Chapter 14
Biofertilizers: Microbes for Agricultural Productivity

Fatemeh Salimi and Javad Hamedi

Abstract The world has witnessed explosive population growth, which requires an efficient food supply. To this end, various efforts have been performed in agriculture among which the applying chemicals with anti-pest and fertilizing activity was a strategy of choice. However, implementation of such compounds has shown to have serious drawbacks, from the reduction of naturally occurring organisms which control the pests to the concerns arisen from environmental pollution. Therefore, the discovery and development of biological strategies have attracted much attention. Accordingly, there are well-known plant growth-promoting microorganisms (GPMs) with great potentials in improving the growth of plants via providing nutrition and alleviating biotic and abiotic stresses. Herein, a comprehensive study was performed to gather together the most updated knowledge on these mechanisms.

Keywords Agricultural applications · Biofertilizers · Microbiomes · Sustainability

14.1 Introduction

Different soil properties such as its texture, structure, and nutritional ingredients directly influence plant growth, among which, the latter property shows great importance. There are non-minerals (hydrogen, oxygen, and carbon), and minerals (macronutrients and micronutrients) obligatory for the growth and development of plants. Carbon dioxide and water provide non-minerals for the plant (Taiz 2010), while the latter is obtained from the soil. Since plants consume large amounts of some macronutrients such as nitrogen, phosphorus, and potassium, these components exist

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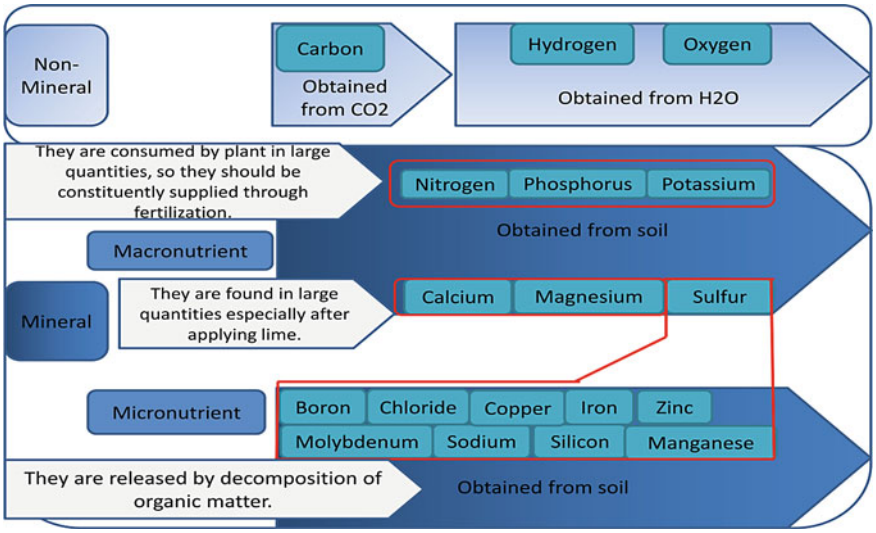

Fig. 14.1 Essential mineral and non-mineral nutrients for plant growth

in very low quantity in nutrient-depleted soil, while other macronutrients like calcium and magnesium are found abundantly in the soil under normal conditions, especially whenever lime is applied to reduce soil acidity. Macronutrients, e.g., sulphur, and micronutrients including boron, copper, iron, chloride, manganese, molybdenum, and zinc are released by the slow procedure of organic matter decomposition (Hänsch and Mendel 2009; Yadav et al. 2021) (Fig. 14.1).

Bioavailability of these nutrients for plants is dependent on their amounts in the soil, the soil composition, as well as its physicochemical characteristics like nutrient and water retention, and oxygen content of the soil which are influenced by soil texture, the proportion of soil ingredients or components like sand, silt, clay and organic matter (Bronick and Lal 2005). High content of clay and organic matter leads to a considerable level of nutrient and water retention, and in some cases, it results in waterlogged soil and thus depletion of oxygen content. In this condition, aerobic respiration and nitrate production are ceased. On the other hand, nutrient uptake becomes difficult in soil containing a high level of sand, due to their leaching and entering to groundwater (Galloway et al. 2008). Soil structure, aggregation of soil particles, determines productivity; because it directly affects the movement of water and oxygen, availability of nutrient, and microbial activity within the rhizospheric regions of plants (Bronick and Lal 2005). Soil pH is also an influential factor in the bioavailability of nutrients. The macronutrients and micronutrients show less bioavailability at high and low pH levels. The slightly acidic pH range due to promoting root growth, nitrogen fixation, and sulfur conversion to sulfate, releasing minerals, and increasing carbonates, sulfates, and phosphates solubility, is suitable for plant growth (Taiz 2010). In sum, the soil contains essential elements, and its physical, chemical, and biological characteristics influence plant growth (Fig. 14.2).

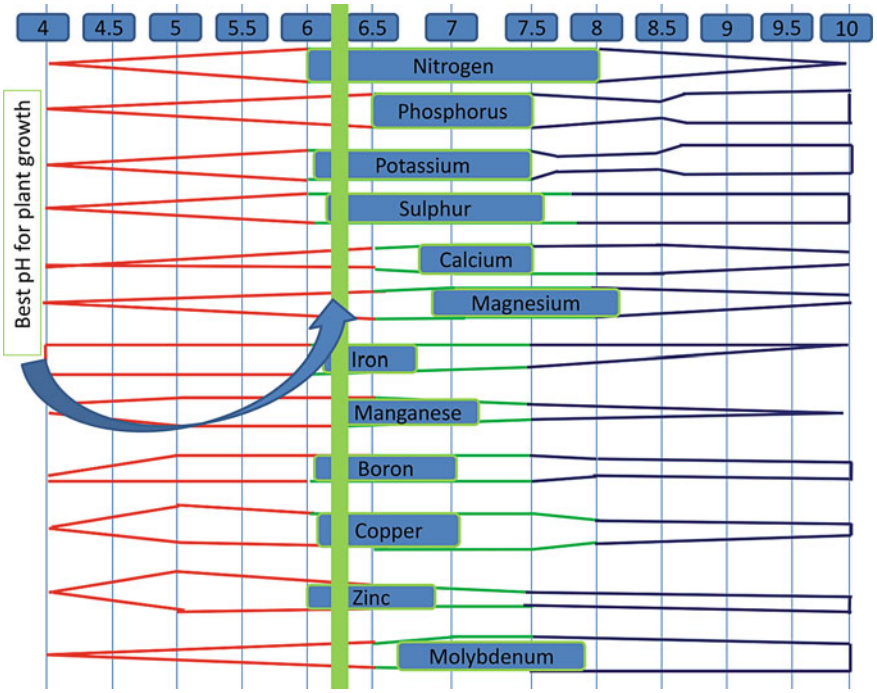

Fig. 14.2 Availability of macro and micronutrients in various pH. Distance of upper and lower lines indicates its bioavailability for living organisms

14.2 Fertilizers as Plant Growth Boosters

It has been predicted that the world population will be undoubtedly increased to 10 billion around 2050, with an annual increase of 97 million (Conway 2012). Therefore, the inevitable increasing demand for food and the falling ratio of cultivable lands will be a serious challenge. According to FAO, an unacceptably high portion of people are undernourished (FAO 2010). To ameliorate this need, fertilizers and pesticides have been consumed to achieve enhanced productivity through promoting plant growth and preventing product loss due to pests.

14.2.1 Chemical Fertilizers

Chemical fertilizers, cost-effective formulation of nitrogen, phosphate, and potassium, have been widely used over centuries. These fertilizers provide nutrition in the form of bioavailable organic/inorganic salts, and their application appeared as a quick improvement in plant growth. In addition to nutrients including, phosphate, nitrate, ammonium, and potassium salts, heavy metals like Hg, Cd, As, Pb, Cu, Ni,

and Cu; natural radionuclides like ^{238}U, ^{232}Th, and ^{210}Po also exist in chemical fertilizers. Netherlands, Egypt, Japan, China, UK, Germany, France, USA, Italy, India, Greece, Indonesia, and Turkey are using 665.5, 624.8, 373.2, 301.5, 287.5, 205.4, 180.1, 160.8, 126.4, 121.4, 115.4,106.9, and 100.4 kg per hectare chemical fertilizer (N + P + K), respectively (Savci 2012).

Over time, it has been revealed that prolonged usage of chemical fertilizers has resulted in the gradual loss of their effectiveness and corresponding environmental problems were also emerging (Kumar et al. 2021; Sharma et al. 2021; Xiang et al. 2012). In this situation, more chemical fertilizers should be applied to achieve the same productivity. Also, the production process of chemical fertilizer results in the release of hazardous substances including sulfur oxides, nitric oxides, and fluorine compounds into the environment. Continuous application of chemical fertilizers can profoundly modify the salinity and pH of the soil, decline soil mineral and its water retention capacity, which can lead to gradual deterioration. It has been observed that extended or continuous supply of a considerable amount of nitrogen fertilizer can cause plant tissue softening, leading to increased sensitivity of plants to pests and diseases (Adesemoye et al. 2010; Chen 2006).

Whenever excessive amounts of chemical fertilizers are applied, and plants cannot assimilate them; several physical and chemical procedures are activated to decrease their concentrations in soil. Rinsing out, leaching, volatilization, immobilization, replacement, precipitation, and microbiological conversions are some of these strategies which may lead to the dispersion of hazardous chemicals into the air and aquatic ecosystems and cause long-lasting serious environmental problems, e.g., eutrophication of waters.

Considerable rate (50–70%) of nitrogenous contamination is due to nitrogenous fertilizer. Unabsorbed nitrogenous fertilizers are converted to nitrate through microbial nitrification. Generated soluble nitrate reaches the depth of soil and enter the groundwater due to its high solubility and negative charge. Leached nitrate, reactive nitrogen species, nitrites, and nitrosamines in ground and surface waters are some of the life-threatening compounds. High levels of nitrates, nitrites, and nitrosamines can aggregate in crops and adversely impose human and animal health. High-level nitrate (more than 50 mg NO^{3-}/L) in drinking water cause inflammation of the digestive and urinary systems, methemoglobinemia, and some related diseases in infants and ruminant animals. Also, it increases the risk of metabolic diseases including cancers, respiratory disorders, cardiovascular ailments, goiter, congenital disabilities, digestive system disease, and the rate of infections with West Nile virus, malaria, and cholera (Galloway et al. 2008).

High buffering feature of soil has resulted in less obvious effects of chemical fertilizers on it; however, over time its deterioration occurs, and in turn, leads to decreased soil quality, normal structure, and composition due to losing its buffering potential. Also, the accumulation of toxic substances in the soil is lethal for living organisms, like microorganisms and earthworms. This biologically passive soil possesses negligible organic matter and less liberation of nutrients as much as biologically active soil, and because of this, interactions of living organisms are disrupted in this condition (Chandramohan et al. 2013). Prolonged consumption of nitrogen fertilizers

like ammonium sulfate, which produces acid, dramatically reduces soil pH. Also, the diversity of microbial species in the rhizospheric regions are changed in acidic soil. In this condition, activities of plant growth-promoting microorganisms like decomposition of organic matter and symbiotic interaction with plants are reduced or inhibited. Ammonium sulfate fertilizer extremely acidifies the soil, and its extensive use will result in Mg deficiency (Fageria et al. 2010).

One of the most problematic matters during applying chemical fertilizers is groundwater contamination (Galloway et al. 2008). Nitrates are generated from the breakdown of nitrogen fertilizers, and because of their water-solubility feature, they easily move within the soil and can persist in that position for a long time leading to ecosystem deterioration (3).

Besides detrimental effects on human and animal life, terrestrial and aquatic ecosystems are affected by the extensive use of chemical fertilizers. For instance, large fractions of nitrogen fertilizers are oxidized, and nitrogen monoxides and nitric oxides are generated through denitrification process, which results in the depletion of the ozone layer and increases the probability of skin cancer. Also, nitric acid can be created after using urea and ammonium salt, the most current forms of ammonium, and in combination with sulphuric acid leading to the generation of acid rains which adversely affects ecosystems and results in erosion and depletion of the soil. High levels of water-soluble potassium impose an adverse effect on the soil pH and structure as well as seed germination. In this condition, uptake of other minerals and nutrients is ceased and the quality of the crop is declined (Savci 2012).

Rampant fertilization using phosphorous fertilizers disrupts its balance and leads to the accumulation of phosphorous in sites of application. Applied ammonium phosphates and superphosphates containing calcium phosphate, readily relocate under acidic conditions and plants could consume them as the phosphorous source. While in alkaline conditions, phosphorous compounds are potently attached in the phosphorus retarded reactions between soluble phosphates, and aluminum, iron, manganese, and calcium ions. The result of these reactions (including phosphate sorption, adsorption, retention, precipitation, or immobilization) is the production of insoluble and unleachable salts. Therefore, they cannot travel within the soil tissue. Therefore, these processes profoundly decrease the availability of plants to phosphorous. This process makes the continuous application of phosphate fertilizers inevitable. Excessive and accumulated phosphates accelerate eutrophication in terrestrial and aquatic ecosystems, which can impose lethal effects on their inhabitants.

Eutrophication creates an oxygen-free environment that is not suitable for drinking and profoundly reduce living species in the marine ecosystems as well as causes proliferation of unwanted species and unfavorable odor (Chislock et al. 2013). In addition, trace amounts of cadmium, chromium, lead, uranium, and radium exist in phosphate fertilizers. The prolonged application of this fertilizer can enhance the concentrations of these pollutants. These hazardous compounds pollute the soil and water, and whenever they enter the surface water or are absorbed by plants, can, in turn, enter into the human body through the food chain and create life-threatening problems (Fig. 14.3) (Khan et al. 2018).

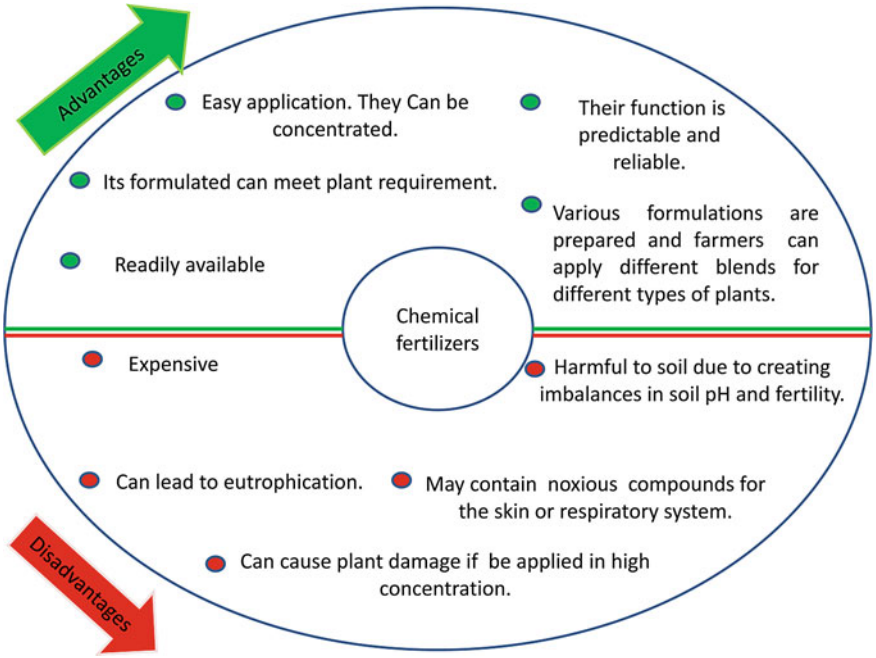

Fig. 14.3 Advantages and disadvantages of chemical fertilizers

Although extensive usage of chemical fertilizers has adverse effects on human and animal health and ecosystems, the inevitable need for chemical fertilizers by the agriculture industry is obvious. Lack of their use can lead to insufficient nutrients for the world population. Therefore, there is a pivotal need for alternative fertilizers which sustainably and eco-friendly flourish agricultural yield.

14.2.2 Organic Fertilizers

Conventional agriculture via chemical fertilizers and devastating agricultural imposes adverse effects on human and animal health, microbial habitats, and beneficial insects. It leads to the deterioration of soil and terrestrial ecosystems, and ozone layers. Also, it has been proven that its efficiency is unsustainable (Fricke and Vogtmann 1993; Mäder et al. 2002). Therefore, alternate farming methods are being applied to recover soil quality and ameliorate environmental degradation. One of these new eco-friendly approaches with self-sustainability features is organic agriculture. FAO/WHO has defined organic agriculture as a comprehensive production management system which uses it, the health of agro-ecosystem that is characterized by improved biodiversity, balanced biogeochemical cycles, and enhanced animal and microbial activities in soils. In organic agriculture, sustainable management practices

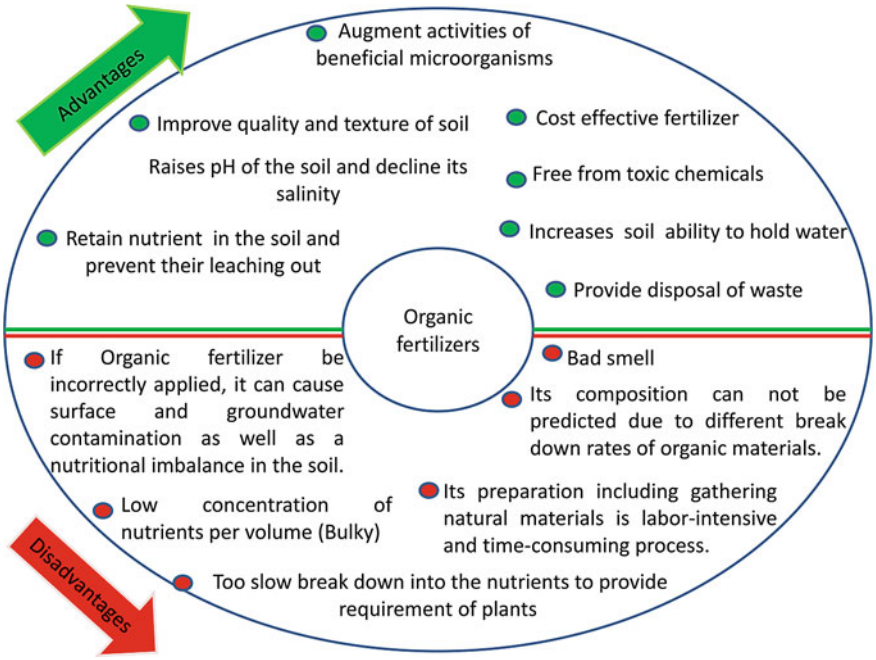

Fig. 14.4 Advantages and disadvantages of organic fertilizers

in all aspects of farm management by considering regional conditions and applying locally adapted systems such as agronomic, biological, and mechanical methods should be implemented (FAO/WHO 2015).

In this regard, natural means like growing cover crops, mainly leguminous species, are applied. Organic fertilizers like composts and manure act in a different way in comparison to chemical fertilizers, e.g., they supply nutrients in a lower concentration (like potassium) (Fricke and Vogtmann 1993; Hernández et al. 2010) and slower release rate (like nitrogen because of low rate of mineralization) (Hernández et al. 2010). In sustainable agriculture, maximum yield cannot be achieved by solely relying on organic fertilizers. Therefore, through the combined usage of chemical–organic fertilizer along with biofertilizers, production yield can be maximized in a sustainable approach (Qin et al. 2015; Shafi et al. 2012; Song et al. 2015). The advantages and disadvantages of organic fertilizers are presented in (Fig. 14.4).

14.2.3 Biofertilizers

Applying manure, crop rotation with legumes, and water managing to increase soil's nutrient content through their naturally occurring microorganisms are some of the ancient strategies to improve the fertility of lands (Franche et al. 2009; Morrison

and Cozatl-Manzano 2003; Suthar et al. 2017). Microbial fertilizers were developed and commercialized in the late nineteenth century for the first time (Bashan 1998; Kilian et al. 2000) to ameliorate the unfavorable aspects of excessive usage of chemical fertilizers. Applying biofertilizer results in a reduced application of chemical fertilizer (Singh and Adholeya 2003).

Biofertilizers and green manure, intercrop, or organic supplemented chemical fertilizers are not the same concepts (Bhattacharyya and Jha 2012; Halpern et al. 2015). They are formulated with different types of beneficial microorganisms or their latent forms, which once applied, colonize the rhizospheric region or the inner parts of the plant and perform agriculturally-important activities including conversion of nutritionally critical nutrients from unavailable to bioavailable form (nitrogen and phosphate), mineralization of sulphur, zinc, potassium, and iron, producing degrading enzymes, antibiotics and phytohormones, induction of resistant mechanisms in the plants, secretion of growth hormones through which enhance crop yield up to 10–40% (Ahemad and Kibret 2014; Bhardwaj et al. 2014; Bhattacharjee and Dey 2014; Gaur 2010; Kour et al. 2019b, 2021; Lugtenberg and Kamilova 2009; Mishra et al. 2013a; Owen et al. 2015). Since the biofertilizers have low cost and renewable properties, they can be supplied along with chemical fertilizers to cut down their use, intensify their beneficial activities, and reduce their deteriorating activity on the ecosystem. Biofertilizer provides various inorganic substances with low bio-availability. Therefore, this strategy is more cost-effective for farmers than synthetic fertilizers (Kour et al. 2020). Co-application of phosphate solubilizing bacteria and bacteria with potassium solubilizing activity along with rocks containing low soluble phosphate and potassium enhanced yield and assimilation of nitrogen, phosphate, and potassium by various plants in phosphate and potassium limiting conditions (Han and Lee 2005; Han et al. 2006; Vassilev et al. 2006a). There are several types of biofertilizers, including microorganisms with the ability of nitrogen fixation, phosphate solubilization, phosphate mobilization, and promotion of plant growth (Mondal et al. 2020; Yadav 2021). It is proven that combined application of chemical, organic, and biofertilizers can meet the increasing need of enhancing world population to foods at a time when agriculture-based industries are facing various environmental concerns (Suthar et al. 2017).

However, despite the tremendous application of biofertilizers, their application is limited due to many factors such as the unpredictability of results, difficult identification, and traceability of microbial strains in the field, limited knowledge about interactions among microbial cells and plants, and the technical process of large scale production (Bashan et al. 2014; Lucy et al. 2004; Owen et al. 2015). In addition to bacterial grazers, especially naked amoeba, nematodes can also modify the effectiveness of microbial inoculum (Malusa et al. 2010). One of the important obstacles in applying biofertilizers is that behavioral characteristics of microorganisms are changed in a microbial community compared to a situation that they exist in pure culture. These constraints are encouraging reasons to conduct extensive and comprehensive studies on biofertilizers (Fig. 14.5).

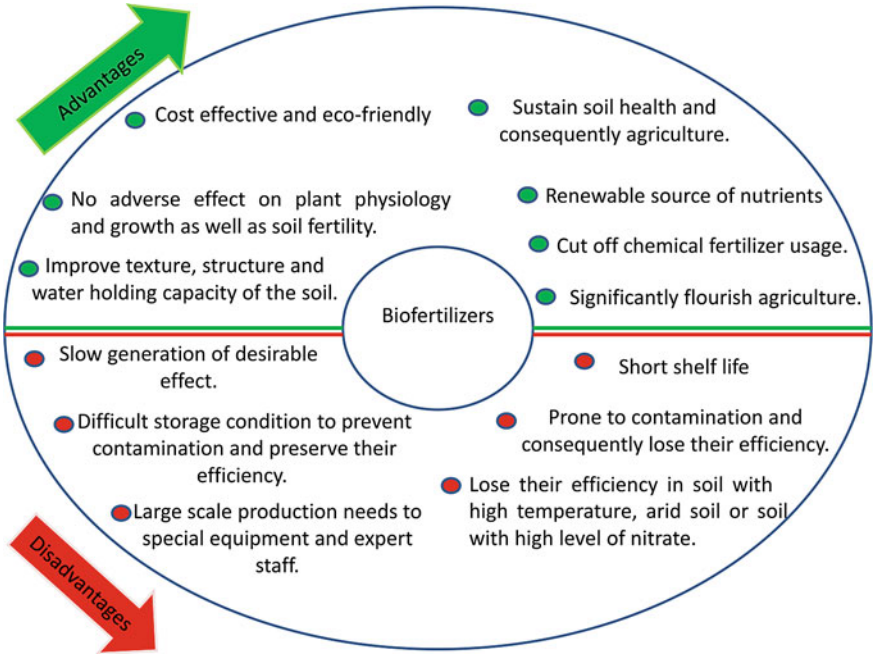

Fig. 14.5 Advantages and disadvantages of biofertilizers

14.2.3.1 Major Constituents of Biofertilizers

Each type of biofertilizer contains various beneficial microorganisms, including plant growth-promoting rhizobacteria, fungal strains like mycorrhiza and cyanobacteria (Table 14.1). These microorganisms can suppress the adverse effects of phytopathogenic organisms and abiotic stresses on plant growth and development via various strategies such as facilitating nitrogen, phosphate, iron, zinc, and potassium acquisition, modifying phytohormone levels, which, in turn, can flourish agriculture in an eco-friendly manner (Kour et al. 2019a; Singh et al. 2020; Thakur et al. 2020; Tiwari et al. 2020, 2021). According to the mechanisms by which biofertilizers augment plant growth, they are divided into various groups including biofertilizers containing microorganisms with nitrogen fixation ability (*Rhizobium, Bradyrhizobium, Azospirillum,* and *Azotobacter*), phosphate mobilization ability (*Mycorrhiza*), growth promotion activity (*Pseudomonas*), phosphorous solubilization capability (*Bacillus, Pseudomonas, Aspergillus*, *Penicillium*, *Fusarium, Trichoderma*, *Mucor*, *Ovularopsis*, *Tritirachium,* and *Candida*), and biofertilizers with compost enriching activities (*Humicola fuscoatra*, *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus ochraceus*, *Fusarium solani*, and *F. oxysporum*).

Biological fertilizers can be made either by a single microorganism or a mixture of them. Results would be better if applying single microorganism which shows simultaneous mechanisms to promote plant growth, e.g., simultaneous capability of

Table 14.1 Active agents in various types of commercial biofertilizers

S. no.	Commercial biofertilizers
1	Nitrogen-fixing biofertilizers containing *Rhizobium, Bradyrhizobium, Azospirillum* or *Azotobacter*
2	Phosphate solubilizing biofertilizers containing *Bacillus, Pseudomonas* or *Aspergillus*
3	Phosphate mobilizing biofertilizers containing *Mycorrhizae*
4	Plant growth-promoting biofertilizers containing *Pseudomonas* sp.
5	Phospho-bacterium and *Mycorrhizae*
6	*Rhizobium* and phosphobacterins
7	Cyanobacteria
8	*Aspergillus niger* 1107 phosphate solubilizing fungus
9	*Bacilluscereus* strain RS87
10	*Azoarcus* and *Zoogloea*
11	*Vesicular arbuscular mycorrhiza*
12	*Azospirillium brasiliense*
13	*Azospirillium amazonense*
14	*Acetobacter diazotrophicus*
15	*Derxia gummosa*
16	*Torulospora globasa*
17	*Thiobacillus*
18	*Trichoderma* sp.
19	*Paecillomyces* sp.
20	*Pseudomonasfluorescens*

Source Mahanty et al. (2017)

phosphate solubilization and biological control of filamentous fungi (Vassilev et al. 2006a), simultaneous ability to solubilize phosphate and biological control of *Trichoderma* strains (Altomare et al. 1999), simultaneous assimilation of both inorganic and organic nitrogen along with phosphate or other micro- and macro-elements by arbuscular mycorrhiza fungi (AMF) (Hawkins et al. 2000; Smith and Read 2008) have been reported. On the other hand, biofertilizers containing mixed microbial inoculum can promote plant growth through various strategies. These microbial strains are produced in individual fermentation processes and then mixed with each other (De Roy et al. 2014). For instance, the co-application of *Penicillium* spp. and AMF, *Penicillium* and *Rhizobium* spp., AMF with rhizobia, *Rhizobium* and phosphate solubilizing bacteria, AMF/Rhizobium/phosphate solubilizing fungus can be named, which leads to enhanced growth of cereals (Babana and Antoun 2006; Kucey 1988) and legumes, respectively (Alagawadi and Gaur 1988; Downey and Van Kessel 1990; Rice et al. 2000; Wang et al. 2011).

In some cases, the yield of fertilized plants by biofertilizer containing arbuscular mycorrhiza fungi and free-living bacteria with nitrogen fixing ability or various

PGPR was equal to plants which were fertilized with chemicals (Adesemoye et al. 2008; Malusa et al. 2007; Wu et al. 2005; Xavier and Germida 2003). Biofertilizers with several plant growth-promoting mechanisms can obtain more acceptance from farmers and markets who prefer to use multifunctional products and present a product for several purposes, respectively (Vassilev et al. 2006b).

Plant Growth-Promoting Rhizobacteria (PGPR)

Rhizospheric bacteria are divided into three groups according to their interaction with plants, including commensal, parasite, and beneficial association (Rai et al. 2020). In commensalism, bacteria harmlessly colonize on the root surface (Verma et al. 2017). No observable effect is imposed on the physicochemical properties of plants (Beattie 2007). Phytopathogenic rhizobacteria establish a parasitism interaction with host plants through producing phytotoxic substances which adversely affect the physicochemical properties of plants. Beneficial rhizobacteria are considered an available part of the rhizospheric reign. They grow in the vicinity of host plants and stimulate their growth through multifaceted activities like solubilizing the nutrients, fixing atmospheric nitrogen, producing phytohormones and lytic enzymes, stimulating the growth of beneficial microorganisms like mycorrhizae, limiting phytopathogens, acting as biocontrol agents (Franco-Correa et al. 2010), removing phytotoxic substances or alleviating salinity, drought, and flooding stresses (Bashan and De-Bashan 2010; Khalid et al. 2004), thus they are identified as plant growth-promoting rhizobacteria (PGPR).

A few of their characteristics like high adaptability to various environmental conditions, fast growth rate, and considerable ability to degrade an extended spectrum of natural and xenobiotic compounds lead to their successful competition with autochthonous, and especially phytopathogenic microorganisms. Rhizobacteria with aggressive root colonization potential, ability to stimulate plant growth, and biocontrol activities can be considered as PGPR (Vessey 2003).

According to their association with root cells of the plants, PGPR are divided into extracellular plant growth-promoting rhizobacteria (ePGPR) and intracellular plant growth-promoting rhizobacteria (iPGPR) (Martínez-Viveros et al. 2010). ePGPR frequently exist within the rhizoplane or in the spaces between the cells of the root cortex, while iPGPR are exclusively present within the specialized nodular structures of root cells. ePGPR belong to *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium, Micrococcus*, *Pseudomonas,* and *Serratia* genera (Gray and Smith 2005), while iPGPR belong to *Frankia*, *Allorhizobium*, *Azorhizobium* (*Azorhizobium caulinodans*) (Dreyfus et al. 1988), *Bradyrhizobium* (*Bradyrhizobium japonicum*) (Guerinot and Chelm 1984), *Mesorhizobium* (*Mesorhizobium chacoense* (Velázquez et al. 2001), *Mesorhizobium pluriforium* (de Lajudie et al. 1998), *Sinorhizobium* (*Sinorhizobium arboris* (Nick et al. 1999), *Sinorhizobium fredii* (Chen et al. 1988), and *Sinorhizobium medicae* (Rome et al. 1996)), and *Rhizobium* (*Rhizobium cicero* (Nour et al. 1994), *Rhizobium etli* (Segovia et al. 1993), *Rhizobium fredii* (Scholla and Elkan 1984), *Rhizobium galegae* (Lindström 1989), *Rhizobium gallicum* (Amarger et al. 1997), *Rhizobium giardinii* (Amarger et al. 1997)) genera.

Fungi

Fungal biofertilizers comprise a single fungus or mixture of fungal strains and through direct or indirect mechanisms improve the growth of plants and yield of crops (Devi et al. 2020). Mycorrhizal hyphae extend into the soil through infecting plants growing in their vicinity and impose their improving effect on plant growth by penetrating nutrient depletion zone and increasing bioavailability or mobility of elements (Chiariello et al. 1982).

A mycorrhiza, a fungal distinct morphological structure, generates mutualistic association with roots of host plants like herbs, shrubs, trees, aquatic, xerophytes, epiphytes, hydrophytes, many crops, and forest tree species (Rai et al. 2013). In this association, roots of host plants are intracellularly or extracellularly colonized, either by endomycorrhizal fungi or ectomycorrhizal fungi, respectively. Applying biofertilizers containing mycorrhizal fungi probably results in carbon storage in soil via altering kinetic properties of the root, improving its ability to uptake nutrients which consequently leads to improved quality of soil organic matter to support more agricultural productivity (Smith and Smith 1997).

They are various endomycorrhiza, they are categorized into arbuscular, ericoid, arbutoid, monotropoid, and orchid mycorrhizae. Arbuscular mycorrhizal (AM) fungi are frequent in the terrestrial ecosystem from the arctic to the tropics (Gerdemann 1968). Studies have shown their high occurrence in symbiotic association. They possess high diversity due to the diversity of plant species, soil characteristics, and seasonal conditions (Smith and Smith 2012). They belong to the Glomeromycota phylum (Schuessler et al. 2001). *Gerdemannia*, *Acaulospora*, *Scutellospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Archaeospora*, *Geosiphon*, *Paraglomus*, and *Sclerocystis*are AM forming genera. After arbuscular mycorrhiza colonization on the root cortex, highly branched structures like arbuscules are formed inside the cells where nutrient exchange occurs between plant and fungus (Balestrini et al. 2015).

Fungi alleviate biotic stresses like parasitic fungi and nematodes (Duchesne et al. 1989) and abiotic stresses due to drought, salinity, and flooding and enhance the resistance of plants against heavy metals, promote plant productivity and agricultural productivity mainly in low-nutrient and stressful conditions through mobilizing P, supplying macro and micronutrients like P, Ca, Zn, S, N which are inaccessible to plant roots (Augé et al. 2015; Meier et al. 2015; Porcel et al. 2012; Rana et al. 2019a; Sharma et al. 2019). It has been revealed that AM symbiosis induces the expression of Pi transporters in plants (Walder and van der Heijden 2015; Xie et al. 2013). Fungal hyphae have a higher penetration ability than plant roots which able them to obtain nutrients which are away from plant roots. In turn, plants supply carbohydrates for AM fungi (Allen 2011).

Trees with ectomycorrhizal (ECM) fungi inoculated roots due to rapid absorbing and accumulating nitrogen, phosphorus, potassium, and calcium show better growth parameters than nonmycorrhizal plants. These fungi accelerate the degradation of the complicated minerals and organic matter in the soil and their transmission to the tree. Also, they enhance the tolerance of trees to biotic and abiotic stress including,

drought, high temperatures, extreme pH of the soil, and toxins. *Pisolithus tinctorius* and *Piriformospora indica* are ECM fungi with multifaceted plant growth-promoting activities (Schwartz et al. 2006). There are many fungal biofertilizers such as fungal species which exert their plant growth improving effects through solubilizing phosphorous (*Aspergillus* spp., *A. tubingensis*, *Penicillium* spp., *Fusarium* spp., *Trichoderma* spp., *Mucor* spp., *Tritirachium* spp., and *Candida* spp.), solubilizing potassium (*Aspergillusfumigates*, *A. niger*, *A. terreus*, and ectomycorrhizal fungi), solubilizing zinc (*Saccharomyces* spp., *Oidiodendron maius* and *Aspergillus niger*, *Penicillium simplicissimum*), mobilizing phosphate (ectomycorrhiza and *Arbuscular mycorrhiza*), and enriching compost (*Trichoderma* spp., *Penicillium* spp., *Aspergillus* spp., *Pleurotus* spp., *Chaetomium* spp., *Humicola fuscoatra*, and *Fusarium* spp.) (Lian et al. 2008; Whitelaw 2000; Raj 2007).

Cyanobacteria

Biofertilizers containing microalgae, especially cyanobacteria promote plant growth and soil condition via various strategies. They improve the nutrient quantity of soil through secreting phytohormones like auxin (*Nostoc*, *Hapalosiphon*), gibberellin, vitamins like vitamin B12 (*Cylindrospermum* sp. *Tolypothrix tenuis*, *Nostoc muscorum*, and *Hapalosiphon fontinalis*), amino acids (Rodríguez et al. 2006a, 2006b; Roger and Pierre-Adrien 1982), fixing nitrogen and releasing nutrients after their death and decomposition. *Anabaena azollae* with lignolysis ability release phenolic compounds once applyied as biofertilizer. *Nostoc linkia*, *Anabaena variabilis*, *Aulosira fertilisima*, *Calothrix* sp., *Tolypothrix* sp., and *Scytonema* sp. are nitrogen-fixing cyanobacteria, which can be utilized for rice cultivation (Prasad and Prasad 2001). *Anabaena* fixes nitrogen (up to 60 kg/ha/season) in association with water fern *Azolla* and enhances organic matter content in the soils (Moore 1969).

Cyanobacteria contain considerable amounts of macro and micronutrients, as well as amino acids. They can be considered as a suitable alternative for chemical fertilizers to alleviate their environmental polluting effects (MM 2001). Algae via producing organic acids can increase the bioavailability of soil phosphate. The physicochemical properties of soil can be improved via algal biofertilizers. They enhance water holding capacity and aeration of soils through their jelly structure and filamentous structure, respectively. Soil salinity is reduced after their application. They can act as viable biocontrol agents by preventing the growth of weeds. Since cyanobacteria are capable of degrading various kinds of pollutants and possess simple growth requirements, they can be applied to rehabilitate deteriorated ecosystems (Subramanian 1996).

14.2.3.2 Biofertilizer's Mechanism of Action

As mentioned, microbial cells in biofertilizers improve plant growth via various mechanisms which will be discussed hereafter (Tables 14.2, 14.3, and 14.4) and (Fig. 14.6) also summarized these mechanisms.

Table 14.2 Plant growth-promoting activities of microbial cells by increasing bioavailability of nutrients

Microorganisms	Mechanism of action for plant growth improvement
Azoarcus sp.	Nitrogen fixation
Azorhizobium	
Azospirillum sp.	
Azotobacter sp.	
Beijerinckia sp.	
Burkholderia sp.	
Frankia sp.	
Gluconacetobacter diazotrophicus	
Herbaspirillum sp.	
Bacillus polymyxa	
Cyanobacteria	
Paenibacillus sp.	Potassium solubilization
Bacillus sp.	
Aspergillus fumigates, Aspergillus niger, Aspergillus terreus	
Acidothiobacillus ferrooxidans	
Phyllobacterium sp.	Phosphate solubilization
Rhizobium leguminosarum	
Mesorhizobium mediterraneum	
Bradyrhizobium sp.	
Bradyrhizobium japonicum	
Arthrobacter sp.	
Burkholderia sp.	
Enterobacter asburiae	
Acinetobacter sp.	
Flavobacterium sp.	
Microbacterium pseudomonas	
Rhodococcus sp.	
Erwinia sp.	
Aspergillus tubingensis, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus awamori*, *Aspergillus fumigates*, *Aspergillus tubingensis*, *Aspergillus melleus*	

(continued)

Table 14.2 (continued)

Microorganisms	Mechanism of action for plant growth improvement
Penicillium bilaji, *Penicillium albidum*, *Penicillium italicum*, *Penicillium simplicissimum*, *Penicillium frequentans*, *Penicillium oxalicum*, *Penicillium rubrum*, *Penicillium expansum*, *Penicillium citrinum*	
Fusarium moniliforme, *Fusarium udam*	
Trichoderma viridi, *Trichoderma harzianum*, *Trichoderma virens*, *Trichoderma asperellum*	
Mucor ramosissimus, *Mucor mucedo*, *Mucor hiemalis*	
Tritirachium album, *Tritirachium egenum*	
Candida krissii, *Candida scotti*	
Ectomycorrhiza	Phosphate mobilizing biofertilizer
Arbuscular mycorrhiza	
Acaulospora spp.	
Scutellospora spp.	
Enterophospora, *Gerdemannia*, *Gigaspora* sp.	
Saccharomyces spp.	Zinc solubilising biofertilizers
Oidiodendron maius	
Penicillium simplicissimum	
Aspergillus niger	

Sources Hayat et al. (2010), Meena et al. (2017)

14.3 Making Nutrient Available for Plants

Biofertilizers augment plant growth through enhancing the bioavailability of nutrients in the rhizospheric regions gradually. They increase nutrient availability and prevent nutrient leaching out via fixing nitrogen, solubilizing phosphate, potassium, and zinc and producing siderophores as well as decompose organic material (Prasad et al. 2021).

14.3.1 Fixation of Nitrogen

Nitrogen is a critical macronutrient for plant growth and productivity, which plants require to construct macromolecules like proteins and nucleic acid. Most portion of nitrogen (78%) exists in the atmosphere as N_2 which is an unavailable form for plant assimilation. N_2 should be converted to bioavailable organic form (ammonia) to

Table 14.3 Plant growth-promoting activities of microbial cells by producing or modulating phytohormones

Microorganisms	Mechanism of action for plant growth improvement
Azobacter sp.	Cytokinin synthesis
Bacillus sp.	
Rhizobium leguminosarum	
Bacillus sp.	Auxin synthesis
Bacillus sp.	Gibberelin synthesis
Sphingomonas sp.	
Paenibacillus polymyxa	
Pseudomonas fluorescens	
Rhizobium leguminosarum	
Paenibacillus sp.	Indole acetic acid synthesis
Rhizobium leguminosarum	
Aeromonas veronii	
Agrobacterium sp.	
Alcaligenes piechaudii	
Azospirillum brasilense	
Azotobacter sp.	
Comamonas acidovorans	
Enterobacter cloacae, Enterobacter sp.	
Bradyrhizobium sp., *Bradyrhizobium japonicum*	
Mycobacterium sp.	
Kluyvera ascorbata SUD 165	
Serratia mercescens	
Azospirillum brasilense	
Bacilluscirculans P2, *Bacillus* sp. P3, *Bacillusmagaterium* P5 *Bacillus*. sp. Psd7	
Streptomycesanthocysnicus	
Azospirillumlipoferum strains 15, *Pseudomonasaeruginosa* Psd5 *Pseudomonaspieketti* Psd6, *Pseudomonas fluorescens* MTCC103	

Sources Hayat et al. (2010), Meena et al. (2017)

Table 14.4 Plant growth-promoting activities of microbial cells by inhibiting phytopathogens and increasing resistance of plant

Microorganisms	Mechanism of action for plant growth improvement
Bacillus sp.	Siderophore production
Chryseobacterium sp.	
Phyllobacterium sp.	
Pseudomonas fluorescens	
Rhizobium sp.	
Streptomyces sp.	
Mesorhizobium loti MP6	
Pseudomonas tolaasii	
Serratia mercescens	
Kluyvera ascorbata SUD 165	
Rhizobium meliloti	
Bradyrhizobium sp.	
Bradyrhizobium japonicum	
Pseudomonas sp.	ACC deaminase synthesis
Rhizobium sp.	
Alcaligenes sp.	
Bacillus pumilus	
Enterobacter cloacae	
Pseudomonas cepacia	
Pseudomonas putida	
Pseudomonas sp.	
Variovorax paradoxus	
Bacillus sp.	Induction of plant stress resistance
Mycobacterium sp.	
Pseudomonas sp.	
Rhizobia sp.	
Rhizobia sp.	Hydrogen cyanide production
Bacillus sp.	Antibiotic production
Pseudomonas sp.	
Pseudomonas sp.	Chitinase and β-glucanases production
Sinorhizobium sp.	

Sources Hayat et al. (2010), Meena et al. (2017)

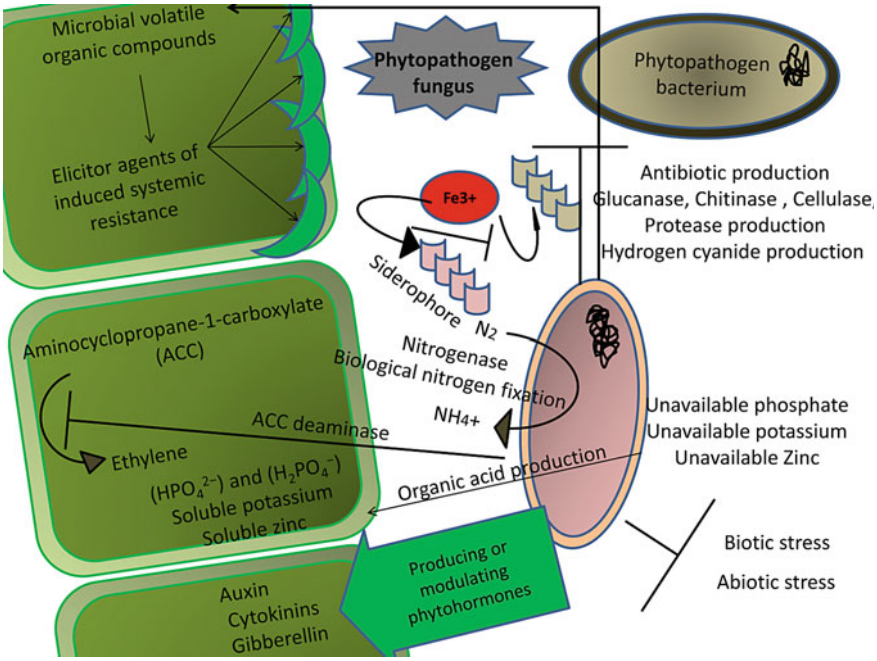

Fig. 14.6 Various mechanisms by which microbial cell forming biofertilizers improve plant growth

compensate the loss of N from soils or ecosystems by a well-known process known as biological nitrogen fixation (Tairo and Ndakidemi 2013). This process, exclusively, can be performed by nitrogen-fixing organisms, also known as diazotrophs, including bacteria and cyanobacteria through an oxygen-sensitive enzymatic complex known as nitrogenase system (Rana et al. 2020; Smith and Newton 2013). A considerable amount of ammonia (2.5×10^{11} kg) is annually produced through this system (Schlesinger and Bernhardt 2013). The amount of biologically fixed nitrogen can be affected by environmental conditions or different plant-microbe combinations. Biological fixation of nitrogen tremendously declines volatilization, leaching, and denitrification process. Biological nitrogen fixation can be done via free-living and symbiotic microorganisms. Some nitrogen-fixing microorganisms possess intimate endophytic associations with host plants and some nitrogen fixers, who live in close association in the rhizospheric region, and do not form intimate endophytic symbioses.

The nitrogen fixing ability of Rhizobia species like *Rhizobium*, *Allorhizobium Sinorhizobium*, *Bradyrhizobium*, *Azorhizobium*, and *Mesorhizobium*), as endophytes of leguminous plants, have been extensively studied (Gopalakrishnan et al. 2015; Laranjo and Oliveira 2014; Rana et al. 2019b). N_2-fixing endophytes are highly found in the legume class, but are not restricted to this class (Carvalho et al. 2014). Recently, many investigates have reported the isolation of endophytes from various non-leguminous plants. Restriction of a specific compartment has not been observed

in the endophytes of non-legumes. It has been observed that they colonize in various parts of plants, including, roots, stems, and leaves. The obligative or facultative association among these microorganisms and host plants can be created. The stomata or cracks at the site of lateral root emergence are sites through which these microorganisms can enter into various tissues of plants (Glick 2015; Gaiero et al. 2013). Entering endophytes into the plant's tissues caused a more favorable environment for the plant in the rhizospheric region (Reinhold-Hurek 2011). Since, they easily access to nutritional elements and low concentration of oxygen needed for nitrogenase activity. In return, the endophytes encourage the productivity of the host plants by fixing nitrogen and supplying compounds with growth-promoting activity. In recent years, the number of identified endophytic diazotrophs has been significantly enhanced. Various bacteria belonging to different genera, such as *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, and *Serratia*, have been reported to augment the growth of plants via various strategies (Glick 2015)

Nitrogen-fixing microorganisms can be either symbiotic or non-symbiotic. Symbiotic microorganisms (*Rhizobium*, *Frankia,* and *Azolla*) form a symbiotic relationship with leguminous and non-leguminous plants (Ahemad 2010; Glick 2015), while asymbiotic nitrogen fixers are free-living (*Azotobacter*, *Beijerinckia*) or endophytic (*Gluconacetobacter*, *Azospirillum,* and *Herbaspirillum*) microorganisms (Bhattacharyya and Jha 2012). There are many nitrogen-fixing microorganisms including *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Acetobacter*, *Azomonas*, *Beijerinckia*, *Bacillus*, *Clostridium*, *Enterobacter*, *Erwinia*, *Derxia*, *Desulfovibrio*, *Corynebacterium*, *Campylobacter*, *Herbaspirillum*, *Klebsiella*, *Lignobacter*, *Mycobacterium*, *Rhodospirillum*, *Rhodopseudomonas*, *Xanthobacter*, *Mycobacterium,* and *Methylosinus* which are associated with non-legumes(Wani 1990).

Some symbiotic bacteria with nitrogen fixing ability have been extensively studied, which includes *Rhizobia, Bradyrhizobium*, and *Frankia*. A large portion (70–80%) of biological fixation of nitrogen is performed by symbiotic microorganisms (Ishizuka 1992). Below are the descriptions of some symbiotic nitrogen fixers.

14.3.1.1 Rhizobium

Rhizobium is the best-known group of microorganisms which via symbiotic relationship with legume crops fixes nitrogen (50–100 kg/ha) and belongs to *Rhizobiaceae* family which consists of *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium*, *Rhizobium*, *Sinorhizobium*, *Devosia, Methylobacterium, Ochrobactrum,* and *Phyllobacterium, Burkholderia,* and *Cupriavidus* genera. Commercial rhizobial fertilizers, for legume crops, were first introduced in the 1890s. *Rhizobium* is an aerobic, non-sporulating, Gram-negative, rod-shaped, and fast-growing bacterium which forms nodules in the leguminous plant (Allito and Alemneh 2014; Lindström et al. 2006; Lindström and Martinez-Romero 2007).

Plant roots attract the rhizobia via their exudates. They colonize the roots of leguminous plants. An infection thread passes the root cortex toward a cluster of dividing cells that will become a plant root primordium. In this symbiotic relationship, the plant produces flavonoids that induce rhizobial nod genes and subsequently signal nodulation through the expression of *nod, nol, and noe* genes, leading to nodule formation, wherein nitrogen is biologically fixed. Besides N_2-fixing activity, rhizobia augment plant growth through mobilizing inorganic and organic P. In selecting biofertilizers, the high ability of their strains to fix nitrogen and compete with phytopathogens microorganisms should be considered. Under unfavorable conditions (acidic or basic soil) in which the population of symbiotic rhizobia is low (100 rhizobia per gram of soil), inoculation with compatible rhizobia is likely to prove highly advantageous. While in the presence of high densities of the rhizobial population, the inoculation is likely to be unnecessary, and investment in inoculation practice might be wasted (Abdel-Lateif et al. 2013; Abiala et al. 2013).

14.3.1.2 Bradyrhizobium

One important group of the symbiotic nitrogen fixers is *Bradyrhizobium.* They are slow-growing soil-dwelling microorganisms which fix nitrogen, and in turn, use carbohydrate derived from plants in a symbiotic association. It has been shown that nodule formation and availability of nutrients in the soil can be enhanced once the application of inoculum consists of *Bradyrhizobium* and certain PGPRs probably due to auxins and siderophores production which consequently promote plant growth and increase its tolerance to the phytopathogens (Youssef and Eissa 2014).

14.3.1.3 Frankia

Many commercial drugs are derived from these bacteria. Recent investigations have revealed the significant importance of actinobacteria in producing medically (Salimi et al. 2018a, b, 2019), industrially (Imanparast et al. 2018), and agriculturally important compounds or enzymes (Hamedi and Mohammadipanah 2015). One of these outstanding genera is *Frankia. Frankia,* a N2-fixing actinobacteria, fix nitrogen via nodulation of actinorhizal plants (more than 280 species of woody plants) including the Elaeagnaceae, Casuarinaceae, Datisticaceae, Coriariaceae, and Myricaceae families, whereas nodulation occurs occasionally in Betulaceae, Rhamnaceae, and Rosaceae (Benson and Clawson 2000). These plants are woody trees or shrubs except for *Datisca* and can impose a pivotal role in agroforestry and land reclamation. The *Frankia* genus belongs to the family Frankiaceae. These bacteria produce differentiated structures, vesicles, where nitrogen is biologically fixed. Some parameters like the age of the microbial inoculum, its concentration, and preservation strategy may greatly affect inoculum efficiency.

Plant survival and performance can be improved through inoculation and nodulation before seedling transplanting (Prat 1992). They infect host plants through

two various strategies: intracellular and intercellular root invasion (Wall and Berry 2007). Earlier occurs via the signal exchange between *Frankia* and the host plant which results in root curling and invagination of *Frankia* growing filaments into them and their subsequent encapsulation by a cell wall deposit. Besides nitrogen fixation, *Frankia* via releasing plant growth regulators, hydrogen cyanide, siderophores or increasing availability of phosphate improves plant growth. *Frankia* inoculum can be preserved as lyophilized or frozen in glycerol (Fontaine et al. 1986; Franche et al. 2009).

14.3.1.4 Gluconoacetobacter Diazotrophicus

Gluconoacetobacter diazotrophicus also known as *Acetobacter diazotrophicus*, fixes nitrogen in a symbiotic association with sugarcane as the host plant. It belongs to the Acetobacteriaceae family. Its inoculation leads to cut off chemical N fertilizer usage for at least two successive years (Muthukumarasamy et al. 2002).

14.3.1.5 *Cyanobacteria* and *Azolla*

Nitrogen can be fixed in plant roots via associate interaction by heterocystous cyanobacteria, including *Nostoc* and *Anabaena.* A significant amount of nitrogen (36% of global N2 fixation) is symbiotically fixed via an aquatic cyanobacterium, *Trichodesmium* (Gallon 2001). Cyanobacterial nitrogen fixation in heterocysts fulfills the nitrogen requirement of plants, and in turn, the plant supply carbohydrates derived from their photosynthetic activity. Asymbiotic association can be generated among cyanobacteria and fungi, liverworts, ferns, as well as flowering plants (Roychowdhury et al. 2014). Until the end of the 1970s, symbiosis of *Azolla–Anabaena* was the crucial nitrogen source to cultivate rice in China. Also, it can serve as an applicable source of nitrogen. It imposes its growth-promoting on plants via producing phytohormones like auxin, indole acetic acid, and gibberellic acid and providing a considerable level of iron, zinc, phosphorus, potassium, molybdenum, and other micronutrients. It has been reported inhibitory effects of three cyanobacteria including *Anabaena oryzae*, *Nostoc calcicola*, and *Spirulina* sp. on galls and egg masses (Al Abboud and Alawlaqi 2014; Mishra et al. 2013).

The effect of microorganisms, that non-symbiotically fix nitrogen, on agricultural productivity and yield is tremendous. Non-symbiotic nitrogen fixers compensate their access to plant derived nutrients decreasing their distance from the host (rhizoplane) or entering into the plants (endophytes). *Azotobacter* sp., *Azospirillum*, *Azoarcus* sp., *Gluconacetobacter diazotrophicus*, *Herbaspirillium* sp., *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum, Rhodopseudomonas,* and *Xanthobacter* are non-symbiotic nitrogen fixers (Saxena and Tilak 1998). In the following, some of these non-symbiotic nitrogen fixers are presented in detail.

14.3.1.6 Azotobacter

Free-living microorganisms like *Azotobacter, Clostridium*, *Rhodospirillum,* and *Klebsiella* which are known as asymbiotic nitrogen fixers are present in the rhizospheric region. *Azotobacter*, as a well-known asymbiotic nitrogen fixer belongs to the family *Azotobacteriaceae*, establishes a mutual relationship with plants in which they fix nitrogen and absorb organic compounds from plant exudate. It has been reported that *Azotobacter* through producing and releasing vitamin B and various plant hormones like gibberellins, naphthalene acetic acid (NAA), promotes root growth and minerals uptake and simultaneously inhibits root pathogens (Mathivanan et al. 2015). The *Azotobacter* has been found in the rhizospheric region of some crop plants like vegetables, sugarcane, rice, maize, bajra, and plantation crops. *A.chroococcum*, via multiple mechanisms other than nitrogen fixation like production of vitamin, growth substance, antifungals, and siderophore (Martinez-Toledo et al. 1988), promotes plant growth. *Azotobacter indicum* through producing a lot of antifungal compounds inhibits some pathogenic fungi in the rhizosphere hence considerably decreases the seedling mortality (Martin et al. 2011). Occurrence of *Azotobacters* has been reported in soils with neutral or alkaline pH. *A. chroococcum* is a common species in arid soils. Other reported species include *A. vinelandii*, *A. beijerinckii*, *A. insignis*, and *A. macrocytogenes.* A sizable proportion of root colonized *Azotobacter* penetrates the root tissues and lives in an associate relationship with the host plants. However, any visible nodules or outgrowth on root tissue is not created (Bhat et al. 2015).

14.3.1.7 Azospirillum

Azospirillum with ten species including *A. lipoferum, A. brasilense*, *A. amazonense*, *A. halopraeferens*, *A. irakense, A. largimobile, A. doebereinerae, A. oryzae,* and *A. melinis* is one of the non-symbiont nitrogen-fixing bacterial genus (20–40 kg/ha) (non-nodule forming bacteria), belong to the *Spirilaceae* family and colonizes a great variety of annual and perennial plants (Mehnaz 2015). In these microorganisms, nitrogen fixation occurs under microaerophilic conditions. These bacteria have an interrelationship with roots of corn, wheat, sorghum, and other grasses (Montañez et al. 2012) especially plant with C4 dicarboxylic pathway of photosynthesis which their growth and nitrogen fixation occurs in the presence of the aspartic and malic acid as well as the organic salts (Mishra and Dash 2014). It seems that *Azospirillum* is not limited to a specific plant and can be considered as a general root colonizer; therefore, they are suitable for pearl millet, sorghum, maize, sugarcane, etc. *Azospirillum* can increase the growth of different crops including, sunflower, carrot, oak, sugarbeet, tomato, eggplant, pepper, cotton, wheat, and rice due to fixing nitrogen, as well as producing growth-promoting compounds including, IAA, gibberellins, and cytokinin by which development of root and nutrient (N, P, and K) uptake are enhanced. It has been shown that maize inoculation with *A. brasilense* sp. 245 enhanced the production of various phytohormones which had been led to a substantial enhancement of

maize growth. It has been shown that commercial production and field application of *Azospirillum*is simple. Its inoculum can be cost effectively produced and applied as peat formulation (Steenhoudt and Vanderleyden 2000).

14.3.1.8 Nitrogen-Fixing Endophytes

There is increasing evidence which proves the presence of endophytic nitrogen-fixing bacteria (10^8 CFU per g of dry weight). They cause no disease and damage. Nitrogen-fixing endophytes are bacteria belonging to *Azoarcus*, *Gluconacetobacter,* and *Herbaspirillum* genera. These bacteria successfully multiply and spread within plant tissues (Rana et al. 2019c). They colonize on the root cortex of host plants like rhizospheric bacteria. Then, using hydrolytic enzymes, they penetrate endodermis to colonize the steel, from which they may be subsequently translocated to the aerial parts, and in turn, will systemically spread in xylem vessels and shoots. Endophytic diazotrophs colonize the apoplast, like the intercellular spaces, the xylem vessels, and lignified xylem parenchyma, as well as dead cells, such as those comprising lysigenous aerenchyma in rice and kallar grass. *G.diazotrophicus* and *H. frisingense* are some examples of endophytic nitrogen-fixing bacteria in sugar cane and C4- gramineous plant *Miscanthus sinensis*, respectively (Franche et al. 2009). *Azoarcus*, is also an endophytic nitrogen fixer, which can enter into the host plant (*Leptochloa fusca* L Kunth) and live endophytically. Biofertilizer containing *Azoarcus* can efficiently be used under salinity stress in soils with low fertility.

14.3.2 Phosphate Solubilizing Activity

Phosphate is the second indispensable macronutrient for growing plants. The low frequency of its soluble form limits the growth of terrestrial plants that require phosphate to synthesize macromolecules and perform the transfer of energy, respiration, photosynthesis, and signal transduction (Hesham et al. 2021; Khan et al. 2010; Subrahmanyam et al. 2020). Phosphate abundance is 400–1200 mg kg^{-1} of soil. Phosphate application can deeply affect crop yield due to its fundamental role in the growth and reproduction processes of plants. In general, chemical phosphatic fertilizers are applied to supply phosphates to the soil. Studies have shown that a low portion of phosphatic fertilizers (30–35%) is utilized by the plants, while its significant portion (65–70%) is turned into insoluble, immobilized, or precipitated forms and consequently unavailable to the plants. Therefore, available phosphate is less than plant requirement (Angus 2012). Aluminum and iron phosphates, as well as calcium phosphates, are most of the insoluble phosphate forms in acidic and alkaline soils, respectively. The insoluble forms are found as inorganic material like apatite or organic forms such as phosphomonoesters, phosphotriesters, and inositol phosphate (Mahdi et al. 2012). The abundance of phosphate in soluble form is usually very negligible (1 ppm) (Goldstein 1994).

Monobasic phosphoric acid (HPO_4^{2-})] and dibasic [dihydrogen phosphate ($H_2PO_4^-$)] are less frequent, soluble, and bioavailable forms of phosphate in the soil. Insoluble phosphate compounds (both organic and inorganic) should be converted to bioavailable form to avoid continuous usage of phosphate chemical fertilizer and its degrading effect on the ecosystem and also to elevate agricultural yields in soils with less bioavailable phosphorous. This crucial requirement can be provided using a biofertilizer containing phosphate solubilizing microorganisms (Rodríguez et al. 2006a, 2006b). Acidification of soil via producing low molecular weight organic acids like glycolic acid, citric acids, gluconic acid, 2-ketogluconic acid, malonic acid, oxalic acid, succinic acid, and propionic acid which make inorganic phosphorus into their soluble form occur by the activity of these bacteria. They play a crucial role in providing phosphorus to the plants. Hydroxyl and carboxyl groups are existing organic acids with low molecular weight. They can chelate the cations bound to phosphate and convert insoluble phosphorous to its bioavailable form (Glick 2012).

Phosphate solubilizing bacteria belong to various genera including, *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Acetobacter*, *Flavobacterium*, *Arthrobacter*, *Enterobacter*, *Beijerinckia*, *Microbacterium*, *Rhizobium*, *Mesorhizobium*, *Flavobacterium*, *Rhodococcus*, *Serratia*, *Phyllobacterium*, and *Erwinia*cansolubilize insoluble and inorganic phosphate compounds such as rock phosphate, dicalcium phosphate, tricalcium phosphate, hydroxyl apatite (Goldstein 1986; Otieno et al. 2015; Rodriguez and Fraga 1999a). Higher frequency of bacteria with phosphate solubilizing activity is commonly found in the rhizospheric regions in comparison with nonrhizosphere soil due to a higher amount of organic substrates in rhizospheres which attract phosphate solubilizing bacteria (PSB) (Youssef and Eissa 2014). In addition, PSB augment the growth of plants via inducing biological nitrogen fixation by nitrogen-fixing microbial cells (Mohammadi and Sohrabi 2012).

Also, various P containing organic substances can be applied as a promising P resource for requirements of plants after mineralization process where organic P is hydrolyzed to its inorganic form by means of enzymes like phosphatase (phosphohydrolases) (Rodriguez and Fraga 1999), phytase (Richardson 1997), phosphonoacetate hydrolase (McGrath et al. 1998), D-α-glycerophosphatase (Skraly and Cameron 1998), and C-P lyase (Ohtake et al. 1998). A considerable level of acid phosphatases is created by rhizospheric microorganisms like bacteria belong to *Rhizobium* (Abd-Alla 1994), *Enterobacter*, *Serratia*, *Citrobacter*, *Proteus,* and *Klebsiella* (Thaller et al. 1995), as well as *Pseudomonas* (Gügi et al. 1991) and *Bacillus* genera (McComb et al. 2013).

14.3.3 Potassium Solubilizing Activity

Potassium (K) is the third essential element necessary for the growth of plants. Orthoclase, mica, illite, and muscovite are the insoluble source of K in soil. Soluble potassium exists in very low concentration in the soil (Parmar and Sindhu 2013; Yadav

et al. 2020b). In soluble-potassium limiting conditions, plant growth and production rate significantly declined. To enhance the bioavailability of potassium to plants, a combination of high K containing clay mineral with K solubilizing bacteria can be applied to meet the K requirement of plants in the agricultural industry (Shrivastava et al. 2016). These microorganisms via producing organic substances solubilize potassium, thus enhance the concentration of soluble K in soil solution. *Frateuria aurantia*, *Bacillus edaphicus*, *Paenibacillus glucanolyticus*, *Bacillus mucilaginosus*, *Acidothiobacillus* sp., *Pseudomonas* sp., *Burkholderia* sp., and *Paenibacillus* sp. are well-known as potassium mobilizing microorganisms (Prakash and Verma 2016; Priyadharsini and Muthukumar 2016; Raghavendra et al. 2016; Rawat et al. 2016). Also, these microorganisms produce diverse amino acids, compounds with plant growth-promoting activity (IAA and gibberellic acid), and vitamins, by them plant growth and productivity are increased (Dotaniya et al. 2016)

14.3.4 Zinc Solubilization

Zn is one of the crucial elements which is required in low amount. Deficiency of zinc declines the growth and yield of crops. Hence, Zn containing fertilizers, with soluble zinc sulfate ($ZnSO_4$), are currently used. On this matter, applying Zn solubilizers extremely affect the reproduction and quality of crops. The reaction of used zinc fertilizers with soil constituents converts them into bioavailable. Several events, including cation exchange of acidic soil, chemisorption in alkaline soil (Zn-$CaCO_3$) or making a complex with organic ligands immobilize the zinc in soil and decline its abundance in soil. Most well-known biofertilizers contain microorganisms which supply significant macronutrients like nitrogen, phosphate, and potassium, while the unmet need to micronutrient like Zn also negatively affects plant growth, development, and productivity. Therefore, biofertilizers containing Zn solubilizing microorganisms like *B. subtilis*, *Thiobacillus thiooxidans*, and *Saccharomyces* sp. are severely required. It has been shown that better responses can be achieved through co-application of these strains with Zn fertilizers or Zn containing oxides (Zinc oxide, Zinc carbonate, and Zinc sulfide) (Samoon et al. 2010).

14.3.5 Iron Sequestration

Iron is one of the pivotal growth elements for nearly all living organisms, including animals plants, bacteria, and fungi (Rajkumar et al. 2010). In the presence of oxygen, iron mainly presents as ferric iron (Fe^{3+}) and probably generate insoluble hydroxides and oxyhydroxides. Therefore, a large amount of the iron is not in bioavailable form for plant and bacterial assimilation (Rajkumar et al. 2010). The low abundance of bioavailable iron in terrestrial ecosystems creates an extreme competition. Plants frequently produce and excrete water-soluble organic compounds (siderophores)

with low molecular weight, that chelate Fe^{3+} and maintain it in solution. The root surface receives Fe^{3+} from siderophores and reduces it to Fe^{2+} and consequently absorbs it. Also, bacteria produce and release siderophores, high-affinity iron chelator, to scavenge iron by the formation of soluble Fe^{3+}complexes. They can be considered as agents with iron solubilizing activity from minerals or organic compounds in iron-limiting conditions (Ahemad and Khan 2011; Rajkumar et al. 2010). They can be categorized into two types including extracellular and intracellular iron chelators. Enterobactin is one of the strongest siderophores (Hider and Kong 2010).

Microbially derived siderophores also can be applied by plants; hence they can augment the growth of plants under iron limited condition. Plants can assimilate iron provided by microbial siderophores via diverse strategies like chelating and releasing iron, direct uptake of siderophores-Fe complexes, or ligand exchange reaction (Thomine and Lanquar 2011). Several isolates belonging to *Pseudomonas*, *Enterobacter*, *Bacillus*, and *Rhodococcus* genera are siderophore producing microorganisms. Siderophores producing *Phyllobacterium* strain, *Pseudomonas fluorescens* C7, and *Chryseobacterium* sp C138 promote the growth and quality of strawberries and *Arabidopsis thaliana*, respectively (Parray et al. 2016). More importantly, biofertilizer containing siderophore producing microorganisms like *Pseudomonas, Bacillus* sp. and *Streptomyces* can be applied as biological agents for biocontrol. They limit the reproduction and activity of phytopathogens via producing high iron affinity siderophores. Through this mechanism, phytopathogens like *Fusarium oxysporum* cannot meet their iron requirement, and therefore, their reproduction will be limited (Bashan and De-Bashan 2005; Saraf et al. 2014).

14.3.6 Production of Volatile Organic Compounds

Producing volatile organic compounds (VOCs) is one of the interesting strategies which is applied by biofertilizers to promote plant growth and its resistance towards fungal pathogen and pathogenic nematodes as well as abiotic stresses. Acetoin, 2,3-butanediol cyclohexane, 2-(benzyloxy) ethanamine, benzene, methyldecane-1-(*N*-phenylcarbamyl)-2-morpholinocyclohexene, dodecane, benzene(1-methylnonadecyl), 1-chlorooctadecane, tetradecane, 2,6,10-trimethyl, dotriacontane, and 11-decyldocosane are some of these compounds (Effmert et al. 2012; Kanchiswamy et al. 2015; Ryu et al. 2003).

Rhizobacterially-produced VOCs act as signaling molecules to trigger the plant responses and form plant–microbe interactions and elicitor agents of induced systemic resistance (Ryu et al. 2003; Sharifi and Ryu 2016). Reported VOC producing microorganisms are *Bacillus subtilis* GB03, *B. amyloliquefaciens* IN937a, *Pseudomonas*, *Serratia*, *Arthrobacter*, and *Stenotrophomonas* and *Enterobacter cloacae* JM22. It has been reported that some of these VOC producing microorganisms can promote the growth of *Arabidopsis thaliana* (Choudhary et al. 2016).

Some rhizospheric microorganisms including *Rhizobium*, *Pseudomonas*, *Bacillus*, and *Aeromonas* genera can produce hydrogen cyanide (HCN), a bioactive compounds with an adverse effect on the reproduction of phytopathogens or weeds whose usage will protect the host plants (Ahmad et al. 2008; Das et al. 2017; Flury et al. 2017; Nandi et al. 2015; Sivakumar et al. 2012; Zachow et al. 2017). It has been found that HCN production is common in *Pseudomonas* (88.89%) and *Bacillus* (50%) genera (Ahmad et al. 2008). Since, produced HCN has no adverse effect on host plants, HCN producing microorganisms can act as biocontrol agents. In most cases, these microorganisms also produce compounds with antibiotic activity or cell wall degrading enzymes which along with HCN can synergistically suppress the growth of phytopathogens. On the other hand, low level of HCN cannot effectively prevent the proliferation of most fungal phytopathogens (Ramette et al. 2006) but can prevent phytopathogens to become resistant (Olanrewaju et al. 2017). HCN impose its toxicity effect due to the inhibitory effect on cytochrome c oxidase and other critical metalloenzymes (Nandi et al. 2017). Recently, it has been revealed that promoting the activity of HCN on the growth of plants is mostly related to its role in increasing the bioavailability of phosphate for the pioneer plants (like French sorrel) living in oligotrophic alpine environments (like granite-based substrate) (Rijavec and Lapanje 2016).

14.3.7 Production of Hydrolytic Enzymes

Many microorganisms can improve plant growth by suppressing the growth of their pathogens. Production of hydrolytic enzymes such as chitinase, glucanase, protease, and cellulase (Suyal et al. 2021; Yadav et al. 2016). Producing hydrolytic enzymes are one of the critical strategies by which microorganisms control pathogen growth (Jadhav and Sayyed 2016; Jadhav et al. 2017). A wide range of polymeric compounds like chitin, proteins, cellulose, and hemicellulose in the cell wall of the targeted phytopathogens can be hydrolyzed via these enzymes (Mabood et al. 2014).

14.3.7.1 Chitinase Production

Chitin is an insoluble unbranched β-1,4-β-linked polymer of *N*-acetyl-D-glucosamine $(C8\ H_{13}O_5N)_n$ and is the second most plentiful naturally occurring polymer (Huang et al. 2005). Chitinase producing microorganisms can be considered as promising biological control agents and prevent fungal related plant diseases. Chitinase producing fluorescent *Pseudomonas* and *Streptomyces* sp. isolates can control ragi blast disease and sheath blight disease in rice, respectively (Chaiharn et al. 2018; Negi et al. 2017). Chitinasescategorized into three classes according to their mode action: β-1,4-*N*-acetyl-glucosaminidases, endochitinases, and exochitinases. Chitin degradation can be achieved through endochitinases via randomly cleaving at internal sites of chitin micro-fibril orexochitinases via progressive release of diacetylchitobiose in

a stepwise manner without releasing monosaccharide or oligosaccharides (Harman et al. 1993; Manocha and Balasubramanian 1994).

14.3.7.2 Glucanase Production

β-1,3-Glucanases-producing microorganisms like *Paenibacillus terrae* NK3-4 can efficiently degrade another important cell wall component of fungi and yeasts, β-1,3(1,6)-Glucans (Simmons 1994; Yu et al. 2019). This polysaccharide consists of a β-1,3-linked backbone with some branches via β-1,6-linkages. They are classified into two groups, according to their mode of action: sequent removing of glucose residues from non-reducing end or randomly breakdown of linkage at random sites and releasing smaller oligosaccharides can be conducted via exo- or endo-β-1,3-glucanases, respectively. (Jadhav and Sayyed 2016).

14.3.7.3 Cellulase Production

Bacillus cereus, *Bacillus subtilis*, *Bacillus thuringiensis,* and *Streptomyces* sp. can impose their biocontrol activity via degrading the 1,4-β-D-glucosidic bonds in cellulose (Patagundi et al. 2014; Sadeghi et al. 2017). Cellulose consists of β-D-glucose units which are bonded via 1,4-β-linkages. These microorganisms also play a pivotal role in nature through the recycling of this abundant polymer. The rigid, insoluble, crystalline cellulosic microfibrils are formed via abundant intra- and intermolecular hydrogen bonds. Various hydrolytic enzymes including endoglucanases, exo-glucanases, and β-glucosidases are involved to completely degrade cellulose into β-glucose (Lynd et al. 2002).

14.3.7.4 Protease Production

Protease or proteinase plays a crucial role in degrading the cell wall of phytopathogenic fungi. This enzyme degrades the protein matrix where chitin and/or fibrils of β-glucan (major components of the cell walls) are present. The polymer is hydrolyzed to peptide chains and/or their amino acids by this enzyme. Also, several proteases via inactivating extracellular enzymes of phytopathogenic fungi, suppress their growth (Al-Askar et al. 2015; Jadhav and Sayyed 2016).

14.3.8 Production of Hormones

Phytohormones, plant hormones, are organic substances that affect physiological, biochemical, and morphological characteristics in plants including growth, differentiation, and development of cells, tissues, and organs (Damam et al. 2016; Peleg and

Blumwald 2011). These compounds can be considered as chemical signals to communicate cellular activities in higher plants (Voß et al. 2014), and their synthesis is tightly regulated. Some well-known examples are auxins, ethylene, gibberellins, abscisic acid (ABA), and cytokinins, which play a critical role at very low concentrations (<1 mM). They are active in plants in a short period of time and are mainly produced in special parts of the plant and transferred to another part. Since these chemical compounds affect the growth of the plant, they are also recognized as regulators of plant growth. Under stress conditions, plants or their rhizospheric microorganisms produce or modulate phytohormone levels to coordinate various signal transduction pathways and consequently ameliorate the adverse effects of environmental stresses (Kazan 2015).

Microbial produced phytohormones are known as exogenous phytohormones. Microbial production of plant hormones such as auxin and cytokinins are reported by a lot of rhizospheric microorganisms (Ahemad and Khan 2011). Also, phytohormones improve defense response of plants through stimulating cell division, extension, differentiation, photosynthesis, and pigment formation, inducing seed and tuber germination, increasing the development rate of xylem and root (Gupta et al. 2015; Ljung 2013; Spaepen and Vanderleyden 2011). *Azospirillum* is a well-known plant growth-promoting bacterium with the ability to excrete phytohormones including gibberellins, cytokinins, and auxins (Tien et al. 1979).

Root surface area and its length can be enhanced by microbial IAA, which enables the plant to achieve more nutrients from the soil (Ahemad and Khan 2012b). The plant cell wall is affected by rhizobacterial IAA and its loosening lead to facilitated exudation of plant exudates, which assure sufficient bacterial growth (Ahemad and Khan 2012a). Inoculating auxin-synthesizing *Bacillus* spp. positively affects the growth of *Solanum tuberosum* (Ahmed 2010). Seed germination, floral induction, development of flower and fruit, and growth of leaf and steam are affected by another pivotal phytohormone, gibberellin. Gibberellin-producing *Sphingomonas* sp. LK11 positively affects plant growth characters (Khan et al. 2014). It has been reported that cytokinin-producing *Bacillus subtilis* strains caused draught resistance of inoculated plants. It has been shown that *Bacillus amyloliquefaciens* RWL-1, an endophyte, synthesize ABA. Hence, it has the ability to enhance the salinity tolerance of *Oryza sativa*. Biofertilizers containing phytohormone producing or modulating microorganisms can offer economic and ecological advantages to boost agricultural production (Shahzad et al. 2017).

Ethylene is a significant phytohormone that affects the ripening of fruits and the abscission of leaves (Reid 1981). Elevated level of aminocyclopropane-1-carboxylate (ACC) synthesis, that is, the precursor of ethylene, is observed in plants under stress conditions like low temperature, drought, flooding, infections with pathogens, and the presence of heavy metals which creates physical or chemical perturbation in various tissues of plants (Li and Glick 2005), and for this reason, wounding hormone is its other name (Salisbury 1992). Increased level of ethylene halts the growth of stem and root, fixation of nitrogen in legumes, and causes premature senescence and consequently decreases the yield. In this regard, there are some rhizospheric microorganisms which produce aminocyclopropane-1-carboxylate deaminase, a pyridoxal

phosphate-dependent enzyme. This enzyme can hydrolyze the precursor of ethylene, ACC, to ammonia and α-ketobutyrate then use them as nitrogen and carbon sources. Therefore, ACC deaminase producing microorganisms via reducing the level of ethylene precursor, ACC, improve the growth of plants in the presence of biotic and abiotic stresses (Glick 2014). So, plant growth-promoting microorganism's ability to hydrolyze ACC possesses profound significance in declining the adverse effect of environmental stressors.

Synthesis of this enzyme is induced in the presence of ACC. ACC deaminase is encoded by *AcdS* gene which is found in Actinobacteria, Deinococcus-Thermus, three classes of α, β, and γ Proteobacteria, various fungi belonging to Ascomycota and Basidiomycota, and in some Stramenopiles (Nascimento et al. 2014). Of possessing microorganisms, different bacteria (*Alcaligenes* sp., *Bacillus pumilus*, *Pseudomonas* sp., and *Variovorax paradoxus,* as well as *Azoarcus*, *Azorhizobium caulinodans*, *Azospirillum* spp., *Gluconacetobacter diazotrophicus*, *Herbaspirillum* spp., *Burkholderia vietnamiensis.*) and some yeast (*Hansenula saturnus* and *Issatchenkia occidentalis*) (Minami et al. 1998; Palmer et al. 2007), as well as fungi (*Penicillium citrinum*, *Trichoderma asperellum*, and *Phytophthora sojae*) (Jia et al. 1999; Singh and Kashyap 2012; Viterbo et al. 2010) and archaea like *Pyrococcus horikoshii* (Fujino et al. 2004; Singh et al. 2015) can be considered. Also, it has been known that even certain plants like *Arabidopsis thaliana* (McDonnell et al. 2009) are ACC deaminase producing organisms.

These ACC deaminase producing microorganisms efficiently augment the growth rate, physiological characteristics, and quality of plants, especially in presence of salinity stress. ACC deaminase synthesizing *Pseudomonas putida* UW4 reduced post-submergence ethylene production. Therefore, it has been concluded that plant response to environmental stressors can be modified by rhizospheric bacteria (Ravanbakhsh et al. 2017). According to other studies, a salt-tolerant bacterium with ACC deaminase activity, *Enterobacter* sp., was isolated from the rice field and was reported to promote rice seedling growth in salinity stress (Sarkar et al. 2018). In another study, ACC producing endophyte, *Pseudomonas migulae* 8R6, limited phytoplasma-induced damages, and consequently *Flavescence dorée* disease in periwinkle (Gamalero et al. 2017).

It has been revealed that a salt-tolerant endophyte SMR20 with the capability to produce ACC deaminase, *Brachybacterium paraconglomeratum*, isolated from *Chlorophytum borivilianum* reduced salt stress-induced damage in the host plant and delayed chlorosis and senescence and improved yield. In addition, this bacterium modifies the levels of indole-3-acetic acid and abscisic acid in plants (Barnawal et al. 2016).

14.4 Environmental Stress Relief

Various biotic (bacterial and fungal phytopathogens, pests, and herbivores) and abiotic stresses (hostile conditions of ecosystem like drought, water logging, extreme

temperatures, salt stress, oxidative stress, air pollution, heavy metals, pesticides, and unfavorable soil pH) affect plant growth and its productivity during their growth and development which lead to a reduction of agricultural yield (Rastegari et al. 2020a, 2020b; Suman et al. 2016; Yadav et al. 2020a). Reactive oxygen species (ROS) including H_2O_2, O_2^-, and OH^- radicals are generated in stress conditions and their elevated level created oxidative stress, which consequently imposes its deleterious effect on plants (CH 2018). They can alleviate this oxidative stress through various strategies like producing and accumulating poly-sugars, proline, glycine-betaine, abscisic acid, and up-regulating enzymatic and nonenzymatic antioxidants, like superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, ascorbic acid, α-tocopherol, and glutathione (Agami et al. 2016).

In these conditions, plant inoculation by biofertilizers can provide more protection against these stresses through microbial activities, including the release of substances that can improve soil structure, inhibiting the causative agents of biotic stress via modulating phytohormones and inducing systemic resistance (Yang et al. 2009). In addition, beneficial microorganisms can ameliorate the toxic effect of heavy metal on plants through various strategies like binding mechanisms. It has been reported that *Pseudomonas putida* and *Pseudomonas fluorescens* alleviate the toxic effect of cadmium contamination on barley plants through their ability to scavenge cadmium ions from soil (Baharlouei et al. 2011).

Microbial induced modifications lead to increased survival and productivity of plants. Some examples have validated the protective effect of biofertilizers, e.g., *Azospirillum* inoculation enhanced the growth of wheat and faba beans under saline stress. One group of microbial substances which alters the structure of soil, and imposes an improving effect on the growth of plants in the presence of water stress is a microbial polysaccharide. For example, inoculation of sunflower and wheat plants with exopolysaccharide producing *Rhizobium* sp. and *Pantoea agglomerans* resulted in better growth parameters in comparison with uninoculated plants. It has been reported that *Pseudomonas* strains via enhancing the assimilation of Mg^{2+}, K^+, and Ca^{2+}, declining uptake of Na^+, and enhancing the synthesis of endogenous indole acetic acid have improved asparagus seedling growth and seed germination in presence of water and salt stresses. Several reactive oxygen species generated during water stress cause injury to the photosynthetic mechanisms of the plant (Heidari and Golpayegani 2012). In this regard, biofertilizer containing *Pseudomonades*, *Bacillus lentus*, and *Azospirillum brasilense* through increasing expression of enzymatic antioxidants and enhancing the amount of chlorophyll in leaves ameliorate this stress. Therefore, biological fertilizer can augment the photosynthetic activity of the plant, and improve its physiological properties in presence of unfavorable conditions (Heidari and Golpayegani 2012).

Improving leaf water status, especially in the presence of salt and drought stresses, is another strategy to improve plant growth (Ahmad et al. 2013; Naveed et al. 2014). Biofertilizers via improving stomatal conductance of plant leaf enhance its ability in utilizing water and surviving under drought conditions. *Pseudomonas aeruginosa* has improved the growth of *Vigna radiata* plant in drought conditions (Naveed et al. 2014; Sarma and Saikia 2014). It has been demonstrated that *Bacillus megaterium*

and *Pantoea agglomerans* enhanced the ability of maize roots to absorb water under salinity stress (Gond et al. 2015; Marulanda et al. 2010). *Azospirillum brasilense* also has enhanced the salt resistance of the jojoba plant rooting (Ahmad et al. 2013; Gonzalez et al. 2015; Naveed et al. 2014)

Biotic stresses impose their deleterious effect on co-evolution, dynamics of population, nutrient cycling in the ecosystem, ecology of natural habitat, and health of horticultural plant (Gusain et al. 2015). *Bacillus* spp. (like *Paenibacillus polymyxa* strains B2, B3, and B4, *Bacillus amyloliquefaciens* strain HYD-B17, *B. licheniformis* strain HYTAPB18, *B. thuringiensis* strain HYDGRFB19, *P. favisporus* strain BKB30, and *B. subtilis* strain RMPB44) and *Pseudomonas* sp. Decrease the growth and activity of phytopathogens by their antimicrobial metabolites or competition.

Pseudomonas sp. and rhizobacteria produce antimicrobial compounds and proteins with toxic properties against *Gaeumannomyces graminis* var. tritici (inducing wheat take-all) and crop insect pests, respectively (Strange and Scott 2005).

Phenazines, phenazine-1-carboxylic acid, phenazine-1-carboxamide, pyrrolnitrin, pyoluteorin, 2,4diacetylphloroglucinol, rhamnolipids, oomycin A, cepaciamide A, ecomycins, viscosinamide, butyrolactones, N-butylbenzene sulfonamide, pyocyanin are antifungal agents produced by *Pseudomonas* strains also produce antibacterial compounds (pseudomonic acid and azomycin), antitumor antibiotics (cepafungins and FR901463). Karalicine is an antiviral derived from *Pseudomonas* (Ramadan et al. 2016). Surfactin, iturins, and bacillomycin are antibacterial compounds derived from *Bacillus* sp. (Wang et al. 2015).

B. cereus UW85 and *B. thuringiensis* subsp. *kurstaki* HD-1 can be applied as biological agents to control the damping-off of alfalfa and gypsy moth, respectively (Broderick et al. 2000; Handelsman et al. 1990). *Photorhabdus* and *Xenorhabdus* inhibit harmful insects. *Pseudomonas* sp. and *Bacillus subtilis* produce siderophores that can be considered as biocontrol agents, which limit fungal pathogens like *Fusarium* wilt that is produced by *Fusarium oxysporum* in potato (Haggag et al. 2015).

Tolerance of plants to the attack of herbivores can be elevated by symbiosis with rhizobacteria. It is likely that these bacteria via supplying additional nitrogen make synthesizing cyanogenic defense compounds possible. These synthesized compounds repel leaf-chewing herbivores (Godschalx 2017). Also, chitinases and β-glucanases-producing microorganisms like *Sinorhizobium fredii* KCC5, *Pseudomonas fluorescens* LPK2, and *Pseudomonas spp.* via degrading chitin and β-1,4-*N*-*N*-acetylglucosamine inhibit *Fusarium udum* (causative agent of fusarium wilt), *Rhizoctoniasolani,* and *Phytophthoracapsici* (destructive crop pathogens), respectively (Ramadan et al. 2016).

14.5 Factors Influencing the Efficiency of Biofertilizers

One of the considerable properties which has resulted in limited application of biofertilizers is their unpredictable function under different agro-environmental conditions,

which may seriously reduce their plant growth promotion potential. There are some less investigated factors including the production process of biofertilizers, interaction of host plant and microbial strains, competition condition of the soil, which involved in this phenomenon (Bashan 1998; Owen et al. 2015). Reducing the unfavorable properties of the biofertilizers and improving the influential factors on biofertilizer efficiency can encourage the farmers to use them. Biofertilization program by selecting strains according to their high root colonization, abundant sporulation, or growth augmentation in pot experiments without considering affecting factors like field conditions can lead to non-conclusive results.

14.5.1 Effect of the Plants on the Efficacy of Biofertilizers

One of the determinant factors on biofertilizer efficiency is the interaction of microbial biofertilizers with plants. Crop species and environmental conditions of the field are two main factors that govern the interaction between microorganisms and plants (Dodd and Ruiz-Lozano 2012). Promoting and inhibitory effects of plants are significantly depending on their phenological growth phase and physiological and nutritional status. These situations directly affect the release of compounds from the roots. These compounds in plant exudates impose quantitative and qualitative modifications in the rhizospheric region due to the growth promotion or inhibition of specific rhizospheric bacterial communities (Dennis et al. 2010; Hartmann et al. 2009; Uren 2000; Van Overbeek and Van Elsas 2008). In P-deficiency, plants stimulate hyphal branching and colonization of AM fungi through releasing inducing chemicals (Akiyama et al. 2002; Akiyama et al. 2005). Genistein, a phenylpropanoid compound, and phenolic acids are influential compounds in root exudates that stimulate AMF root colonization and change soil microbial communities, respectively (Cesco et al. 2010; Qu and Wang 2008).

14.5.2 Effect of Soil Conditions on the Efficacy of Biofertilizers

Another critical factor in successful biofertilizer establishment is the soil in which microbial cell is introduced. Chemical (pH, nutrient content) and physical (texture) properties of soil profoundly affect biofertilizer establishment, colonization, and plant growth-promoting activities (Fierer and Jackson 2006; Girvan et al. 2003; Lauber et al. 2008). Acidic soils have a less diverse microbial community in comparison to neutral soils because of the narrow pH growth tolerance of bacterial taxa (Fierer and Jackson 2006; Rousk et al. 2010). *Acaulospora* species are exclusive to acidic soils in the tropics. Therefore, broad-range microbial species like *Glomus intraradices* can assure the efficiency of biofertilizers. Because it has been adapted to

an extended range of environmental conditions, especially harsh situations including, unfavorable pH and temperature and less available nutrients (Antunes et al. 2011).

14.5.3 Effect of Interaction of Soil Microorganisms with Autochthonous on the Efficacy of Biofertilizers

Successful fertilization can be achieved by characterizing microorganisms, their activities, and the interrelationship between them and soil particles. However, there is limited knowledge about the ecological interactions of autochthonous soil microorganisms and microorganisms derived biofertilizers (Malusà et al. 2016). Once microbial cells forming biofertilizers are introduced in the soil, they are faced with an extreme competition condition due to the presence of indigenous microorganisms. Therefore, the exact evaluation of ecological interactions among indigenous soil microorganisms and introduced microbial inoculants is so critical in determining the biofertilizer's efficiency in the short- and long-period of time. For this purpose, various indicators of soil microorganisms, including their biomass, activity, community structure, and diversity should be comprehensively analyzed (Trabelsi and Mhamdi 2013).

It must also be noted that the observed relationships between inhabitant and inoculant microorganisms would depend considerably on the methods used to show the dynamics of terrestrial microbial communities (Trabelsi and Mhamdi 2013). Simultaneous use of metagenomic approaches and culture-dependent methods can lead to the identification of a number of microbial taxa. However, identification of their related function is still very complicated. The evaluation of coding genes of significant enzymes or main genes in the interaction process between the introduced and native microorganisms may help to gain such information.

It has been revealed that diverse taxonomical or functional classes of autochthonous soil microorganisms are affected by complex inoculum through various strategies. Roots colonization of biofertilizers can be achieved through successful competition with indigenous microorganisms. In these competitive conditions, the ability to produce biofilm or having motility can be advantageou for colonizing root and competition. Therefore, to use these beneficial characteristics, a sufficient amount of microbial cells should be present to produce specific chemicals and consequently gain these properties through turning on quorum sensing (Gera and Srivastava 2006). Some of the soil habitants like protozoan grazing, bacterial grazer, fungi, and insects in soil adversely affect biofertilizer efficacy through reducing their number and colonization ability (Finlay 1985). It has been observed that the population of nematodes has been increased after applying biofertilizers (Malusá et al. 2012). Colonization of wheat rhizosphere by *Pseudomonas* species and *Bacillus subtilis* was greatly declined by nematode species (e.g., *Caenorhabditis elegans*, *Acrobeloides thornei*, and *Cruznema* sp.) (Knox et al. 2003).

14.5.4 Effect of Farmers' Practices on the Efficacy of Biofertilizers

Another important factor in biofertilizer efficacy is the overall fertility or nutritional conditions of the soil. Some microbial inocula like *Glomus* are more mostly found in fertile soils, with a great amount of nutrients (Hayman and Stovold 1979). In most cases, applying a large amount of chemical fertilizers for a long term increases the nitrogen, phosphorus, and/or potassium accumulation in the soil, modifies interaction of plant and microbial cells, and simultaneously imposes adverse effects on colonization, establishment, and activities of soil microbial communities including autochthonous soil microorganisms and biofertilizer microbial cells (Gosling et al. 2006). For example, low levels of AMF colonization have been observed in maize, soybean, and wheat which were grown on chemically fertilized soils (Duan et al. 2010). The genera *Scutellospora*, *Acaulospora,* and *Gigaspora* are more frequent in soils with low level of nutrients (Hartmann 2006; Johnson et al. 2005; Mäder et al. 2002; Oehl et al. 2004). Long-term applications (10 or 90 years) of chemical phosphate fertilizers or irrigation with wastewater result in P-accumulation in the soils, which declined colonization and population of AM fungi (Cheng et al. 2013; Ortega-Larrocea et al. 2001). Moreover, in the presence of sufficient bioavailable nitrogen and phosphate, the growth of AM fungi is more likely suppressed, they show mutualistic symbiosis in soil with sufficient bioavailable nitrogen, along with limited P (Johnson et al. 2010).

Therefore, it has been proposed that to achieve efficient colonization of microbial inoculum and their corresponding activities, the quantity of applied chemical fertilizers must be reduced (by 20–50%) (Adesemoye et al. 2009; Jeffries et al. 2003). It was shown that most efficiencies of biofertilizer, which consists of two strains of *Pseudomonas fluorescens* on wheat, were achieved when it is applied via 25% of recommended NPK fertilizers dose (Shaharoona et al. 2008).

In most cases, organic fertilizers like manure, compost, stillage, and vermicompost extract stimulated colonization and growth of several microbial communities via plant growth-promoting activities (Canfora et al. 2015; Esperschütz et al. 2007; Toljander et al. 2008). Although some organic fertilizers reduce AMF richness like sewage sludge (Esperschütz et al. 2007; Toljander et al. 2008), application of some agrochemicals such as aliette, ridomil, benomyl, and benlate has some adverse effects on AM fungi development (Sukarno et al. 1996).

14.5.5 Other Factors Affecting the Efficacy of Biofertilizers

It has been shown that colonization and mycorrhiza-mediated nutrient uptake are detrimentally influenced by tillage, monoculture, and intensive agriculture detrimentally (Perron et al. 2001). Method of inoculum application has a significant role in its efficiency (Date 2001; Deaker et al. 2004). To achieve the best results, new machines

are developed or existing machines are adapted to efficiently applying various physical forms of biofertilizers in specific conditions, e.g., inoculation of horticultural crops or big trees by liquid biofertilizer containing AMF is performed (Malusà et al. 2016; Malusa and Sas 2009).

Biofertilizers can be introduced through the foliar application, treating seeds, mixing with soil or organic matter like vermicompost, spraying through hydraulic atomization system (Bhattacharjya and Chandra 2013; Świechowski et al. 2012). Each of these strategies has a considerable effect on biofertilizer efficiency.

The number of delivered spores and their efficacy is influenced by water volume and adjuvants (Bailey et al. 2007). According to the possible recovery period of PGPR (30–40 days after inoculation), it has been suggested that it is suitable to repeat biofertilizer inoculation (2–4 times) during the growing season, with an interval of 3–4 weeks (Bashan et al. 1995).

In some cases, the activities of biofertilizers are slower than that of chemical fertilizers. The activity of a biofertilizer depends on various factors including inoculant delivery system, skill of farmers, viability of biofertilizer under adverse climatic, transport condition, and storage management. Generally, developing biofertilizers needs high investment costs. Moreover, occurring mutation during the production process is deemed as a major limitation of biofertilizers. It must also be noted that low awareness of the farmers can cause poor resource generation by the industries (Singh et al. 2016).

14.6 Production Process

As mentioned previously, biofertilizers can have one or more microbial strains. Preparing the microbial inoculum has a considerable effect on the final product efficiency and quality (Bashan et al. 2014; Stephens and Rask 2000). Recently, the application of complex microbial consortia in diverse annual and horticultural crops has attracted more attention due to favorable results on legumes and non-legume plants. For example, rhizobia with arbuscular mycorrhizal fungi (Alagawadi and Gaur 1988), (AMF), Rhizobium and phosphate solubilizing bacteria (Wang et al. 2011), Rhizobium and a phosphorus solubilizing bacteria (Prasad and Chandra 2003), AMF with free-living bacteria with a nitrogen-fixing ability (Adesemoye et al. 2008; Barea et al. 2002) and biofertilizer simultaneously containing AMF and various PGPR for diverse annual and horticultural crops (Malusa et al. 2007) are successful examples of biofertilizer with complex inocula. In selecting strains to prepare a consortium for a biofertilizer, it should be noted that the applied strains not only should not inhibit the growth of each other, but also coapplication of them must lead to higher colonization (Vestergård et al. 2008).

In designing a biofertilizer for a specific agrogeographical condition, adaptation of the microbial cells to ecosystem conditions should be investigated (Malusá et al. 2012; Zoppellari et al. 2014). Along with the selection of suitable and efficient microbial cells, the type of fermentation process which profoundly affects the shelf

life of a biofertilizer is also important. In this period, the microbial cells in inoculant should preserve their survival and plant growth-promoting activities at an acceptable level (Bashan et al. 2014). One of the critical involved factors in biofertilizer shelf life is a multistep process, which is called formulation.

A suitable formulation containing additives (Bashan et al. 2014; Herrmann and Lesueur 2013; Malusá et al. 2012) which by protecting microbial cells during storage and transportation enhance their persistence in soil or even improve the microbial cell efficacy using nodulation elicitors (Legume biofertilizers) (Smith and Smith 2012) and colonization and establishment inducing metabolites like strigolactones assures biofertilizer efficiency (Manikandan et al. 2010; Skorupska et al. 2010). It has been shown that biofertilizer in the shape of granular inoculants represents better results under unfavorable conditions of the soil (Rice et al. 2000). Easier distribution of microbial cells in liquid inoculants can lead to their shorter shelf life (Bashan et al. 2014; Date 2001; Stephens and Rask 2000). Encapsulation of PGPR in alginate or other polymers with various compositions and structures was introduced (Vassilev et al. 2005); however, limit industrial application was seen (Bashan et al. 2014; John et al. 2011).

14.7 Fermentation Process

In order to commercialize a biofertilizer, suitable and efficient microbial strains should be selected and characterized. Large-scale production of these strains should be performed and the desired inoculants must be prepared (Sethi and Adhikary 2012). Mass production of microbial cells for biofertilization can be produced through fermentation processes including, submerged fermentation (SmF) and solid state fermentation (SSF) (De Roy et al. 2014).

Selecting a suitable and affordable nutrient medium is an essential prerequisite for a successful biofertilizer production process. Cost-effective substrates like liquid synthetic media, vegetable extracts, soluble sugars, fruit and dairy by-products, and wastewater can be used in submerged fermentation for large-scale production of biofertilizers (Subramaniyam and Vimala 2012). Low-cost substances or even industrial waste like agro-industrial wastes can be used as the substrate to mass production of microbial cells for biological fertilizers through SSF. In this fermentation, microorganisms are grown on solid materials such as sterilized peat or calcinated clay without the presence of free water (Gowthaman et al. 2001). In this strategy, tight contact between microorganisms and agro-industrial wastes provides the highest substrate concentrations for fermentation.

Also, fertilizers can be produced without a complicated formulation process by mixing microbial cells with agricultural by-products. Industrial wastes, like whey, molasses, bagasse, paper pulp, wheat bran, rice, and rice straw, vegetable and fruit wastes can be efficiently applied as low-cost substrates to solubilize insoluble, inorganic, and low-grade phosphate rocks by various fungi in solid state fermentation (Mendes et al. 2015; Pandey et al. 1999). Prepared biofertilizer using agro-industrial

waste materials through solid state fermentation can enrich the soil with organic substances, minerals, and bioactive compounds. It has been revealed that a mixture inoculum of bacterial and fungal microorganisms accelerates the mineralization process of solid waste and their action results in the highly nutrient-rich final product (Cariello et al. 2007; Singh et al. 2011).

Agricultural residues which are treated by microorganisms have a significant ability to reinstate fertility and microbial diversity of disturbed soils. Some fermentation parameters, including pH, temperature, and incubation period, should be optimized in a pilot-scale study to achieve the best production yield.

14.8 Biofertilizer Formulation

The formulation process is a crucial multistep approach in commercializing new biofertilizers and directly affects their efficiency, stability, and quality (Bashan et al. 2014). During the formulation process, microbial cells (one or more strains) are mixed with certain carriers which preserve the cells and their activities under not optimized storage conditions (unfavorable temperature and light exposure) (Herrmann and Lesueur 2013). Good formulation assures successful multiplication of microbial cells, extending their shelf life, and enhances their activity to a higher rate after inoculation to the host plants (Arora et al. 2010). Since formulated biofertilizer largely consists of substances as carriers, they significantly affect the success or the failure of the inoculation.

Being non-toxic, non-pollutant, biodegradable, and biocompatible are critical prerequisites of carriers for formulation. They should be stable and preserve microbial survival under harsh conditions, having adjustable pH, sufficient shelf life, fine grinding in order to mix with other constituents (nutrients, adjuvants), and consist of low-priced substances (Catroux et al. 2001; Herrmann and Lesueur 2013). In desirable formulation, controlled release of microorganisms into the soil can be achieved. Also, applying this formulation can be performed via standard seeding machinery (Malusá et al. 2012). Adherence of microbial cells on the seeds can be improved by using adhesive material, which is known as stickers. Organic, inorganic, or synthetic substances can be used as carriers. Generally, they are categorized into four key classes including soils (inorganic soil peat, clays, coal, and lignite), herbal waste (farmyard manure, wheat bran, charcoal, composts, cellulose, soybean meal, soybean and peanut oil, press mud, and corn cobs), inert materials (ground rock phosphate, vermiculite, perlite, bentonite, calcium sulfate, polyacrylamide, and alginate), and lyophilized microbial cultures and old dried bacteria (Bashan 1998).

It is possible that a formulated biofertilizer be made from a mixture of mentioned carriers. Besides, the carriers and stickers, some macro- and micronutrients such as carbon or mineral resources, hormones, and fungicides, which are known as additives may be added during biofertilizer formulation (Arora and Mishra 2016). Additives, like skim milk (Vassilev et al. 1997), xanthan (Lorda et al. 2007), or sodium alginate (Tittabutr et al. 2007) provide nutrient and moisture, as well as inactivate toxic

compounds, delay inoculant desiccation and improve its quality, stability, and shelf life (Manikandan et al. 2010).

Five formulations are currently used for biofertilizers, including peat formulations, liquid formulations, granules, and freeze-dried powders as well as the most recent strategy of stabilization (Bashan 1998).

14.8.1 Peat Formulations

Peat is composed of partially decayed vegetation that is accumulated for a long time. Microbial cells can successfully grow on it as a nutrient-enriched and protective environment (Bashan et al. 2014). Used peat in the formulation process should have acceptable content of organic matter and water retaining capacity. They must be free from toxic substances for microorganisms, plants, animals, and humans), should be cost-effective, highly adsorptive, and simply sterilizable. Peat processing steps include drainage, sieving, and drying (~5%). Drying must be performed at the lowest possible temperatures to avoid the release of hazardous compounds. After the drying process, they are passed through a 250-μm sieve, and their pH is adjusted at pH 6.5–7.0 by liming (Roughley 1976). Then sterilization of the prepared peat is conducted, and a sufficient quantity of liquid inoculum is added to the peat to achieve a final moisture content of 40–55%. Microbial inoculated peat is incubated for a period of time to allow bacterial multiplication in the carrier which is known as maturing or curing and has a tremendous effect on bacteria survival during storage and on seeds (Okon and Baker 1987). In order to increase the uniformity of biofertilizer coverage on seed, sticking agents including polymeric materials like polysaccharides (like Arabic gum or carboxymethylcellulose), polyalcohol derivatives, or caseinate salts are incorporated into the peat (Albareda et al. 2008; Stephens and Rask 2000). These adhesive agents should be free from hazardous compounds for plant seed or microorganisms, be dispersible in water, and improve microbial cells' survival rate and adherence to the seeds.

Microbially treated peat is generally applied on-site on the seeds just before sowing. The seed coating by microbial inoculated peats can be performed using cement mixers, and mechanical tumbling machines (Schulz and Thelen 2008). Some peat characters like its undefined and complex content, which are source-dependent, poor controllability on the quantity of microorganisms applied per seed, its costly processing, and probable release of toxic substances during its sterilization interfere in the consistent quality of peat formulations and influence the growth and survival of microbial cells (Bashan et al. 2014; Tittabutr et al. 2007). Extensive use of peat poses an adverse impact on the environment and ecosystem where it has been extracted (John et al. 2011). Cork industry derived compost by better ability in preserving the survival of various microbial cells in rhizospheric soil or on the seeds can be a suitable alternative to peat (up to six months) (Albareda et al. 2008).

14.8.2 Liquid Formulations

Easy handling and application either on seeds or in soil are some of the properties which make liquid formulations popular. These are various types of these formulations including, aqueous (broth cultures), mineral or organic oils, oil in water, or polymer-based suspensions (Herrmann and Lesueur 2013). Its physical form makes adding nutrients and cell protectants, including sucrose, glycerol, and Arabic gum, possible, which can improve its performance (John et al. 2011; Sahu and Brahmaprakash 2016). In comparison to peat formulations, they are easily sterilized, compatible with machinery on large farms, and eventually, these features enhanced their field efficacy (Bashan et al. 2014). Biofertilizer in liquid formulations needs more specific storage conditions (cool temperatures) (Stephens and Rask 2000).

14.8.3 Granule Formulations

Another formulation can be made using marble, calcite, and silica grains. For this purpose, created granules are wetted with an adhesive and coated with the microbial cells (Bashan et al. 2014). To have a high-quality end product, a microbial culture containing suitable microbial cells should be prepared (Herrmann and Lesueur 2013). Being less dusty and easier and controllable handling and application are some of the granule advantages compared to peat formulation, although the bulkier size of granules allows cost-effective transport and storage. To avoid direct contact of microbial cell coated granules with the chemicals or pesticides, they are put in a furrow near to the seed to facilitate lateral root interactions (Bashan et al. 2014; Herrmann and Lesueur 2013). Granular inoculants have more survival rate under unfavorable soil conditions such as soil acidity, moisture stress, or cool, wet soils than other formulations (Rice et al. 2000).

14.8.4 Freeze-Dry Formulations

Biofertilizers can be formulated in shapes of freeze-dried powders using various nontoxic and cost-effective, organic, inorganic, or synthetic carriers (Bashan et al. 2014). These carriers physically or nutritionally through supplying a protective surface or a specific substrate, respectively, provide temporarily niche for microbial cells of biofertilizers in soil. Therefore, they have an enormous significance (Arora et al. 2010). These carriers should have high moisture absorption capability, pH buffering capacity, and being sterilizable to assure delivering the right number of viable cells in good physiological condition (Bashan et al. 2014).

14.8.5 Cell Immobilization Formulations

Cell immobilization is a new promising approach which has also been implemented in the formulation of biofertilizer preparations (Jain et al. 2012; Stockwell et al. 2011). In this approach, microbial cells are attached, entrapped or immobilized into a matrix through various strategies including flocculation, adsorption on surfaces, covalent bonding to carriers, cross-linking of cells, and encapsulation in a natural or synthetic polymer gel-like polysaccharides, a protein material, polyacrylamide and polyurethane (Cassidy et al. 1996). Chemical compounds should have the ability to interact with other constituents for encapsulation. Polyacrylamide and alginate are the two commonly used chemicals in preparing encapsulated biofertilizers. Among these, alginate is a naturally occurring polymer with biodegradability and nontoxic feature. To perform encapsulation using alginate, microbial cells should be dispersed into the alginate solution and the microbial cells incorporated alginate matrix is prepared through dropping mixed solution into cationic solution. Shelf life and inoculation efficacy can be extended via adding nutrients and other additives (Malusá et al. 2012). Then dried beads are packaged (Date 2001). Many strategies including spray drying, solvent extraction/evaporation, coacervation, extrusion, emulsion technique, thermal gelation, pre-gel dissolving methods have been presented to precisely define the size, shape, and texture of the beads (Park and Chang 2000).

Encapsulation through protecting cells (bacteria, fungal spores, or small fragments of hypha) in a nutritive shell against mechanical and environmental stresses (like unfavorable pH, temperature, organic solvent, or toxins), as well as predators, assures biofertilizer efficiency (Bashan 1998; Jain et al. 2012; John et al. 2011) and contamination can be minimized through providing aseptic conditions. PSB encapsulation enhanced their efficiency in P solubilization (Jain et al. 2012). Due to a concentrated situation, low volume, and extended shelf life of encapsulated biofertilizers, their transportation, and storage (room temperatures) are easier than other formulations (John et al. 2011). Once the introduction of the encapsulated microbial cell into the soil occurs, they slowly degrade the capsules, which gradually released into the soil (Bashan 1998). In these regards, smaller beads (microencapsulation) enhance the application efficacy via providing direct contact with seeds (John et al. 2011). High production costs and technical handling are the limitations of this formulation. Gels consist of chemical components like fluidized bed, magnesium silicate, or cellulose-based gel revealed some promise, but none have been adopted on-farm (Jawson et al. 1989).

14.9 Advances in Formulation

It has been revealed that limited formulations cannot meet the need of diverse microorganisms to present new biofertilizers with better efficiency, stability, shelf life, lower cost, easier application, handling, and storage. Therefore, an extended

range of materials including organic (water sludge, composts, sawdust, sugarcane bagasse, whey, coal, or enriched agro-industrial residues) and inorganic substances (clays, lapillus, volcanic pumice, or diatomite earth) are being evaluated to develop new carriers (Albareda et al. 2008; Malusá et al. 2012). Some of them have many drawbacks, e.g., sludge wastewater due to the presence of hazardous heavy metals (Malusá et al. 2012).

Various formulated biofertilizers can be applied through different routes. For instance, the seed can be directly inoculated by dry biofertilizers or be soaked with water, then mixed with peat powder (sprinkle method). In another approach, suspended biofertilizer can be added to the seeds and mixed with them (slurry method). Biofertilizer with peat formulation can be suspended in water and sprayed into the furrow during sowing. Moreover, biofertilizer and adhesive can be supplied as slurry to seeds and coated with ground material like lime. Finally, soil can directly be treated by biofertilizers (Bashan 1998).

14.10 Packaging and Quality Control

The nature of the packaging material for biofertilizers can affect its quality. These materials should allow the exchange of oxygen while limiting the water passage. The packaging should minimize biofertilizer contamination during storage and transportation (Roughley 1976). Unfavorable quality and unreliable efficiency under field conditions are the critical factors in biofertilizer failure to gain farmer's acceptance (Herridge 2008; Tarbell and Koske 2007). For example, it has been revealed that 90% of all commercial legume biofertilizers have no practical effect on the legume production yield (Catroux et al. 2001). Contamination is another extensive problem in commercialized biofertilizers. Herrmann and Lesueur (2013) analyzed 65 commercial biofertilizers among which only 37% are containing "pure" and the remaining products (63%) were contaminated with one or more bacterial strains (Herrmann and Lesueur 2013). It has been reported that a significant portion of the commercialized biofertilizers (40%) do not contain pure strains or do not have the claimed strains. Lack of facilities to produce and store high-quality inoculants generates these problems and often leads to inconsistent field results (Bashan 1998). In this regard, systems of quality control are greatly required for ensuring that efficacious biofertilizers are entered into the markets. Quality control and quality assurance systems remove low-quality inoculants from markets. Therefore, consistent results can be obtained in field conditions and better global acceptance can be achieved (Bashan 1998; Bhattacharyya and Jha 2012).

In addition, sufficient information including the name of the microorganisms, guaranteed numbers, nutrients, and other used components content, registration information, lot number, expiry date, dosage and method of application, instructions for disposal, precautions of use of commercialized biofertilizers should be represented on its label to evaluate the quality and the efficacy of a biofertilizer by farmers and make sure to purchase an effective product (Gemell et al. 2005; Husen et al. 2016).

Accordingly, there is a great need to educate manufacturing workers and farmers to assure the quality requirements and a successful crop inoculation, respectively. To prepare biofertilizers with a stable and reproducible efficacy under a wide range of field conditions, it should contain pure isolates and contains no opportunistic pathogens for human, animals, and plants and possesses long term shelf life and its microbial cells should be potently propagated under an extended range of environmental condition (Catroux et al. 2001; John et al. 2011; Lupwayi et al. 2006). A list of several commercial biofertilizers is presented in (Table 14.5).

14.11 Conclusion and Future Prospects

Since the emergence of civilization, 10,0000 years ago, increasing the quality and quantity of agricultural products is one of the main concerns of humans. To achieve these goals, chemical fertilizers have been extensively used; however, their unfavorable effects were revealed on ecosystems. Although organic fertilizers have no adverse effect on soil and its organisms, their labor-intensive and time-consuming preparation makes them unsuitable for application on large scale as a commercial approach. Therefore, it needs promising, safe, and commercial alternatives without environmental adverse effects. Biofertilizers can be considered as cost-effective fertilizers that act in an eco-friendly manner without imposing adverse effects on plant growth and terrestrial and aquatic micro- and macro-organisms, improve soil fertility and its texture, and therefore, can flourish agriculture-related industries. But effective large-scale production and storage strategies should be invented and applied to produce biofertilizers that can be resistant and effective in a wide range of environmental conditions like high temperature and aridity.

Table 14.5 Commercial biofertilizers, their company and microbial strains

Product	Company	Microbial strains
Cell-Tech	Novozymes	Rhizobia
Nitragin Gold	Novozymes	Rhizobia
TagTeam	Novozymes	rhizobia + *Penicillium bilaii*
Accomplish	Loveland Products, Inc	PGPR + enzymes + organic acids + chelators
Nodulator	BASF Canada Inc.	*Bradyrhizobium japonicum*
NodulatorN/T	BASF Canada Inc.	*Bacillus subtilis MBI 600* + *Bradyrhizobium Japonicum*
Nodulator PRO	BASF Canada Inc.	*Bacillus subtilis* + *Bradyrhizobium japonicum*
Nodulator XL	BASF Canada Inc.	*Rhizobium leguminosarum biovar viceae 1435*
Bioboots	Brett-Young Seeds	*Delftia acidovorans*
Bioboots (soybean)	Brett-Young Seeds	*Delftia acidovorans* + *Bradyrhizobium* sp.
EVL coating	EVL Inc.	PGPR consortia
Nitrofix	LabiofamS.A.	*Azospirillum* sp.
Bioativo	Institutode Fosfato Biológico (IFB) Ltda.	PGPR consortia
VitaSoil	Symborg	PGPR consortia
Azotobacterin	JSC "Industrial Innovations"	*Azospirillum brasilense* B-4485
Mamezo	Tokachi Federation of Agricultural Cooperatives (TFAC)	rhizobia (in peat)
R-Processing Seeds	Tokachi Federation of Agricultural Cooperatives (TFAC)	rhizobia (coated legume seeds)
Hyper Coating Seeds	Tokachi Federation of Agricultural Cooperatives (TFAC)	rhizobia (coated grass legume seeds)
Life	Biomax	PGPR consortia
Biomix	Biomax	PGPR consortia
Biozink	Biomax	PGPR consortia
Biodine	Biomax	PGPR consortia
Grotop PSB Powder	MD Biocoals Pvt. Ltd.	Phosphate Solubilizing Microorganisms (*Bacillus* sp.), Powder 10^7–10^9 cfu g^{-1} and Liquid 10^9 cfu ml^{-1}

(continued)

Table 14.5 (continued)

Product	Company	Microbial strains
Bio Promoter	Mani Dharma Biotech Private Limited, Tamil Nadu	*Bacillus megaterium* + *Aspergillus niger*
Multiplex Nalapak	Multiplex Bio-Tech Pvt. Ltd., Karnataka	Homogenous mixture of *Azotobacter* + *Azospirillium* + phosphate solubilizer + potash mobilizer
Ambiphos	Ambika Biotech & Agro Services, Madhya Pradesh	Phosphate solubilizing microorganism (*Aspergillus niger*)
Biophos	Biotech International Limited, Delhi	*Bacillus megaterium* var. *Phosphaticum*
BioP-P	Sundaram Overseas Cooperation, Gujarat	Phosphate solubilizing microorganism (2×10^8 CFU g^{-1})
PSM	Shree Biocare India, Shree Biocare Solution Pvt Ltd, Gujarat	Phosphate solubilizing microorganisms
Multiplex Sagar (Compost Poly Culture)	Multiplex Bio-Tech Pvt. Ltd., Karnataka	Homogenous mixture of *Azospirillum* + *Trichoderma* + *Pleurotous*
Enriched compost Culture	Organic Biotech Pvt Limited, Maharastra	*Trichoderma harzianum* + *Aspergillus* + *Penicillium*
Bio-manure Culture	Uno Natural and Greens Private Limited, Tamil Nadu	*Trichoderma harzianum* + *Aspergillus*
LignoBiocompost Culture	Peak Chemical Industries Limited, West Bengal	*Trichoderma resei*, *Phanerochaete chrysosporium* and *Aspergillus awamori*

Sources Kabaluk et al. (2010), Pal et al. (2015)

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