Chapter 2 Biofertilizers: The Role in Sustainable Agriculture



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Abstract The global rise in the human population presents a significant challenge to the world's food security. There is a huge gap between production and consumption (7.2 million tonnes nutrient deficit) due to the expanding population and agricultural land's shrinking over time. Therefore, crop production must be significantly boosted in the next few decades to meet the growing population's food demand. Chemical fertilizers have been extensively used to enable the crop outputs to bridge the lacunae between production and consumption, which ultimately seriously damaged both the natural ecosystems and human health. Therefore, biofertilizers' exploitation is, to a certain extent, considered a substitute for chemical fertilizers in the agricultural industry because of their significant potential to improve food production and safety. Biofertilizers are substances that include cells of different varieties of beneficial microorganisms that could become critical components of advanced nutrient management. Organisms typically used as constituents of biofertilizers are nitrogen fixers (N fixer), phosphorus solubilizers (P-solubilizer) and potassium solubilizers (K-solubilizer) or a mixture of fungi and moulds. These possible biofertilizers play a vital role in the production and sustainability of soils and safeguard the environment by being eco-friendly and cost-effective for producers.

Keywords Biofertilizer \cdot Sustainability \cdot Chemical fertilizers \cdot Agriculture \cdot Food security

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2.1 Introduction

Since the evolution of the human race, agriculture has been a primary survival tool for human generations. The agriculture sector generates food, fodder and other nonwood products on which a significant fraction of the world's population relies (Hervé et al., 2016; Kaur et al., 2018, 2018b). Given the population explosion, we need to set up appropriate plans and protocols to promote sustainable agriculture to meet our demands (Bhardwaj et al., 2014; Singh et al., 2014). Traditional methods are sensu stricto just to the farmer's families and the local village communities as it only engages food and feed production at the domestic level (Jehangir et al., 2017; Pandey, 2018). However, with the advent of innovative technologies, the production of agriculture output per hectare increases. Sustainable agriculture is a sensu latu concept to grow crops to their threshold limit while simultaneously protecting the environment (Barragán-Ocaña & Del-Valle-Rivera, 2016). There is a stochastic change in agricultural practice methodology as people have been paying more attention to safeguarding the environment while working to amplify the farm yield. Various hormones, fertilizers and even innovative approaches to fertigation are being used to increase crop production (Campos et al., 2019; Umesha et al., 2018a). Though yield may be amplified with increased application of synthetic chemicals, the overdose of synthetic fertilizers would diminish the utility of living conditions through pollutant and biomagnification or ecological amplification (Uosif et al., 2014). But that would compromise environmental sustainability. So, without compromising the future generation's needs, the current generation could attain sustainability in the agricultural sector by using eco-friendly products and environmentally sound technologies (Calabi-Floody et al., 2018; Umesha et al., 2018b; Wang et al., 2015).

Biofertilizer is one such product that helps us in achieving sustainable agriculture. Biofertilizers are the amalgamation of live or latent cells of competent phosphate-solubilizing strains, N₂-fixing or cellulolytic microorganisms, mainly used to apply to seeds and seedlings, etc. (Agarwal et al., 2018; García-Fraile et al., 2015). They play a significant role in escalating soil fertility by fixing atmospheric nitrogen and converting it into usable products. They also promote root growth by producing necessary hormones and antimetabolites and help in soil mineralization and nutrient decomposition (A, B., Ak, M., M, G., G, G., P, P., ... B, J., 2009; Kumar et al., 2017). They are economical and can be used as supplements to synthetic fertilizers. Microflora like bacteria, fungi and blue-green algae are used as the principal ingredients of biofertilizers. To improve their shelf-life, these should be packed in material like peat and lignite powder. This way, biofertilizers have an utmost significance in sustaining agriculture and a safe environment (Agarwal et al., 2018). They can be grouped into different categories (Kumar et al., 2017) based on their service in sustainable agriculture (Table 2.1).

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no.	Biofertilizers	Examples
1.	Nitrogen fixing	Frankia, Anabaena, Rhizobium (symbiotic)
		Anabaena, Azotobacter, Clostridium (free-living)
		Azospirillum (associative symbiotic)
2.	Phosphorus mobilizing	<i>Glomus</i> sp., <i>Gigaspora</i> sp., <i>Scutellospora</i> sp. and <i>Sclerocystis</i> sp. (arbuscular mycorrhiza)
		R. solani (orchid mycorrhiza)
		<i>Laccaria</i> sp., <i>boletus</i> sp., <i>Pisolithus</i> sp., <i>amanita</i> sp. (ectomycorrhiza)
		Pezizellaericae
3.	Phosphorus solubilizing	Bacillus circulans, B. megaterium var. phosphaticum, B. subtilis, P. striata (bacteria)
		Aspergillus awamori, Penicillium sp. (Fungi)
4.	Biofertilizers	Bacillus sp. (for zinc and silicate
	(micronutrients)	Solubilizers)
5.	Rhizobacteria (plant growth promoting)	P. fluorescens (promoting plant growth)

Table 2.1 Categories of biofertilizers

2.1.1 Rhizobium

It is the most extensively studied genus to carrying the function of N_2 fixation (Odame, 1997). This genus's strains *are* symbiotically associated with leguminous crops, the essential food components. Apart from the ingredient of meals, legumes possess the potential to improve soil health via N_2 fixation (Laranjo et al., 2014), with different strains of this genus.

2.1.2 Azospirillum

Genus Azospirillum – a free-living genus – fixes the atmospheric nitrogen at a rate of 20–40 kg ha⁻¹y⁻¹ (Bashan, 1993). Strains of this genus are used as biofertilizers in various economically important crops like corn, rice, wheat, etc., which are (Döbereiner, 1997; Reinhold & Hurek, 1989; Sundaram et al., 1988). There is a proven fact that there is an increase in agricultural production and soil properties by applying *Azospirillum* strains Motsara et al. (1995).

2.1.3 Azotobacter

Azotobacter is an important genus promoting the synthesis of active secondary compounds like heteroxins, vitamins, gibberellins, etc. and thus significantly improves plants' root growth. This genus's species show intolerance to fluctuations of pH, salts and temperature (Jaga & Singh, 2010; Rao, 1986). Growth and crop yield of Triticum aestivum have increased with the augmentation of *Azotobacter* species (Ei-Lattief, 2016). Along with some yeast strains, it shows many improvised results (Ahmed et al., 2011). Some well-known methods of application of *Azotobacter* species are seed sipping and seedling root dipping.

2.1.4 Phosphorus-Solubilizing and Phosphorus-Mobilizing Microbes

Biofertilizers containing P-solubilizing and phosphorus-mobilizing microbes can make the accumulated phosphates readily available for plant growth progress (Goldstein, 1986). PSB also alters the status of soil nutrient structure (Blake, 1993).

2.2 Biofertilizers: Why their Need Is Inevitable?

In contemporary times there is a trend of environmental hazards and threats to sustainable agriculture due to the extensive use of chemical fertilizers. Given this, biofertilizers' continued application proves very economical, eco-friendly, efficient and productive to marginal and small farmers over chemical fertilizers. There are primarily two reasons which push the agriculturists for frequent use of biofertilizers:

- · Usage of biofertilizers is a front runner in increased crop productivity.
- Increased usage of chemical fertilizer augments the damage in soil texture with accompanying environmental problems.

2.3 How Biofertilizers Work

Bacteria, fungi and other microorganisms, though distributed heterogeneously, are omnipotent in the world. The microbes that are present in the rhizosphere and can promote growth and development are accordingly termed as plant growth-promoting bacteria (PGPB). These days several PGPBs are available as biofertilizers at a commercial scale (Calvo et al., 2014). PGPB plays a very prominent role in plant growth and soil fertility maintenance in the following ways (Fig. 2.1):

Under certain stress conditions, PGPBs may produce and supply important hormones like auxins, cytokinin and gibberellin, which directly regulate plant growth. PGPBs also aid plants by providing essential elements like nitrogen (N) and phosphorous (P) and enhance potassium (K) intake, etc. Since these activities promote plant growth directly, they are called direct ways.

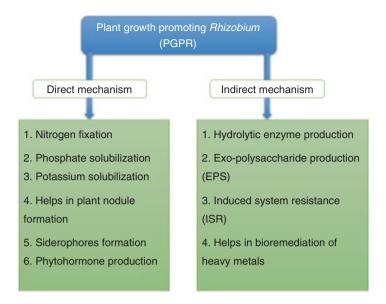


Fig. 2.1 Direct and indirect mode of action

PGPBs also promote plants' growth indirectly via different pathways, e.g. protecting against the deleterious effects of plant pathogens (Gamalero et al., 2009).

2.3.1 Direct Way

Nitrogen (N_2) fixation is the best-studied direct way of growth promotion in plants by PGPBs. Nitrogen belongs to the category of essential nutrient element for plants. Though the atmosphere contains 78% nitrogen as dinitrogen (N_2) , plants cannot take up and use it in this form. The plant available forms (PAFs) of nitrogen are ammonia and nitrates mainly produced by microorganisms via biological nitrogen fixation (BNF). Plants can assimilate the nitrates and ammonia via assimilation pathways, i.e. ammonium assimilation and nitrate assimilation, respectively (Tairo & Ndakidemi, 2013). The nitrogen-fixing microorganisms (also called diazotrophs) possess a unique enzyme complex known as dinitrogenase which acts on atmospheric nitrogen and converts it into ammonia (Smith et al., 2013). Diazotrophs can be free-living and symbiotic. The symbiotic category includes *Rhizobiaceae* members that share a symbiosis with plants belonging to *Leguminosae* (Ahemad & Khan, 2012a, 2012b, 2012c, 2012d).

Symbiotic nitrogen-fixing rhizobacteria collectively called *Rhizobia*, e.g. *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium* and *Mesorhizobium*, belong to the *Rhizobiaceae* (*Alphaproteobacteria*) family. They create a symbiotic relationship with their hosts (legumes) by infecting their roots. A complex interplay

of chemical signalling between the host and symbiont is required to establish this relationship, which results in the root nodule formation *Rhizobia* resides intracellularly as symbiont (Allito et al., 2015). Simultaneously, the non-symbiotic nitrogenfixing rhizobacteria form a non-obligatory relationship with the non-leguminous plants (Verma et al., 2010). All diazotrophs carry nitrogen fixation by a complex molybdenum-iron dinitrogenase system, consisting of dinitrogenase reductase with a cofactor of Fe (iron) and dinitrogenase with Fe and Mo (molybdenum) as its cofactors (Smith et al., 2013). However, many free-living bacteria like A. vinelandii contain iron-iron or vanadium-iron cofactors in response to molybdenum depletion. Ferredoxin reduces dinitrogenase reductase, which in turn reduces dinitrogenase, followed by reduction of dinitrogen (N_2) to ammonia (NH_3) (Santi et al., 2013). The genes responsible for nitrogen fixation are called Nif genes found in free-living and symbiotic nitrogen fixers (Black et al., 2012). Nif genes consist of two types of structural and regulatory genes. The former is responsible for Fe protein activation, biosynthesis of Fe-Mo cofactor and donation of electrons, and the latter accountable for enzyme functionality and synthesis (Ahemad & Kibret, 2014).

Maximum legume plants develop de novo lateral root organs to accommodate the symbiotic *Rhizobium*, called "root nodules". The process of development of root nodules in legumes is an essential feature associated with nitrogen fixation. It involves de-differentiation of differentiated root cortical cells following bacterial infection to the roots. The de-differentiated cells then differentiate into root nodules harbouring the nitrogen-fixing microbes (Suzaki et al., 2015). The schematic representation of plant-bacteria interaction has been presented in Fig. 2.2.

For the proper formation of nodules, two regulatory events, viz. infection of bacteria in epidermis and organogenesis of the cortex's nodule (Suzaki & Kawaguchi, 2014), must be well synchronized. Nod factors produced by rhizobia are chemically lipochoto-oligosaccharides which initiate the symbiotic relation between the

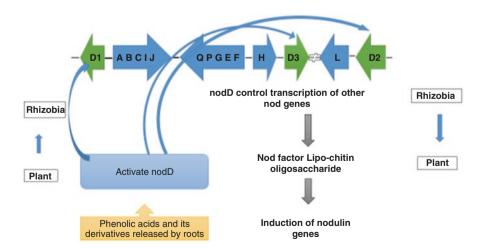


Fig. 2.2 Diagrammatic representation of plant-bacteria interaction

rhizobium and host plants (Maillet et al., 2011). The amount of plant ethylene has been reported to increase after the infection with Rhizobium spp., which prevents further rhizobial disease and nodule development (Abeles et al., 2012). Several rhizobial strains restrict the increase in ethylene production by synthesizing "rhizobitoxine", small molecule that inhibits the function а of enzyme 1-aminocyclopropane-1-carboxylate (ACC) synthase - the main regulatory enzyme in the ethylene biosynthesis pathway (Nascimento et al., 2012). The limit on ethylene production because of rhizobitoxine causes an increase in the number of nodules formed on host plant roots and enhances symbiosis (Vijayan et al., 2013). Some rhizobial strains decrease the ethylene concentration by producing the enzyme ACC deaminase, which breaks down some ACC before its conversion to ethylene. This ethylene reduction results in increased formation of nodules and plant biomass by 25–40% (Zahir et al., 2011). About 10% of rhizobial strains in the field naturally have ACC deaminase, so the number of nodules formed by Rhizobia strains without ACC deaminase can be enhanced through transformation via genetic engineering (Glick, 2014). The same approach was used in the Sinorhizobium meliloti strain lacking this enzyme which was transformed with the ACC deaminase gene from Rhizobium leguminosarum by. viciae. This transformation reportedly increased the number of nodules significantly by approximately 35% and the biomass of alfalfa by 40% compared to the control of wild strain (Glick, 2012, 2015).

Phytohormones produced and supplied by plant-associated microflora can stimulate growth and development in the host plant by modulating its endogenous hormone levels (Gray, 2004; Van Loon, 2007). Auxins (indole-3-acetic acid), gibberellins (GAs) and cytokinins are among the most significant plant growth regulators produced by associated microbes. It is reported that 80% of associated rhizospheric microbes of different crops synthesize auxins as their secondary metabolites (Ahemad & Khan, 2011). They do so through the tryptophan-dependent and tryptophan-independent pathways. The following three tryptophan-dependent auxin synthesis pathways are known:

- (i) Indole-3-pyruvic acid (IPyA) pathway present in *Rhizobium*, *Bradyrhizobium* and *Azospirillum*
- (ii) Indole-3-acetamide (IAM) pathway found in certain pathogenic bacteria such as *Pseudomonas syringae* and *Agrobacterium tumefaciens*
- (iii) Tryptamine pathway found in species like *Bacillus megaterium* and *Bacillus licheniformis*

IAA from a rhizobacterial source has been recognized as the primary causal molecule of pathogenesis or phytostimulation (Ahemad & Khan, 2012b; Mahanty et al., 2017). In addition to IAA, cytokinin modulation is reported to be involved in phytostimulation. For example, enhancement of seedling growth in *Arabidopsis thaliana* and *Proteus vulgaris* has been reported via cytokinin synthesis by *Bacillus megaterium* (Ortíz-Castro et al., 2008). Diverse genera of bacteria like *Azospirillum*, *Bacillus, Klebsiella, Proteus, Pseudomonas, Xanthomonas*, etc. include wellcharacterized cytokinin-producing species. Besides cytokinin and ethylene, gibberellin (GA) production has also been observed in plant-associated bacteria and fungi. GA-producing bacteria are used to boost seed germination rate even though bacterial GA's precise function is not understood (Goswami et al., 2016).

2.3.2 Indirect Way

As mentioned above, PGPB may also indirectly promote the growth of plants with which they are associated, e.g. by reducing the adverse effects of pathogenic fungi or bacteria on plant growth and development. Scientists are also promoting the use of PGPB as an eco-friendly alternative to chemical fungicides.

Antibiotics

PGPBs protect plants from many pathogen attacks by producing antibiotics acting against the pathogens (mainly fungi). They make different antibiotics under different conditions, with many of them studied in detail. Some of these antibiotic-producing strains have also been commercialized. Scientists have also modified these antibiotic-producing strains of PGPBs to produce antibiotics under laboratory conditions (Devine et al., 2017).

Cell Wall-Degrading Enzymes

Some plants defend themselves against the pathogenic fungus by producing enzymes involved in cell wall degradation, e.g. such as chitinase, which hydrolyses chitin in the fungal cell wall. Similarly, some bacteria (used as biocontrol agents) produce enzymes like cellulases, chitinases, glucanases, lipases and proteases, which can degrade many pathogenic fungi's cell walls (Kim et al., 2015). Hence PGPBs control fungal diseases and prevent yield loss which is a promising and eco-friendly way.

Hydrogen Cyanide

Many PGPBs like *Rhizobium*, *Pseudomonas* and *Bacillus* produce hydrogen cyanide to control many diseases. The lower levels of hydrogen cyanide do not allow fungal pathogens to attack plants and enhance plants' resistance against the diseases. The hydrogen cyanide works by inhibiting the cytochrome-c oxidase and its other metabolites. Some bacteria also produce antibiotics and HCN that act synergistically against fungal pathogens and prevent the development of resistant pathogenic fungi (Olanrewaju et al., 2017; Ramette et al., 2006).

2.4 Methods of Application of Biofertilizers to Crops

2.4.1 Seed Treatment

In this method, selected biofertilizers are mixed with water and gently combined with the seed mass with the help of an adhesive like gum acacia, jiggery solution, etc.; however, before sowing, the seeds are shade dried on a clean sheet or piece of cloth.

2.4.2 Seedling Root Dip

In this method, selected biofertilizers are mixed with water, and the seedling roots are dipped in the mixture for about 8–10 hours before transplantation. This method is usually used for transplanting crops such as rice.

2.4.3 Soil Treatment

Recommended biofertilizers and compost are mixed in the ratio of 1:50 by weight and kept overnight. This mixture is mixed with soil at the time of sowing seeds or transplanting the seedlings.

2.5 The Role of Biofertilizers in the Alleviation of Environmental Stresses

Plants, being sessile, are vulnerable to a plethora of stress (abiotic and biotic) at any given instant, interrupting normal metabolism seen as abnormal physiology. During stress, the peculiar features in plants are the excessive formation of reactive oxygen species (ROS), which include both free radicals and non-radical molecules. These extreme ROS molecules damage cellular lipids, hence damaging cells, and cause metabolic disorders and variations in senescence. Several plant growth-promoting rhizobacteria (PGPR) are known to help plants alleviate stress. For example, rhizobacteria maintain the cytoplasmic osmolarity under drought stress by producing various osmolytes like glycine betaine, proline, ectoine, and trehalose. The production of glycine betaine helps plants tolerate droughts, frost and salinity stresses simultaneously.

As mentioned above, several rhizobacterial species may produce growth regulators like ethylene and cytokinin, thus promoting crop plants' growth under stress. *Pseudomonas alcaligenes*, *P. aureofaciens*, *P. aurantiaca* and *P. chlororaphis* have plant hormones under unfavourable conditions in saline arid soils (Verma et al., 2017; Yadav et al., 2018). Further, alleviation of heat stress in plants by cytokininproducing PGPB was isolated from the rhizosphere's soil (Arkhipova et al., 2007). Liu et al. (2013) also reported that a cytokinin producer, *Bacillus subtilis*, improved the tolerance to drought stress. Forchetti et al. (2010) showed that *Bacillus pumilus* and *Achromobacter xylosoxidans* which are drought-tolerant endophytic bacterial strains produce salicylic acid. Similarly, inoculation with rhizobacterial strains enhances the growth parameters of sunflower under conditions of water stress.

Inoculation of cucumber plants with bacteria like *Pseudomonas fluorescens*, *P. extremorientalis*, *Stenotrophomonas rhizophila* and *Serratia plymuthica* increases dry weight appreciably up to 62% compared to the control. The improvement in

growth and salt tolerance has been reported to be the IAA production under a salt environment. Further, the fruit yield of the cucumber was also enhanced under controlled conditions (Egamberdieva, 2011). Timmusk et al. (2014) showed that wheat treatment with PGPB under drought stress increased biomass to 78% higher than untreated plants. Enhanced biomass and root architecture modifications under drought stress were reported when inoculated with PGPB strain (Bresson et al., 2014) *Phyllobacterium brassicacearum* (STM196).

2.6 Some Factors Limiting the Use of Biofertilizers

- Lack of regulatory acts and facilities regarding testing of samples: One of the potential limitations to the use of fertilizers is a scarcity of facilities provided by institutions for testing biofertilizer samples. Further, there is a lack of government involvement in this area which is a potential eco-friendly alternative to chemical pesticides. In biofertilization, future research should be focused on options available to confront the issues and propose valid frameworks for the development of eco-friendly practices that allow advancement on the efficiency and subsequent supply of product for the industry in the global economies. Furthermore, their application's technical tests must authenticate their safe use at the worldwide level.
- Biofertilizers' insufficient popularization and low level of farmer acceptance: Among farmers, biofertilizers have not gained the required popularity. However, it comes with various potential benefits for crops, especially under stresses. This non-acceptance seems to be the lack of awareness among the farmers compared to their synthetic counterparts. Other problems such as lack of timely financing, experts' involvement and biofertilizers' non-availability also hinder their popularity and acceptance.

2.7 Conclusions

Biofertilizers form a significant component of organic farming in modern agricultural practices in terms of being a sustainable alternative to chemical fertilizers, linked with various environmental hazards. However, to popularize the biofertilizers' status, increased demand and awareness about its uses are yet to be created. Biofertilizer technology, a significant part of sustainable agriculture, has to be proper for farmers' and planters' social and infrastructural situations. It should be economically feasible for all farmers, renewable, adaptable to prevailing local conditions and satisfactory from the society's cultural patterns, practically implementable and productive. Acknowledgement The authors are thankful to the University Grants Commission for providing fellowship to corresponding author and grateful to the Department of Botany, the University of Kashmir, for sharing their facilities and those who made their literature available for the compilation of this manuscript.

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