HCN (Mishra et al. 2015; Tewari and Arora 2016), δ-endotoxins or Cry protein (Loper and Gross 2007; López-Pazos et al. 2009), exopolysaccharides (Tewari and Arora 2014a), biosurfactants (Banat et al. 2010), microbial components including homoserine lipopolysaccharides, lactones. flagella, and volatiles like acetoin and 2,3-butanediol (Lugtenberg and Kamilova 2009; Ahemad and Kibret 2014). δ-Endotoxins or Cry protein show toxic influence upon ingestion by various classes of insects/nematodes including Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera, Isoptera, Orthoptera, Siphonaptera, and Thysanoptera (Schünemann et al. 2014). Exopolysaccharides produced by beneficial microbes protect them from pathogens through their bio-filming ability, protecting in a hydrated and nutrient-rich local environment. Biosurfactants are amphiphilic low molecular weight surface-active compounds that are important for reducing surface tension at air/water interface and interfacial tension at oil/water interfaces (Sachdev and Cameotra 2013). Biosurfactants form channels in pathogens' cell wall and disturb their cell surface properties (Raaijmakers et al. 2006). Rhamnolipids and cyclic lipopeptides are the best-known biocontrol biosurfactant types (Debode et al. 2007). Another mechanism of biocontrol reported by microbes is triggering of induced systemic resistance (ISR) in plants. Triggered ISR in plants leads to increased cell wall protection; alteration of physiological and metabolic pathways to enhance synthesis of biocontrol-active compounds in plants, for example, phenolic compounds at the site of infection; and formation of structural barriers with heavy deposition of callose to restrict entry of pathogens. ISR also challenges the pathogen via higher accumulation of pathogenesis-related proteins (PR proteins) or even

peroxidase, phenylalanine ammonia lyase, phytoalexins, polyphenol oxidase, and/or chalcone synthase as defensive compounds (Compant et al. 2005). The other way of microbial biocontrol is nutrient chelation (like Fe), through production of siderophores which chelate the ferric ion, depriving the pathogenic fungi of the micronutrient as fungal siderophores show low affinity toward iron, also solubilization of phosphorous and zinc by beneficial microbes depriving the pathogens of these nutrients (Whipps 2001; Compant et al. 2005). Niche exclusion is another mechanistic approach of beneficial microbes in excluding the pathogens from the rhizosphere and inhibiting their pathogenesis (Pathak et al. 2017).

9.2.3 Amelioration of Abiotic Stress

Dynamics in climate has inflicted agriculture due to soil erosion, inception of marginal lands, loss of pioneer or keystone species, compromised quantitative and qualitative crop yield, and soil contamination and infertility. Catalyzing the scenario, anthropogenic activities have a menacing effect and result in and shrinking of fertile lands. The State of the World's Land and Water Resources for Food and Agriculture (SOLAW 2011) flagged the warning that almost 25% of the global land has been totally degraded, 8% being in the moderate, 38% stable or slightly, and 10% as improving. Coming up with eco-friendly strategy is the exigency to restrain the land loss and ameliorate the quality of soil. Microbial inoculations have emerged as a promising trend in uplifting the quality of soil in comparison to chemical fertilizers (Carvajal-Muñoz and Carmona-Garcia 2012).

Abiotic stresses are the major problems faced by the world limiting the productivity of agriculture. Various abiotic stresses include salinity, drought, flood, pH, and temperature which challenge the growth of plants and fertility of soil. Microbial inoculants have paved their way in ameliorating the aforementioned stresses ensuring the food security. Microbes influence the biological, physical, and chemical properties of soil by forming stable aggregates with pore spaces trapping water and nutrients under healthy and stressed conditions (Helliwell et al. 2014).

Salinity is a major global problem degrading 240,000 square miles of land, with loss of 7.7 square miles of land in arid and semiarid areas every day (Qadir et al. 2014). Saline conditions induce various physiological and biochemical changes in plants and render the soil infertile for cultivation. The adverse salinity symptoms include osmotic imbalance, reduced photosynthetic rate, inhibited seed germination, ethylene stress, disturbed microbial population and plant-microbe interactions, Na⁺ toxicity, Ca⁺², K⁺ deficiency, and oxidative stress due to reactive oxygen species (ROS) (Wang et al. 2016a, b; Negrão et al. 2017; Mishra et al. 2017). Microbes have shown tolerance against salinity and have been reported as ameliorators of stressed plants and soils. The initial stress-combating strategy includes efflux of Ca⁺², K⁺ by synthesis of compatible solutes/osmoregulators such as soluble sugars, amino acids and derivatives, and tetrahydropyrimidines (Arora et al. 2006; Fernandez-Aunión et al. 2010; Tewari and Arora 2013). Osmoregulators stabilize the osmotic balance across the membrane, maintain the turgor pressure, and ensure the correct folding of

proteins (Kim et al. 2014; Mishra et al. 2018). In addition, under saline conditions, EPS-producing bacterial inoculants encounter Na⁺ toxicity by immobilizing the ions stabilizing the soil ionic balance. Additionally, these biopolymers aggregate soil particles trapping water molecules, hydrating soil, and maintaining water retention capacity under salinity stress (Sandhya et al. 2009; Tewari and Arora 2014a, b). EPS-producing fungi are also responsible for mitigating stress constraints by entangling and enmeshing soil particles in macroaggregates formed through EPS adhesion or hyphal networks (Bossuyt et al. 2001). AMF modify the soil structure through production of a glycoprotein glomalin, adept at increasing hydrophobicity and forming soil aggregates with attachment sites and chelated nutrients (Rillig et al. 2002; Rillig and Mummey 2006; Wright and Anderson 2000). Ethylene stress in plants under saline conditions is mitigated through the synthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which acts as a sink to ACC (a precursor to ethylene), thus mitigating the senescence-related problems in plants (Saleem et al. 2018).

Drought has recently been a recurrent phenomenon due to increasing climate change and related anthropogenic activities. Drought restricts the growth of plants and fertility of soil leading to major productivity loss across the globe. Drought disturbs the plant water potential, impairs seed germination, stunted root and shoot growth, lowered water and nutrient content in plants and soils, disturbed C/N ratio, loss in population of beneficial microbes, etc. (Geng et al. 2017; Fahad et al. 2017). Microbial inoculants facilitate the amelioration of drought stress by production of cytokinin, antioxidants, ACC deaminase, EPS, and other growth-associated metabolites. Cytokinins increase the abscisic acid (ABA) content of plants which further closes the stomata to reduce foliar water loss (Ngumbi and Kloepper 2014). ACC deaminase acts in a way similar to that discussed in salinity stress. Antioxidants act in reducing the damaging effects of ROS, thus securing the cell, membranes, and biomolecules (Grover et al. 2011). EPS produced by microbes help in maintaining the hydrological balance of the soil through aggregation of soil particles trapping water molecules, thereby increasing nutrient uptake (Sandhya et al. 2009). Molecular mechanism behind the tolerance includes upregulation of marker drought response genes (Gagné-Bourque et al. 2015).

Flooding is another abiotic stress faced globally, negatively impacting the growth and yield of lands and plants. Waterlogging causes anoxia in the plant rhizosphere leading to stomatal closure, reduced photosynthesis, stunted growth, and shutdown of oxidative phosphorylation leading to anaerobic fermentation (Liao and Lin 1994). Inoculation with microbes has shown adaptive stratagem supporting growth of plants even under anoxic conditions. The tolerance mechanisms include synthesis of ACC deaminase to sink ethylene, use of rhizobia to support nodulation and nitrogenase activity as they can use nitrogenous oxides as terminal electron acceptor and enhance nodule formation, and trapping of nutrients where mycorrhizal associations have found to spread their mycelial extensions increasing the absorption power of roots (Harley and Smith 1983; Tewari and Arora 2016).

Global warming and related anthropogenic activities have altered the temperature of the earth with a much hotter climate and the temperature continuing to increase. With lesser rainfall, increasing temperature has given rise to the problem of heat stress. Under the stress condition, soils lose their water due to excessive evaporation and get prone to erosion by wind and water. Plants also show attenuated growth due to lowered water and nutrient uptake, impaired photosynthesis, and increased leaf senescence (Fahad et al. 2017). To promote growth of plants and to revive the fertility of soils, microbes have been a sustainable solution configuring the tolerance mechanism. Microbes induce production of osmoregulants to regulate the osmotic equilibrium across the membranes preventing plasmolysis and increased synthesis of heat shock proteins (HSPs) which could tolerate temperature stress and regulate the biological enzymatic mechanisms. HSPs like chaperons instruct the correct folding of proteins ensuring the proper enzymatic functioning of plants/microbes even under high temperature stress (Münchbach et al. 1999: Ali et al. 2014) and accumulation of trehalose to protect against thermal injury and fungi; trehalose inhibits protein denaturation and supports aggregation maintaining the normal conformation under stressed conditions. Contrasting to heat stress, cold stress is also a climatic extremity which adversely effects the growth of plants. At lower temperatures cellular metabolism of plants disrupts, destabilizing the membrane fluidity and nucleic acids leading to incorrect transcription, translation, and degradation (Phadtare 2004). Under such conditions, cold-tolerant microbes can be inoculated to counteract the adversity. Tolerance mechanisms of cold-tolerant microbes include increased concentration of unsaturated fatty acids in cell membrane to enhance the fluidity (Vorachek-Warren et al. 2002), cold shock proteins (CSPs) like chaperons ensuring the correct folding of proteins, normal RNA metabolism (Barria et al. 2013) and accumulation of trehalose (Li et al. 2009).

pH stress is another challenge which limits the productivity of agriculture. Extremities in pH lead to abnormal growth and respiration rate, loss of beneficial plant-microbe interactions, reduced chelation of nutrients, and loss of essential anions (Sakano 2001). Shift from normal pH shows substantial changes in gene expression and cell biochemistry mediated by pH-sensitive cellular signaling cascades (Arst and Peñalva 2003). Microbes initiate the adapting responses to support growth even under stressed environment. Halophilic or alkaliphilic bacteria get adapted to high alkalinity by retaining their cellular pH in the surrounding of 9–11. Controlling their metabolic activities, these alkaliphilic microbes use proton transfer systems in their cytoplasm to maintain osmotic balance and cellular vitality (Horikoshi 1999; Torbaghan et al. 2017). With the onset of pH stress, microbes start producing enzymes supporting their tolerance and growth. AMF extend their hyphae to absorb more nutrients and also protect the roots of plants securing from stress (Chen et al. 2006). Biofilming/flocculation (composed of EPS) is also a mechanism by which microbes form a local environment protecting from outer pH extremes.

9.3 Microbe-based Inoculant Types

Microbial inoculants contain agriculturally advantageous microorganisms which due to their plant growth-promoting attributes, better tolerance under adverse conditions, and eco-friendly nature (unlike their chemical counterparts) are playing significant roles in crop production in a sustainable way (Vessey 2003). A wide range of beneficial microbial diversity (*Bacillus*, pseudomonads, rhizobia, blue-green algae, Trichoderma, mycorrhiza, endophytes, etc.) are now being used as bioinoculants (Sarma et al. 2015; Egamberdieva et al. 2018). The indispensable properties of such green inoculants have now been commercialized successfully in the market, and they are being exploited as biofertilizers and biopesticides with no harmful effects (Malusá et al. 2012). According to the reports of Market Data Forecast (2018), the current market of microbial soil inoculants at global level has been stated up to USD 396.07 million in 2018 and is expected to rise at annual growth rate of 9.5% reach the projection of USD 623.51 million 2023 to by (www.marketdataforecast.com/market-reports/microbial-soil-inoculants-market-5373/). Various kinds of bioinoculants are being used nowadays such as solid or liquid with bacterial or fungal cells or both, pure culture, and consortia- and metabolite-based (Reddy and Sarayanan 2013; Mishra and Arora 2016). The seeds are either bioprimed with microbial suspension followed by air-drying, inoculant is film coated on the surface of the seed, or the seeds are pelleted with the help of additives (O'Callaghan 2016). Some of inoculants being used nowadays with their mechanism of action are mentioned in the table with their key constitutional microbial communities (Table 9.1). However, a tripartite interaction between the microbiota, the soil, and the host is of utmost importance for a microbial inoculant to be better adapted to field conditions in order to ensure growth-promoting effects on plants (Baez-Rogelio et al. 2017). Therefore, detailed dynamics of the interaction and the mass production of bioinoculant plays a crucial role and serves as a prerequisite in order to understand the fate of the microbe in field conditions (Terrazas et al. 2016). The section describes various bacterial, fungal, and other types of microbe-based inoculants that are being successfully used in market as biofertilizers and biopesticides along with suitable examples of plant growth promotion (Table 9.2), hence confirming them as stepping stones for next green revolution, achieving the demand of sustainability of agroecosystems.

9.3.1 Bacterial Inoculants

At present, bacterial-based bioformulations are flourishing in comparison to other types of inoculants and are dominating the industry of microbe based formulations. Major bacterial inoculants contain genera of PGPR such as rhizobia, *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Herbaspirillum*, *Burkholderia*, and *Bacillus* (Glick 1995; Probanza et al. 1996; Artursson et al. 2006; Adesemoye and Kloepper 2009). Rhizobiaceae comprise of a group of Gram-negative diazotrophic rhizobacteria, and have been well recognized as efficient nitrogen (N₂) fixers by

	mid to omorphicate .			
S. no	Mechanisms involved	Associated microbes	Role in agriculture	References
Direct 1	Direct plant growth promotion			
Phytoh	Phytohormone production			
-i	Auxin	Bacillus, Bradyrhizobium, Enterobacter cloacae, Paenibacillus, Rhizobium, Azospirillum brasilense, A. lipoferum, P. fluorescens, P. chlororaphis, P. auruginosa, Saccharomyces cerevisiae, B. megaterium, B. marinus, Sphingomonas sp., Microbacterium sp., Mycobacterium sp., Piriformospora indica	Modifies the structure of plant roots inducing branching of roots and root hair formation, promotes growth of plants by enhancing nutrient and water uptake	Tsavkelova et al. (2005), Sirrenberg et al. (2007), Spaepen and Vanderleyden (2011), Mohite (2013) and Xu et al. (2014)
ci	Gibberellic acid (GA)	Pseudomonas monteilii, Azotobacter spp., Pseudomonas spp., Sphaceloma, Neurospora, Phaeosphaeria, P. fluorescens, B. subtilis, P. stutzeri, Stenotrophomonas maltophilia, P. putida, Rhizobium spp., Bradyrhizobium loti, Sinorhizobium fredii and Rhizobium loti, Sinorhizobium fredii and Rhizobium etli, Acetobacter diazotrophicus and Herbaspirillum seropedicae	GAs initiate various developmental processes in plants including stem elongation and germination increasing plant growth and yield	Karadeniz et al. (2006) and Sivasakthi et al. (2013), Pandya and Desai (2014), Ambika et al. (2015), Desai (2017), Patel and Saraf (2017) and Salazar- Cerezo et al. (2018)
ю.	Cytokinins	Azotobacter spp., Rhizobium spp., Pantoea agglomerans, Rhodospirillum rubrum, P. fluorescens, B. subtilis, P. polymyxa	Promote growth of plants under various conditions by maintenance of diverse aspects including embryogenesis, development of roots and shoot meristems, nodule formation, apical dominance	Glick (2012), Dawwam et al. (2013), Olanrewaju et al. (2017) and Numan et al. (2018)

 Table 9.1
 Mechanisms of plant growth promotion by microbes

Nutrie	Nutrient assimilation			
	N ₂ fixation	Rhizobia spp., Azospirillum spp., S. meliloti, R. leguminosarum, Bradyrhizobium sp., Pantoea, Bacillus, Klebsiella, Gluconacetobacter diazotrophicus, Rhizobium etli, P. putida, Azotobacter	Helps in fixing of nitrogen for plants increasing their growth and nodulation and also maintaining the fertility of soil	Helman et al. (2011), Glick (2012) and De Souza et al. (2015)
6	Phosphate (P) chelation	 P. fluorescens, B. megaterium, Enterobacter, Pantoea, Klebsiella, Enterobacterium, Gordonia, Rhodococcus, Arthrobacter, Serratia, Chryseobacterium, Gordonia, Phyllobacterium, Delftia, Kushneria, Aspergilli, Penicillium, Alternaria, Arthrobotrys, Aspergillus, Arthrobotrys, Aspergillus, Arthrobotrys, Aspergillus, Cephalosporium, Cladosporium, Curvularia, Cunninghamella, Chaetomium, Glomus, Helminthosporium, Micromonospora, Mortierella, Myrothecium, Oidiodendron, Paecilomyces, Penicillium, Phoma, Pichia fermentans, Populospora, Saccharomyces, Schizosaccharomyc	Availing P to plants, supporting their growth and metabolism and also enriching the quality of soil	Postma and Lynch (2010), Zhu et al. (2011), Tajini et al. (2012), Srinivasan et al. (2012), Sharma et al. (2013), Zhao et al. (2014), David et al. (2014), Istina et al. (2015) and Adnan et al. (2017)
				(continued)

Table 9	Table 9.1 (continued)			
S. no	Mechanisms involved	Associated microbes	Role in agriculture	References
ю.	Potassium (K) solubilization	 B. mucilaginosus, B. circulanscan, B. edaphicus, Burkholderia, A. ferrooxidans, Arthrobacter sp., Enterobacter hormaechei, Paenibacillus mucilaginosus, P. frequentans, Cladosporium, Aminobacter, Sphingomonas, Paenibacillus glucanolyticus 	The availability of potassium to plants adjusts the osmotic balance under stress, promotes photosynthesis, and also enhances productivity of soil	Zhang and Kong (2014), Ahmad et al. (2016), Setiawati and Mutmainnah (2016), Meena et al. (2016) and Etesami et al. (2017)
4	Iron chelation (siderophore production)	Acinetobacter baumannii, P. aeruginosa, Staphylococcus aureus, K. pneumoniae, E. coli, Bacillus spp., Rhizobium, Serratia, Mycobacterium, Nocardia, Rhodococcus, Streptomyces spp., Enterobacteriaceae, Arthrobacter spp., P. stutzeri	Chelated Fe is used by plants and microbes for their metabolic actions, acts as catalyst in various biological processes and also helpful for biofilm formation leading to stress tolerance under various inhospitable conditions	Ahmed and Holmström (2014) and Saha et al. (2016b)
5.	Other micronutrients	Anabaena, Trichoderma asperellum, Providencia sp., B. juncea, Pseudomonas, Rhizobium, Azotobacter, Azospirillum	Enhance the nutrition level of plants for their better growth and biofortified products	Yaseen et al. (2013), Adak et al. (2016) and Garg et al. (2018)
Other 1	Other metabolites			
	Exopolysaccharides (EPS)	P. fluorescens, E. hormaechei, P. migulae, Rhizobium sp., P. polymyxa, Azotobacter vinelandii, Xanthomonas sp., Azotobacter, P. anguilliseptica, Ganoderma lucidum, Agaricus blazei, Cordycep ssp., Lentinus edodes, Grifola frondosa, P. tenuipes	 Help in tolerance under various stress conditions by regulating osmotic balance, water and nutrient content Selbmann et al. (2003), Mahapatra and Banerjee (2013), Viscardi et al. (2016), Niu et al. (2017) and Mohammed (2018) 	nditions by regulating osmotic balance, anerjee (2013), Viscardi et al. (2016),

4	Biosurfactant	Bacillus sp., B. subtilis, B. lichentformis, P. aeruginosa, S. aureus, E. coli, B. amyloliquefaciens, Penicillium, Candida bombicola, Candida lipolytica, C. ishiwadae, C. batistae, A. ustus, Ustilago maydis, Trichosporon ashii	Help in amelioration of heavy metal polluted soils and promote growth of plants	Bodour et al. (2003), Nitschke et al. (2005), Bhardwaj et al. (2013), Rajesh et al. (2017), Prasad et al. (2018) and Sena et al. (2018)
Indirect	Indirect plant growth promotion			
	HCN	Bacillus sp., Paenibacillus sp., B. thuringiensis, P. stutzeri, Staphylococcus sp., Pseudomonas sp.	Shows antagonism against various phytopathogens including <i>Rhizoctonia</i> solani, F. oxysporum, F. proliferatum, F. graminearum, Colletotrichum capsici, P. syringae pv. syringae z1, P. syringae pv. coronafaciens z1238, Erwinia carotovora pv. carotovora z87, Xanthomonas campestris pv. campestris z1352, Macrophomina phaseolina	Passari et al. (2016), Rijavec and Lapanje (2016) and Tewari and Arora (2016)
6	Antibiotic production	 P. fluorescens, P. chlororaphis, Penicillium, Pseudomonas sp., P. aeruginosa, P. brassicacearum, P. protegens, B. cepacia, Serratia plymuthica, Ochrobactrum intermedium, Pantoea ananatis, P. agglomerans, B. cereus, B. thuringiensis, Trichoderma, Aspergillus 	A wide array of antibiotics suppress phytopathogens and indirectly help in plant growth promotion	Mavrodi et al. (2012) and Kawaguchi and Inoue (2012), Wang et al. (2015, 2016a, b), Pandey et al. (2018), Sun et al. (2016), Cheng et al. (2015) et al. (2016) and Yao et al. (2015)
				(continued)

Table 9	Table 9.1 (continued)			
S. no	Mechanisms involved	Associated microbes	Role in agriculture	References
ю.	Bacteriocins	B. cereus, B. thuringiensis, B. clausii, B. subtilis, B. licheniformis, P. putida, Lysinibacillus	 Help in suppression of phytopathogens including Agrobacterium tumefaciens, Candida tropicalis, A. solani, A. niger, A. fumigatus, A. flavus, Cryphonectria parasitica, Monilia sitophila, M. hiemalis, P. digitatum, and Rhizopus sp. 	Smitha and Bhat (2013), Grinter et al. (2012), Mouloud et al. (2013) and Subramanian and Smith (2015)
4	Lytic enzymes	 S. plymuthica, Paenibacillus sp., Streptomyces sp., B. cepacia, S. marcescens, Lysobacter enzymogenes 	These enzymes help in hydrolysis of various polymers of pathogens reducing their virulence factor suppressing their pathogenic activity directly	Singh and Singh (1989), Frankowski et al. (2001) and Pal and McSpadden (2006)
5.	δ endotoxins or Cry protein	B. thuringiensis	Suppresses disease and yield loss by controlling caterpillars, beetles, mosquitoes, blackflies, lepidopteran, coleopteran, and some homopteran pests	Loper and Gross (2007), López-Pazos et al. (2009), Palma et al. (2014), Rubio-Infante and Moreno-Fierros (2016) and Mukhija and Khanna (2018)
.9	Volatile organic compounds (VOCs)	P. fluorescens, B. amyloliquefaciens, B. megaterium, P. protegens	Inhibit the growth of disease-causing fungi (<i>R. solanacearum</i> , <i>F. oxysporum</i> f. sp. cubense, <i>S. sclerotiorum</i> , <i>Aspergillus</i> , <i>Penicillium</i> spp.)	Yuan et al. (2012), Giorgio et al. (2015), Manaa and Kim (2018) and Van Agtmaal et al. (2015)
7.	Induced systemic resistance (ISR)	Bacillus, S. marcescens, Burkholderia phytofirmans, P. denitrificans, P. putida, P. fluorescens	Triggering of ISR in plants by microbes activates their defense system and helps in combating phytopathogens	Compant et al. (2005), Choudhary et al. (2007) and Beneduzi et al. (2012)

×.	Biosurfactants	Ustilago maydis, Candida bombicola, B. subtilis, B. licheniformis, B. amyloliquefaciens, B. atrophaeus	The antibacterial and antifungal activities of CLPs (cyclic lipopeptides) are effective against phytopathogens including <i>Colletotrichum dematium</i> , <i>R. solani, P. ultimum, F. graminearum</i>	Yu et al. (2002, D'aes et al. (2010) and Sarwar et al. (2018)
6	Exopolysaccharides (EPS)	Burkholderia gladioli, Bacillus, Pseudomonas, P. polymyxa	EPS forms a protective biofilm layer across the plants and microbes protecting from pathogens and also chelate nutrients	Haggag (2010) and Upadhyay et al. (2011)
10.	Niche and nutrient exclusion	Pseudomonads, Bacillus, Fusarium, Trichoderma species, Cladorrhinum foecundissimum, Glomus intraradices, Idriella bolleyi, Pythium mycoparasiticum, Trichoderma hamatum, T. virens	Excludes availability of nutrients (N, P, K, Fe) and increase niche competition inhibiting the growth of phytopathogens	Whipps (2001), Winding et al. (2004) and Hibbing et al. (2010)

ו מווה א		able y.z. various microbe-based inoculants available unrougnout the globe and their roles	ind their roles	
	Name of			
S. no	bioinoculants	Associated microbes	Applications	Keterences
Fungal	Fungal inoculants			
	PlantShield [®]	Trichoderma harzianum Rifai strain KRL-AG2	Used as a biopesticide against Fusarium, Pythium, and Rhizoctonia	https://ohioline.osu.edu/ factsheet/SAG-18
5	Bioten WP; Tenet WP; Remedier WP	T. gamsii strain ICC 080	Fungicide controlling soilborne pathogens	BRAD (2010)
ë.	Nutri-Life Root- Guard TM	Arthrobotrys conoides, Purpureocillium lilacinus, and Pochonia chlamydosporium	Bio-balancer maintaining the ratio of beneficial and detrimental organisms in the root zone	http://www.nutri-tech.com.au/
4	Polyversum [®] and Technical DV 74	Pythium oligandrum DV 74	Used as fungicide for many food crops, ornamental plants, and turfs acting against almost 20 pathogenic fungi including Alternaria, Botrytis, Fusarium, etc.	https://www3.epa.gov/
5.	BioMal WP (22359)	Colletotrichum gloeosporioides f.sp. Malvae	As a herbicide to control round-leaved mallow infection in crops	PMRA (2006)
6.	Acceleron [®] B-300 SAT and JumpStart [®]	Penicillium bilaiae	Increases plants ability to uptake nutrients significantly increasing yield of corns by an average of 3 bushels per acres, stress tolerance	Novozymes [®] https://www.novozymes.com/
7.	Ambiphos	Aspergillus niger	Chelates undissolved phosphorous and makes it available to plants	Ambika Biotech & Agro Services, Madhya Pradesh; Pal et al. (2015)
8.	Met52 EC and Met52/BIO1020	Metarhizium anisopliae	Efficient biological insecticides used for control of ticks, whiteflies, and black vine weevil (pests), applicable for many crops	Novozymes [®] https://www.novozymes.com/

 Table 9.2
 Various microbe-based inoculants available throughout the globe and their roles

	Glomus trantcum var. tenuinypharum var.	Intensitying mycorrhizal colonization,	Symborg
MycoUp, Kesid HC, Resid MG	nov.	nutrient and water from soul, resistance against external stressing factors, much efficient under saline conditions and deteriorated lands	http://www.symborg.com/
	Gliocladium fungi	Improving seedling emergence and promoting plant growth, stress tolerance,	Vedara http://verdera.fi/
TRICHOSOIL	T. harzianum	Prominent efficacy against Plant uncesses Prothium, Sclerotium (damping-off	Lage y Cía. S.A http://www.lageycia.com/
Bacterial inoculants		phytopathogens	
MON 89034	<i>B. thuringiensis</i> Cry1A.105, and Cry2Ab2 insecticidal protein	Plant-incorporated protectant used to target pests such as Ostrinia nubilalis, Diatraea grandiosella, D. crambidoides, Helicowerta real Dradinema nebris	BRAD (2008)
		D. saccharalis, Spodoptera frugiperda	
Nutri-Life B.Sub TM	B. subtilis	Aids in plant growth promotion and production of phytoalexins	http://www.nutri-tech.com.au/
	Iron- and sulfur-mobilizing bacteria	Conversion of insoluble S and Fe to absorbable forms, aids in balancing the soil pH, increase in flowers, fruits, grains number and size, stimulating plant restriction processes	International Panaacea Ltd. (http://www.iplbiologicals. com/)
Novodor Flowable Concentrate (24068)	B.thuringiensis subsp. Tenebrionis	Biocontrol action against Colorado potato beetle larvae on potatoes and tomatoes and elm leaf beetle	PMRA (2006)
Actinovate [®]	Streptomyces lydicus WYEC 108	Biofungicide against powdery mildew, Botrytis, Pythium, Rhizoctonia,	Novozymes [®] https://www.novozymes.com/

Table 9	Table 9.2 (continued)			
S. no	Name of bioinoculants	Associated microbes	Applications	References
			Fusarium, Phytophthora, Verticillium, potential siderophores, and chitinase producer complementing plant growth	
9	Mycostop®	<i>Streptomyces</i> ray bacteria isolated from Finnish Sphagnum peat	Biocontrol against damping-off, wilt, and root diseases caused by <i>Fusarium</i> , <i>Phytophthora</i> , <i>Alternaria</i> , and <i>Pythium</i> fungi, also resistive against <i>Rhizoctonia</i> sp. and <i>Botrytis</i> sp. phytopathogens,	Verdara http://verdera.fi/ www.Jallemand.com/
7.	VitaSoil WP	Selected rhizosphere microorganisms (unspecified)	Helping in recycling nutrients in infertile soils, stimulating biological regeneration, applicable for horticultural crops, strawberry, maize, cereals, and woody plants	Symborg http://www.symborg.com/
×.	Rizos®	B. subtilis UFPEDA 764	Stimulates metabolite production affecting germination, larger radicular growth, improved plant's resistance to stress, enhanced yield	Lallemand http://labfarroupilha.com/
9.	Onix®	B. methylotrophicus UFPEDA 20	Elevating plant growth and yield even under stress conditions	Lallemand http://labfarroupilha.com/
10.	Azos®	A. brasilense abv-5	Decreases the concentration of ethylene of leaf boosting photosynthetic activity, increases water and nutrient absorption, provides resistance against water stress	Lallemand http://labfarroupilha.com/
11.	LIKUIQ® + ADD-IT AZUL	Bradyrhizobium elkanii strains	Specific for soybean, increases adhesion of rhizobia to seed promoting the infection of plant's roots	Lage y Cia. S.A. http://www.lageycia.com/

12.	GRAMINOSOIL	Azospirillum	Efficient in plant growth promotion of maize, sorghum, and wheat crops	Lage y Cía. S.A. http://www.lageycia.com/
Geneti	Genetically modified organisms (GMOs)	(GMOs)		
	Transgenic soybean	Agrobacterium tumefaciens strain CP4	Herbicide tolerance conferred by expression of 5-enolpyruvylshikimate-3- phosphate synthase (EPSPS) isolated from the bacterium	Phillips (2008)
2.	Corn	Bacillus thuringiensis	Tolerance to insect pests due to expression of insecticidal protein Cry1Ab	
3.	Plum	Virus	Resistance to plum pox virus through incorporation of coat protein gene	
4.	Phytaseed TM Canola (MPS 961-965)	Azospirillum brasilense, Azotobacter vinelandii along with bacterial genes (idi, crtE, crtB, crtI, crtY, crtW, and crtZ)	Engineered for phytase degradation for enhancement of phosphorous level	Yu et al. (2008) and Fujisawa et al. (2000)
5.	Sorghum	Azospirillum, arbuscular mycorrhizal fungi	Improvement of N and P status to increase the yield	Patidar and Mali (2004) and Faten et al. (2016)
9.	Chickpea	Arbuscular mycorrhizal fungi	Helps in chelation of Fe, Zn, Ca, Cu, Mn, and Mg	Pellegrino and Bedini (2014)
Other	Other microbe-based inoculants			
-:	Madex	Granulosis viruses	Active in pest control against <i>Cydia pomonella</i>	Erayya et al. (2013)
5	Gypcheck	Nuclear polyhedrosis viruses	Shows biocontrol action against Lymantria dispar	Erayya et al. (2013)
3.	Multiplex Nalapak	Consortia of <i>Azotobacter + Azospirillum +</i> phosphate solubilizer + potash mobilizer	Promotes plant growth by production of phytohormones IAA, GA, and cytokines; also improves the quality of soil	Multiplex Bio-Tech Pvt. Ltd., Karnataka; Pal et al. (2015)
4.	QuickRoots®	B. amyloliquefaciens and T. virens	Microbial seed inoculant enhancing availability of N and K to stimulate expanded roots increasing growth and yield of plants	Novozymes [®] https://www.novozymes.com/
				(continued)

Name of bioinoculantsName of Associated microbesApplicationsReferences References5.Accomplish®Viable microorganisms plus enzymes, organic acids, and chelatorsIncreasing availability of nutrientsLoveland F García-Fra6.Micro-Blaze®Biosurfactant-producing microbes (unspecified by manufacturers)Used as bioremediation agent degrading hydrocarbons, pollutants, and other organic wastesInproving root size and branchingGarcía-Fra7.Micro-Blaze® AGROSpore-forming microbes (unspecified by manufacturers)Improving root zone and increasing uptake of moisture and nutrients (N and P) for better plant growth, degradation of toxic chemicals, prevention of diseaseshttp://micro8.Arka MicrobialNPK chelating microbes (unspecified)Early seed germination, increased seedling vigor, yield increase of plants, increased N, P, K availabilityhttp://www	Table 9	Table 9.2 (continued)			
Viable microorganisms plus enzymes, organic acids, and chelatorsIncreasing availability of nutrients stimulating root size and branchingBiosurfactant-producing microbes quidUsed as bioremediation agent degrading hydrocarbons, pollutants, and other organic wastesAGROSpore-forming microbes (unspecified by manufacturers)Uptocarbons, pollutants, and other organic wastesAGROSpore-forming microbes (unspecified by manufacturers)Improving root zone and increasing uptake of moisture and nutrients (N and P) for better plant growth, degradation of toxic chemicals, prevention of diseases1NPK chelating microbes (unspecified) seedling vigor, yield increase of plants, increased N, P, K availability	S. no	Name of bioinoculants	Associated microbes	Applications	References
Biosurfactant-producing microbesUsed as bioremediation agent degrading hydrocarbons, pollutants, and other organic wastesAGROSpore-forming microbes (unspecified by manufacturers)Improving root zone and increasing 	5.	Accomplish [®]	Viable microorganisms plus enzymes, organic acids, and chelators	Increasing availability of nutrients stimulating root size and branching	Loveland Products, Inc. García-Fraile et al. (2015)
Spore-forming microbes (unspecified by manufacturers)Improving root zone and increasing uptake of moisture and nutrients (N and P) for better plant growth, degradation of toxic chemicals, prevention of diseasesNPK chelating microbes (unspecified)Early seed germination, increased seedling vigor, yield increase of plants, increased N, P, K availability	.9	Micro-Blaze [®] Emergency Liquid Spill Control	Biosurfactant-producing microbes (unspecified by manufacturers)	Used as bioremediation agent degrading hydrocarbons, pollutants, and other organic wastes	http://micro-blaze.com/
NPK chelating microbes (unspecified) Early seed germination, increased seedling vigor, yield increase of plants, increased N, P, K availability	7.	Micro-Blaze [®] AGRO	Spore-forming microbes (unspecified by manufacturers)	Improving root zone and increasing uptake of moisture and nutrients (N and P) for better plant growth, degradation of toxic chemicals, prevention of diseases	http://micro-blaze.com/
	8.	Arka Microbial Consortium	NPK chelating microbes (unspecified)	Early seed germination, increased seedling vigor, yield increase of plants, increased N, P, K availability	http://www.iihr.emet.in/

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establishing a symbiotic and mutualistic relationship with leguminous plants (Sessitsch et al. 2002). Rhizobia have been known to be used as biofertilizers with crops since more than a century (Arora et al. 2017). Nobbe and Hiltner (1896) patented "Nitragin," the first biofertilizer in the market, followed by a number of microbial products containing rhizobia as a prime constituent (Arora et al. 2017). There have been many studies that reported to substantiate the role of live rhizobialcontaining inoculants as a valuable option to be used with legumes instead of chemical N fertilizers (Arora et al. 2010). Biofertilizers witnessed substantial growth and have been projected to reach up to USD 2653.48 million by 2023 at an annual growth rate of 14.42%. Nitrogen fixers as bioinoculants cover the highest share in market among other biofertilizers and accounted up to 73% in 2017 with highest market in North America (www.marketresearchfuture.com/reports/bio-fertilizersmarket-1386). In various countries, liquid formulations of free-living nitrogen-fixing bacteria Azotobacter and Azospirillum including cyanobacteria have also been commercialized (Vendan and Thangaraju 2006). In the future we need to further explore the role and enhnce the share of formulations of rhizobia commercially for an eco-friendly supply of nitrogen (Arora et al. 2017). Along with the symbiotic nitrogen fixers, there is also a dense population of free-living N assimilators including pseudomonads, Azoarcus, Beijerinckia, cyanobacteria (Nostoc and Anabaena), Klebsiella, Pantoea, Azotobacter, Azospirillum, Bacillus, Burkholderia, Herbaspirillum, and Gluconacetobacter diazotrophicus, which enhance the N content of the soil-enriching productive potential of the habitat (Mehnaz et al. 2001). Karimi et al. (2018) studied the potential of *Azospirillum* species as biofertilizers on wheat plants under semiarid conditions. Results of field trials showed an increment in the yield of inoculated plants by 18% in comparison to uninoculated plants. Rawat et al. (2013) expounded that a total of 78.8 kg ha⁻¹ year⁻¹ N was added to soil under inoculation of Azotobacter and Rhizobium to soybean-wheat crop rotation. Soil Shakti, Soil Gold, Premium Azotolus, and Premium Azoto from International Panaacea Ltd. (IPL) are some of the N-fixing Azotobacter sp.-based inoculants available in market.

Phosphate-solubilizing microbial inoculants have also gained much attention and currently hold the second in market share of global biofertilizers accounting for 14.6% (Novozymes). The solubilization of rock phosphates in the culture filtrates because of production of gluconic acid, lactic acid, formic acid, citric acid, oxalic acid, 2-ketogluconic acid, and malic acid by 19 strains of phosphate-solubilizing fluorescent pseudomonads including *Pseudomonas fluorescens*, *P. poae*, *P. trivialis*, and *Pseudomonas* spp. was reported (Vyas and Gulati 2009). Some of PSB-based products marketed commercially are FOSFOSOL, containing *Penicillium janthinellium*, which is used at big scale in Colombia, and P Sol B in India that contain *Pseudomonas striata* possessing broad approach in agriculture (Moreno-Sarmiento et al. 2007).

Potassium, the third most important element required by plants, is also known to be solubilized by bacteria such as *Bacillus extroquens*, *Clostridium pasteurianum*, and *Thiobacillus* (Shanware et al. 2014). Reports show that their exopolysaccharides and inorganic and organic production are found to solubilize potassium (Groudev

1987). It is reported that India ranks fourth in the world, whereas countries like the USA, China, and Brazil are on the top in total consumption of potassium bioinoculants (Investing News Network 2015). Symbion-K, K Sol B, and BioSol-K are some bioproducts that are available in the market and are effectively known for their potassium-mobilizing ability.

Chelation of Fe by microbes has supported the inoculation of plants with irontrapping microbes influencing the growth of plants under various habitable and stressed conditions. Many studies favor the microbe-mediated uptake of Fe, showing utilization of microbial inoculants even at low concentrations that consequently enhances the agricultural productivity (Fageria 2009; Saha et al. 2016a). Commercially available bioformulations used for uptake of Fe by plants are rare, and one of the recently recognized in India is Fe Sol B traded under Agri Life Bio Solutions (Mishra and Arora 2016).

Zn-solubilizing microbial inoculants are also widespread and are being used to improve the phytoavailability of Zn in soil and foliage with an aim to enhance yield of crops (White and Broadley 2009). *Bacillus, Pseudomonas, Gluconacetobacter*, and *Acinetobacter* are among prominent genera which are used as Zn solubilizers. Among them bacilli are the most significant candidates which are being used as Zn bioinoculants. *B. aryabhattai* MDSR7 and MDSR14 have been studied and found to increase the concentration of Zn in edible portions of soybean and wheat and can be successfully utilized as a biofertilizer or in biofortification (Ramesh et al. 2014). The study by Kamran et al. (2017) also supports the Zn solubilization by rhizobacteria comprising of *Pantoea dispersa, Enterobacter cloacae*, and especially *Pseudomonas fragi* to wheat resulting in increased plant biomass.

The application of microbes under biotic stress is now a popular trend to reduce the use of chemical pesticides and promote agricultural productivity. With the above discussed mechanisms of biocontrol, there are numerous reports of microbial inoculants suppressing the pathogenicity and in return providing growth-stimulating nutrients and metabolites. Microbial inoculants have successfully curbed phytopathogens such as Sclerotium rolfsii, Pythium, Ralstonia solanacearum, Fusarium oxysporum, Rhizoctonia solani, Fusarium udum, Macrophomina and Phytophthora (fungi), Meloidogyne incognita, Panagrellus redivivus. Bursaphelenchus xylophilus, Heterodera glycines (nematodes), lepidopteran, beetle, redheaded pine sawfly, and Douglas-fir tussock moth (Tian et al. 2007; Sanchez et al. 2005; Maksimov et al. 2011). Omara et al. (2017) found that combined inoculation with Methylobacterium strains (Methylobacterium aminovorans and M. rhodinum), Bradyrhizobium japonicum (St. 110), Bacillus megaterium var. phosphaticum, and T. viride attenuated the pathogenicity of R. solani in soybean seedlings and also resulted in increased nodule number, NPK %, seed index, and seed yield. In lineation, Qiao et al. (2017) propounded that inoculation of tomato with strain B. subtilis PTS-394 stimulated ISR in the plant through production of lipopeptides and polyketides suppressing the adversity of F. oxysporum. Nematicidal activity of the microbial inoculants was discussed by Xiang et al. (2017) where in vitro trials showed elevated mortality rate of H. glycines on inoculation of B. velezensis strain Bve2, B. safensis strain Bsa27, and B. mojavensis strain Bmo3 to soybean plant. Antibacterial activity of 2,4 DAPG (from Pseudomonas sp.) against black rot causative agent Xanthomonas campestris pv. campestris (Xcc) was assessed by Mishra and Arora (2012). Chin-A-Woeng et al. (1998) opined the combating property of antibiotic, pyrrolnitrin, produced by P. fluorescens BL915 strain against R. solani in cotton plant. Multifarious approaches regarding microbial quenching of phytopathogens also include biocontrol of Macrophomina phaseolina using siderophore-producing R. meliloti and exopolysaccharide-producing fluorescent pseudomonads (Arora et al. 2001; Tewari and Arora 2016); suppression of charcoal rot of chickpea by pyocyanin-producing fluorescent pseudomonads (Khare and Arora 2010; Khare et al. 2011); antifungal potential of fluorescent Pseudomonas isolates PGC1 and PGC2 against R. solani and *Phytophthora capsici* by producing chitinase, β -1,3-glucanase, and also non-enzymatic antifungal metabolites (Arora et al. 2007); protection against M. phaseolina in Mucuna pruriens using combined inoculation with Ensifer meliloti RMP6 Ery⁺Kan⁺ and *Bradyrhizobium* sp. BMP7 Tet⁺Kan⁺ (Aeron et al. 2011); antagonism by Bacillus pumilus against Gaeumannomyces graminis var. tritici in wheat and nematicidal activity of Azospirillum lipoferum against Heterodera avenae in wheat (Bansal et al. 1999); anti-parasitism evinced by Bacillus cereus strain S2 against M. incognita (mortality rate 90.96%) and Caenorhabditis elegans (77.89%) (Gao et al. 2016); and enterotoxin-like binary protein-producing strain Pseudomonas protegens strain 15G2 exhibiting nematicidal potential against Pristionchus pacificus, P. redivivus, and Acrobeloides sp. (Wei et al. 2014). At global level, annual production of biopesticide is accounted for over 3000 tons and has been estimated to increase with a rate of 10% every year (Kumar and Singh 2015; Damalas and Koutroubas 2018). According to reports of Hubbard et al. (2014), over 225 microbial biopesticides are being manufactured in 30 OECD countries. On the other hand, North American Free Trade Agreement (NAFTA) countries (USA, Canada, and Mexico) have been reported to use around 45% of the biopesticides sold in the world with Asia having only 5% share (Bailey et al. 2010). Sudo-Shield™ designed using *P. fluorescens*, aids refurbishment of plants affected by damping off, rot and wilt diseases (http://www.nutri-tech.com.au/), Pest Managemnt Regulatory Agency (PMRA) registered biopesticide products (with Canadian registered number) include Dygall (21106) comprising Agrobacterium agrobacter strain 84 for preventing crown gall disease, Bioprotec CAF (26854) Bioprotec 3P (27750) DiPel 2X (26508), DiPel WP (11252), Thuricide HPC (11302), Novodor Flowable Concentrate (24068) with B. thuringiensis as active ingredient and used for control of various lepidopteran insects, Colorado potato beetle larvae, elm leaf beetle, , Bio-Save[®] 10LP3 incorporating strain *P. syringae* strain ESC 10 targeting biological decay, Bloomtime Biological[™]3, Bloomtime Biological[™] FD3, Pantoea agglomerans strain E325 as the biocontrol organism mitigating Fireblight (Erwinia amylovora) (https://ohioline.osu.edu/). An efficient competent hostile strain of Bacillus subtilis was isolated and studied for deforming the structure of six pathogenic fungi by Chaurasia et al. (2005) under in vitro conditions. Some of the formulations based on these genera available commercially include BlightBan, Biocoat, Bio-Save, and Cedoman (Mishra and Arora 2016). Tewari and Arora (2016) also reported a useful strain of *Pseudomonas aeruginosa* that causes ample increase in the yield of sunflower crop under arid and saline conditions by diminishing the incidence of charcoal rot disease in the plant.

Abiotic stresses like drought, flood, and salinity are well-known global constraints of agricultural sector. Mitigation of these stresses through application of bioinoculants has been proposed in several studies by different researchers around the world. Azospirillum, Bacillus, Pseudomonas, and Rhizobium sp. are known to impart drought tolerance in various plants. B. subtilis provided tolerance to Platycladus orientalis from drought by increasing levels of ABA and enhanced conductance of stomata (Liu et al. 2013). In case of flooding stress, Bradyrhizobium *japonicom*, a member of rhizobia, showed a positive response on the growth of soybean under flooding conditions by enhancing its nitrogen-fixing capability (Kadempir et al. 2014). Also capability of *Azospirillum* to thrive under submerged conditions could be exploited as a bioinoculant under flood stress (Sahoo et al. 2014). These studies suggest that PGPR could be used to attenuate the negative effects of stress caused by drought and flood. Likewise salinity stress was found to be reduced by many bacterial species such as *Bacillus*, *Pseudomonas*, *Enterobacter*, Р. aurantiaca TSAU22, Pseudomonas extremorientalis TSAU6. and P. extremorientalis TSAU20. P. extremorientalis increased growth of wheat seedlings by up to 52% in comparison to control under 100 mM salinity (Egamberdieva 2009). Enterobacter sp. EJO, procured from a halophytic condition, enhanced growth of Arabidopsis and tomato under 200 Mm NaCl stress (Kim et al. 2014). Bacillus amyloliquefaciens SQR9 have been observed to improve salinity stress (100 mM Na Cl) in maize seedlings along with increased chlorophyll and glutathione content and peroxidase and catalase activity (Chen et al. 2016). Tewari and Arora (2018) depicted the role of EPS-producing strain under saline conditions, where Pseudomonas aeruginosa PF23^{EPS+} showed maximum production of salicylic acid and biocontrol against M. phaseolina up to 500 mM NaCl, while mutant strains were found to be deficient in SA production and salt tolerance.

This explains that the products containing bacteria as main constituents can play a key role in agricultural sustainability and thus can be further used as active ingredients in many biological products. However, it is required to screen and select rhizobacteria in order to make a broad-spectrum efficient microbial bioformulation.

9.3.2 Fungal Inoculants

In addition to bacteria and their beneficial relationship with plant roots, fungi are also known to form mutualistic relationships with plants, particularly involved in transfer of nutrients and their cycling. Mycorrhizal fungi play an important role being symbiotic partners of most of the plant species (Adesemoye and Kloepper 2009) and help augment protection against environmental stresses, biological control of harmful pathogens, plant growth, and soil fertility by extending their hyphae in the soil matrices and increasing surface area of root (Adesemoye et al. 2009). Hyphae of mycorrhizal fungi are also known to improve quality of soil by directly affecting soil

aggregation, stabilizing aeration and water dynamics (Rillig et al. 2002). They are able to access insoluble P from soil matrices which otherwise is inaccessible in absence of these beneficial fungi and hence known as excellent soil renovators (Smith and Read 1997). It has also been reported that the growth rate and germination of mycorrhizal fungi get directly affected by bacterial communities and are also highly responsible for bacterial community compositions in rhizosphere (Adesemoye et al. 2009). With the discovery of Beauveria bassiana, Metarhizium and Trichoderma spp., more efforts are now being made in order to develop commercial fungal preparations; however, they are still to be explored further for development of novel bioinoculants (McCoy 1990). Ectomycorrhizal fungi (EMF) and arbuscular mycorrhizal fungi (AMF) are important tools that are known to enhance the production of crops and also to protect crops from adverse conditions (Pal et al. 2015; Lenoir et al. 2016). ECM are reported to enhance growth of trees as these are known to break down complex organic compounds and minerals as compared to other fungi (Pal et al. 2015). The most common inoculum used in this class is the *Pisolithus* tinctorius which is used as vegetative mycelium with peat or clay as carrier (Schwartz et al. 2006). *Piriformospora indica* is another example of ECM used as growth promoter for plants and provides tolerance to environmental stresses (Tejesvi et al. 2010). AMF are the major plant habitants and are also notable P mobilizers, increasing soil amino acid and organic acid contents (Bolduc and Hijri 2011). AMF are also reported as K solubilizers, trapping K from mineral or inorganic sources (Sangeetha 2012; K chelation activity of AMF is found to be directly proportional to P solubilization (Cardoso and Kuyper 2006). Spores of AMF as inocula are known as the most reliable bioinoculants, whereas fragments of colonized roots are also used (Biermann and Linderman 1983). Rhizophagus (formerly Glomus) intraradices and Funneliformis (formerly Glomus) mosseae have commercially been used as inoculants in Europe and the USA (Kruger et al. 2012). Other fungal biofertilizers belong to Penicillium, Aspergillus, Chaetomium, and Trichoderma species and are known to increase plant biomass in different ways. Zn-solubilizing fungi (ZSF) such as *Penicillium citrinum* and *Aspergillus niger* in addition to *Aspergillus candidus* are also known for their Zn-solubilizing ability (Anitha et al. 2013; Shaikh and Saraf 2017). JumpStart[®], a fungal biofertilizer containing *Penicillium bilaii*, is commercially being used to promote P uptake in wheat and canola with yield increment of ~6% (Harvey et al. 2009).

Biocontrol activity of *Trichoderma* spp. as biopesticides is well documented since 1930 (Ha 2010; Vinale et al. 2008) and is the most studied biocontrol fungal agent competing with many other organisms for nutrients and space. Nowadays, it has been commercialized successfully by companies like BioWorks, USA, with sales of worth several million dollars (Harman 2011). *Trichoderma harzianum* ATCC 20476 was the first registered fungus with the Environmental Protection Agency (EPA) for biocontrol of plant diseases in 1989 (Junaid et al. 2013). The current scenario of agricultural revolution affirms the availability of wide array of these microbe-based biopesticides throughout the globe with felicitous features in diluting the biotic stress (due to phytopathogens) (Mishra et al. 2015). Global data suggests that 27% of biopesticide products commercially available in market are

fungal (Woo et al. 2014). Nutri-Life Tricho-ShieldTM is a talc-based bioformulation of *T. harzianum*, *T. lignorum*, and *T. koningii* with excellent property of maintaining a balance between desirable and non-desirable microbes in soil and on plants surface. *Aspergillus flavus* AF36 under product name Alfa guard and manufactured by Circle One Global, USA, competes with aflatoxin producing strains of *A. flavus* and inhibits them along with controlling disease in cotton (Junaid et al. 2013). BioMal WP (22359) with *Colletotrichum gloeosporioides* f. sp. *malvae* as active constituent functions as effective herbicide controlling round-leaved mallow in field crops (PMRA 2006).

Fungal inoculants, like their bacterial counterparts, are also known to alleviate drought stress in many plants and are known to increase their growth under stress conditions. Effect of AMF on growth of plant sainfoin (Onobrychis viciifolia Scop.) has been studied by Jing et al. (2014). The fungus G. mosseae was found to increase the growth and drought tolerance of the plant by increasing water, N and P content in it. Similarly three treatments of Funelliformis sp., another AMF, were reported to increase growth of strawberry plants under drought-stress with increase in root colonization by the fungus (Boyer et al. 2015). Likewise, mycorrhizal fungi are known to provide resistance to plants under submerged or flooded conditions by forming symbiotic associations with them (Wang et al. 2011; Wu et al. 2013). Rhizophagus irregularis, another AMF, are reported to increase root hydraulic conductivity with higher expressions of aquaporins in tomato plant under waterlogging conditions, IAA playing the key role in absence of oxygen (Calvo-Polanco et al. 2014). AMF are known to provide tolerance under salt stress in plants and are well known as bioameliorators of saline-stressed soils (Yano-Melo et al. 2003). Mung bean plants when inoculated with AMF were reported for enhanced growth under different dilutions of seawater, in comparison to uninoculated plants (Rabie 2005). Also co-inoculation of AMF (e.g., Glomus clarum) with nitrogen-fixing bacteria (e.g., Azospirillum brasilense) is known to increase salinity tolerance in some leguminous plants like Vicia faba. Similarly, T. harzianum was also observed to alleviate salinity stress in wheat and rice plants when their seeds were bioprimed with the fungus (Rawal et al. 2013). Hence, from abovementioned examples, we conclude that fungal bioinoculants have substantial application in agricultural field to increase productivity and can be successfully used in restoration of infertile land, and further exploitation of their characteristic features would result in important commercial products which could curb many agronomic problems.

9.3.3 Genetically Engineered Microbes (GEMs)/Genetically Modified Organisms (GMOs)

In the present era, genetic engineering is emerging as an exciting technique comprehending the desired characters in microbes analogous with the demand. Construction of GEMs through recombinant DNA techniques has been a known fact since 1970s (Vidaver et al. 2013). With the rising divergent agricultural

constraints, the need shifts to use condition-specific microbial strains, instead of applying basic plant growth promoters. GEMs have been popular through their beneficial traits including effective biocontrol agents (specific for various diseases), improving plant growth and productivity (Amarger 2002), degradation of pollutants (Dutta et al. 2003), as biosensors or biomarkers in determining the load of pollutants in soil and water (Belkin 2003), extraction of enzymes and other important phytohormones and encountering biotic and abiotic stresses (Viebahn et al. 2005). In the year 1971, Ananda Mohan Chakrabarty firstly introduced genetically engineered microbe Pseudomonas putida as superbug in the area of oil degradation (Ezezika and Singer 2010). 24th April, 1987 flagged as benchmark, when Advanced Genetic Sciences, Inc. (AGS), successfully conducted field trials of the GEM product FrostBan[®] (on strawberry), comprising of genetically engineered P. syringae, deleting the gene responsible for protein involved in ice nucleation (Lindow and Panopoulos 1988; Smith 1997). Subsequently, biopesticidal transformation systems were constructed by Crop Genetics International (CGI) using B. thuringiensis (Bt) and Clavibacter xyli ssp. cynodontis (endophyte of Bermuda grass, maize, etc.). Bt Cry1Ac δ -endotoxin was delivered in maize tissue by incorporating the associated transgenic sequences to Clavibacter xyli and then introducing them to plants, diluting the vulnerability to corn earworm and European corn borers (Turner et al. 1991; Lampel et al. 1994). The success story of the product did not gain much acknowledgment due to lack of consistent delivery, uncontrolled spread of the GEM, and also impaired plant yield (John Turner, personal communication 2011). Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) between 1991 and 1996 registered GEM comprising of B. thuringiensis d-endotoxins encapsulated in P. fluorescens (Shand 1989; Mycogen 1998). Bt crops with successful incorporation of Cry gene popularized and the area planted with genetically modified crops increased from million hectares in 1997 to about 66 million hectares in 2011 (James 1999, 2011). Commercial Bt products account for 90% of global microbial pest control agents (MPCAs) and about 80% of all global biopesticides sold (Whalon and Wingerd 2003; Castagnola and Jurat-Fuentes 2012). Each year about 13,000 tonnes of Bt products are produced using aerobic fermentation technology throughout the globe (WHO 1999). Presently, there are almost 63 genetically modified microbial pesticides (Vidaver et al. 2013) including bacteria, fungi, and viruses. Timms-Wilson et al. (2001) successfully inserted genes encoding phenazine-1-carboxylic acid (PCA) in P. fluorescens enhancing the survivability in microcosm systems. Delaney et al. (2001) corroborated increment in DAPG production by genetically engineered microbe as compared to the wild strain of P. fluorescens F113, inhibiting greater-fold pathogenicity of Pythium in incidence of damping-off disease. Furthermore, biodegradation and bioremediation are also additional aspects where GEMs have rooted in augmenting the process. Genetic manipulations catalyzing the degradation and remediation processes are impregnated, enhancing the microbial potential. Friello et al. (2001) engineered multiplasmid-containing Pseudomonas with the increased potential of oxidizing aliphatic, aromatic, terpenic, and polyaromatic hydrocarbons. Similarly, Monti et al. (2005) engineered 2,4-dinitrotoluene degradation pathway genes from

Burkholderia sp. strain DNT to P. fluorescens ATCC 17400, encompassing the ability of degrading DNT even at cold temperatures. Wu et al. (2006) used Escherichia coli as cloning host to assimilate genes from Comamonas sp. strain CNB-1 for 4-chloronitrobenzene and nitrobenzene reduction. This biotechnological approach has also been a popularly nominated solution to heavy metal stress amelioration. Renninger et al. (2004) speculated the tac-lac promoter controlled cloning of genes encoding polyphosphate production, in P. aeruginosa, thereby potentially remediating uranium contamination. P. fluorescens HK44 became the first genetically engineered bacterium approved by US EPA with the ability to degrade poly-aliphatic hydrocarbons (PAHs) (Ripp et al. 2000). Recombinant technology has also been implied to baculoviruses with the aim to enhance their insecticidal efficiency. Genome of baculoviruses is engineered by cloning several insecticidal proteinaceous toxins including juvenile hormone esterase, PTTH, melittin, trehalase, fungal insecticidal protease, and scorpion and mite toxins (Eravva et al. 2013). The examples discussed in the same study are toxin from Androctonus australis (foreign gene) into virus BmNPV (nuclear polyhedrosis virus (NPV) against Bombyx mori, toxin 34 from Pyemotes tritici (foreign gene) into virus zHPV against Heliothis zea, and neurotoxin from spider (foreign gene) into virus AcMNPV and HvJHE against Spodoptera frugiperda. Due to cost ineffectiveness and challenge to native wild species, this technique is yet steps away to reach farmlands. Therefore, work has to be done to overcome the problems and implement this technique to achieve next green revolution.

Transgenic approaches have also been applied in biofortification of foods to enhance the nutrient levels and counteract the problem of hidden hunger. Genetic manipulation is implied in various crops incorporating bacterial pathways into crops to exploit alternative pathways of nutrient assimilation. Improvement of rice protein quality has been achieved by targeting bacterial enzymes like aspartate kinase, dihydrodipicolinate synthase (DHPS) (Yang et al. 2016), and *E. coli* aspartate aminotransferase (Zhou et al. 2009). Provitamin A content of wheat is increased by incorporation of bacterial *PSY* and carotene desaturase genes (*CrtB*, *CrtI*) (Wang et al. 2014). Similarly, many transgenic legumes and pulses have been biofortified using essential genes from bacteria (other examples in Table 9.1).

9.3.4 Other Microbial Inoculants

The application of bacterial and fungal bioinoculants has been very useful in improving plant health. However these cell-based bioinoculants have their limitation in field conditions. New strategies and applications of using other inoculants such as use of microbial metabolites or other additives along with cell-based bioinoculants could enhance their performance. Apart from this, these bioinoculants can also be used in hostile conditions such as countering abiotic and biotic stresses. With the peaking impact of chemicals in agriculture, the resuscitating solutions are needed to maintain the dynamism so that there can be better and sustainable means to tackle the challenges being faced by plants. Direct application of microbial metabolites to

plants is now an emerging strategy with propitious results along with cell-based inoculants (Arora and Mishra 2016). Microbial metabolites, viz., phytohormones, flavonoids, osmoprotectants, EPS, and biosurfactants, are of great importance (Morel et al. 2015). The application of metabolites has shown more concentrated and unidirectional results and functionality in comparison to traditional microbial inoculants. These metabolites are assimilated as additives/adjuvants or carriers in bioformulations or are exogenously applied to rhizospheric regions, which may enhance growth-promoting attributes in both plants and the inoculated microbe.

Among microbial metabolites, production of phytohormones is the most utilized trait providing substantial aid in development of microbial inoculants; even when present at low concentration, they induce cell proliferation and expansion (Perrot-Rechenmann 2010; Daviére and Achard 2013). Although plants and inoculated microbes do synthesize phytohormones, yet studies indicate using precursor of their biosynthesis, or exogenous application may enhance crop productivity by several folds (Arora et al. 2017). Auxins, GA, cytokinins, and jasmonic acid are the important phytohormones which reflect significant growth-promoting traits upon external inoculation. Auxins are the profoundly studied phytohormones regulating the plant growth by increasing the root volume, therewith incrementing the active sites for chelation of minerals and nutrients; also auxins induce establishment of beneficial plant-microbe symbiosis and expression of genes involved in plant colonization specifically under stress conditions (Spaepen et al. 2007; Morel et al. 2012) and biocontrol (Khare and Arora 2010). Their exogenous application has been corroborated proving that it may increase nodulation rate, shoot weight, and yield in many crops (Morel et al. 2016). Evidently, Zahir et al. (2010) revealed that L-tryptophan application to mung bean along with Rhizobium phaseoli strain enhanced the auxin biosynthesis supporting increased nodulation, growth, and yield.

Bacterial EPS has been categorized as an ample microbial metabolite with wide spectrum of agricultural benefits including plant growth promotion, bacterial survival, soil reclamation, plant-microbe interactions under hospitable and stress conditions, and also synthesis of biopolymers. There are pronounced reports on plant growth-promoting activity of EPS-producing microbes where EPS served as plant and microbe protector (from biotic and abiotic stresses), salinity and heavy metal sequester, hydrator in drought, soil aggregator (trapping moisture and nutrients), nutrient chelator, etc. (Tewari and Arora 2014a). With these multitudinous applications, crude EPS has been directly, or through bioformulations, applied to plants and soils enhancing their action abilities (Arora et al. 2017). Haggag et al. (2015) showed that 200 ppm of crude EPS purified from *Paenibacillus polymyxa*, on its foliar and seed application, elicited resistance against powdery mild dew and leaf rust in wheat plant. Liang et al. (2016) propounded 80% of free radical scavenging rate in EPS from *P. mucilaginous* TKU32 combating salinity stress and elevating growth. Tewari and Arora (2014b) checked the efficacy of EPS-augmented talcbased bioformulation on yield of sunflower and found positive results even under saline conditions. Impregnation of crude EPS in bioformulations thus can be an efficient delivery system/carrier assuring slow and constant microbial release, in the new generation bioinoculants (Arora and Mishra 2016).

Biosurfactants have paved their way in microbial green revolution by improving soil quality through removal of heavy metals and hydrocarbon contaminants (Sun et al. 2016), antimicrobial activity against plant pathogens (Nihorimbere et al. 2011; Khare et al. 2011), quorum sensing, facilitating important plant-microbe communication (Berti et al. 2007; Rosenberg and Ron 1999), and adjuvants in fungicides, insecticides, and herbicides (Rostas and Blassmann 2009). Moldes et al. (2011) found 58.6-62.8% reduction of octane hydrocarbon by biosurfactant from Lactoba*cillus pentosus* explaining biodegradation property, replenishing the soil. Kim et al. (2011) demonstrated insecticidal activity by biosurfactants (from strain of Pseudomonas) against green peach aphid (Myzus persicae). There are also reports of different types of biosurfactants being used as adjuvants by many pesticide manufacturing companies (Mulqueen 2003). Jeneil Biotech Inc. company, USA, is manufacturing a rhamnolipid (purified from P. aeruginosa)-based product Zonix Biofungicide[™] substantial for biocontrol against downy mildew, late blight, black rot, and all phytophthora and pythium diseases (http://www.jeneilbiotech.com/) (Thavasi et al. 2011).

Microbial osmoprotectants, lipochitooligosaccharides (LCO), and flavonoids are also being employed as inoculants to enhance stress tolerance, symbiotic association, and nodulation, respectively (Oldroyd 2013; Kaya et al. 2013; Morel et al. 2016). Dolatabadian et al. (2012, 2013) postulated increased nodulation, nitrogen fixation efficiency, growth parameters, and osmo-balancing metabolisms under salt stress in *Glycine max-Bradyrhizobium japonicum* association, upon inoculation with genistein-type flavonoid. Dyna-Start Max[™] is a LCO-based product manufactured by company Loveland (http://www.lovelandproducts.com/) specifically for plant growth promotion of soybean and peanuts. Ratchet[®] and Torque[®] are other LCO-associated bioformulations. Nutri-Life BAM[™] is a formulation blending lactic acid bacteria, purple non-sulfur bacteria plus beneficial yeasts, and also microbial exudates administered to increase propitious bacterial population in root zones and increase nutrient availability, better yield, and quality of plants (http://www.nutritech.com.au/).

Viruses have been noted as rancid elements imperiling the health and productivity of plants. But to the exception, there are reports where viruses have also affirmatively affected the agriculture through drought and cold tolerance and coping with biotic stress (Xu et al. 2008; Roossinck 2013). Viruses including Baculoviridae, Iridoviridae, Poxviridae, Parvoviridae, *Reoviridae*, Picornaviridae, and *Rhabdoviridae* are reported to cause infections in insects substantiating their role in biocontrol (Kalawate 2014). Among these the family of *Baculoviridae* is attaining much attention in the field of bioinsecticides (Harrison and Hoover 2012) with more than 20 species and 30 different products registered as commercially marketed bioinsecticides (Rao et al. 2015) providing resilience against Lepidoptera, Hymenoptera, Diptera, Neuroptera, Coleoptera, Trichoptera, Crustacea, and mites. Baculoviruses have strongly grasped the insecticidal market with the contribution of 60% of the total 1200 known insecticidal viruses, with potential of acting against approximately 30% of the food and fiber crops' pests (Erayya et al. 2013). The same study highlighted some of the majority of baculovirus-based insecticidal products used worldwide including Capex against *Adoxophyes orana* (Czechoslovakia), Agrovir Germany 1990 against *Agrotis segetum* (Germany), and Gypcheck against *Lymantria dispar* (USA). Furthermore, the viral product market in Europe and the USA is also proportionate, preceding China, including some of the available products: Granupom (AgrEvo), Carpovirusine (NPP-Calliope), Carposin (Agrichem), Virin-Gyap (NPO Vector), and CYD-X (Thermo Trilogy) (Mishra and Arora 2016). Six virus-based registered microbial biopesticides have also been reported (Steinwand 2008) of which phage products of USA's leading company Omnilytics have been popularly used in biocontrol against *Xanthomonas campestris* pv. *vesicatoria* (Frampton et al. 2012; Schofield et al. 2012; Mishra et al. 2015).

The other emerging strategy for microbial inoculants application is incorporation of mixed cultures in the bioformulations to achieve multifaceted approach toward plant growth promotion and stress adaptation. The three-way action system of using consortia involves (1) different microbes operating diverse tasks at the same time. (2) allocating stability among the cells against the dynamics of environment, and (3) furcating the functions into different modules of consortia reducing the load on single strain (Jia et al. 2016). Becerra-Castro et al. (2012) posited that upon the application of mixed cultures including Bacillus pumilus 28-11, Alcaligenes faecalis 212-2, Micrococcus luteus 212-4, and Enterobacter sp. 214-6, the degradation of nalkanes and polycyclic aromatic hydrocarbons (PHA) from oil-contaminated soil was enhanced. Saxena et al. (2015) reported the dual inoculation of two microbial species: one phosphate-solubilizing bacteria *Bacillus* sp. and the other free-living phosphate-solubilizing fungi Aspergillus niger S-36 improving the growth and yield of chickpea. Rubiya (2006) developed "Multigeneric diazotrophic co-flocs" constituting Azospirillum, Azotobacter, and Rhizobium, improving growth and yield of rice. Abd-Alla et al. (2014) reported the co-inoculation of AMF and rhizobia to enhance yield and productivity of crops because of higher nutrient uptake. Similarly, Zhu et al. (2016) found elevated alfalfa yield under saline conditions upon combined inoculation with AMF and nitrogen-fixing bacteria (Arora et al. 2017). Singh et al. (2014) also opined the benefits of co-inoculation with rootnodulating Rhizobium sp. RASH6^{Chl + Kan+} and phosphate-solubilizing P. fluorescens PB6^{Amp + Str+} incrementing growth parameters and nodulation of chickpea. Therewith, apart from traditional inoculants, these advanced combinations and techniques can be a better picture of sustainable agriculture.

Mixing of bioinoculants with micro-/macronutrients or other additives including secondary metabolites or substrates is done to enhance the growth of plants. Nutrients including phosphates, K rocks, S, or insoluble Zn are amended in the bioformulations along with their solubilizing microbes enhancing their PGP activity (Abou-el-Seoud and Abdel-Megeed 2012). Similarly, chitin has also been used as an additive together with microbes showing biocontrol activity, resulting in their amplified chitinase activity and also acting as efficient carbon source for plants (Arora et al. 2007). Nutrients such as amino acids and sugars are also supplemented in the bioformulations serving as source of C and N (Arora and Mishra 2016). Omer (2010) reported higher plant growth promotion of bean seeds on application of talcyeast and cellulose-added clay-based powdered bioformulation in comparison to

without enriched bioformulations. Singh et al. (2014) concluded that the enrichment of sawdust-based bioformulation with molasses both in mono- and co-inoculation increased the shelf life and efficiency of *Rhizobium* sp. and P-solubilizing *P. fluorescens*. Aeron et al. (2011) also supported that CMC-amended rhizobial inoculant enhanced growth and protection of *Mucuna pruriens* against *M. phaseolina*.

9.4 Conclusion and Future Perspectives

In the context of climate change, increasing population, land degradation, and biotic and abiotic stress conditions, the demand for sustainable and cost-effective alternative methods is the prime call for securing the future needs. In lineation to the requirement, microbial inoculants have been the focus and the most potential ecologically acceptable candidates which can overcome the use of agrochemicals. Microbial inoculants have been in the market since long. The use of rhizobia as biofertilizer for legumes is reported since 1896 and the use of *B. thuringiensis* for biocontrol of pests since the 1930s (Russo et al. 2012). With the strategic advancement, the application of beneficial soil microbes can be included in bioremediation, phytoremediation, nutrient assimilation, soil aggregation and plant growth promotion (Baez-Rogelio et al. 2017). The future perspectives of microbial applications include advancement of both theoretical and technological concepts through implementation of various multidisciplinary technologies including omics, 3D printing, nanotechnology, and synthetic biology. The damage caused by chemicals (pesticides and fertilizers) demands the implementation of organic farming and introduction of eco-friendly technologies to sublimate the toxicity persisting in the environment from unsustainable actions. Synergism of both organic farming technologies and microorganisms can be the most efficacious proposal in enhancing the agricultural production as well as ameliorating the quality of soil. Products of microbial inoculants have gained attention among the farmers, yet there are many loopholes in the technology which are required to be fixed so as to further improve it. With little knowledge about uncultured microbes, the spectra of microbial inoculants can be widened by further studying these unknown entities and introducing their beneficial properties to agriculture.

The application of microbial inoculants to plants involves complex interaction between the microbe and cultivar which needs to be explored further to gain information about the ecosystem biology. This detailed information can be used to improve the microbe-cultivar adaptation relationship by designing the inoculants as per the demands of the plants. Furthermore, each plant has its own natural microbiome, which can also be detailed to formulate the biofertilizers depending on the need of the plant (Hunter 2016). The influential action of microbial inoculants also depends on the delivery methods deciding their vicinity in rhizosphere, availability to plants, maintenance of desired characteristics, and also the shelf life and cost of the bioformulation. Though there are many theoretical studies regarding natural carriers, cell encapsulation, nano-carriers, additives, and metabolite-based

bioformulations, yet the practical potentialities of the strategies are still in the primary stage and need to be further advanced. Future studies could also include designing the bioformulations with multiple desirable traits like plant growth promotion and bioremediation in one consortium. Transfer of technology from lab to land demands the overcoming of in vitro and in vivo experimental failures and popularizing the microbial inoculants in farmers' fields.

In conclusion, it can be reported that commercialization and application of microbial inoculants is gaining attention, but their use is still lacking far behind the use of agrochemicals. Farmers are more influenced by conventional methods and do not risk the acceptance of new technologies. Thus, governmental policies can be key player in educating the farmers about green eco-friendly technologies. The microbial technology should successfully reach the fields, so as to establish a synergism between agricultural productivity and environmental sustainability. The already popular strategy of microbial inoculants could further be realized by decoding the plant-microbe communication and initiating the wide-scale application reducing the unsustainable means. Microbial inoculants are efficient models promoting plant growth by diverse mechanisms, and their cost-effectiveness and lesser maintenance are required to be further fine tuned to use them for ushering in a new era of sustainable agriculture. It is also important to convince the farming community by ensuring the consistency so that the substitution becomes easier and the target of next green revolution is achieved.

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