

# Chapter 15

## Development of Biofertilizers and Microbial Consortium an Approach to Sustainable Agriculture Practices



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**Abstract** Globally, there is excessive use of chemical fertilizer beyond the soil and crop threshold limits which had a deleterious effect on the soil ecosystem. So, now agriculturalists are switching from agrochemical practices to agro-biotechnological practices by using soil microbes as a source of fertilizers. In developed countries, soil microbial communities have been considered as the prime factor for sustainable agricultural practices for the last few decades. The activities and the interaction of these soil microorganisms have been proven to promote plant growth, soil quality, and productivity and maintain the biogeochemical cycle, earth geochemical stability, and climatic conditions of the earth system. Biofertilizers are the formulation of the beneficial microbial strains (bacteria, fungus, and algae) packed on the carrier for mobilization. Biofertilizers can fix the atmospheric nitrogen and mineralize the soil's organic matter. Biofertilizers inoculants may be single species-specific or in the combination of different compatible strains. Microbial consortia are the symbiotic interactions of combinations of two or more compatible microbial strains. A microbial consortium improves the productivity of crop and soil in extreme stress conditions much better than the single-strain inoculants. Therefore, microbial fertilizers and consortium are the best solution to achieve sustainable agricultural practices worldwide.

**Keywords** Biofertilizers · Microbial consortia · Symbiotic interaction · Compatible microbial strain

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## 15.1 Introduction

In sustainable agricultural practices, the soil microbial community has earned a great importance over the past decades. Activities of soil microbes were applied in all the spheres of sustainable ecosystem processes and biotechnological developments (Lladó et al. 2017; Tamayo-Vélez and Osorio 2018). International organizations, policymakers, and practitioners have raised the interest to explore the soil microbiota applications, especially in the field of bioremediations, food and agricultural science, and industrial (Chibuzor et al. 2018; Chuks Kenneth et al. 2019; Company et al. 2010; Madigan et al. 2009; Odoh 2017; Sam et al. 2017; Zabbey et al. 2017; Zuroff and Curtis 2012). Organic farming is a distinctive sustainable agricultural practice which improves the overall crop yield and soil microbiota conditions and lowers the soil deteriorations. A sustainable agricultural practice is an agro-biotechnological method where the existing food requirements are fulfilled without affecting the food security of future generations. The increasing human populations have increased the demand for food thereby pressurizing soil resources to increase the yield per unit area. In 2010, food and agriculture organization reported that the need for agro products would increase by 60% till 2030.

Soil microbial communities are considered most important part of the soil ecosystem. The soil microbes have the ability to increase the food production and help in balancing the earth's climate and biogeochemical cycles (Hansel et al. 2008; Tringe et al. 2005).

The increase in global requirement for food prompted the excessive utilization of chemical fertilizers in agricultural field beyond the threshold limits of crops and soil (Liu et al. 2017; Sun et al. 2015). Therefore, scientists discovered the possible way to replace the agrochemical methods of agricultural practices with the agro-biotechnical approaches which involves the use of soil microorganisms as biofertilizers or microbial consortium. The application of soil microbes in agriculture solves the plant growth problems and fulfils the global needs for sustainable agricultural practices (Hung et al. 2015; Odoh 2017). Biofertilizers and microbial consortium are eco-friendly, affordable, and renewable source of nutrients for the plants, so they have achieved the global acceptance in organic farming. The objective of this chapter is to summarize the development and application of biofertilizers and microbial consortium and their role in sustainable agriculture practices.

## 15.2 Biofertilizers

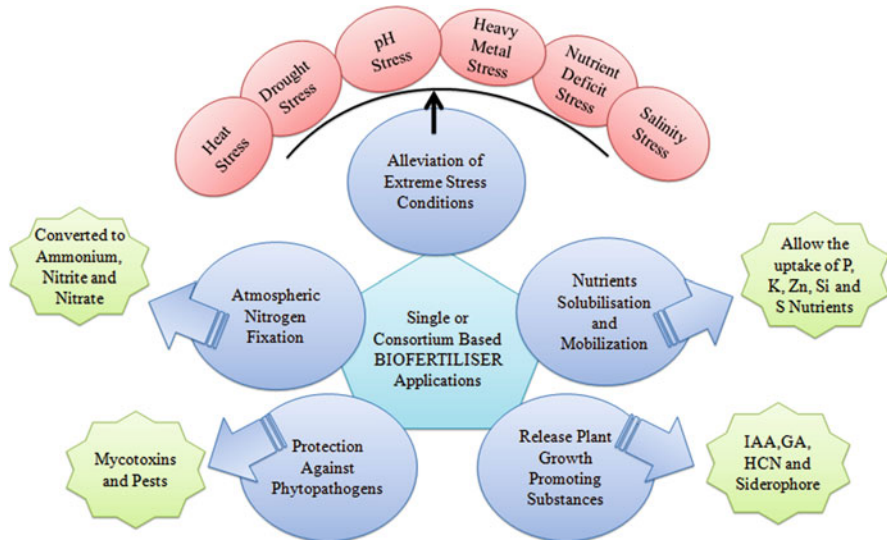
Sustainable agriculture practices can be used to reduce the excessive utilization of chemical fertilizers by replacing them with biofertilizers (Mishra and Dash 2014). Usually, the biofertilizers term is translated in different ways like all the things from plant extracts to green manures and through animal manures (El-Ramady et al. 2018).

The advancement in knowledge of interaction between the plants and soil microbes clarified the concept of biofertilizers. In 2003, Vessey defined biofertilizers as a substance composed of beneficial microorganisms which increases the supply of essential nutrients and minerals to the host plant and thus promotes the host plant development (Vessey 2003). Further, the biofertilizers are determined as the substances containing living microorganisms, which improve the growth of host plant different mechanisms. In addition to the above definition, the substances containing beneficial microorganisms that are utilized against plant pathogens are called as biopesticides or biofertilizers (Fuentes-Ramirez and Caballero-Mellado 2006). Similarly, there are phyto-stimulators and rhizoremediators which improve the plant growth by secreting the plant hormones and biodegrade the organic pollutants respectively, but not every microbial formulation can be considered as biofertilizers directly (Bhattacharyya and Jha 2012; Somers et al. 2004).

The scientific view of biofertilizers is the single microorganism which has the ability to promote the plant growth, but in the agricultural context, biofertilizers are substances composed of different microbial strain(s) which are used for various soil and plant improvement applications. The biofertilizers can also contribute in the improvement of soil microorganism by the addition of useful substances. It was reported that the term “biofertilizer” should not be misinterpreted for biostimulants which are obtained from non-living microbial cell or microbial extract (Malusá and Vassilev 2014; Reddy 2014).

### ***15.2.1 Role of Biofertilizers in Agriculture***

The major role of biofertilizers is stimulating the growth of plants without affecting the environment and increasing the crop yield (Mishra et al. 2013) (Fig. 15.1). Studies had reported that with biofertilizer inoculations in field increase the crop yield approximately by 16% compared to non-inoculated field (Schütz et al. 2018). Microbial biofertilizers improve the structure and fertility of soil by maintaining the soil microbial loads (Rashid et al. 2016). Biofertilizers also improve the plant-water relationship, provide strength to the crops to withstand the abiotic and biotic stress conditions, and protect the crops from various pests and soil-borne diseases like disease caused by mycotoxins (Bhattacharjee and Dey 2014; Simarmata et al. 2016; Xiang et al. 2012). Therefore, biofertilizers are considered commercially as the most effective method in sustainable agricultural practices, but there are some limitations like lack of storage, appropriate materials for production, and transportation facilities, highly sensitive towards temperatures, and most importantly having short shelf life (Patil and Solanki 2016). On the other hand, microbial biofertilizers need to be applied in higher concentration to crops for its effective usage, and their results are observed only after their longer usage. The results of biofertilizers are dependent on the soil conditions of the applied zone (Jangid et al. 2012). Scientists are still working to develop new approaches or technologies to defeat the limitations of biofertilizers in the agricultural systems (García-Fraile et al. 2015).



**Fig. 15.1** Schematic representation of the role of the single or consortium-based biofertilizer applications

## 15.2.2 Types of Microbial Fertilizers

There are different types of microbial fertilizers utilized for sustainable agricultural practices. They are grouped according to the microorganism they carry (Itelima et al. 2018). The types of microbial fertilizers are discussed briefly in the following section.

### 15.2.2.1 Nitrogen Biofertilizers

Nitrogen is considered as the most important nutrient for the crop productions and overall development of plant growth (Thilakarathna et al. 2016). Nitrogen is defined as macronutrient which is the key component of the chlorophyll molecules and also plays a crucial role in most of the enzymatic process in plant cells (Wagner 2011). Nitrogen is most abundantly present in the earth atmosphere, but this atmospheric form of nitrogen is not available for plants and animals due to its triple bond structure which makes its stiff and unbreakable (Figueiredo Mdo et al. 2013).

The most efficient method used by the plants to uptake the atmospheric nitrogen is through the process called biological nitrogen fixation. Microbes involved in biological nitrogen fixation are basically classified as symbiotic and non-symbiotic. In microbial nitrogen fixation process, the atmospheric nitrogen form is converted to the most usable form of nitrogen such as ammonia by the action of nitrogenase enzyme. This ammonia form of atmospheric nitrogen is easily utilized by plants (Galloway et al. 2003; Tairo and Ndakidemi 2013; Vicente and

Dean 2017). The symbiotic nitrogen fixation is basically carried by *Rhizobium* bacteria, which have mutual symbiotic relation with the root nodules of leguminous plants, and the non-symbiotic nitrogen fixation is carried out by the free-living microorganisms like *Cyanobacteria*, *Azotobacter*, and *Azospirillum* species (García-Fraile et al. 2015).

### Symbiotic Nitrogen Fixer

The symbiotic nitrogen-fixing bacteria belong mainly to the Rhizobiaceae family and consist of the following genera: *Allorhizobium*, *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, and *Sinorhizobium* (Patel and Sinha 2011) generally known as *Rhizobia*. The *Rhizobium* develops the mutualistic relationship with the leguminous plants through the formation of the extra structures of root termed as nodules. Inside the root nodules of leguminous plants, the nitrogen fixation process occurs which change the atmospheric nitrogen into the ammonium through the special enzyme called nitrogenase and is further effectively utilized by the plants cells (Shrimant Shridhar 2012). It was reported that the use of rhizobial biofertilizers in the pulse crop field increases the crop yield because of the symbiotic action between host and pulse crop. *Rhizobium* biofertilizers have the ability to fix 15–20 kg N ha<sup>-1</sup> with 20% increase in crop yields of leguminous plants. The efficiency of these nitrogen biofertilizers depends upon the *rhizobium* strains and the host plant involved; thus in the process of formation of nitrogen biofertilizers, the compatibility of these organisms must be a prime consideration. It was reported that rhizobium fertilizers can fix the 30–643 kg N ha<sup>-1</sup> in soybean, 25–100 kg N ha<sup>-1</sup> in green gram, 126–319 kg N ha<sup>-1</sup> in groundnut, 125–143 kg N ha<sup>-1</sup> in black gram, and 77–92 kg N ha<sup>-1</sup> in pigeon pea (Gopalakrishnan et al. 2015). Similarly, the symbiotic relationship between the vegetable crops and *rhizobium* is also achieved. The most commonly reported vegetables are *Pisum sativum*, *Medicago sativa*, *Trifolium* sp., *Phaseolus vulgaris*, *Lotus corniculatus*, *Cicer arietinum*, and *Glycine max* (Verma et al. 2010). *Rhizobium*, *Mesorhizobium*, and *Bradyrhizobium* have been reported to enhance the growth of legume and supply the nitrogen to the legume plants in the soil populated with metals (Bramhachari et al. 2018). The signature members of the Rhizobiaceae family were reported to secrete the molecules like L-aminocyclopropane-1-carboxylatedeaminase, siderophores, and indoleacetic acid (Wdowiak-Wróbel et al. 2017). It has been observed that the strains of rhizobium which has the ability to secrete L-aminocyclopropane-1-carboxylatedeaminase resulted in the better physiology, growth, and quality of mung bean crops in saline soil conditions.

Further, one more microorganism, *Frankia*, can be used for symbiotic nitrogen biofertilizers. *Frankia* are the gram-positive free-living soil bacteria and have the symbiotic relationship with the actinorhizal plants (Mus et al. 2016). *Frankia* produces root nodules with the actinorhizal plants which are anatomically, morphologically, and functionality different from that of the root nodules of leguminous plants (Hoche et al. 2009). Application of *Frankia*-based nitrogen biofertilizers in the arid

soil environments has shown positive impact on actinorhizal trees and also improves the soil fertility of the degraded land (Diagne et al. 2013). In India, South America, China, and Senegal, an agriculturally important tree *Casuarina* was treated with the *Frankia*-based biofertilizers which has shown increase in growth and biomass (Sayed 2011).

### Free-Living Non-photosynthetic Nitrogen Fixer

Among the soil bacterial communities, only *Azospirillum* and *Azotobacter* groups are identified as the potent biofertilizers ability in cereals and legume crops (Gupta et al. 2016). *Azotobacter* microbes are aerobic, free-living bacteria which have the ability to fix approximately 20 kg N ha<sup>-1</sup>/year (Bikash Bag et al. 2017; Mahanty et al. 2017). The most common *Azotobacter* species which are used as biofertilizers to fix atmospheric nitrogen in non-legume crops are *A. beijerinckii*, *A. vinelandii*, *A. chroococcum*, *A. nigricans*, and *A. paspali* (Chandra et al. 2018; Wani et al. 2013). The use of *Azotobacter* sp.-based biofertilizers in maize crops resulted in the improvement in the stem base diameter, plant height, and dry and fresh organic matter content (Iwuagwu et al. 2013). It has been reported that the spraying of *Azotobacter* sp. biofertilizers at oat, clove, and wheat crops increases their dry organic matter by 13–19%, 14–27%, and 10–23%, respectively, compared to control condition (without *Azotobacter* biofertilizer) (Sethi and Adhikary 2012).

In the study conducted by Gothandapani et al. (2017), it was reported that *Azotobacter* species secretes the other useful substances which can improve the growth and development of plants. The beneficial molecules produced by *Azotobacter* species are auxins, cytokines, gibberellins, nicotinic, pantothenic acid, and vitamin B which improve the germination of seeds. Further, it has observed, increase in the seed germination by 20–30%, overall crop yield and provide protection against pathogenic rhizospheric microbes, in the crops inoculated with *Azotobacter* sp. (Mahato and Kafle 2018; Vikhe 2014).

### Free-Living Photosynthetic Nitrogen Fixer

Most commonly used free-living photosynthetic microorganism as nitrogen fixer biofertilizers is *Blue green algae* (BGA) or *Cyanobacteria*. They are generally found in lakes, rivers, ponds, and water streams and have the ability to fix the atmospheric nitrogen into the ammonium and nitrogenous compounds (Singh et al. 2016). Among the BGA, the most commonly used genera for the biofertilizer are *Nostoc*, *Cylindrospermum*, *Anabaena*, *Calothrix*, *Stigonema*, *Aulosira*, and *Tolypothrix* which consists of heterocyst, a modified thick-walled nitrogen-fixing cell (Kumar et al. 2010b). Studies have reported that along with heterocyst containing BGA, some non-heterocyst containing unicellular (*Dermocapsa*, *Aphanothece*) and filamentous (*Trichodesmium*, *Oscillatoria*) genera of *Cyanobacteria* also has the ability to fix the atmospheric nitrogen (Berrendero et al. 2016). According to the study of

Rathod et al. (2018), these cyanobacteria secrete the beneficial substances like antifungal and antibacterial compounds along with some vitamins and amino acids and therefore have the ability to promote the growth of the plants. Likewise, blue green algae have the ability to convert the insoluble phosphate form to soluble phosphate form, thereby increasing the phosphorous availability in the soil for crops (Rai et al. 2019). In India, generally *Aulosira fertilissima* is considered to be the most effective cyanobacterial nitrogen fixer-based biofertilizers for rice crops (Thingujam et al. 2016). *Cyanobacteria* fixes 20–40 kg N ha<sup>-1</sup> of atmospheric nitrogen; thus they are considered best alternative against the previously used chemical fertilizers (Issa et al. 2014; Singh et al. 2016).

### Associative Nitrogen Fixer

*Azospirillum* sp. is aerobic, free-living, and non-nodulated bacterium which has the potential to fix the atmospheric nitrogen. The *Azospirillum* sp. is reported vital for the growth of crops in greenhouse and trial fields (Vurukonda et al. 2016). In agricultural or wild crops, *Azospirillum* sp. usually grows at the surface and inside the roots, and this type of association is known as rhizosphere association (Gangwar et al. 2017). *Azospirillum* sp.-based biofertilizers are proposed for the non-legume crops like paddy, oilseeds, banana, millets, chilly, coconut, oil palm, sugarcane, and cotton (Pathak et al. 2018) and can fix 20–40 kg N ha<sup>-1</sup> atmospheric nitrogen into the soil. It has reported that *Azospirillum* sp.-based nitrogen fixer biofertilizers fix approximately 50% of nitrogen for sugarcane crops (Saranraj and Sivasakthivelan 2013). In barley crop, the salt stress was reduced by using *A. brasilense*-based biofertilizers. According to Atta et al. (2018)s studies, these microorganisms seems to secrete various plant hormones which have the ability to modify the physiological and morphological characteristics of applied crops.

#### 15.2.2.2 Phosphorus Biofertilizers

Phosphorous is the second most essential macronutrient, which is readily absorbed by the plants for the overall growth and development of plants. Phosphorous is involved in various plant metabolic pathways (Sharma et al. 2013). The majorly available phosphorous forms in soil are insoluble phosphate and soluble phosphate, determined on the basis of organic and inorganic compounds. Nearly 90% to 98% phosphorous present in soil is not utilized by the plants, while some form of phosphorus is absorbed such as H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and HPO<sub>4</sub><sup>-</sup> (Sharma 2011; Vijayabharathi et al. 2016). According to Sharon et al. (2016), the generally used way to tackle with the insufficiency of phosphorous in soil is through the utilization of the phosphate mineralized fertilizers in the form of monopotassium phosphate or monocalcium phosphate, but the use of these chemical fertilizers for long term has the negative effects on the soil ecosystem. In the acidic soil condition, the phosphorus is bonded with aluminium and iron, and similarly in alkaline soil conditions, it is chemically

bonded with calcium and magnesium ions, thereby resulting in the unavailability of the phosphorous for plants in soil (Mehrvarz et al. 2008; Ranjan et al. 2013).

So the best approach in sustainable agricultural practices is utilization of the microbial-based biofertilizers which has the ability to convert the insoluble phosphate form to soluble phosphate form and increases the availability of phosphorous in the soil (Barea 2015). Bacterial strains utilized as phosphate biofertilizers are *Agrobacterium* sp., *Pseudomonas* spp., and *Bacillus circulans*, while there are some bacteria which have been reported for phosphorous solubilizing activity such as *Azotobacter*, *Burkholderia*, *Erwinia*, *Bacillus*, *Rhizobium*, *Enterobacter*, *Bradyrhizobium*, *Paenibacillus*, *Serratia*, *Thiobacillus*, *Salmonella*, *Ralstonia*, and *Sinomonas* (Alori et al. 2017; Elias et al. 2016).

Interestingly, even some fungal strains are reported to have phosphorous solubilization and mobilization abilities. The microbial fungal strains detected for phosphorous mobilization activity are *Achrothcium*, *Fusarium*, *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Myrothecium*, *Pichia fermentans*, *Yarrowia*, *Saccharomyces*, *Curvularia*, *Arthrobotrys*, *Rhizopus*, *Cephalosporium*, *Trichoderma*, *Oidiodendron*, *Schwanniomyces*, *Populospora*, *Glomus*, *Phoma*, *Micromonospora*, *Paecilomyces*, *Torula*, and *Mortierella* (Alori et al. 2017; Pal et al. 2015).

### 15.2.2.3 Plant Growth-Promoting Biofertilizers (PGPB)

Microorganisms of this types of biofertilisers improve the overall growth of plants by secreting various active compounds like siderophores, cyanides, plant hormones (gibberellic acid and indoleacetic acid), antibiotics, chitinase, and volatile organic compounds (Majeed et al. 2015). These agroactive compounds are produced in large amount and have the ability to enhance the morphological features of the host plant (Gouda et al. 2018). The plant growth-promoting biofertilizers are based on the rhizobacteria which belong to the following genera like *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Rhizobium*, *Achromobacter*, *Pseudomonas* sp., *Flavobacterium*, *Enterobacter*, *Arthrobacter*, *Bradyrhizobium*, *Amorphosporangium*, *Xanthomonas*, *Erwinia*, *Cellulomonas*, and *Bacillus* (Mohammadi and Sohrabi 2012; Vejan et al. 2016). In the study of Anwar et al. (2016), some *actinomycetes* strains produce the agroactive compounds which promote the plant growth and development.

Some plant growth-promoting rhizobacteria (PGPR) show dual functional properties like biofertilizers and biopesticides. For instance, *Burkholderia cepacia* have been detected with biocontrol activities of *Fusarium* sp. which synthesizes the fungal mycotoxins while they also have the ability to secrete the siderophores which improves the growth of maize crops during the iron deficiency conditions (Bhattacharyya and Jha 2012). There are two groups of PGPR based on the affinity with the roots of plants: extracellular PGPR (ePGPR) which is found in rhizospheric region between the cells of cortex or at the rhizoplane and intracellular PGPR (iPGPR) which are found inside the root nodules (Ahemad and Kibret 2014). These PGPR microorganisms enhance the growth of plants either directly or indirectly. In direct method, the PGPR secrete the phytohormones like GA,



siderophores, and IAA which improve the soil nitrogen and phosphorus content. While in indirect method, the PGPR secrete the secondary metabolites like antibiotics and lytic enzymes which provide protection to the host plants towards the various phytopathogens and also enhance the induced systemic resistance activity (Beneduzi et al. 2012; Bhattacharyya and Jha 2012). The soyabean crops inoculated with the *Azotobacter chroococcum*- and *Pseudomonas fluorescens*-based phosphorous biofertilizer improve the phosphatase activity around the roots (Rotaru 2015). *Pseudomonas* microbial strains are reported to secrete the toxic secondary antimicrobial compounds like pyoluteorin, viscosinamide, pyrrolnitrin, and phenazines which create negative effects on the various organisms (Flury et al. 2017).

#### 15.2.2.4 Potassium Biofertilizers

Potassium is considered the third most essential macronutrient for developmental and growth process of plant cells. Potassium plays a vital role in enzymatic reactions, degeneration of sugar, photosynthesis reaction, and protein formation (Basak and Biswas 2009). The total percentage of potassium available in soil is estimated to be in the range of 0.04–3%. In the soil, potassium is available in various forms such as exchangeable potassium, non-exchangeable potassium, mineral potassium, and solution potassium, but among these, mineral potassium form is most abundantly present with 90–98% in the soil which is not accessible for the host plants (Etesami et al. 2017). It has reported that microorganisms such as fungi, bacteria, and *actinomycetes* secrete the various beneficial compounds like polysaccharides, organic acids, exchange reactions, acidolysis, chelation, and complexolysis (Etesami et al. 2017; Mishra et al. 2018).

The unavailable potassium ions of soil react with the  $\text{Si}^{4+}$  ions and form the metal-organic complex thereby releasing the available potassium form into the soil solution. The biofilm has reported to solubilize potassium from anorthite and biotite (Das and Pradhan 2016). Bacteria responsible for the solubilization of potassium are the following: *Bacillus circulans*, *Burkholderia* sp., *Paenibacillus mucilaginosus*, *Cladosporium* sp., *Paenibacillus glucanolyticus*, *Acidithiobacillus ferrooxidans*, *Bacillus edaphicus* and *Enterobacter hormaechei*, *Arthrobacter* sp., *Sphingomonas* sp., *P. frequentans*, and *Aminobacter* sp. (Meena et al. 2016). The commercially available brands for potassium mobilizing biofertilizers are Biosol-K, K Sol B<sup>®</sup>, and Symbion-K which are made up of *Frateruria aurantia* and considered to be effective biofertilizers for growth and development of plants (Mishra and Arora 2016). The microbes involved in potassium solubilization method are observed to have positive effects on the development and growth of plants such as cucumber, cotton, tomato, tobacco, rape, sorghum, chili, pepper, sudan grass, and khella (Meena et al. 2016). According to Bashir et al. (2017) studies, inoculation of soil with the potassium solubilizing biofertilizers enhances the potassium uptake by plants and indigenous activities of soil microbes, improves crop and soil qualities, and reduces the utilization of mineralized potassium fertilizers.

### 15.2.3 Biofertilizer Production

In order to prepare the best and effective quality of biofertilizers, the following each steps as shown in the Fig. 15.2 need to be carried out carefully in the defined environment (Mohod et al. 2015). Total eight steps are involved in the production procedure as follows: searching and isolating the effective microbial strains, discovering the characteristic of the selective microbes at the proper growth conditions and medium, scaling up the production of selective microbial biomass, choosing the appropriate carrier to load microbial culture, formulating the bioinoculant, testing at the field, industrial level of production experiments, and developing the quality control, transportation, and storage systems (Shaikh and Sayyed 2015; Stamenković et al. 2018).

Selected microbes for biofertilizers production must have certain defined characteristics features for their effectiveness and usefulness at agricultural fields. The characteristic features are as follows: should be easy to replicate in bulk, should be compatible with the natural rhizosphere microorganism, must have high rhizosphere competences, should have the ability to enhance the overall development and growth of crops through various mechanisms or by releasing agroactive compounds, and should not cause any negative effects to the ecosystem (Nakkeeran et al. 2006).

The selective media which are used for the mass production of selective microorganism should be easily accessible in the market, inexpensive, and provide all the essential nutrients in the defined amount (Glick 2020). Biofertilizer production involves the fermentation techniques like solid state, liquid, and semisolid for the large-scale productions. It has been reported that chemical-defined media are needed for the maximum growth of selective microbes as they can alter the ratio of the substance affecting the multiplication of microorganisms (Stamenković et al. 2018).

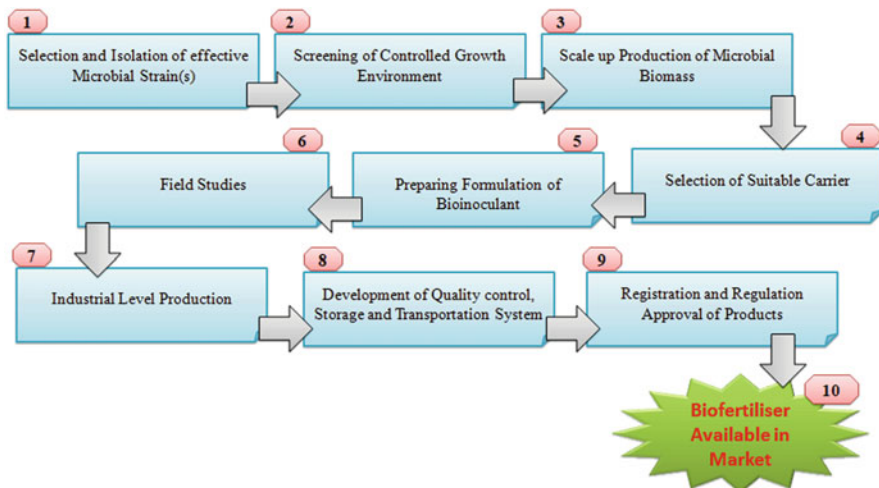


Fig. 15.2 Flow chart of the biofertilizer production procedure

The selection of the suitable carrier is done on the basis of the desired form or quality of end product and microbial strains utilized for biofertilizer production. The next steps is encapsulation of the growth of selective microbial strains or preparation of liquid formulations. Then at last the prepared formulation of biofertilizer is tested at field level and should pass the defined requirements like having positive effect on the growth and yield of crop and having no toxic effect at the ecosystem. After qualifying the minimum defined requirements of biofertilizer, they are applied for registration to grant the approval (Backer et al. 2018; Bashan et al. 2014). The approved biofertilizer formulation is then packed, and the packets should have information mentioning product name, microbial strain (s) composed off, preferred plant name, manufacture and expiry date, producer name and address, and proper instruction and precaution to be followed by the consumers (Bhattacharjee and Dey 2014; García-Fraile et al. 2015).

#### 15.2.4 Quality of Biofertilizer

The parameter of determining biofertilizers quality before commercialization is the most important, so there is need to be performed at each production level carefully (Sethi and Adhikary 2012). In the nations like the USA and European Union, the quality and the production parameters are not clearly defined, but in the nations where sustainable agricultural practices are performed, the rules and regulation of quality control is well defined. The Chinese quality control is defined on the basis of the eight parameters, and among them, the density of the microbial strains used is considered the most important. The eight parameters followed in China are as follows: water and carbon content, size of carrier, amount of microbial load, expiry period, appearance, and contamination. These above parameters are defined for the different microbe groups such as *rhizobium*, phosphorus solubilizing bacteria, silicate solubilizing bacteria, nitrogen fixing bacteria, multistrain consortia, and organic and inorganic phosphorus. For bacterial based liquid formulating biofertilizers, the microbial content must be in the range between  $>0.5 \times 10^9$  cfu mL<sup>-1</sup> and  $>1.51 \times 10^9$  cfu mL<sup>-1</sup>, and in solid product, the microbial content ranges between  $>0.1 \times 10^9$  cfu g<sup>-1</sup> and  $>0.3 \times 10^9$  cfu g<sup>-1</sup>. According to the parameters approved, the total organic load the biofertilizer should contain is 18–20% irrespective of their phenotypic form and the shelf life at least half a year.

Similarly in India, seven regulatory parameters are defined for the biofertilizers, and the seven parameters are as follows: the phenotypic form, contamination level, size of the carrier, the minimum organic load, pH, water content, and the efficiency parameters. These parameters are defined for the following groups of microorganisms in India like *Rhizobium* sp., *Azospirillum* sp., *Azotobacter* sp., mycorrhizal biofertilizers, and phosphate solubilizing bacteria. For bacteria-based biofertilizer, the minimum amount of organic load in solid carrier system is  $5 \times 10^9$  cfu g<sup>-1</sup> and  $1 \times 10^8$  cfu mL<sup>-1</sup> for liquid carrier system. In case of mycorrhizal fungal based

biofertilisers, 1 g of prepared biofertiliser must compose of at least 100 viable propagules (Sekar et al. 2016).

### 15.2.5 Application of Biofertilizers

Biofertilizers are applied either directly to the soil or indirectly to seeds, seedling, leaves, etc. (Chen 2006). Each type of approaches has some merits and demerits based on the parameters like type of crop, inoculants used, environmental conditions, and some technical problems from farmers' side (Mahmood et al. 2016). Biofertilizers need to be applied carefully with certain precautions like used biofertilizer solution should not be kept overnight, should be kept in the range 0–35 °C, and should avoid direct contact to sunlight.

Among the approaches used for applying biofertilizers, the seed treatment approach is generally used as it requires small quantity of inoculation product and is very simple to use (Asif et al. 2018). The three ways by which biofertilizers can be applied on the seeds are slurry, seed coating, and dusting (Malusà and Ciesielska 2014). In dusting, the biofertilizers are mixed with the dry seeds, but this technique is not much effective as the interaction between biofertilizer microorganisms and the seed is weak. In slurry approach, biofertilizers are combined with the wet seeds or the seed can be kept in the slurry overnight (Malusà and Ciesielska 2014). It has been reported that the seeds have to be coated with defined number of microbes so the fixative agents like gums, vegetable oils, carboxy methyl cellulose, solution of sucrose, and some harmless marketable products are utilized (Bashan et al. 2014). Alternatively, 1% milk powder or 25% of molasses solution is mixed with the suspension in case biofertilizers do not have any fixative agent. In the third seed coating approach, seeds are added into the slurry suspension of microorganisms and then further coated its outer covering with some inorganic inert substances like charcoal, lime, talc, dolomite, clay, calcium carbonate, and rock phosphate. This outer coating with inert materials protects the seeds from harmful effects of chemical fertilizers and pesticides and from the unfavourable environmental conditions (Malusà and Ciesielska 2014). The bacterial groups involved in seed treatment processes are *Rhizobium*, *Azospirillum*, phosphorous solubilizing microbes, and *Azotobacter* and can also be with the consortium of microbes. According to BrahmaPrakash et al. (2017) studies, the seed is first covered with nitrogen fixer microbes, and then further phosphorous solubilizing microbes are coated as outer layer to maintain the viable microbial load. In case large numbers of microbial strains are introduced in the soil field directly, then a soil inoculation technique is required. In this technique of soil inoculation, carrier of granules size 0.5–1.5 mm is favoured, and granular form of soil aggregates, peat, talcum powder, and perlite are mostly utilized in this approach.

Soil treatment process provides protection to the microbial fertilizer strains from the harmful effect of fungicides and pesticides and prevents the destruction of seed coats and the loss of biofertilizers during the seeding machinery activities. The soil

inoculation approach improves the chances of interaction between seeds and biofertilizers as compared to seed treatment approach. While there are some technical demerit of this approach like requirement of specialized equipment and high amount of biofertilizers which causes additional transportation and storage problems. In the developed nations, soil inoculation approach with granules is generally employed (Bashan et al. 2014; Deaker et al. 2004).

### **15.3 Introduction of Microbial Consortia**

In nature, microorganisms live in the form of two or more groups called microbial consortium (MC). Therefore, microbial consortium is utilized in the sustainable agricultural practice that has the abilities to perform the activities not possible for individual microorganism. Microbial consortium is formed by the stable symbiotic interaction between the two or more microbial groups for the overall development of crops (Madigan et al. 2009). The microbial consortium has the ability to increase the organic content of soil, make nutrients available for the plants through solubilization and mobilization, and fix the atmospheric nitrogen in the nodules of the leguminous crops (Nutti and Giovannetti 2015). The use and acceptability of microbial consortium in agricultural practices has increased as compared to single strain. Although microbial strains retain their individual characters in the microbial consortium, they still have the ability to respond as a completely different organism in abiotic and biotic stress environment due to their intrinsic beneficial interactions (Nutti and Giovannetti 2015). Microbial consortium has the quorum sensing signalling which helped them to respond and detect the nutrient gradient and microbial density. Quorum sensing mechanism expresses their fascinating biochemical effects that allow their functionality, robustness, stability, and ability to carry out difficult biochemical works.

#### ***15.3.1 Microbial Consortium as Biofertilizers***

In soil ecosystem, the microbial interactions are complex and dynamic. The biofertilization phenomenon is used to improve the growth and provide the nutrients to the crop (Odoh 2017). Microbial consortium is used as biofertilizers and works in similar way with some advantages over single strain biofertilizers. According to Bradáčová et al. (2019), the comparative analysis of single strain and microbial consortium revealed that the application of microbial consortium has the ability to enhance the crop growth and productivity during the extreme environmental situations. Microbial fertilizers are considered important for the sustainable agricultural practices and maintain the soil fertility for a longer period of time. Microbial fertilizers have the ability to fix the atmospheric nitrogen and solubilize soil phosphorous into the form which can be taken up by the plant roots. Microbial

consortium apart from solubilizing the nutrients also has the ability to secrete some bioactive substances like Nod factor and Myc in the signalling pathway (Roberts et al. 2013).

### ***15.3.2 Interaction Between Microbes and Plants***

Soil is the topmost layer of the earth crust and is made up of mixtures of minerals, organic matter, gases, and microorganisms that interacts with each other and supports the living system on the earth. Soil system has physical, chemical, and biological properties. The most important and nutritional rich component of soil system is “soil organic matter” (SOM) for plant growth and development. Soil organic matter contains the larger portion of remnants of animals and plant that help in maintaining the soil flora and fauna. It has reported that humic acid substances contribute approximately 60% of SOM, while soil microbes contribute only 8% of SOM, and the remaining is the non-living component of soil system (Htwe et al. 2019; Liste 2003). SOM component of soil is nutrient rich and considered essential portion for sorption of contaminants and cation exchange mechanism, thus promoting the growth of plants and soil microbes (Chibuzor et al. 2018). It has the ability to control soil erosion, and circulation of water and soil also helps in soil aggregation (Guo et al. 2019).

An omics molecular study has disclosed the extent of soil-plant-microbes interactions in the soil system. The advance approach of omics techniques provides the platform to identify, detect, and quantify the diversity of soil microorganism linked with the particular plant. It has reported that the plants are associated with soil microorganism through the various mechanisms that are obligatory for their existences (Schirawski and Perlin 2018). The microorganisms colonizing the rhizosphere of the plants are generally rhizobacteria and mycorrhizal fungi (Hamilton et al. 2016; Nadeem et al. 2014; Yadav et al. 2015a, b). Plant roots not only act as the host for the various soil microorganisms but also secrete the beneficial compounds which provide nutrition to the microbes even after the plant die. These beneficial compounds have the ability to provide the resistance to the plant against the abiotic and biotic stress conditions.

It has documented that high microbial diversity and less nutrient content in rhizoplane part of the soil system generally cause the competition for survival, ability to improve the growth of crops, and development of the adaption mechanism to the stress conditions (Ngumbi and Kloepper 2016).

The beneficial soil microbes interact with the roots of the plants and improve the plant health and growth by the utilizing the biofertilizers, biostimulant, and biocontrol agents (Glick 2014; Nath Yadav et al. 2016; Rashid et al. 2016). Fungal interaction to plant roots aids in the phosphorus solubilizing and mobilizing, protects the plants from many plant pathogens, and provides access to water availability in the drought conditions (Barnawal et al. 2014).

### 15.3.3 Interaction Among the Bacterial Groups

The interaction between bacteria among the microbial consortium includes the PGPR group like *Pseudomonas*, *Arthrobacter*, *Alcaligenes*, *Burkholderia*, *Klebsiella*, *Bacillus*, *Azospirillum*, *Serratia*, and *Enterobacter*. These PGPR improve the overall growth and development of the crop through various mechanisms (Jambon et al. 2018; Saharan and Nehra 2011). It has reported that the interaction of rhizobacteria with plants improves the ability to segregate the soil pollutants (Chibuzor et al. 2018). The high biochemical and microbial activities have been detected in the rhizospheric environment due to the high availability of nutrients compared to the phylospheric and rhizoplanic components of the soil system (Venturi and Keel 2016).

PGPR also have the ability to improve the nutrient absorption and seed germination, protect the plants from phytopathogens, develop the resistance toward the environmental stress conditions, and increase the shoot and root generations (Odoh 2015). According to Bulgarelli et al. (2012), plant growth-promoting rhizobacteria recruitment process is regulated by the structure of soil microbial communities. It has reported that variation at the genetic level in the plant species is the driving force for the differential recruitment of PGPR communities (Lundberg et al. 2012). These bacterial consortiums are enrolled in the interesting roles like phosphate solubilization, plant development, nitrogen fixation, and the secretion of various plant hormones (Htwe et al. 2019; Odoh 2017).

The bacterial communities of consortium communicate with each other through the chemical signalling process known as quorum sensing (Barriuso 2015). During the quorum sensing, the microbial community's communication and gene expression is regulated by the autoinducers or quorum sensing molecules (QSM). QS signalling is defined as regulatory response to transcribe the particular gene in order to identify the compounds (Venturi and Keel 2016). This QS signalling between the cells are always defined and organized pathogenic activities by adjusting the microbes when the stress conditions are triggered (Jiang et al. 2019). The QSM consists of autoinducing peptide, autoinducer-2, and acyl-homoserine lactone which control the certain biochemical processes like sporulation, biofilm formation, antibiotics productions, releasing out the various virulence factors, and motility (Barriuso 2015; Fleitas Martínez et al. 2018). In this effective cell-to-cell interaction, the high energy-based cost-effective specific tasks are performed only in the presence of the large bacterial population size (Clinton and Rumbaugh 2016).

Additionally, the secretion of nodulation factor (Nod) and volatile organic compounds (VOCs) by *rhizobia* are identified with the ability to assist in the bacterial communications (Hung et al. 2015; Jambon et al. 2018). The VOCs secreted support the long distance communication between the microorganism and plant or between microorganisms and maintain the symbiotic relationship, diffusing the mycorrhizal, harmful microbes and saprophytes (Brilli et al. 2019; Hung et al. 2015; Tyc et al. 2017). VOCs of bacteria improve the plant growth by using acetoin chemical which has the ability to interfere with gene expression of plant and activates the systemic

resistances (Bennett et al. 2012). It has been reported that plant roots react to strigolactones and flavonoids as the signalling molecules or host plant symbiosis (Venturi and Keel 2016).

### ***15.3.4 Interaction Between Bacteria and Fungi***

The interaction between bacteria and fungi is internally modified through the behavioural characters of the communicating partners (Deveau et al. 2018). There is a close association of biophysical and metabolic activities during the co-occurrences of fungi and bacteria that help in the growth of bacteria and fungi mutuality.

The understanding of microbiomes like *Arabidopsis* root microbiome has been resolved through the characterization of bacteria and fungi interaction (BFI) (Bergelson et al. 2019). This is due to the involvement of molecular techniques which provide account of biomes and environment habitats emphasizing on the microbial diversity (Thompson et al. 2017).

The interaction between the PGPR and arbuscular mycorrhizal fungi (AMF) has been reported to enhance the crop development and growth (Pathak et al. 2017). This interaction also improves the nutrients concentration in the soil and propagates the soil microbiota. It has been reported that PGPR and AMF associations are considered as potent biofertilizers and biocontrol agents for sustainable agricultural practices as they reduced the dependency on the chemical fertilizers (Franco et al. 2011; Pathak et al. 2017).

PGPR are categorized based on the intra- and extracellular PGPB, and in the host plant, they promote plant growth either directly by secreting growth-promoting hormones or indirectly by secreting antimicrobial molecules (Kumar Deshwal and Kumar 2013; Zheng et al. 2018). During the mycorrhization, PGPR and mycorrhizal helping bacteria to interact symbiotically with mycorrhizal roots and fungi in order to uptake the nutrients. Scientists have discovered the PGPR and AMF developed plant resistance by inducing the systemic host immune response (Bramhachari et al. 2018; Zamioudis and Pieterse 2012). The application of PGPR and AMF has proven beneficial for the crops grown in the nutrient-limited soil (Gouda et al. 2018). The usage of PGPR like *Pseudomonas* sp., *Bacillus* sp., and AMF either singly or in combination has reported to produce significant improvement in the growth of crops in various fields (Pathak et al. 2017; Philippot et al. 2013).

### ***15.3.5 Merits and Demerits of Microbial Consortium***

In various field of applications, scientists aimed to employ the single pure microbial culture. In spite of the advancement in the microbiology, most of the microbial strains are still not culturable as pure cultures. A co-culture technique has the ability



to share the products of the metabolisms and provide strength in stress environmental conditions. Thus, co-culture approach can be utilized to search for the various potentials of unculturable microorganisms. The merits and demerits of utilization of microbial consortium are as follows:

#### **15.3.5.1 Merits**

In microbial consortium, different complex carbon sources can be used as mixed microbial culture of microbial consortium producing different enzymes that can degrade the substrates in the different manner (Bhatia et al. 2015). According to Shou et al. (2007), microbial consortium mixed culture cross-feeds the nutrients and regulates the nearby environment to promote each other's development and growth.

Higher productivity is reported in case of mutual interaction as complex multiple step reactions are executed faster than the single strains inoculants. Co-culture technique can utilize the unculturable microorganisms (Stewart 2012). Microbial consortium mixed culture inhibits the growth of unfavourable and toxic microorganisms thereby controlling the contamination.

#### **15.3.5.2 Demerits**

The development of the microbial consortium is difficult as the interaction and properties of individual strains of consortia can affect the fermentation process at the industrial level production. During the contamination of microbial consortium, it is difficult to detect the contaminating agent. Unavailability of the prior knowledge of microbial functions, microbial metabolite descriptions, and nutrient demands may restrict the consortium manufacturing process.

Conservation of the microbial consortium through freeze drying is also difficult as the microbial strains have different survival rate at freezing cycles.

### ***15.3.6 Construction of Artificial Microbial Consortium at Industrial Level and Their Interaction***

For constructing of non-natural microbial consortium at the industrial level, there are certain parameters that need to be considered such as (a) appropriate inoculums ratio should be taken to avoid the exhaustion of the energy sources, (b) selected micro-organism should not have common carbon source for energy, (c) the optimum temperature and pH for microbial growth should be in the physiological range, (d) selected microbe strains should be from the same species as they have the same metabolic behaviour which makes them more compatible, (e) must have prior understanding of the nutrients requirement to design the culture medium

suitable for the growth of different strains, and (f) different *in silico* approaches like flux base analysis (FBA) and constraint-based reconstruction and analysis (COBRA) can be utilized for the better understanding of various complex interactions of microbial groups (Schellenberger et al. 2011).

The efficiency of microbial consortium is based on the mutual interactions of individual strains. Microbial strains have reported different types of phenotypic interactions with one another such as (a) growth of the microbial strains is inhibited at the particular distance from the competitor strains due to the secretion of antibiotics in extracellular environment, known as distance inhibition interaction; (b) there is the formation of the dark precipitation zone when the microbial strain grows larger in size to interact with other strains, known as zone line interactions; (c) in this type of interaction, microbial strains grow enough to contact with other strains with no proof of secondary metabolite release and is known as contact inhibition interactions; and (d) when one microbial colony is taken up by the other colony, it is called overgrowth interaction (Bertrand et al. 2013).

### 15.3.7 Microbial Consortium in Stress Environment

The climatic factors create obstacle in the agricultural practices as the estimated increase in temperature, drought, and salinity causes abiotic stress condition in the crops, thereby affecting the productivity of crops (Grover et al. 2011; Larson 2013). Plant-associated microbial groups have gained attention as they have the ability to enhance the crop productivity and provide resistance in the stress conditions (Mapelli et al. 2013). Therefore, microbial fertilizers especially consortium application is the best way to mitigate the abiotic stress conditions of plants and improve the growth of the crops in the unfavourable conditions (Jain et al. 2013). Table 15.1 lists the microbial consortiums associated with crops at different extreme environmental conditions. Under 2,4-DNT stress conditions, the microbial consortium degrading 2,4-DNT constitutes of *Pseudomonas*, *Burkholderia*, *Ralstonia*, *Variovorax*, and *Bacillus* spp. has reported to increase the root length of *Arabidopsis* (Thijs et al. 2014).

Application of *R. tropici* and *A. brasilense* co-culture on the bean has no adverse effect of nod gene transcription and salinity situations (Dardanelli et al. 2008). In Jain et al. (2013) studies, the microbial consortium is composed of *Trichoderma* (THU0816), *Rhizobium* (RL091), and *Pseudomonas fluorescens* (PHU094) that has activated the expression of antioxidant enzymes such as peroxidase and superoxide dismutase in stress. In salinity condition, the paddy crops were inoculated with the PGPR-based microbial consortium of *B. pumilus* and *P. pseudoalcaligenes* in has increased the availability of essential nutrients like nitrogen, potassium, phosphorous and reduced the sodium and calcium availability in soil (Jha and Subramanian 2013). Under salt stress, the growth of *P. vulgaris* bacteria is improved with use of *A. brasilense* and *Rhizobium* consortium (Dardanelli et al. 2008; Smith et al. 2015). Microbial consortium of AMF and *B. thuringiensis* increases the production of

**Table 15.1** Microbial consortiums associated with crops at different extreme environmental conditions

Microbial strain(s)	Crop	Extreme environmental conditions	Result	Strategies adapted	References
<i>R. etli</i>	<i>Phaseolus vulgaris</i>	Drought	Crop becomes resistant to drought stress and the nitrogen amount, dry weight of nodules and also functionality of nodule reduced	Upregulation of oxidase in Bacteroides	Talbi et al. (2012)
AMF	<i>Hemarthria altissima</i> , <i>Leymus chinensis</i>		Increases the photosynthetic rate and rate of passage of CO <sub>2</sub> entering	Improves the activities of antioxidant enzymes	Li et al. (2019)
<i>Azospirillum brasilense</i>	<i>Glycine max</i>		Enhanced the plant traits leading the crop resistant to water scarcity stress	Fixation of atmospheric nitrogen and secretion of IAA	Hungria et al. (2015)
	<i>Zea mays</i>	Salinity	Reduces the proline concentration and promotes the plant growth	Alters the selectivity of Na <sup>+</sup> , Ca <sup>++</sup> , and K <sup>+</sup> ions	Fukami et al. (2017, 2018)
<i>Achromobacter piechaudii</i>	<i>Populus</i> sp., <i>Lycopersicon esculentum</i>	Heavy metal and salt	Promotes the root hair formation and also enhances the root and shoot growth	Synthesises of IAA	Carmen and Roberto (2011), Fahad et al. (2015)
<i>Sinorhizobium arbores</i>	<i>Acacia Senegal</i> , <i>Cajanus cajan</i>	Heat	Retain the stability of metabolic actions	Enzyme production like chitinase, esterase, and glucanase	Kumar et al. (2010a), Räsänen et al. (2001)
<i>Brevibacillus brevis</i>	<i>Cotton crop</i>		Development and growth of plants	Production of ARA, IAA and ammonia	Nehra et al. (2016)
<i>B. subtilis</i> , <i>B. megaterium</i> , <i>B. thuringiensis</i>	<i>Triticum aestivum</i> L, <i>Cicer arietinum</i>	pH, drought, temperature	Increases in the relative water content (RWC), accumulation of biomolecules like sugar, proteins	Phytohormone biosynthesis	Khan et al. (2019)
PGPR and AMF	<i>Avena sativa</i>	Hydrocarbon pollution and saline-alkali soil	Improvement in the soil quality and removal of petroleum hydrocarbon	Boost the activities of urease, dehydrogenase, sucrose	Xun et al. (2015)

proline and lowered the risk of oxidative damage to triglyceride in *Zea mays* in drought conditions. In this microbial consortium, *B. thuringiensis* provide the nutrients to the plant, and AMF improves the stress tolerance (Armada et al. 2015). Inoculation of microbial consortium combination of *Anabaena* sp. with *Providencia* sp. and *Anabaena* with *Azotobacter* in maize hybrids has reported to evoked defence response of plant and increases the zinc mobilization (Prasanna et al. 2015).

## 15.4 Impact on Soil Microorganism

The physiochemical, functional, and structural properties of the soil and soil microorganisms are affected by the uses of biofertilizers (Javoreková et al. 2015). The application of PGPR-based biofertilizer has different effects like some may enhance the growth and others may inhibit the growth, while few of them has neutral or no effect on the microbial growth (Castro-Sowinski et al. 2007).

According to Javoreková et al. (2015) and Rastogi and Sani (2011), the microbial shifts can be evaluated by using the techniques such as terminal restriction fragment length polymorphism (t-RFLP), denaturing gradient gel electrophoresis (DGGE), the community level physiological profiling (CLPP), amplified ribosomal DNA restriction analysis (ARDRA), and single-strand conformation polymorphism (SSCP), with the usage of BIOLOG<sup>®</sup> plates.

Trabelsi et al. (2011, 2012) have used t-RFLP techniques to demonstrate the application of *rhizobium gallicum8a3*, and *Sinorhizobium meliloti 4H41* has influenced the diversity of *Actinobacteria*,  $\gamma$ - and  $\alpha$ -*proteobacteria*, and *Firmicutes*.

Application of the co-culture of *Azospirillum brasilense (40 and 42 M)* strains has altered the community level physiological profiling (CLPP) of microbes related to rice crop (de Salamone et al. 2010). Similarly, the application of *Rhizobium leguminosarum bv. viciae* has also affected the CLPP-associated microorganisms with fababean crop (Siczek and Lipiec 2016). Through SSCP techniques, it has been determined that the application of *Sinorhizobium meliloti L33* strain has increased the number of  $\alpha$ -*proteobacteria* and reduced the number of  $\gamma$ -*proteobacteria* in the rhizospheric region of *Medicago sativa* plant (Wang et al. 2018). The *Stenotrophomonas acidaminiphila BJI* is the probiotic strain which has been reported to improve the bacterial growth in the *Vicia faba* rhizosphere polluted with chlorothalonil (Zhang et al. 2017).

## 15.5 Regulatory Issues of Biofertilizers

Commercialization of the first biofertilizer product was done by the Nobbe and Hiltner in the year 1895 with the Rhizobia-based products under the trade name “Nitragin.” In India, the first rhizobium-based biofertilizer was commercialized by

N.V. Joshi for the growth of legume crops (García-Fraile et al. 2015). Through the 95 years of plan, the Agricultural Ministry started promotion and vulgarization of biofertilizer production, designing of standard protocols for different types of biofertilizer, providing hands on training, and applications (Ghosh 2004). The Central and State Government initiated different propagandas to encourage agricultural practitioners to shift from chemical to biological fertilizers and increase the biofertilizer production by giving subsidies and grants at various levels.

The most dominant players in the microbial fertilizers market are Novozymes A/S (Denmark), Camson Bio Technologies Limited (India), Gujarat State Fertilizers and Chemicals Limited (India), Lallemand Inc. (Canada), and Rhizobacter Argentina S.A. (Argentina). In Asian countries, the government subsidies and policies are strongly promoting the biofertilizer market and targeted for green and sustainable agricultural practices. For the development and production of microbial fertilizers and biopesticides, nearly US \$1.5 billion has been consumed (García-Fraile et al. 2015). From the last decades then, farmers have shifted from chemical to organic agricultural. In India recently, the demand of production and utilization of biofertilizers has increased, and about 100 Indian private and public firms are established for the production of biofertilizers (García-Fraile et al. 2015; Pindi and Satyanarayana 2012). The average consumption rate of biofertilizers in country is high in comparison to its production rate. The highest production proportion is by Agro Industries Corporations followed by national Biofertilizers Development Centres, State Agricultural Departments, Private Sectors, and State Agricultural Universities (Mazid and Khan 2014).

## 15.6 Regulatory Issues of Microbial Consortium

The developed nations follow the strict protocols and regulations for the application of the microbial consortium. Before commercializing the product, the foremost step is the successful registration in which the product should meet the specific requirements as mentioned in the guidelines. Prior to the registration, the microbial consortium formulation must have a suitable carrier like biochar or alginate which allows the microbial cells to attach to the seeds during the sowing (Bashan 2016). While in the liquid formulation, microbial consortiums are sprinkled in the seed furrows or can spray on the seeds before sowing.

The microbial viability, biological activity, and survival of the microbial strains can be ensured by the product lifespan and storage. There should be clear knowledge on chronic and the acute applications of the microbial consortium. For example, in acute applications, the microbial consortium is used for a limited time; it can focus on the particular crop development stage and during the abiotic stress conditions. Unlikely in the chronic applications, the microbial consortium is used at the regular interval of spraying or slow release through seed treatment method (Backer et al. 2018). The regulation of microbial consortium is not clear in the American and European countries. Therefore, it is necessary to unified standard protocol,

regulatory, and characterization of the microbial consortium across the world and most importantly in Asian and African countries as they have high potential for agricultural practices and large population of uneducated people which are employed for agroactivities.

## 15.7 Global Biofertilizer Market

The first biofertilizer registered for crop inoculation more than 100 years is *Rhizobium* sp.-based biofertilizer known as Nitragin which is currently available in the market (O'Callaghan 2016). It has been reported that in the available fertilizers in the market, only 5% microbial-based fertilizers are registered for agricultural practices (Verma et al. 2019). Table 15.1 presents the list of some commercial available biofertilizers around the globe. Owen et al. (2015) reported that rhizobial biofertilizers are the most demanded biofertilizer of approximately 80% in the market, while the phosphate-solubilizing biofertilizers and mycorrhizal fungal-based biofertilizers constitute approximately 15% and 7%, respectively, of the total market demand.

It has been expected in the forecast period of 2018–2023 that the global biofertilizers market demand will be reached approximately 2304 million till 2023 through the cumulative annual growth rate of 10%. The biofertilizer commercialization based on the geographical region has divided into Africa, Europe, North America, Middle East, Asia-Pacific, and Latin America. The Asia Pacific biofertilizers are the rapidly growing biofertilizers in the market as countries like India and China have the large area population along with the increasing economics (Table 15.2).

## 15.8 Global Efforts on Sustainable Agropractices

Across the globe, the increasing environmental issues have led to the loss in agricultural productivity; therefore it demands to develop the global action to overcome these losses. The International Code of Conduct on Pesticides Management was released in 2014 by the combined approval of the World Health Organization and Food and Agricultural Organizations to collect and document the number of deaths from the agrochemical users globally. The death rate due to the usage of chemical fertilizers by the agropracticers is continuously increasing in India despite of following the instructions and protocol strictly and also because of the ill-informed practitioners. The chemical fertilizers available in the market are generally classified as Class 1 chemicals. The government targeted to increase the economic status of the nations by focusing to improve the agricultural practices, but the situation has worsened due to the irregular campaigning in the search to regenerate the agricultural sector. According to the available data, Nigeria produced

**Table 15.2** List of the few microbial biofertilizers available in the market across the globe

Product	Organismal consortium	Company	Country
Amnite A100 <sup>®</sup>	<i>Azotobacter, Bacillus, Rhizobium, Chaetomium, Pseudomonas</i>	Cleveland biotech	United Kingdom
Bioativo <sup>®</sup>	<i>PGPR consortia, organic matter</i>	Embrafos Ltd.	Brazil
Bactofil A10 <sup>®</sup>	<i>A. brasilense, B. megaterium, P. fluorescens, A. vinelandii</i>	Agro bio Hungary kft	Hungary
Biozink <sup>®</sup> , biomix <sup>®</sup> , biodine <sup>®</sup>	<i>Azotobacter, phosphobacteria, P. fluorescens</i>	Biomax	India
Life <sup>®</sup>	<i>PGPR consortia</i>		
Calosphere		Camson Bio Technologies Ltd.	
Symbiom-N	<i>Rhizobium, Acetobacter, Azospirillum, Azotobacter</i>	T. Stanes and Company Ltd.	
Bio super	<i>Cellulomonas, Bacillus, Pseudomonas, Rhodococcus</i>	SKS Bioproducts Pvt Ltd.	
Ceres <sup>®</sup>	<i>P. fluorescens</i>	Biovitis	France
Galtrol <sup>®</sup>	<i>Agrobacterium radiobacter strain 84</i>	AgBioChem	USA
BioYield	<i>B. amyloliquefaciens, B. subtilis</i>	Gustafson, Inc., Dallas	
TagTeam <sup>®</sup>	<i>Penicillium bilaiae, Rhizobium</i>	Novozymes	
Hyper coating seeds <sup>®</sup>	<i>Legume seed and rhizobium</i>	Tokachi Federation of Agriculture Cooperatives (TFAC)	Japan
Inomix <sup>®</sup> biostimulant	<i>B. subtilis, B. polymyxa</i>	LAB (Labiotech)	Spain
VitaSoil <sup>®</sup>	<i>PGPR consortia</i>	Symborg	
Nodulator <sup>®</sup>	<i>Bradyrhizobium japonicum</i>	Lallen and plant care BASF Inc.	Canada

about 25% of pesticides with 99% of death due to the use of pesticides in the developing nations (Ojo 2016). This is due to the insufficient education, regarding the use of toxic and cheaper chemicals, careless handling, and unsafe protocol. Similarly, the Taihu Lake in China has been contaminated due to the runoff of fertilizers, pesticides, and herbicides from the agricultural fields. In spite of restriction on DDT and HCH by the Chinese government, still the traces of these toxic chemicals are traced in the sediments (Feng et al. 2003). This is causing harmful effect on the health of the humans and environment and disturbed the biodiversity structures. Thus, it triggers the WHO and FOA to prohibit the use of chemical and hazardous products in the agricultural sectors and also aware the people about the microbial-based fertilizers for the sustainable agricultural practices. The utilization of the microbial fertilizers is considered the best solution to overcome the environmental issues like eutrophication and soil contamination with agrochemical fertilizers.

## 15.9 Prospects and Challenges of Biofertilizer Application

Microbial fertilizers are considered as the potent source of nutrients to the plants and have achieved global recommendation and acceptances for its usage in the sustainable agricultural productions. Its applications are well noticeable in the European, Asian, and American countries, while its applicability is not completely established in the African countries. This is due to the shortage of proper infrastructure, awareness, skilled manpower, and regulatory protocols. These factors have created restrictions in the sustainable agropractices; therefore the advantages of biofertilizer usage like nitrogen fixation, nutrient uptake, enhancing the crop yield and affordability are not achieved.

## 15.10 Conclusions

Biofertilizer and microbial consortium are considered potent tools in sustainable agricultural practices as they are a renewable, supplementary, and environmentally friendly nutrients source for plants. They are an essential part in the integrated plant nutrient system as they convert the unusable form of beneficial soil nutrients to become usable without causing harmful effects on the natural ecosystem (Alley and Vanlauwe 2009). Microbial fertilizers are a vital element in improving crop productivity and soil fertility and also increasing the growth of crops during the abiotic stress in extreme environments. Development and application of microbial consortium signify the importance of microbial inoculants in the upcoming years. Despite a large number of PGPR microbes are known for their growth-promoting action, very few are designed to biofertilizers or microbial consortium. Thus, the development of new techniques is required to expand the applications of microbial fertilizers and establish sustainable agriculture practices.

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