

Sukhminderjit Kaur
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Gurvinder Singh Kocher *Editors*

Metabolomics, Proteomes and Gene Editing Approaches in Biofertilizer Industry

 Springer

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ISBN 978-981-99-3560-4

ISBN 978-981-99-3561-1 (eBook)

<https://doi.org/10.1007/978-981-99-3561-1>

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Preface

Constant deterioration of soil quality due to extensive use of pernicious chemical fertilizers has become a global concern, which not only restrains crop yield and quality but also has critical ecological consequences. Because of the toxic and non-biodegradable properties of such synthetic fertilizers, the quality of arable land is declining day by day along with the nutrient content of vegetation all around the world. Soil microbes are important in sustainable agriculture as they help to increase soil health and fertility. These microscopic organisms can satisfy the need for higher yields by providing adequate nutrients, biologically controlling pests and disease as well as stimulating plant growth. Rhizospheric microbes are also emerging as an efficient and eco-friendly alternative to counteract the negative impacts exerted by the overuse of chemical fertilizers. As a result, beneficial rhizospheric bacteria with different functional capacities are utilized as bio-fertilizers to sustainably increase the output of ecosystem.

The endeavour of the book entitled *Metabolomics, Proteomes and Gene Editing Approaches in Biofertilizer Industry* is to present details of cutting-edge research in the field of bio-fertilizer and plant-microbe interaction that will help readers to understand how microbes play a significant role as bio-fertilizers. The process of bio-fertilizer development, the process of testing its efficacy for commercial use and the potency will also be elaborated in this volume using suitable figures. The information in this book regarding the secretion of various secondary metabolites responsible for nutrient management will be helpful for designing bio-formulations to assist plant growth.

We believe this book will help to provide a substantial number of pieces of evidence that underline the genomic basis of nutrient management by microbes. Essential information will be provided regarding the genomic and proteomic background of bio-fertilizers. This collective work is distinct because of our focus on diverse emerging technologies which are high-throughput, scalable and applicable to different countries regardless of their socio-economic conditions. We consider this as a sincere effort to highlight the underutilized potential in advanced technologies in

abatement of dynamic issues in sustainable bio-fertilizer. This book will improve the current state of knowledge and should invoke researchers and innovators to take ahead the current inter-disciplinary knowledge into technologies that are readily available and effectively minimize hazards associated with chemical fertilizers.

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Rishon LeTsiyon, Israel
Maunath Bhanjan, India
Ludhiana, Punjab, India

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Acknowledgements

Biofertilizers are the key to sustainable agriculture and it has become the foremost priority of soil microbiologists to mine the soil microbial diversity with respect to their spatiotemporal, biochemical and physiological structure through modern techniques like metagenomics, proteomics, metabolomics and the like. Hence, the knowledge in these areas is vast and often does not reach every hand. An edited book like the present one provides updated knowledge to a researcher, and it is equally challenging to compile different studies in a book. First of all, we would like to acknowledge all the contributor authors of chapters in this book for bringing such an exhaustive compilation for a wider readership. All the editors gratefully acknowledge their parent organization for all the support and encouragement rendered. Thanks are also due to all those researchers whose original work has made the basis of this compilation; without the endless efforts of the researchers, science and society could not progress. We are also thankful to Springer for bringing this compilation to the scientific fraternity.

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Constraints in Biofertilizer Industry and Future Scope

1

Jupinder Kaur, Baby, and Taman

Abstract

Soil microbes are important in sustainable agriculture as they help to increase soil health and fertility. These microscopic organisms can satisfy the need for higher yields by providing adequate nutrients, biologically controlling pests and disease, as well as stimulating plant growth. Rhizospheric microbes are also emerging as an efficient and ecofriendly alternative to counteract the negative impacts exerted by the overuse of chemical fertilizers. As a result, beneficial rhizospheric bacteria with different functional capacities are utilized as biofertilizers to sustainably increase output of agroecosystem. Though the research aspect and field application of microbial inoculants in agriculture is gaining momentum, we are not still able to replace or decrease dependence on agrochemicals. Even though there is an immense amount of interest among farmers regarding the utilization of beneficial microorganisms, still there are many constraints in the application and dissemination of this technique. Thus, every aspect of the biofertilizer industry with respect to agriculture production should be scrutinized to recognize concerning checkpoints and bottlenecks. Therefore, in this chapter, the types of biofertilizers, beneficial attributes of microbes responsible for plant growth promotion, challenges encountered during production, application, as well as popularization of biofertilizer technology along with future scope have been discussed.

Keywords

Biofertilizer commercialization · Crop productivity · PGPR · Sustainable agriculture

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S. Kaur et al. (eds.), *Metabolomics, Proteomes and Gene Editing Approaches in Biofertilizer Industry*, https://doi.org/10.1007/978-981-99-3561-1_1

1.1 Introduction

Environmental stress is becoming a significant problem, and as a result, crop output is decreasing remarkably quickly. Additionally, because we rely too much on synthetic fertilizers to fulfill the enormous mandate for food from the rising inhabitants, businesses have been forced to develop fertilizers that might be harmful (Mahanty et al. 2017). However, using too many chemical inputs has reduced soil fertility and increased the susceptibility of crops to disease. Given the risks associated with chemical fertilizers, biofertilizers are viewed as a safe alternative that greatly minimizes the ecological impact. Inexpensive and safe for the environment, biofertilizers have been shown to considerably increase soil fertility when used over the long term. Biofertilizer technology must be adequate for the social and infrastructural demands of its users, economically feasible, renewable, useable by all farmers, stable over the long term, and acceptable to a variety of social groups in order to be a key component of sustainable agriculture.

Farmers are experimenting with various crop feeding techniques to assist fulfil the increased food demand brought on by the ongoing growth of the global population. By 2030, the FAO predicts a 60% rise in demand for agricultural goods. So, one of the key issues faced by society in the twenty-first century is how to increase productivity while protecting the environment (Hailu et al. 2008). For optimal outcomes, various crops require different biofertilizers. Later, scientists discovered that using a certain mix of biofertilizers produces greater outcomes than using a single or individual fertilizer. The grain yield and overall dry weight of field-grown maize were significantly increased (up to 115%) by *Azotobacter* and *Azospirillum* inoculation. Similar to this, it has been documented that inoculating rice seedlings with *Azospirillum* and *Azotobacter* species increased rice yield from 2–3 t/ha to 3.9–6.4 t/ha and successfully substituted inorganic nitrogen fertilizer. Another study looked at how different nitrogen fertility levels affected the impact of rice root inoculation on production. Unexpectedly, even with the lowest level of inorganic N fertilization, a good yield was found. It is possible to save 50% of the expensive phosphate fertilizer by using phosphorus-solubilizing bacteria as biofertilizers, which might boost sugarcane output by up to 50%.

One of the main pulse crops cultivated in many nations throughout the world is the chickpea. Numerous chickpea growth metrics were greatly improved above the control when fungal and bacterial strains (*Aspergillus niger* S-36 and *Bacillus* sp. RM2) were inoculated singly or together. However, it was shown that combining the inoculations of bacterial and fungal species was more successful than doing it alone in each case (Saxena et al. 2015). Conferring to Mohammadi et al. (2011) research, biofertilizers dramatically improved chickpea nutrient absorption. In another study, two cyanobacterial species *Oscillatoria angustissima* and *Nostoc entophyllum* were introduced into the soil to function as biofertilizers for pea plants. These biofertilizers dramatically improved pea plants' growth, germination rate, and photosynthetic pigment fraction (Mohammadi et al. 2011). Additionally, microbial inoculants protect plants against abiotic stress and pathogens. Therefore, using bio-inoculants in agriculture is the best and most sustainable option.

1.2 Biofertilizers

Fertilizer production and use have increased dramatically in order to boost agricultural yields. Thus, to supply the rising demand for chemical fertilizers, the industry must increasingly absorb the rising cost of raw materials used for fertilizer preparation, the majority of which are naturally nonrenewable. We are now in a position where agricultural development must move forward with increased speed. Chemical fertilizers, however, are insufficient to provide all of the nutrient requirements for cultivation. This has opened the door for integrated plant nutrition, which makes careful and coordinated use of chemical and synthetic nutrient sources in addition to biofertilizers and other organic fertilizers.

Biofertilizers are developed from latent and live microorganisms (bacteria, fungi, algae, yeast, etc.) which, when used on soil, seeds, or plant parts, promote the development of plants and preserve ecological equilibrium (Chakraborty and Akhtar 2021; Gosal et al. 2017). Rhizospheric microbes as biofertilizers are emerging as an effective and sustainable substitute to counteract the negative impacts exerted by the overuse of chemical fertilizers. Beneficial rhizospheric microbes help in the sustainable intensification of agroecosystem productivity with their diverse functional abilities (plant growth promoting traits). These microbes (PGPR) play a very crucial part in improving soil physical properties, fertility by several direct and indirect mechanisms (Table 1.1).

The main direct technique used by rhizobacteria or helpful microorganisms is the supply of nutrients like nitrogen, phosphorus, zinc, etc. The biological nitrogen fixation process reduces dinitrogen to ammonia. It involves the nitrogen-fixing bacteria *Rhizobium*, *Frankia*, *Azospirillum*, *Azotobacter*, etc., using a complicated enzyme system known as nitrogenase to transform atmospheric nitrogen into nitrate that plants can use (Bhattacharyya and Jha 2012). Phosphorous and other nutrients which are present in insoluble and unavailable forms in soil are also solubilized by these microorganisms. Another trait of bacteria used as biofertilizers is the production of hormones that promote plant development. Gibberellins, IAA, and other plant hormones are produced by the microorganisms to encourage plant development (Kaur and Gosal 2015; Kaur and Gosal 2017). Apart from these, siderophore production (chelating or isolating iron via iron-binding protein molecule knowns as siderophores) and ACC deaminase activity are two of the primary methods

Table 1.1 Direct and indirect mechanisms of plant growth promotion exhibited by rhizobacteria

Beneficial microorganisms (mechanism of action)	
Direct mechanisms	Indirect mechanisms
Biological nitrogen fixation	Siderophore production
Phosphorus solubilization	Competition
Plant hormone production	Antibiotic and lytic enzymes production
Siderophore production	HCN production
ACC deaminase activity	Toxin production
Micronutrient solubilization	Induced systemic resistance

utilized by rhizobacteria for the enhancement of plant development (assist plants to cope with stressful circumstances). Siderophore production is also listed as an indirect method of plant improvement as siderophores protect the plant from pathogens by iron sequestration. Induced systemic resistance reduces disease severity in various plants (Walters et al. 2013). The other secondary method shown by PGPR includes competition (competition with the disease-causing microorganisms for nutrients and habitats which protect host plants from various diseases caused by those pathogens) and the production of antibiotics, toxins, lytic enzymes, and HCN. HCN is a volatile substance with antifungal properties (Rijavec and Lapanje 2016). It is extensively utilized as a biological control agent in agriculture. Numerous bacteria-producing HCN can be used as biocontrol or biofertilizers to boost yields. Some examples of microorganisms used in the preparation of biofertilizers are *Azotobacter*, *Azospirillum*, *Bacillus*, *Actinomyces*, *Rhizobium*, *Pseudomonas*, Cyanobacteria, microalgae, arbuscular mycorrhiza fungi (AMF), etc.

1.3 Types of Biofertilizers

Biofertilizers are an effective substitute for intensive farming techniques and are produced from a range of microorganisms, such as bacteria, fungi, and algae. These not only preserve the soil's biodiversity but are also cost-effective and environmentally friendly (Kaur and Vishnu 2022). Biofertilizers can be categorized according to the types of microorganisms involved (Table 1.2).

1.3.1 Bacterial Biofertilizers

Beneficial bacteria when employed as a biofertilizer colonize the plant's interior or rhizosphere and help to promote development by converting nutritionally needed components (nitrogen, phosphorus, zinc, iron, sulfur, etc.) from unavailable form to available form through biological processes including nitrogen fixation and rock phosphate solubilization, etc. (Gosal and Kaur 2017; Kaur and Vishnu 2022). These bacterial biofertilizers are cheaper, easily available, and eco-friendly for farmers. Their application enhances productivity and soil health and safeguards the environment. There are many different kinds of bacterial biofertilizers, including those that fix nitrogen, solubilize phosphorus, produce PGPR, and provide micronutrients. A brief discussion of each of these bacterial biofertilizers is provided below.

1.3.1.1 Nitrogen-Fixing Bacteria

Nitrogen is present in the environment in enormous amounts, yet it is a restrictive nutrient for plants because it cannot be directly utilized as such by them. Nitrogen is required to be fixed, i.e., converted into plant utilizable forms to get assimilated by plants. However, certain microorganisms (diazotrophs) are capable of significant nitrogen fixation by forming various associations with plants in rhizospheric soil (Table 1.3).

Table 1.2 Types of biofertilizers (Singh et al. 2014a, b)

Bacterial biofertilizers	Nitrogen fixers	Symbiotic nitrogen fixers (<i>Rhizobium</i> , <i>Frankia</i>)
		Free living nitrogen fixers (<i>Azotobacter</i> , <i>Beijerinckia</i> , <i>Klebsiella</i> , <i>Clostridium</i>)
		Associative nitrogen fixers (<i>Azospirillum</i>)
	Phosphate solubilizers	<i>Bacillus</i> , <i>Pseudomonas</i>
	Plant growth-promoting rhizobacteria (PGPR)	<i>Bacillus</i> , <i>Pseudomonas</i>
	Micronutrient (silicate, sulfur, zinc, iron, etc.)-providing bacteria (<i>Bacillus</i> , <i>Pseudomonas</i>)	
Fungal biofertilizer	Phosphate solubilizer (<i>Penicillium</i> , <i>Aspergillus</i> . Etc.)	
	Phosphate mobilizer	Arbuscular mycorrhiza (<i>Glomus</i> , <i>Scutellospora</i> , <i>Gigaspora</i> , <i>Acaulospora</i> , and <i>Sclerocystis</i>)
		Ectomycorrhiza (<i>Amanita</i> , <i>Laccaria</i> , <i>Pisolithus</i> , <i>Boletus</i>)
		Ericoid mycorrhiza (<i>Pezizella ericae</i>)
		Orchid mycorrhiza (<i>Rhizoctonia solani</i>)
Algal biofertilizer	Symbiotic	<i>Anabaena azollae</i>
	Nonsymbiotic	<i>Anabaena</i> , <i>Nostoc</i>
Consortium or composite biofertilizer	Mixing of different compatible microorganisms with PGP activities	

The association of nitrogen-fixing bacteria with plant can be nonsymbiotic, symbiotic, or associative. This property provides effective absorption of nitrogen by plants and reduces losses due to denitrification, leaching, and volatilization (Thomas and Singh 2019). In the laboratory, the simplest way for isolation of nitrogen-fixing bacteria is to use nitrogen-free medium. On nitrogen-free medium, only those bacteria will grow which utilizes atmospheric nitrogen as their nitrogen source.

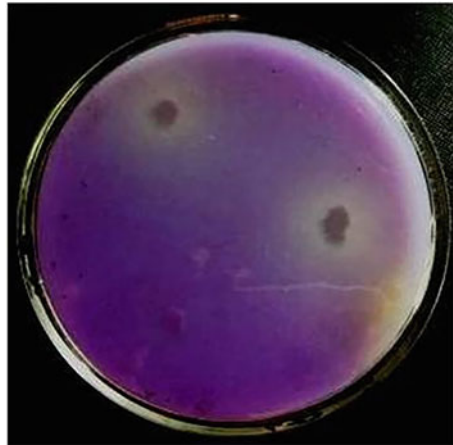
1.3.1.2 Phosphorous-Solubilizing Bacteria

Phosphorous is the second most common limiting nutrient required for the growth and productivity of crops. Most of the phosphorous is present in insoluble organic form in soil which cannot be utilized by plants. So, it needed to be solubilized for plants to take up. Those microbes which have the ability to convert the insoluble unavailable form of phosphorous into soluble available form (orthophosphates) are known as phosphate-solubilizing microorganisms (PSMs). Since phosphorous is often quite reactive, cations (Ca^{2+} , Mg^{2+} , Fe^{3+} , and Al^{3+}) precipitate it to immobilize it. In such forms, it cannot be directly utilized by plants, so it is converted into soluble forms by bacteria like *Pseudomonas*, *Rhizobium*, *Bacillus*, *Agrobacterium*, etc. (Kaur and Gosal 2018).

Table 1.3 Different types of associations exhibited by nitrogen-fixing bacteria with plant

Nitrogen-fixing bacteria		
Free living bacteria	Symbiotic bacteria	Associative bacteria
These are also known as nonsymbiotic nitrogen fixers.	Symbiotic nitrogen-fixing bacteria fix nitrogen with the help of the host plant	Associative nitrogen fixers form loose association with the roots of plants for the process of nitrogen fixation
Present freely in the rhizosphere and fix atmospheric nitrogen in the soil without any association with the host plant	<i>Rhizobium</i> is the symbiotic nitrogen-fixing bacteria	<i>Azospirillum</i> is an example of associative nitrogen fixer
<i>Azotobacter</i> is the most common example of free-living nitrogen-fixing bacteria	<i>Rhizobium</i> enhances nodulation, nitrogen fixation, and yield in legume crops	This bacterium forms close association with the plants like wheat, maize, and millets
<i>Azotobacter</i> is generally used for crops like wheat, rice, and maize	<i>Frankia</i> is another example of symbiotic nitrogen-fixing bacteria	It also produces growth-promoting hormones like IAA and gibberellins
It modifies the root structure of plants making them disease resistant and increasing their yield	<i>Frankia</i> form nodules in the roots of nonleguminous plants for the process of nitrogen fixation	<i>Azospirillum</i> application as biofertilizer can be extended to arid soils in order to protect crops from droughts

Fig. 1.1 Phosphate solubilization zones shown by the bacterial isolates on modified Pikovskaya's Agar



In the laboratory, for the isolation of phosphate-solubilizing bacteria, culture media with the insoluble inorganic form of phosphorous are used. The zone around the colony of those microorganisms is indicative of their ability to dissolve the insoluble inorganic form of phosphorus (Fig. 1.1).

1.3.1.3 Micronutrients Providing Bacteria

Plants also need some key micronutrients, such as silica (Si), potassium (K), iron (Fe), zinc (Zn), etc., for healthy growth, in addition to macronutrients. For plants

including wheat, eggplant, black pepper, and cucumber, soil-dwelling bacteria like *Bacillus edaphicus*, *Paenibacillus glucanolyticus*, and *Bacillus mucilaginosus* can also be utilized as biofertilizers for the provision of micronutrients. A deficiency of micronutrients in the soil can lead to a lower yield of agricultural crops. Potassium and zinc are vital micronutrients required for the optimum growth of plants. Farmers have utilized a variety of potassium and zinc fertilizers to overcome K and Zn deficiencies in crops. However, because they are converted into insoluble complex forms after a few days of fertilizer application in the soil, their use places a strain on the economy and ecology. The use of potassium and zinc-solubilizing rhizobacteria is a necessary substitute for all of these methods (Kamran et al. 2017). Similar to this, by introducing protons and organic acids to the media, microorganisms may hydrolyze silicates and aluminum silicates, while they are metabolizing these protons and organic acids.

1.3.1.4 Plant Growth-Promoting Rhizobacteria (PGPR)

A category of bacteria known as PGPR increases plant output and growth through a number of processes, including phytohormone synthesis, phosphate solubilization, and nitrogen fixation (Gosal et al. 2017). The use of PGPR is known to enhance plant development in a variety of ways while preserving natural flora, something that synthetic fertilizers are unable to do. Numerous PGPR community types may be found in the rhizospheric soil, and they have a positive effect on crop productivity. *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Mycobacterium*, *Mesorhizobium*, *Flavobacterium*, etc., are some typical examples of PGPR genera that demonstrate plant growth-promoting activity (Kaur 2021).

Many research and reviews have found that inoculating plants with PGPR promotes plant development, increases yield, solubilizes P (phosphorus) or K (potassium), and helps in absorption of N (nitrogen) and other elements. Furthermore, several studies have shown that PGPR inoculation promotes root development, resulting in a root system with a larger surface area and a greater number of root hairs. PGPR also protects plants from a variety of illnesses, either by direct contact with the pathogen or by inducing host resistance. Apart from that, the primary contribution of the PGPR in agroecosystems is the generation of antibiotics and other plant growth-stimulating chemicals. The use of such bacteria as eco-friendly biofertilizer aids in the reduction of the usage of costly fertilizers. According to recent research findings, inoculating agricultural soils with PGPR increases productivity compared to uninoculated soil. PGPR is an environmentally friendly and cost-effective strategy for enhancing plant development and mitigating stress situations (Basu et al. 2021).

1.3.2 Fungal Biofertilizers

Millions of fungal species are inhabitants of soil, which are crucial for decomposition, biological control, and ecosystem regulation. Likewise bacteria, fungi can also

be used as biofertilizers. Fungi have no role in the provision of nitrogen as the process of biological nitrogen fixation is limited only to prokaryotes. But by functioning as phosphate solubilizers or mobilizers, they play a crucial part in the supply of the macronutrient phosphorous. There are many species of fungi that solubilize phosphorous by converting unavailable insoluble (organic) forms to available soluble (inorganic) forms of phosphorous. Such fungi are termed as phosphate-solubilizing fungi. The process of phosphate mobilization is carried out by mycorrhiza.

Mycorrhiza is a relationship between fungus and plant roots that is mutually advantageous for both parties. Fungi rely on plants for food and energy, while the hosts get nutrients from the fungi. Fungal hyphae enhance the soil's surface area and stretch the plant roots for effective nutrient exchange and uptake by the plant. The formation of specific fungal structures inside the plant cell, such as arbuscules or vesicles, is a hallmark of the development of mycorrhizal symbiosis. During the presymbiotic phase, it starts with spore germination, germ tube enlargement, hyphae branching, and direct interaction with the host plant. During the symbiotic stage, the fungus colonizes the root cortex and produces arbuscules that allow bidirectional nutrient transfer between the hosts. According to certain theories, plants might use the nutrient-dependent regulation of AM colonization as a crucial feedback mechanism to either promote or prevent fungal colonization depending on their needs (Debnath et al. 2019).

The application of mycorrhizal fungi as biofertilizers improves soil quality, soil porosity, aeration, aggregate formation, water dynamics, and resistance to abiotic stress. The mycorrhizal fungi help the host plant grow and defend it from pathogenic assaults.

1.3.3 Algal Biofertilizers

Recent studies have demonstrated the potential for using a variety of photosynthetic microorganisms, including cyanobacteria and microalgae, as biofertilizers and soil conditioners. Blue-green algae and *Azolla* (nonsymbiotic) are the two most common types of algae used as biofertilizers. Small nitrogen-fixing super plants called *Azolla* float unrestrainedly and have scaly leaves and floating roots. *Azolla* is widely recognized for its symbiotic relationship with the nitrogen-fixing *Azollae* and *Anabaena* (Kaur and Purewal 2019). Besides fixing nitrogen, blue-green algae also fix phosphorus, zinc, potassium, sulfur, and other micronutrients. They are heterocystic and have filaments, which are advantageous for nitrogen fixation.

1.3.4 Consortium or Composite Biofertilizers

Consortium biofertilizer is a term used to describe the utilization of two or more microbial cultures as a biofertilizer to preserve soil health and improve plant output as a result of various growth processes. According to research, consortia are more

efficient than single microorganisms. The use of two or more microbial cultures in combination produces quicker and better results. This could be a result of bacteria interacting and cooperating with one another in the rhizosphere. The microbial cultures to be used as a consortium should be checked for their compatibility with each other before their use as biofertilizers (Dal et al. 2020).

1.4 Biofertilizer: Constraints and Their Potential Solutions

To get higher agricultural productivity in a sustainable manner, the use of microbial based products as biofertilizers is currently gaining popularity. These biofertilizers are being used by farmers for many crops (cereals, legumes, fodder, etc.). Despite the fact that there is an immense amount of interest among farmers regarding the utilization of beneficial microorganisms, still there are many constraints in the application and dissemination of this technique.

The rhizobacterial microorganisms after their isolation are screened for various plant growth-promoting traits before their recommendation for use in the farmer's field. But there are a number of difficulties in translating their use from the lab to the field. An initial laboratory screening is necessary for creating a novel PGPR strain that is an efficient bio-inoculant. This screening depends on certain direct and indirect processes of PGPR that promote plant development. Effective plant growth promotion in the field cannot be guaranteed by primary screening of isolated axenic culture plants for PGPR characteristics alone. Additionally, pure culture isolates with fewer *in vitro* growth-promoting behaviors might have different strategies for promoting plant growth. These processes are not thoroughly known, making it challenging to screen for such isolates under normal circumstances. As a result, because of their inadequate *in vitro* performance, such valuable strains displaying these pathways may get out (Sessitsch and Mitter 2015). The widespread use and implementation of PGPRs demands resolving a number of crucial concerns and overcoming a number of difficulties and limitations as below:

- Carrier based
- Marketing based
- Field application based
- Quality control based
- Biosafety based
- Biological based
- Technological and infrastructure based
- Regulation based
- Finance based

A brief description of these limitations and their potential solutions is discussed below.

1.4.1 Carrier-Based Constraints

An appropriate carrier must be used when applying the biofertilizer in the field due to the short shelf life of the bio-inoculant agent. Therefore, one of the main obstacles to its widespread usage in fields is the lack of a suitable carrier. The use of poor-quality carrier material will decrease the efficiency of biofertilizers. Peat, charcoal, lignite, and other suitable carriers are utilized to manufacture biofertilizers. The majority of these carriers are not available in developing nations like India; they once again present technological challenges (Jaborova et al. 2020). Although it is acknowledged that among the possible carriers, peat is the most suited, the problem is that it has a shelf life of less than 6 months. But the biofertilizer should have a number of other qualities to demonstrate its effectiveness as a carrier. It should be inexpensive, have a great concentration of biological matter and water-holding ability, and have a longer period of microbial retention. For a biofertilizer to be of high quality, the carrier must be practically sterile, contain no moisture, be toxin-free and nonpolluting, and have a nearly neutral pH (Bashan et al. 2014). Thus, only charcoal may be utilized as a carrier because it is easily accessible in the Indian market. Biochar can also be utilized as a good carrier for biofertilizers due to its capacity to enhance soil and plant health (Backer et al. 2018).

Carrier-based biofertilizers have a 6-month shelf life. The initial count of microbes in carrier-based biofertilizer is around 10^8 cfu/ml. This count keeps on decreasing gradually. These are not popular among farmers due to difficulties in their method of application. Additionally, carrier-based biofertilizers are not heat and UV resistant. So, the only way to mitigate the disadvantage of carrier-based biofertilizers is the use of liquid biofertilizers. Liquid biofertilizers are liquid formulations of microorganisms along with nutrients and cell protectants. These biofertilizers have a higher shelf life (more than a year) and are tolerant to higher temperatures and ultraviolet radiations. These are very well adopted and popular among farmers as they are very simple and easy to use relative to carrier-based biofertilizers (Sahu and BrahmaPrakash 2016).

1.4.2 Marketing-Based Constraints

The lack of appropriate transportation and storage services is one of the main obstacles to establishing a biofertilizer product for the market. Farmers are also not well-convinced of the benefits of using biofertilizers for sustainable agriculture as opposed to dangerous agrochemicals. As a result, there is less demand for such eco-friendly goods. Due to a shortage of technically skilled people, the development of extension centers does not aid in raising awareness among farmers. The fact that agricultural crops are grown under numerous physical, chemical, and environmental conditions, (including various temperatures, rainfall, soil, and crop varieties) poses a significant problem for the producers of biofertilizers. These conditions typically differ from farm to farm, sometimes even within the same region (Thomas and Singh 2019).

The effectiveness of biofertilizers differs as a result of these changes. Before any microbial goods get to the point of commercialization, a broad plan is followed in every state within a nation. The agriculture ministry or department gives the all-clear to place orders mostly from their own producing facilities. Biofertilizer packets are shipped from producers to a number of districts. Before these packets are delivered to the field, a network of extension workers is engaged (Barea 2015). The microorganisms used as bio-inoculants are subjected to high temperatures throughout this course, which might cause them to become inactive or die, making them low- or poor-quality biofertilizers. These subpar packages will henceforth be detrimental to the farmers as well as the total crop production.

The National Biofertilizer Development Centre was founded in Ghaziabad (India) to help with this marketing limitation, and the following were added to improve marketing in three ways:

1. State governments working with villages and district-level authorities.
2. State marketing federation working with farmers and cooperatives.
3. Cooperation between the state's agriculture and industry is facilitated via agroservice centers.

1.4.3 Field Application-Based Constraints

When we use biofertilizer under field conditions, the establishment of inoculum in root region takes time. Therefore, the crop's reaction to biofertilizers is often very slow and ineffectual. As a result, farmers only adopt biofertilizers to a limited extent. In field application, the purity of the inoculants and the inoculation methods are extremely important. Due to the detrimental after properties of artificial pesticides and the current adverse abiotic circumstances, biofertilizer efficiency is decreased (Parnell et al. 2016). Another significant factor in the decline of biological activity is environmental stress, such as salt and drought in some regions. Both biotic and abiotic stressors are placed on the inoculants (Arora et al. 2010). Other factors that together contribute to the poor performance of the biofertilizers include the soil's acidity and alkalinity, the use of pesticides, and high concentrations of nitrate in the soil, which limit the ability of the bio-inoculants to fix nitrogen. Many soils lack other essential minerals like P, Cu, Mo, and Co and have harmful concentrations of heavy metals like Cd, Hg, and Cr. These factors reduce the biological potential of biofertilizers.

Rhizobacteria used as biofertilizer work via a number of processes. The first stage in promoting plant development is the microbe's colonization of the plant roots. To encourage a positive plant-microbe association, this intricate process requires the ability of bacteria to compete in the rhizosphere soil for a suitable niche. The presence and survival of the microorganisms within the host plant are influenced by abiotic parameters such as soil type, temperature, pH, radiation, oxygen content, availability of nutrients, and the degree of contact with the native soil microbiota. Consequently, climatic conditions necessary for a certain variety of farmed crops

determine whether the field application of biofertilizers will be successful or not (Dineshkumar et al. 2018). Thus, it is strongly advised to identify region-specific microbial strains in order for the used rhizobacterial inoculants to function as effectively as possible.

Biofertilizers are usually used in small amounts per acre. Due to competition with the already present soil micro- and macrobiota, their numbers are insufficient to allow for efficient rhizosphere colonization in a field (Atieno et al. 2020). Broad-spectrum biocide fumigants are often used to fumigate the soils surrounding high-value crops. These fumigants have an impact on the soil's microbial community. The soil microbial community and the beneficial interactions that assist host plants in mobilizing and acquiring nutrients are significantly harmed by long-term fumigation. As a result, the PGPR inoculant's ability to colonize the rhizosphere is reduced.

1.4.4 Quality Control-Based Constraints

Quality control is the most crucial feature that farmers look for in a biofertilizer. Living microorganisms have a relatively short shelf life since they are natural goods (Meena et al. 2020). The presence of substandard or fake products might contribute to the failure of any microbial based product in the field. In many places, no quality checks are followed for biofertilizers. The production of biofertilizers without proper microbiological techniques will lead to the development of less efficient biofertilizer formulation. So, setting up quality control criteria for biofertilizers is very important in direction to establish the effectiveness of these fertilizers in encouraging plant development. To maintain the quality of biofertilizers, there are BIS (Bureau of Indian Standards) norms. These BIS norms are also known as "Standards for Biofertilizers." These standards specify values for parameters like viable cell count (10^8 cells per gram or per ml), pH, moisture percentage, permissible contamination, shelf life, carrier, marking on the packet, and application.

The Ministry of Agriculture, Department of Agriculture and Cooperation, and the Government of India, New Delhi, vide their order dated March 24, 2006, included biofertilizers and organic fertilizers under section 3 of the Essential Commodities Act, 1955 (10 of 1955), in Fertilizer (Control) Order, 1985. These rules were further amended in respect of applicability, specifications, and testing protocols vide Gazette notification November 3, 2009.

1.4.5 Biosafety-Based Constraints

It is thought that PGPRs are good candidates for sustainable agriculture. The fact that these bacteria shouldn't have any negative impacts on the environment or people is a crucial aspect of PGPRs and other biofertilizer agents. The USA has released standards on biosafety in biological and biomedical laboratories. The Department of Health and Human Services and the World Health Organization created biosafety levels (BSLs) in 1999 to categorize helpful bacteria into several biosafety classes

based on the many types of hazards they represent. Biosafety levels (BSL) are used to determine the protective measures required in a laboratory setting to safeguard staff, the environment, and the general public. Biosafety in Biomedical Laboratories defines the levels (the BMBL). To achieve proper biosafety and biocontainment, a variety of tools, procedures, and laboratory design elements can be combined. These are established by biological risk analyses that are carried out especially for each experimental technique. The communicable agents (BSL-1–4) were divided into four risk classes based on their pathogenicity to human health, mechanism of transmission, and existing therapies. These levels must be strictly followed while dealing with these bacteria (Meena et al. 2020). Preferably, the microbial strains chosen for the creation of biofertilizers should come from the nonpathogenic BSL-1 bacteria with little environmental hazard.

The utmost (highest) level of confinement is BSL-4, while the other three levels are BSL-1, BSL-2, and BSL-3. BSL-1 laboratories are employed for the research of infectious agents or toxins that are not known to reliably cause illness in healthy individuals. They don't need any specialized tools or architectural characteristics and adhere to standard microbiological practices, which are fundamental safety standards. Surfaces that are simple to clean and resistant to the common chemicals used in the lab are examples of standard engineering controls in BSL-1 facilities. BSL-2 laboratories are used to investigate toxins or infectious agents with a moderate degree of risk that might be harmful if eaten, breathed, or exposed to the skin. Design specifications for BSL-2 laboratories must have automated closing and locked doors, eye cleaning stations in case of mishaps, and handwashing sinks. BSL-2 laboratories must also have access to decontamination tools, such as an autoclave, an incinerator, or another technique, depending on the results of the biological risk assessment.

Infectious substances or poisons that might spread through the air and cause potentially fatal infections are studied in BSL-3 facilities. All studies are carried out in biosafety cabinets, which employ tightly regulated airflow or enclosed enclosures to avoid infection. BSL-3 laboratories are built with simple decontamination in mind. As an added safety precaution, these laboratories must utilize regulated, or "directional," airflow to make sure that air flows from non-laboratory regions (such as the corridor) into laboratory spaces. BSL-4 facilities are used to investigate pathogens or toxins that have a high risk of spreading by aerosols and causing serious illness for which there is no cure or vaccination.

1.4.6 Biological-Based Constraints

A difficult task in and of itself is choosing specific PGPR strain(s) for biofertilizer production. The strain(s) should have a wide variety of hosts and not be very selective or targeted (to certain crops). Their selectivity is one of the key challenges that limit them. In contrast to PGPRs, conventional agrochemicals have a tendency to affect the entire resident microbiota. However, because different microbes are present in the field, the quality and effectiveness of these PGPRs inevitably

fluctuates. When selecting suitable isolates, it is important to assess how well they work with a variety of crops in a range of soil conditions and environmental settings (Meena et al. 2020). The strains shouldn't compete with other beneficial bacteria that live in the rhizosphere and should be able to successfully replace the native, inefficient strains (Thomas and Singh 2019). The host plant's roots must be sufficiently colonized by PGPRs for them to be successful as biofertilizers. They also need to be able to create a favorable rhizosphere for plant growth and increase the bioavailability of N, P, K, and antagonistic traits. PGPRs need to have specific qualities in order to be employed as an efficient and successful bio-inoculant. It must be able to endure in soil, work with the crop being inoculated, and interact with both abiotic and biotic soil microorganisms. Any nontarget should be avoided by taking the necessary precautions. These actions will ensure the longevity of the plant growth impact and the successful application of PGPRs as bio-inoculants.

PGPR dynamics, which mostly involves alterations, has a significant role in PGPR colonization. Because different rhizobacteria have different modes of action for promoting plant development, utilizing PGPRs presents additional difficulties. It is recognized that a number of gram-negative rhizobacteria have a biocontrol capability. Due to their incapacity to create spores, they are challenging to formulate. Furthermore, their formulations don't have a longer shelf life, and the desiccation has the tendency to destroy germs (Goswami et al. 2016).

1.4.7 Technical and Infrastructure-Based Constraints

The shelf life of a biofertilizer and the commercialization of an efficient PGPR strain present substantial obstacles. If biofertilizers with a limited shelf life are not used or sold before they expire, they run the risk of being recycled, which would result in a net financial loss for the marketing agency. Considering that biofertilizers include living microbial cells need to be handled with special care and safety measures during storage and transportation. Technical constraints include the potential for product deterioration because of a lower life span or uncontrolled mutations arising throughout fermentation or storage (Itelima et al. 2018). The mutations cause a serious issue that boosts the cost of production and lowers the bio-inoculant's quality while also causing a net decrease in its efficacy. The broad use of bio-inoculants is severely constrained by the inadequate regional availability of soil-specific strains.

Production of biofertilizers and quality control need cutting-edge technology as well as qualified and professional labor. The primary infrastructure-related limitations are a lack of cutting-edge technology, critical technical assistance, suitable equipment, a knowledgeable technical team, and trained personnel (Meena et al. 2020). So, to overcome this constraint, the following measures can be adopted:

- Availability of suitable facilities for the production of biofertilizers.
- Availability of suitable equipment and uninterrupted power supply will decrease the time and need of labor.

- Availability of proper and adequate space for laboratory, production, as well as storage of biofertilizers.

1.4.8 Regulation-Based Constraints

Regulatory constraints include, for instance, the challenges associated with product registration and patent application. The laws are frequently inconsistent and change across various countries and areas. The regulatory procedures are also extremely complicated, and although the costs vary, they are often on the higher side. The steps involved in registering a product's documentation are both substantial and complex. The lack of a clear legal and regulatory definition for "plant biostimulants" is the fundamental reason that there isn't a globally coordinated, uniform regulatory policy (Goswami et al. 2016). The registration of the biocontrol agent is often a two-step process that takes a lot of time and effort. In order to be utilized in any nation, an active component of a biofertilizer often has to get an authorization certificate from the Directorate-General for Health and Consumer Affairs. The item has to receive national approval. The National Commission and Food Safety Authority of each nation will carry out an evaluation and offer necessary comments. After then, there are several rounds of expert examination that might continue for a supplementary 2–3 years. As a result, the entire process of setting up a new company and commercializing biofertilizer takes time and could take several years (Timmusk et al. 2017).

In India, under the statutory provisions of FCO, biofertilizer production and its sales has been regulated and is a mandatory requirement of registration for every manufacturing unit with the State Fertilizer Controller (who is generally the Commissioner or Director of Agriculture Department). Recently, the INM (DoAC & FW-MoA & FW, GOI) had gazette notified the regulations for biostimulants under the FCO 1985 vide the Gazette of India (CG-DL-E-24022021-225,410) Extraordinary, Part -II-Section-3-Sub-section (ii) bearing No. 812 dated February 23, 2021, and bearing S.O. 882 (E) dated February 23, 2021. Each nation has its own rules for biofertilizer registration comprising many steps. The registration process for biofertilizers should be made easier for their sale and adoption at a large scale.

1.4.9 Finance-Based Constraints

A fundamental disadvantage in the large-scale manufacture of biofertilizers is a lack of enough financial resources. Small producers lack the resources to distribute the biofertilizer themselves after it has been produced. This distribution delay lowers the product's quality and impairs its ability to function as a biocontrol agent. In order to promote bioproducts, the "Common Agricultural Policy" in European nations has allocated 30% of its budget to green payments to farmers. To boost the market for bioalternatives for sustainable agriculture, the Indian government has put out a number of policies and strategies. A few of the initiatives that have been formed

are the National Mission of Sustainable Development (NMSA)/Paramparagat Krishi Vikas Yojana, Rashtriya Krishi Vikas Yojana (RKVY), National Mission on Oilseeds and Oil Palm (NMOOP), and Indian Council of Agricultural Sciences (ICAS). The “National Organic Program” (NOP), established by the US Department of Agriculture (USDA), aims to establish standards for agricultural items produced using organic farming techniques and their spread over the anticipated term (Itelima et al. 2018).

Apart from these main constraints, physical and environmental constraints like seasonal demand for biofertilizers, cropping operations of farmers, and characteristics of soil are among other constraints that limit the use of biofertilizers among farmers.

1.5 Future Scope of the Biofertilizer Industry

Global population growth has put enormous stress on the agricultural sector and natural resources to boost food production. Although it has the potential to make the country’s food self-sufficient, the increased use of chemical fertilizers in agriculture actually damages the environment and public health. Despite disdain for sustainability, the green revolution resulted to a significant rise in food production. Future agricultural expansion that is dependent on inorganic fertilizers will result in additional deterioration of soil quality, risk of groundwater contamination, and environmental degradation. An alternate strategy for boosting soil fertility, soil health, and crop output while being environmentally benign has been discovered as using biofertilizers or bioenhancers (Atieno et al. 2020). In order to make nutrients available to the plants for nourishment, biofertilizers provide a biological rescue mechanism that may mobilize nutrients from an unusable state to a usable form.

The biofertilizer market has boomed as a result of rising interest in and government support for organic farming; nevertheless, the quality control of microbe-based biofertilizers has come under scrutiny due to rising demand and limited supply. The production and usage of biofertilizers to increase crop yield and maintain soil health has increased due to our improving understanding of how microorganisms maintain soil fertility. With a CAGR of over 14.3% from 2011 to 2018, the market for biofertilizers reached a value of more than US \$1.8 billion in 2018. Because employing biofertilizers is crucial for organic farming, the worldwide biofertilizer market is growing quickly. It will need a lot of work to investigate success/failure issues for the use of biofertilizers in the Indian setting, with a focus on the requirement for high levels of creativity and engaged engagement in scientific research and development (Meena et al. 2020). In order to maximize the extra potential of sustainable product development without adversely affecting soil health, public demonstration activities will also be required. Another important factor driving demand for these goods is the growing application of regulatory laws by various governments that favor the use of biofertilizers.

1.6 Conclusion

The agricultural industry is crucial in supplying the expanding population's food needs. Although the green revolution in agriculture increased agricultural output, it has also put our environment in peril (soil, water, and air). Therefore, biofertilizers are an appropriate substitute for chemical fertilizers for maintaining agricultural yield and long-term soil fertility as they don't harm the environment or our natural resources. Globally, in the last decade, there has been a tremendous increase in biofertilizer adoption. As people are now becoming more concerned about food safety and quality, more shifts toward these natural biofertilizers are expected in the coming years. To keep up with this trend of biofertilizer adoption among farmers, it is very necessary to take care of various constraints encountered in the application and dissemination of this technique. We should scrutinize various checkpoints and bottlenecks occurring in every aspect of biofertilizer production technology. Thus, for ensuring a greener tomorrow, it is very essential to popularize this technology and educate the farmers about the benefits of using microbe-based biofertilizers.

References

- Arora NK, Khare E, Maheshwari DK (2010) Plant growth promoting rhizobacteria: constraints in bioformulation, commercialization, and future strategies. In: *Plant growth and health promoting bacteria*. Springer, Berlin, Heidelberg, pp 97–116
- Atieno M, Herrmann L, Nguyen HT, Phan HT, Nguyen NK, Srean P, Than MM, Zhiyong R, Tittabutr P, Shutsrirung A, Bräu L (2020) Assessment of biofertilizer use for sustainable agriculture in the great Mekong region. *J Environ Manage* 275:111300
- Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S, Smith DL (2018) Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Front Plant Sci* 9:1473
- Barea JM (2015) Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. *J Soil Sci Plant Nutr* 15(2):261–282
- Bashan Y, de Bashan LE, Prabhu SR, Hernandez JP (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). *Plant and Soil* 378(1):1–33
- Basu A, Prasad P, Das SN, Kalam S, Sayyed RZ, Reddy MS, El Enshasy H (2021) Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. *Sustainability* 13(3):1140
- Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J Microbiol Biotechnol* 28(4):1327–1350
- Chakraborty T, Akhtar N (2021) Biofertilizers: prospects and challenges for future. In: *Biofertilizers: study and impact*, pp 575–590
- Dal Cortivo C, Ferrari M, Visioli G, Lauro M, Fornasier F, Barion G, Panozzo A, Vamerali T (2020) Effects of seed-applied biofertilizers on rhizosphere biodiversity and growth of common wheat (*Triticum aestivum* L.) in the field. *Front Plant Sci* 11:72
- Debnath S, Rawat D, Mukherjee AK, Adhikary S, Kundu R (2019) Applications and constraints of plant beneficial microorganisms in agriculture. In: *Biostimulants in plant science*. IntechOpen, London
- Dineshkumar R, Kumaravel R, Gopalsamy J, Sikder MNA, Sampathkumar P (2018) Microalgae as bio-fertilizers for rice growth and seed yield productivity. *Waste Biomass Valor* 9(5):793–800