Chapter 1
Microbial Biofertilizers: Types and Applications

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Abstract The increased dependency of modern agriculture on excessive synthetic input of chemical fertilizers has caused several environmental problems related to greenhouse effect, soil deterioration, and air and water pollution. Furthermore, there is an imperative need for viable agricultural practices on a global level with reduced energy and environmental problems, for adequate cost-efficient production of food for the increasing human population. Consequently, biofertilizers containing micro-organisms like bacteria, fungi, and algae have been suggested as viable solutions for large-scale agricultural practices which not only are natural, ecofriendly, and economical but also maintain soil structure as well as biodiversity of agricultural land. Besides providing nutrient enrichment to the soil, microbial biofertilizers promote plant growth by increasing efficient uptake or availability of nutrients for the plants and by suppressing soilborne diseases. Biofertilizers supplement nutrients mainly by fixation of atmospheric nitrogen, by phosphorus solubilization, and by synthesizing plant growth-promoting substances. The nitrogen-fixing bacteria of the rhizobia and other groups are used for growth promotion of legumes and additional crops. In addition, blue-green algae (BGA) as well as *Azolla* subsidize in the nitrogen budget of practicable agriculture. Arbuscular mycorrhizal fungi are important for the uptake of phosphorus and several other minerals in many plants. Phosphorus-solubilizing bacteria like *Azotobacter* and *Azospirillum* that fix atmospheric nitrogen can increase the solubility and availability of phosphorus to plants and, thus, crop yield. Further, *Azospirillum* provides additional benefits such as the production of growth-promoting substances, disease resistance, and drought tolerance. Thus, application of microbial biofertilizers is an effective approach in increasing and maintaining the nutrient economy of soil, thereby reducing the use of chemical fertilizers, for a proficient and sustainable agriculture.

Keywords Biofertilizer types · Agrochemicals · Beneficial microbes · Application of biofertilizers

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1.1 Introduction

Fertilizers are natural or man-made chemicals that, when applied on the plant or to soil or by fertigation (applying by irrigation water), can supplement natural soil nutrients and augment crop growth and soil fertility (Edgerton 2009). These make available important macronutrients (nitrogen, phosphorus, potassium, calcium, sulfur, and magnesium) along with numerous micronutrients (zinc, copper, iron, boron, and molybdenum) to plants (Alley and Vanlauwe 2009). A high production demand of standard fertilizers is observed for those that are commonly known as NPK fertilizers and provide nitrogen (ammonia, urea, ammonium sulfate, ammonium nitrate, calcium ammonium nitrate), phosphorus (di-ammonium phosphate, superphosphates, ground rock phosphates), and potassium (potash or potassium chloride, sulfate of potash or potassium sulfate, sulfate of potash magnesia, potassium nitrate, kieserite, Epsom salt). Micro-enriched fertilization, involving the addition of micronutrients to these standard fertilizers, has encouraged agronomic bio-fortification to alleviate malnutrition and micronutrient deficiencies of copper, iron, zinc, iodine, selenium, and fluorine in crop plants (Arnon and Stout 1939). For example, fertilizers with added zinc have been found to increase cereal grain yield by higher seedling establishment and tolerance to environmental stresses (Cakmak 2008). However, one constraint to plant growth is non-availability of nutrients especially nitrogen and phosphorus to plants despite their ample occurrence in soil, as most nitrogen is present in soil organic matter and plants have to compete with soil microbes to obtain it, while phosphorus forms precipitates with iron and aluminum (in acidic soils) or with calcium (in alkaline soils) (Schachtman et al. 1998; Hinsinger 2001).

The exponential growth in human population has demanded a concurrent production and supply of food, particularly from plants. Consequently, a highly productive and intensive agricultural system has been mostly accomplished by the use of synthetic chemical fertilizers of nitrogen and phosphorus (Schultz et al. 1995). However, increased dependence of modern agriculture on an excessive, imbalanced, and steady synthetic input of chemical fertilizers has caused deterioration of soil quality (by making them biologically inert and highly saline) and surface and ground water, and it has further reduced biodiversity and stifled ecosystem functioning (Socolow 1999). The production and transport of chemical fertilizers, which require the use and combustion of fossil fuels, result in airborne carbon dioxide and nitrogen pollution that get deposited into terrestrial ecosystems. Furthermore, excessive supply of chemical fertilizers to soil than used by the crops gets stored in plants and often causes potential losses (by leaching, volatilization, acidification, and denitrification) due to elevated nitrate and phosphorus concentrations in water bodies instigating eutrophication and hypoxia in lakes and estuaries (Vance 2001) and environmental pollution problems by emissions of greenhouse gases like nitrous oxide (N_2O) from fertilizer production and application (Mosier et al. 2004; Nash et al. 2012).

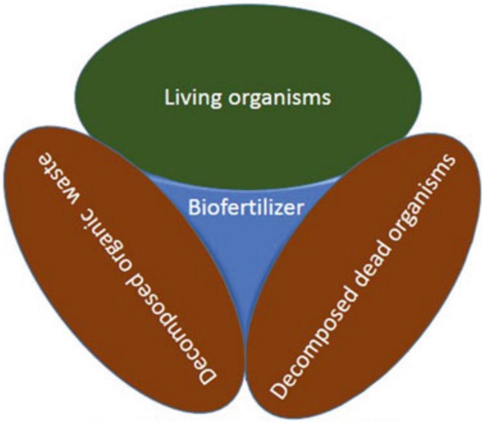

Fig. 1.1 Sources of biofertilizers

Because of the mentioned drawbacks of chemical fertilizers, it is essential to reduce the consumption of chemical fertilizers and pesticides in agriculture without having any adverse effect on crop production by the incorporation and usage of harmless, renewable inputs of fertilizers. The most suitable alternatives for chemical fertilizers are biofertilizers that include organic waste, dead organisms, as well as living organisms (Fig. 1.1). For example, manure and compost are suitable for almost every variety of plants, eggshells have high calcium, and *Stellaria media* (chickweed), *Equisetum* sp. (horsetail), *Azolla pinnata*, *Arctium* sp. (burdock), *Rumex crispus* (yellow dock), *Symphytum officinale* (comfrey), and *Urtica dioica* (nettles) have high nitrogen content. Community waste and sewage sludge provide an inexpensive source of plant nutrition, though these may contain heavy metals and may have adverse effects on crops, consumers, and soil microorganisms (Giller et al. 1998; Graham and Vance 2000). More importantly, biofertilizers can be composed of efficient microbial strains that, by their interactions in rhizosphere, benefit crop plants by the uptake of nutrients. Many bacteria identified as plant growth-promoting rhizobacteria (PGPR), by certain known and unknown mechanisms, can stimulate plant growth. The important known mechanisms exhibited by PGPR that promote plant growth are atmospheric nitrogen fixation, phosphorus solubilization, enhancement of nutrient uptake, or production of plant growth hormones (Bashan et al. 1990; Okon and Labandera-Gonzalez 1994; De Freitas et al. 1997; Bashan 1998; Goldstein et al. 1999). *Achromobacter*, a PGPR, was found to enhance the length as well as number of root hairs and increased nitrate and potassium uptake in *Brassica napus* (oilseed rape), which was evident through the increased dry weights of shoot (from 22% to 33%) and root (from 6% to 21%) (Bertrand et al. 2000). Thus, various types of biofertilizers provide optimum nutrients to crop plant, cause nominal damage to environment, and enhance biodiversity of soil. Their consumption in the future is expected to increase due to overall increase in the demand of fertilizers in order to produce more food on limited arable land and further due to exhausting feedstock/ fossil fuels (energy crisis), increasing chemical-fertilizer cost, depleting soil fertility, concerns about environmental hazards, and an increasing threat to sustainable agriculture. It is predicted that market share of biofertilizers will reach US$1.66 billion by 2022 and will be compounding the annual growth rate of 13.2% during the years of 2015–2022 (Timmusk et al. 2017).

1.2 Microbial Biofertilizers

A biofertilizer of selected efficient living microbial cultures, when applied to plant surfaces, seed or soil, can colonize the rhizosphere or the interior of the host plant and then promote plant growth by increasing the availability, supply, or uptake of primary nutrients to the host. Moreover, in contrast to chemical fertilizers, biofertilizers are more accessible to marginal and small farmers. The most important groups of microbes used in the preparation of microbial biofertilizer are bacteria, fungi, and cyanobacteria, majority of which have symbiotic relationship with plants. The important types of microbial fertilizers, based on their nature and function, are those which supply nitrogen and phosphorus (Table 1.1).

1.2.1 Nitrogen-Fixing Microbes

Nitrogen is most abundant and ubiquitous in the air, yet becomes a limiting nutrient due to difficulty of its fixation and uptake by the plants. However, certain microorganisms, some of which can form various associations with plants as well, are capable of considerable nitrogen fixation. This property allows for the efficient plant uptake of the fixed nitrogen and reduces loses by denitrification, leaching, and volatilization. These microbes can be:

(a) Free-living in the soil (Table 1.1). The assessment of nitrogen fixation by free-living bacteria is difficult, but in some plants like *Medicago sativa*, it has been estimated to range from 3 kg N ha^{-1} to 10 kg N ha^{-1} (Roper et al. 1995). *Azotobacter chroococcum* in arable soils can fix 2–15 mg N g^{-1} of carbon source in culture media, and it further produces abundant slime which aggregates soil. However, free-living cultures of nodulating bacterial symbionts (e.g., *Frankia*) have been found to fix atmospheric nitrogen in the rhizosphere of their host and even non-host plants (Smolander and Sarsa 1990). For *Beijerinckia mobilis* and *Clostridium* spp., inoculation methods of leaf spray and seed soaking stimulated growth in cucumber and barley plants by significant nitrogen fixation and other mechanisms of bacterial plant growth hormone synthesis (Polyanskaya et al. 2002). Free-living cyanobacteria (blue green algae) have been harnessed in rice cultivation in India which can provide up to 20–30 kg N ha^{-1} under ideal conditions (Kannaiyan 2002).

(b) Having symbiotic and other endophytic associations (of rhizobia, *Frankia,* and cyanobacteria) with plants. The nitrogen-fixing efficiency of rhizobia bacteria,

Table 1.1 The important groups of microbial fertilizers

Group of biofertilizers	Sub-group	Examples
Nitrogen-fixing	Free-living	*Anabaena*, *Azotobacter*, *Beijerinkia*, *Derxia*, *Aulosira*, *Tolypothrix*, *Cylindrospermum*, *Stigonema*, *Clostridium*, *Klebsiella*, *Nostoc*, *Rhodopseudomonas*, *Rhodospirillum*, *Desulfovibrio*, *Chromatium*, and *Bacillus polymyxa*
	Symbiotic	Rhizobia (*Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium Mesorhizobium Allorhizobium*), *Frankia*, *Anabaena azollae*, and *Trichodesmium*
	Associative	*Azospirillum* spp. (*A. brasilense*, *A. lipoferum*, *A. amazonense*, *A. halopraeferens*, and *A. irakense*), *Acetobacter diazotrophicus*, *Herbaspirillum* spp., *Azoarcus* spp., *Alcaligenes*, *Bacillus*, *Enterobacter*, *Klebsiella*, and *Pseudomonas*
Phosphorus (microphos)	Phosphate-solubilizing	*Bacillus megaterium* var. *phosphaticum*, *B. subtilis*, *B. circulans*, *B. polymyxa*, *Pseudomonas striata*, *Penicillium* spp., *Aspergillus awamori*, *Trichoderma*, *Rhizoctonia solani*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Microccocus*, *Aereobacter*, *Flavobacterium*, and *Erwinia*
	Phosphate-mobilizing	Arbuscular mycorrhiza (*Glomus* sp., *Gigaspora* sp., *Acaulospora* sp., *Scutellospora* sp., and *Sclerocystis* sp.), ectomycorrhiza (*Laccaria* spp., *Pisolithus* spp., *Boletus* spp., *Amanita* spp.), ericoid mycorrhiza (*Pezizella ericae*), and orchid mycorrhiza (*Rhizoctonia solani*)
Micronutrients	Potassium solubilizing	*Bacillus edaphicus*, *B. mucilaginosus*, and *Paenibacillus glucanolyticus*
	Silicate and zinc solubilizing	*Bacillus subtilis*, *Thiobacillus thioxidans*, and *Saccharomyces* sp.
Growth promoting	Plant growth-promoting rhizobacteria	*Agrobacterium*, *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Actinoplanes*, *Azotobacter*, *Bacillus*, *Pseudomonas fluorescens*, *Rhizobium*, *Bradyrhizobium*, *Erwinia*, *Enterobacter*, *Amorphosporangium*, *Cellulomonas*, *Flavobacterium*, *Streptomyces*, and *Xanthomonas*

Modified from Singh et al. (2014)

an important group of biofertilizers that contains organisms like *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium*, and *Allorhizobium*, can vary till 450 kg N ha^{-1} among different strains and host legume species, in which root nodules are formed (Stamford et al. 1997; Unkovich et al. 1997; Spaink et al. 1998; Vance 1998; Graham and Vance 2000; Unkovich and Pate 2000). The rhizobial biofertilizers can be in powder, liquid, and granular formulations, with different sterilized carriers like peat, perlite, mineral soil, and charcoal (Stephens and Rask 2000). Like rhizobia, *Frankia*, a nitrogen-fixing actinomycete, can also form root nodules in several woody plants (Torrey 1978; Dawson 1986; Benson and Silvester 1993;

Dommergues 1995; Huss-Danell 1997; Wall 2000). This mycelial bacterium forms symbioses with the roots of several non-legume plants like *Casuarina*, *Alnus* (*Alder*) *Myrica*, *Rubus*, etc. These actinorhizal plants are used for timber and fuelwood production, windbreaks, and shelterbelts and in advancing early successional plant community development, mixed plantations, revegetation, and land reclamation (Diagne et al. 2013; Schwencke and Carù 2001). The inoculation of *Frankia* is considered valuable in nurseries and in arid or disturbed environments (Schwintzer and Tjepkema 1990; Sprent and Parsons 2000). Besides, leaves of a few plants (e.g., *Ardisia*) develop special internal cavities harboring symbiotic nitrogen-fixing bacteria like *Xanthomonas* and *Mycobacterium*, and as such, these leaves are source of nitrogen fertilizer to the soil (Miller 1990). Another ecologically important group is that of cyanobacteria—blue green algae (BGA)—some of which like *Trichodesmium*, *Nostoc*, and *Anabaena* contribute to about 36% of the global nitrogen fixation and have been reported to be helpful in enhancing rice-field fertility for the cultivation of rice in many parts of the world (Kundu and Ladha 1995; Gallon 2001; Irisarri et al. 2001). Besides, BGA are also known to be advantageous for possible reclamation of arid environments or ecosystems disposed to flooding (Bashan et al. 1998; Malam Issa et al. 2001). The production and application of BGA is, however, poorly developed, and it should be considered as a biofertilizer for sustainable agricultural practices in various environments (Hashem 2001). Aquatic BGA can further provide natural growth hormones, proteins, vitamins, and minerals to the soil.

(c) Living in rhizosphere (associative/associated) without endophytic symbioses. In comparison to endophytic symbionts, these nitrogen-fixing microbes have less intimate association with roots. These include *Acetobacter diazotrophicus* and *Herbaspirillum* spp. with sugarcane, sorghum, and maize (Triplett 1996; James et al. 1997; Boddey et al. 2000); *Azoarcus* spp. with *Leptochloa fusca* (kallar grass) (Malik et al. 1997); species of *Alcaligenes*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, and *Pseudomonas* with rice and maize (James 2000); and *Azospirillum* with great host specificity comprising a variety of annual and perennial plants (Bashan and Holguin 1997). Several studies have shown that due to nitrogen fixation and production of growth-promoting substances, *Azospirillum* increased the growth and crop yield of wheat, rice, sunflower, carrot, oak, sugar beet, tomato, eggplant, pepper, and cotton (Okon 1985; Bashan et al. 1989; Okon and Labandera-Gonzalez 1994). The inoculum of *Azospirillum* can be inexpensively produced and applied by a simple peat formulation (Vande Broek et al. 2000). The biofertilizer of *Acetobacter diazotrophicus* was found to fix and make available up to 70% of sugarcane crop nitrogen requirement, of about 150 kg N ha^{-1} annually (Boddey et al. 1995).

Thus, the capability of nitrogen fixation in substantial quantity of these microorganisms makes them attractive candidates for their application as biofertilizers.

1.2.2 Phosphorus-Solubilizing Microbes

In soil, the concentration of phosphorus is high, but most of it is present in unavailable forms, which makes it the second most limiting plant nutrient after nitrogen (Schachtman et al. 1998). The phosphorus-solubilizing bacteria (PSB) like *Bacillus* and *Pseudomonas* can increase phosphorus availability to plants by mobilizing it from the unavailable forms in the soil (Richardson 2001). These bacteria and certain soil fungi such as *Penicillium* and *Aspergillus* bring about dissolution of bound phosphates in soil by secreting organic acids characterized by lower pH in their vicinity. The application of the inexpensive rock phosphate with a PSB, *Bacillus megaterium* var. *phosphaticum* to sugarcane, was found to increase sugar yield and juice quality by 12.6%, and it reduced the phosphorus requirement by 25%, thereby further causing a 50% reduction of the costly superphosphate usage (Sundara et al. 2002).

1.2.3 Mycorrhizal Biofertilizers

These are phosphorus-mobilizing biofertilizers or phosphate absorbers. The mycorrhizal fungi form obligate or facultative functional mutualistic symbioses with more than 80% of all land plants, in which the fungus is dependent on host for photosynthates and energy and in return provides a plethora of benefits to its host (Smith and Read 1997; Thakur and Singh 2018). The mycelium of the fungus extends from host plant root surfaces into soil, thereby increasing the surface area for more efficient nutrient access and acquisition for the plant, especially from insoluble phosphorus sources and others like calcium, copper, zinc, etc. (Singh and Giri 2017). Additionally, mycorrhizal fungi are known to enhance soil quality, soil aeration, water dynamics, and heavy metal and drought tolerance of plants and to make plants less susceptible to root pathogens or herbivores (Rillig et al. 2002; Thakur and Singh 2018). This suggests high potential of these fungi for application in agriculture, land reclamation, or vegetation restoration (Menge 1983; Sylvia 1990). Ectomycorrhiza (of Basidiomycetes) forms a mantle on the root surface (of several trees such as *Eucalyptus*, *Quercus*, peach, pine, etc.) and penetrates internally into the intercellular spaces of the cortical region from where it obtains the plant-secreted sugars and other nutrition. The important functions of these fungi are absorption of water and minerals by increasing surface area of roots, solubilizing soil humus organic matter to release and absorb inorganic nutrients, and secreting antimicrobial substances that protect plants from various root pathogens. The importance of ectomycorrhizal symbiosis has been observed for tree plantations in growth and nutrient acquisition, especially for large-scale inoculum practices into nursery or forestry cultivated areas (White 1941; Wilde 1944; Mikola 1970; Smith and Read 1997).

Arbuscular mycorrhizal (AM) fungi like *Glomus* are intercellular, nonspecific obligate endosymbionts (with special structures of vesicles and arbuscules in roots) that, by functioning as an extended root system, harvest moisture and various micronutrients from deeper and distant niches in the soil, besides increasing the mobility and availability of phosphorus to enhance growth and development in host plants. However, unculturability and the obligate nature of AM fungi have made inoculation incompatible with large-scale industrial-scale agriculture, and thus it might require additional research (Wood and Cummings 1992; Ryan and Graham 2002). Nevertheless, the AM inoculation for production of nursery stocks often results in amended and homogeneous crop growth. For agricultural purpose, the ability of fungi for colonization in specific host plants can vary, which can depend on the inoculum source (Biermann and Linderman 1983; Klironomos and Hart 2002). The production of infective propagules by growing inoculum in symbiosis with living host plants or in root organ cultures is a viable mean, but has limitations of high production cost, slow turnover time, and difficulty in excluding root pathogens. AM inoculum is applied as spores (most reliable), fragments of colonized roots (effective for some taxa), or a combination of these and incorporated soil mycelium mixed with carrier substrate like pumice or clay, sand, perlite, vermiculite, soil rite, and soil or glass pellets (Mallesha et al. 1992; Redecker et al. 1995; Gaur and Adholeya 2000; Klironomos and Hart 2002).

1.2.4 Other Mineral-Solubilizing Biofertilizers

Soil-dwelling microorganisms can further be used as biofertilizers to provide various nutrients other than nitrogen and phosphorus such as potassium, zinc, iron, and copper. Certain rhizobacteria can solubilize insoluble potassium forms, which is another essential nutrient necessary for plant growth (Jakobsen et al. 2005). The higher biomass yields due to increased potassium uptake have been observed with *Bacillus edaphicus* (for wheat), *Paenibacillus glucanolyticus* (for black pepper), and *Bacillus mucilaginosus* in co-inoculation with the phosphate-solubilizing *Bacillus megaterium* (for eggplant, pepper, and cucumber) (Meena et al. 2014; Etesami et al. 2017). Another important mineral is zinc, which is present at a low concentration in the Earth’s crust, due to which it is externally applied as the costlier soluble zinc sulfate to overcome its deficiencies in plant. However, some microbes such as *Bacillus subtilis*, *Thiobacillus thiooxidans*, and *Saccharomyces* spp. can solubilize insoluble cheaper zinc compounds like zinc oxide, zinc carbonate, and zinc sulfide in soil (Ansori and Gholami 2015). Similarly, microorganisms can hydrolyze silicates and aluminum silicates by supplying protons (that causes hydrolysis) and organic acids (that form complexes with cations and retain them in a dissolved state) to the medium while metabolizing, which can be beneficial to the plants. For instance, an increase in rice growth and grain yield due to increased dissolution of silica and nutrients from the soil was observed using a silicate-solubilizing *Bacillus* sp. combined with siliceous residues of rice straw, rice husk, and black ash (Cakmakci et al. 2007).

1.2.5 Plant Growth-Promoting Microbes

Besides nitrogen-fixing and phosphorus-solubilizing microbes, there are microbes that are suitable to be used as biofertilizers as these enhance plant growth by synthesizing growth-promoting chemicals (Bashan 1998). For example, rhizospheric *Bacillus pumilus* and *Bacillus licheniformis* were found to produce substantial quantities of physiologically active plant hormone gibberellin (Gutierez-Mañero et al. 2001). However, *Paenibacillus polymyxa* showed a variety of beneficial properties, including nitrogen fixation, phosphorus solubilization, production of antibiotics, cytokinins, chitinase, and other hydrolytic enzymes and enhancement of soil porosity (Timmusk et al. 1999). Further, some species of *Azospirillum* have been reported to produce plant hormones (Bashan et al. 1990; Bashan and Holguin 1997). These indicate the potential of diverse microbes as biofertilizers, which might require additional studies.

The rhizobacterial plant growth-promoting mechanisms of antagonism against phytopathogenic microorganisms include production of antimicrobial metabolites like siderophores and antibiotics, gaseous products like ammonia, and fungal cell wall-degrading enzymes which cause cytolysis, leakage of ions, membrane disruption, and inhibition of mycelial growth and protein biosynthesis (Idris et al. 2007; Lugtenberg and Kamilova 2009). For example, *Pseudomonas* strains can produce antifungal metabolites like phenazines, pyrrolnitrin, pyoluteorin, and cyclic lipopeptides of viscosinamide, which can prevent *Pythium ultimum* infection of sugar beet. *Pseudomonas fluorescens* produces the iron-chelating siderophores like pseudobactin and pyoverdin that bind and take up ferric ions, which makes them better competitors for iron, thus preventing the growth and proliferation of pathogenic microbes like *Pythium ultimum*, *Rhizoctonia batatticola*, and *Fusarium oxysporum* (Cox and Adams 1985; Leeman et al. 1996; Hultberg et al. 2000). *Pseudomonas aeruginosa* produces the siderophores pyoverdine, pyochelin, and salicylic acid and further induces resistance against *Botrytis cinerea* (on bean and tomato) and *Colletotrichum lindemuthianum* (on bean) (De Meyer and Höfte 1997; Audenaert et al. 2002). However, some species of *Pseudomonas* produce extracellular chitinase and laminase that can lyse *Fusarium solani* mycelia. In addition, biofertilizers provide protection against some soilborne diseases, insect pests, and plant diseases; for example, *Azotobacter* pervades the soil with antibiotics which inhibit the spread of soilborne pathogens like *Pythium* and *Phytophthora* (Wani et al. 2013).

1.2.6 Compost Biofertilizers

Compost is a decomposing, brittle, murky material forming a symbiotic food web within the soil, which contains about 2% (*w*/*w*) of nitrogen, phosphorus, and potassium, along with microorganisms, earthworms, and dung beetles. The microbial organic solid residue oxidation causes the formation of humus-containing material, which can be used as an organic fertilizer that sufficiently aerates, aggregates, buffers, and keeps the soil moist, besides providing beneficial minerals to the crops and increasing soil microbial diversity (Yu et al. 2016). Compost is produced from a wide variety of materials like straw, leaves, cattle-shed bedding, fruit and vegetable wastes, biogas plant slurry, industrial wastes, city garbage, sewage sludge, factory waste, etc. The compost is formed from these materials by different decomposing microorganisms like *Trichoderma viridae*, *Aspergillus niger*, *A. terreus*, *Bacillus* spp., several Gram-negative bacteria (*Pseudomonas*, *Serratia*, *Klebsiella*, and *Enterobacter*), etc. that have plant cell wall-degrading cellulolytic or lignolytic and other activities, besides having proteolytic activity and antibiosis (by production of antibiotics) that suppresses other parasitic or pathogenic microorganisms (Boulter et al. 2002). Another important type (vermicompost) contains earthworm cocoons, excreta, microorganisms (like bacteria, actinomycetes, fungi), and different organic matters, which provide nitrogen, phosphorus, potassium, and several micronutrients, and efficiently recycles animal wastes, agricultural residues, and industrial wastes cost-effectively and uses low energy.

1.3 Application Practices of Microbial Biofertilizers

Biofertilizers are mostly supplied as conventional carrier-based inoculants with the advantage of being cheap and easier to produce. The mass production of biofertilizers involves culturing of microorganisms, processing of carrier material, mixing of carrier material with the broth culture, and packing (Fig. 1.2). The ideal carrier materials used in the preparation of biofertilizers must be cheaper, locally

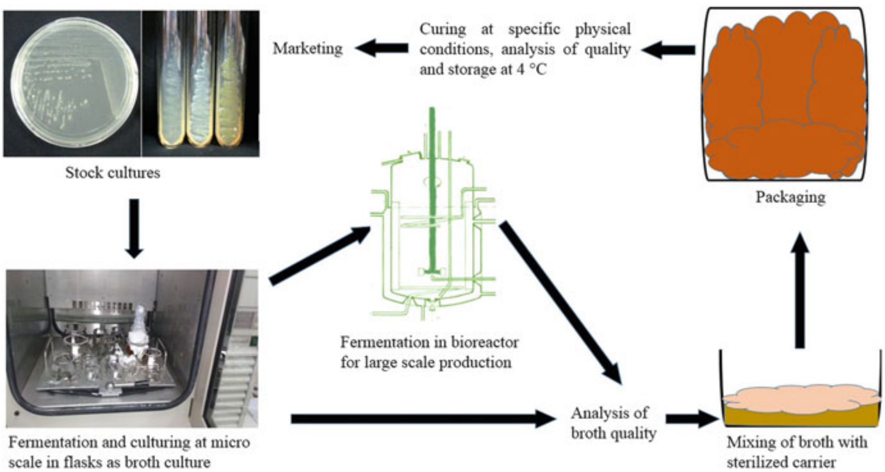

Fig. 1.2 A diagrammatic representation of mass production of bacterial biofertilizers

available, and easier to process; must be non-toxic and organic in structure (so that they remain biodegradable) with high water-holding capacity; and should carry higher bacterial cells and support their survival for longer durations. Some of the commonly used carrier materials in the production of good-quality biofertilizers are neutralized peat soil/lignite, vermiculite, charcoal, press mud, farmyard manure, and soil mixture. However, these can have disadvantages of possessing lower shelf-life, temperature sensitivity, being contamination prone, and becoming less effective by low cell counts. Consequently, liquid formulations have been developed for *Rhizobium*, *Azospirillum*, *Azotobacter*, and *Acetobacter* which although costlier, have the advantages of having easier production, higher cell counts, longer shelf-life, no contamination, storage up to 45 °C, and greater competence in soil (Ngampimol and Kunathigan 2008). Nevertheless, the application practices of microbial biofertilizers include seed treatment, seedling root dipping, and soil application.

1.3.1 Seed Treatment

Seed treatment is a very effective, economic, and most common method implemented for all types of inoculants (Sethi et al. 2014). The seeds are mixed and uniformly coated in a slurry (inoculant mixed with 200 mL of rice kanji) and then shade-dried, before being sown within 24 h. For liquid biofertilizers, depending upon the quantity of seeds, the coating can be done in either plastic bag (if quantity is small) or bucket (if quantity is large). The seed treatment can be done with two or more bacteria (for instance, nitrogen-fixing bacteria such as *Rhizobium*, *Azotobacter*, and *Azospirillum* can be taken along with phosphorus-solubilizing microbes), without any antagonistic effect, and provide maximum quantity of each bacterium on individual seed required for better results (Chen 2006). For example, seed treatment is done for many plants using *Rhizobium* (pulses like chickpea, pea, groundnut, soybean, beans, lentil, lucern, berseem, green gram, black gram, cowpea, and pigeon pea), *Azotobacter* (cereals like wheat, oat, barley; oil seeds like mustard, seasum, linseeds, sunflower, castor; millets like pearl millets, finger millets, kodo millet; forage crops and grasses like bermuda grass, sudan grass, napier grass, para grass, star grass, etc.), and *Azospirillum* or phosphorus-solubilizing bacteria (rice, maize, and sorghum) (Taylor and Harman 1990).

1.3.2 Seedling Root Dipping

This application is common for plantation crops such as cereals, vegetables, fruits, trees, sugarcane, cotton, grapes, banana, and tobacco where seedling roots are dipped in a water suspension of biofertilizer (nitrogen-fixing *Azotobacter* or *Azospirillum* and phosphorus-solubilizing microbial biofertilizer) for sufficient period of time. The treatment time differs for different crops, for instance, vegetable crops are treated for 20–30 min and paddy for 8–12 h before transplantation (Barea and Brown 1974).

1.3.3 Soil Application

In this practice, biofertilizer is applied directly to the soil either alone or in combination. A mixture of phosphate-solubilizing microbial biofertilizer, cow dung, and rock phosphate is kept in shade overnight while maintaining its moisture content at 50% and then applied to the soil (Pindi and Satyanarayana 2012). Some examples of biofertilizers in which soil application is employed are *Rhizobium* (for leguminous plants or trees) and *Azotobacter* (for tea, coffee, rubber, coconuts, all fruit/agroforestry plants for fuelwood, fodder, fruits, gum, spice, leaves, flowers, nuts, and seeds) (Zahran 1999; Hayat et al. 2010).

1.4 Available Microbial Biofertilizers

There are several microbial biofertilizers available as dried or liquid cultures under different trade names in the market, which are used for a variety of purposes including enhancement of plant growth and soil fertility (Table 1.2). For instance, the rhizobia biofertilizers can fix 50–300 kg N ha^{-1} that increases yield by 10–35%, maintains soil fertility, and leaves residual nitrogen for succeeding crops (Davis 1996; Chen 2006). The *Azotobacter* biofertilizer used for almost all crops can fix 20–40 mg N g^{-1} of carbon source that causes up to 15% increase in yield; maintains soil fertility; produces growth-promoting substances such as vitamin B complexes, indole acetic acid, and giberellic acid; and is further helpful in biocontrol of plant diseases by suppressing some of the plant pathogens (Abd El-Lattief 2016; Kurrey et al. 2018). The phosphorus-solubilizing bacterial biofertilizers, which are nonspecific and suitable for all crops, produce enzymes which mineralize the insoluble organic phosphorus into a soluble form, thereby increasing crop yield by 10–30% (Sharma et al. 2013).

1.5 Limitations of Microbial Biofertilizers

Although biofertilizer technology is ecofriendly and possesses a surfeit of advantages, there are some limitations (some of which have been mentioned in Table 1.3) of this technology causing suspicion among stakeholders about its application. The major drawbacks associated with microbial biofertilizers that need immediate attention through further research as well as proper planning include their plant specificity, lower nutrient density (thus, are required in bulk to be made available for most crops), requirement of separate machinery and skill for production and application than that used for chemical fertilizers, difficulty of storage, and more importantly inadequate awareness about their use and benefits among farmers (Malusà et al. 2016). Furthermore, there can be constraints regarding the application or implementation of biofertilizers that affect the technology at stages of production, marketing, or usage (Table 1.3) (Jangid et al. 2012).

Table 1.2 Different microbial biofertilizers available in market and their application

Microbial biofertilizers	Trade names	Application
Azospirillum lipoferum, *Azospirillum brasilense*, and different strains of *Azospirillum*	Biospirillum, Green Plus, Bio-N, Azo-S, ROM, and Spironik	(1) For normal and acidic soils and dry soils (2) For paddy and other crops
Azotobacter chroococcum, different strains of *Azotobacter* (non-symbiotic)	Bioazoto, Bhoomi Rakshak, Kisaan Azotobacter culture, and Azonik	For all crops like wheat, sorghum, barley, maize, paddy, mustard, sunflower, sesamum, cotton, sugarcane, banana, grapes, papaya, watermelon, onion, potato, tomato, cauliflower, chilly, lady finger, rapeseed, linseed, tobacco, mulberry, coconut, spices, fruits, flowers, plantation crops, and forest plants
Gluconacetobacter diazotropicus	Sugar-Plus	For sugarcane
Rhizobium strains (symbiotic, nitrogen fixing)	Biobium, Rhizo-Enrich, Kisaan Rhizobium culture, Rhizoteeka, Green Earth Reap N4, and Rhizonik	Pulses (gram, peas, lentil, moong, urd, cowpea, and arhar), oil legumes (groundnut and soyabeans), fodder legumes (barseem and lucerne), and forest tree legumes (subabul, shisam, and shinsh)
Phosphorus-solubilizing and Phosphorus-mobilizing microbes like *Bacillus megaterium*, mycorrizhal fungi, etc.	Biophos, Get-Phos, MYCO-RISE, Kisaan P.S.B. culture, MycoRhiz, Reap P, and Phosphonive	For all crops
Potassium-mobilizing or potash bacteria like *Bacillus mucilagenosus*	BIO-NPK, Bharpur, BioPotash, Potash-Cure, and Green Earth Reap K	For all crops
Sulfur-solubilizing microbes like *Thiobacillus thioxidans*	Biosulf, Sulf-cure, Sulphonik, S Sol B®, Siron, and MicroS-109	For cereals, millets, pulses, oilseeds, fiber crops, sugar crops, forage crops, plantation crops, vegetables, fruits, spices, flowers, medicinal crops, aromatic crops, orchards, and ornamentals
Zinc-solubilizing microbes	Biozinc, Zinc-Cure, Zinc activator, Zinc extra, and MicroZ-109	For crops like paddy, wheat, pulses, citrus, pomegranate, ginger, etc.
Silica-solubilizing microbes	BioSilica, Silica-Cure, and Silica-109	For crops like cereals, sugar cane, onions, leafy greens, legumes, cucumber, pumpkin, and gourd

Modified from Singh et al. (2014), Biotech International Limited (2018), National fertilizers limited (2018), Biocyclopedia (2018), Indiamart (2018) and International Panaacea Limited (2018)

Table 1.3 The different constraints in biofertilizer technology

Biofertilizer technology constraints	Examples
Technological	(1) Use of less efficient microbial strains and carrier materials (2) Low quality and short shelf-life of microbial inoculants (3) Lack of technically qualified personnel
Infrastructural	(1) Non-availability of suitable production facilities like equipment, space, storage, etc.
Financial and marketing	(1) Non-availability of sufficient funds (2) Less return by sale of products (3) Non-availability of right inoculant (4) Lack of retail outlets or market network for producers
Environmental	(1) Seasonal biofertilizers demand (2) Soil characteristics (3) Simultaneous short-span cropping operations
Human resources	(1) Lack of appropriate training on production practices (2) Unfamiliarity on the quality of the manufactured product (3) Problem in adoption and unawareness of the benefits of technology by farmers (4) Ignorance on the environmental indemnities caused by continuous application of chemical fertilizer

1.6 Conclusion

In modern-day agricultural practices, biofertilizers form an important component of sustainable organic farming in terms of a viable alternative of chemical fertilizers that are associated with various environmental hazards. Biofertilizers can fix and make available atmospheric nitrogen in soil and root nodules, solubilize phosphate (from insoluble forms like tricalcium, iron, and aluminum phosphates) into available forms, sift phosphates from soil layers, produce hormones and antimetabolites to uphold root growth, and decompose organic matter for soil mineralization. This causes increased harvest yields, enhanced soil structure (by influencing the aggregation of the soil particles for better water relation), untainted water sources, and induced drought tolerance in plants (by enhancing leaf water and turgor potential, maintaining stomatal functioning, and increasing root development). However, an increased demand and awareness among farmers and planters about the use of biofertilizers can pave the way for new entrepreneurs to get into biofertilizer manufacturing, which also requires encouragement as well as support from the governments. Biofertilizer technology, which is an inalterable part of sustainable agriculture, has to be appropriate for the social and infrastructural situations of the users, economically feasible and viable, renewable, applicable by all farmers equally, stable in long-term perspective, acceptable by different societal segments, adaptable to existing local conditions and various cultural patterns of society, practically implementable, and productive. Thus, it is apparent that awareness of the significance and economic feasibility of application of biofertilizer technology has to be increased by proper practical training of dealers and farmers.

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