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Review

Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects

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Abstract: The quest for enhancing agricultural yields due to increased pressure on food production has inevitably led to the indiscriminate use of chemical fertilizers and other agrochemicals. Biofertilizers are emerging as a suitable alternative to counteract the adverse environmental impacts exerted by synthetic agrochemicals. Biofertilizers facilitate the overall growth and yield of crops in an eco-friendly manner. They contain living or dormant microbes, which are applied to the soil or used for treating crop seeds. One of the foremost candidates in this respect is rhizobacteria. Plant growth promoting rhizobacteria (PGPR) are an important cluster of beneficial, root-colonizing bacteria thriving in the plant rhizosphere and bulk soil. They exhibit synergistic and antagonistic interactions with the soil microbiota and engage in an array of activities of ecological significance. They promote plant growth by facilitating biotic and abiotic stress tolerance and support the nutrition of host plants. Due to their active growth endorsing activities, PGPRs are considered an eco-friendly alternative to hazardous chemical fertilizers. The use of PGPRs as biofertilizers is a biological approach toward the sustainable intensification of agriculture. However, their application for increasing agricultural yields has several pros and cons. Application of potential biofertilizers that perform well in the laboratory and greenhouse conditions often fails to deliver the expected effects on plant development in field settings. Here we review the different types of PGPR-based biofertilizers, discuss the challenges faced in the widespread adoption of biofertilizers, and deliberate the prospects of using biofertilizers to promote sustainable agriculture.

Keywords: biofertilizer; bioinoculant; PGPR; rhizosphere; sustainable agriculture

Citation: Basu, A.; Prasad, P.; Das, S.N.; Kalam, S.; Sayyed, R.Z.; Reddy, M.S.; El Enshasy, H. Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects. *Sustainability* **2021**, *13*, 1140. https://doi.org/10.3390/su13031140

Received: 24 December 2020
Accepted: 18 January 2021
Published: 22 January 2021

1. Introduction

The advent of the Green Revolution in the latter part of the twentieth century triggered a worldwide boom in the agriculture sector. By introducing new high-yielding seed varieties and increasing the use of synthetic fertilizers, pesticides, and other agrochemicals, the Green Revolution contributed significantly to enhanced plant productivity and crop yields [1]. The global agricultural landscape has drastically changed since then. Rampant overuse of synthetic agrochemicals for enhancing crop productivity has deteriorated the biological and physicochemical health of the arable soil, leading to a declining trend in agricultural productivity across the globe over the past few decades [2–4]. In the present scenario, there is a shrinkage of land resources and the depletion of biological wealth. In order to fulfill the escalating demand for sustainable agriculture, the yield and productivity of agricultural crops need to be concurrently increased with the production of agriculture-related commodities. There is no single or straightforward solution to the above-mentioned intricate, ecological, socio-economic, and technical glitches existing in promoting sustainable agriculture [1].

Promoting sustainable agriculture with a gradual decrease in the use of synthetic agrochemicals and more prominent utilization of the biowaste-derived substances [5,6] as well as the biological and genetic potential of crop plants and microorganisms is an effective strategy to combat the rapid environmental deterioration while ensuring high agricultural productivity and better soil health [7]. In addition to the genetic manipulation of the crop physiology and metabolism for yield enhancement, certain members of the soil microbial community, particularly those residing in the plant rhizosphere, might assist plants in preventing or partially overcoming the environmental stresses [8,9]. Search for eco-friendly alternatives to mitigate the harmful effects of toxic agrochemicals led to the discovery and subsequent use of biofertilizers and other microbial-based products, including organic extracts and vermicompost teas [10–12]. These microbial products are non-toxic, environment-friendly, and act as potential tools for plant growth promotion and disease control. Thus, the biological potential and fertility of soil could be increased, whereas the hazardous effects of agrochemicals could be decreased by employing microbial formulations to fertilize agricultural crops [13–15]. The use of efficient plant growth promoting rhizobacteria (PGPR) as biofertilizers and biological control agents is deliberated as a suitable substitute for minimizing the use of synthetic agrochemicals in crop production [16–19]. This review concisely and holistically provides deeper insights into the various aspects of PGPR-based biofertilizers, their prospects and constraints, and finally the roadmap to their commercialization.

2. Biofertilizers

During the past two decades, the term biofertilizer or bioinoculant has been derived in various ways due to the commendable progress achieved in the studies of the association between microorganisms and plants. A biofertilizer is most commonly defined as "a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant" [16]. Dineshkumar et al. [20] later proposed a modified definition of biofertilizers as "products (carrier or liquid based) containing living or dormant microbes (bacteria, actinomycetes, fungi, algae) alone or in combination, which help in fixing atmospheric nitrogen or solubilizers soil nutrients in addition to the secretion of growth promoting substances for enhancing crop growth and yield".

The microorganisms present in the biofertilizers employ several mechanisms to provide benefits to the crop plants. They can either be efficient in nitrogen fixation, phosphate solubilization, and plant growth promotion or can possess a combination of all such traits [21–24]. Biofertilizers can fix atmospheric N_2 through the biological nitrogen fixation (BNF) process, solubilize nutrients required by the plants, such as phosphate, zinc, and potassium, and also secrete plant growth promoting substances, including various hormones [25,26]. Further, when applied as seed or soil inoculants, biofertilizers can multiply, participate in nutrient cycling, and help in crop production for sustainable farming [27–29].

The microbial inoculants possess several advantages over their chemical counterparts [30–32]. They are eco-friendly, sound sources of renewable nutrients required for maintaining soil health and biology [13,23,29]. Furthermore, they exhibit antagonistic activity against several agricultural pathogens and combat abiotic stresses [8,33–36]. Various microbial taxa have been commercially used as efficient biofertilizers, based on their ability to obtain nutrients from the soil, fix atmospheric N_2, stimulate the solubilization of nutrients, and act as biocontrol agents [37].

3. Plant Growth Promoting Rhizobacteria (PGPR)—The Phyto-Friendly Soil Microbes

Plant rhizosphere, the narrow zone of soil surrounding the root system of growing plants, represents a hotspot for microbial activity in the soil [38]. The rhizosphere is colonized by a wide range of microbial taxa, including both prokaryotes (archaea, bacteria, and viruses) and eukaryotes (fungi, oomycetes, nematodes, protozoa, algae, and arthropods), out of which bacteria and fungi comprise the most abundant groups [39,40] exhibiting fundamental ecological functions. Free-living soil bacteria that thrive in the rhizosphere, aggressively colonize plant roots, and facilitate plant growth are designated as plant growth promoting rhizobacteria (PGPR), a term introduced by Kloepper and Schroth in 1978 [41].

This heterogeneous group of bacteria, representing a vital component of the soil microbiome, is known to produce and secrete various regulatory chemicals in the plant roots' vicinity that aid in plant growth promotion [42,43]. PGPRs influence plants' overall health by contributing to enhanced nutrient acquisition by host plants, protecting against phytopathogenic microbes, and promoting resistance to various abiotic stresses [30,44]. Different PGPR strains are capable of increasing crop yields, exhibit biocontrol, enhance resistance to foliar pathogens, promote nodulation in legumes, and enhance the emergence of seedlings [45–50]. Reported PGPRs include members of the genera *Acinetobacter*, *Aeromonas*, *Agrobacterium*, *Allorhizobium*, *Arthrobacter*, *Azoarcus*, *Azorhizobium*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Delftia*, *Enterobacter*, *Flavobacterium*, *Frankia*, *Gluconacetobacter*, *Klebsiella*, *Mesorhizobium*, *Micrococcus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Streptomyces*, *Thiobacillus*, and others [16,43,44,46,51–53]. An overview of the diverse phytobeneficial effects of PGPRs is represented in Table 1.

3.1. Characteristics of an Ideal PGPR

A rhizobacterial strain is considered to be a putative PGPR if it possesses specific plant growth promoting traits and can enhance plant growth upon inoculation. An ideal PGPR strain should fulfill the following criteria [45]:

(1) It should be highly rhizosphere-competent and eco-friendly.
(2) It should colonize the plant roots in significant numbers upon inoculation.
(3) It should be able to promote plant growth.
(4) It should exhibit a broad spectrum of action.
(5) It should be compatible with other bacteria in the rhizosphere.
(6) It should be tolerant of physicochemical factors like heat, desiccation, radiations, and oxidants.
(7) It should demonstrate better competitive skills over the existing rhizobacterial communities.

Table 1. An overview of the benefits of plant growth promoting rhizobacteria (PGPR) inoculation to plants.

Benefits of PGPR Inoculation to Plants	PGPR Strain(s)	Tested Plant(s)	Reference(s)
Tolerance to drought stress	*Pseudomonas fluorescens* DR11, *Enterobacter hormaechei* DR16, *Pseudomonas migulae* DR35, *Bacillus subtilis*, *Achromobacter piechaudii* ARV8, *Phyllobacterium brassicacearum*, *Paenibacillus polymyxa*, *Rhizobium tropici*, *Azospirillum brasilense*	Foxtail millet (*Setaria italica* L.), Maize (*Zea mays* L.), Bean (*Phaseolus vulgaris* L.), *Arabidopsis thaliana*, Tomato (*Lycopersicum esculentum* Mill cv. F144), Pepper (*Capsicum annuum* L. cv. Maor), Wheat (*Triticum aestivum* L.)	[36,54–59]
Tolerance to salinity stress	*Bacillus pumilus*, *Exiguobacterium oxidotolerans*, *Bacillus megaterium*, *Azospirillum* sp., *Achromobacter piechaudii*, *Eneterobacter* sp. PR14	Brahmi (*Bacopa monnieri* L.), Maize (*Zea mays* L.), Lettuce (*Lactuca sativa* L.), Tomato (*Lycopersicum esculentum* Mill.), Rice (*Oryza sativa* cv. Sahbhagi), Sorghum (*Sorghum bicolor*), Finger Millets (*Eleusine coracana*)	[60–64]
Tolerance to biotic stress (biocontrol)	*Paenibacillus xylanexedens*, *Bacillus amyloliquefaciens*, *Streptomyces* sp., *Ochrobacttrum intermedium*, *Paenibacillus lentimorbus*, *Pseudomonas* spp.	Wheat (*Triticum aestivum* L.), Rice (*Oryza sativa*), Pine (*Pinus taeda* L.), Tomato (*Lycopersicum esculentum* Mill.)	[65–70]
Increased nutrient absorption	*Pantoea* sp. S32, *Paenibacillus polymyxa*	Rice (*Oryza sativa* L.), Habanero pepper (*Capsicum chinense*)	[71–73]
Seed germination enhancement	*Serratia marcences*, *Pseudomonas fluorescens*, *Azospirillum lipoferum*, *Pseudomonas putida*, *Bacillus subtilis*, *Providencia* sp., *Brevundimonas diminuta*	Maize (*Zea mays* L.), Wheat (*Triticum aestivum* L.)	[74–76]
Biostimulation by phytohormone(s) production	*Azospirillum lipoferum*, *Bacillus subtilis*, *Arthrobacter protophormiae*, *Dietzia natronolimnaea*, *Bacillus* sp.	Rice (*Oryza sativa* L.), Tomato (*Solanum lycopersicum* L.), Wheat (*Triticum aestivum* L.)	[46,77–79]
Soil fertility enhancement	*Bacillus subtilis*, *Bacillus cereus*, *Rhizobium* spp.	Poplar (*Populus* sp.), Mung bean (*Vigna radiata* L.)	[80–82]
Bioremediation of heavy metals and pollutants	*Ochrobactrum* sp., *Bacillus* spp., *Pseudomonas* spp., *Pseudomonas fluorescens*, *Bacillus cereus*, *Alcaligenes feacalis* RZS2, *Pseudomonas aeruginosa* RZS3, *Enterobacter* sp. RZS5	Rice (*Oryza sativa* L.), Groundnut (*Arachis hypogaea*), Maize (*Zea mays* L.), Ashwagandha (*Withania somnifera*)	[83–88]
Modulation of plant secondary metabolites	*Bacillus subtilis*, *Azotobacter chroococcum*, *Pseudomonas putida*, *Bacillus pumilus*, *Exiguobacterium oxidotolerans*	Basil (*Ocimum basilicum*), Brahmi (*Bacopa monnieri* L.)	[89,90]

3.2. Mechanisms of PGPR Action

Being the dominant rhizosphere microbial community, PGPRs are actively or passively involved in plant growth promotion. They can act as biofertilizers that promote plants' growth and development by facilitating biotic and abiotic stress tolerance and supporting host plants' nutrition [64,86,91,92]. These beneficial groups of bacteria, through their multifaceted modes of action, including root colonization, positive effects on plant physiology and growth, biofertilization, induced systemic resistance, biocontrol of phytopathogens, etc., offer protection to plants and facilitate plant growth promotion. The detailed mechanisms of PGPR action and their specific contribution to plant growth promotion have been reviewed comprehensively [30,41–44,47–49,51,52,93–102]. The modes of action by which PGPRs promote plant growth have been traditionally classified into direct and indirect mechanisms occurring inside and outside the plant, respectively [51,99] (Figure 1).

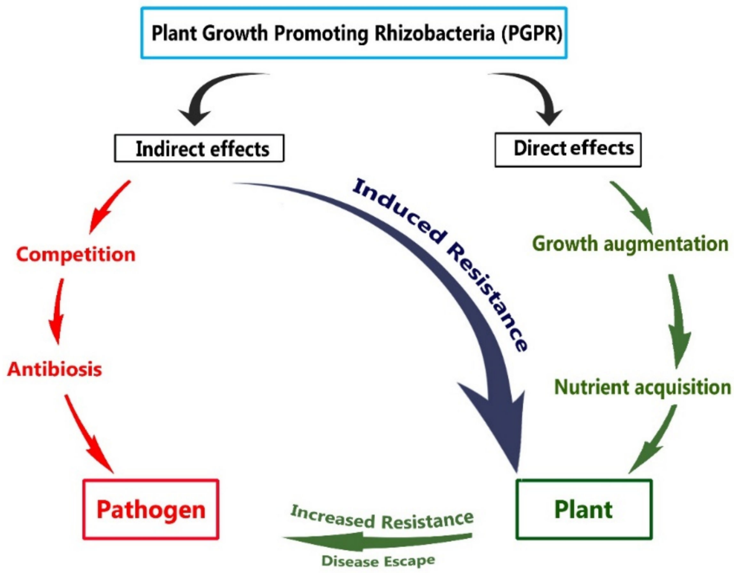

Figure 1. Main overview of interactions between plant growth promoting rhizobacteria (PGPR), plants, and pathogens. PGPRs directly promote plant growth by improving nutrient acquisition by the plant and growth augmentation via regulating phytohormone levels. The indirect effects of PGPRs include suppression of phytopathogens and inducing systemic resistance in plants against a wide range of pathogenic microbes.

Direct modes of PGPR action include improving plant nutrition by providing phytonutrients like fixed nitrogen or solubilized minerals from the soil (like P, K, Zn, Fe, and other essential mineral nutrients) and/or stimulating plant growth and development by regulating phytohormone levels (like auxins, cytokinins, gibberellins, abscisic acid, and ethylene) [44,46,95]. The indirect effects of PGPRs include influencing the plant health by suppressing phytopathogens and other deleterious microorganisms through parasitism, competing for nutrients and niche within the rhizosphere, producing antagonistic substances (like hydrogen cyanide, siderophores, antibiotics, and antimicrobial metabolites) and lytic enzymes (like chitinases, glucanases, and proteases), and inducing systemic resistance in plants against a broad spectrum of root and foliar pathogens [32,81,103,104]. Due to these direct and indirect effects elicited by PGPRs on host plants, they prove to be ideal candidates to be formulated and commercialized as bioinoculants and phytoprotective microbial products. However, the mode and mechanism of PGPR action vary with the host plant type [105]. In addition to this, certain other factors also influence PGPR action, viz. biotic factors like plant genotype, developmental stages, plant defense mechanisms, and presence of other members of the microbial community and abiotic factors like soil type, composition, soil management history, and prevalent environmental conditions [95,106].

4. Global Biofertilizer Market

During the past few decades, the biofertilizer market has seen a global boom in its production and utilization. Due to the unavailability of cultivable land and to cater to the need of the exploding population for agricultural products, the global biofertilizers market has gathered enough momentum. The global biofertilizer market represents a tiny fraction of the synthetic agrochemicals market [107]. The nitrogen-fixing biofertilizers dominate the market with the lion's share of about 80%, followed by the phosphate-solubilizing biofertilizers with a meager 14% share (Figure 2) [107,108]. *Rhizobium* spp., *Azotobacter* spp., and *Azospirillum* spp. are the major nitrogen-fixing biofertilizers available in the market. Although these nitrogen-fixing biofertilizers are primarily used for growing pulses and other leguminous crops, they are also applied to grow selected cereals and cash crops as well [107,109].

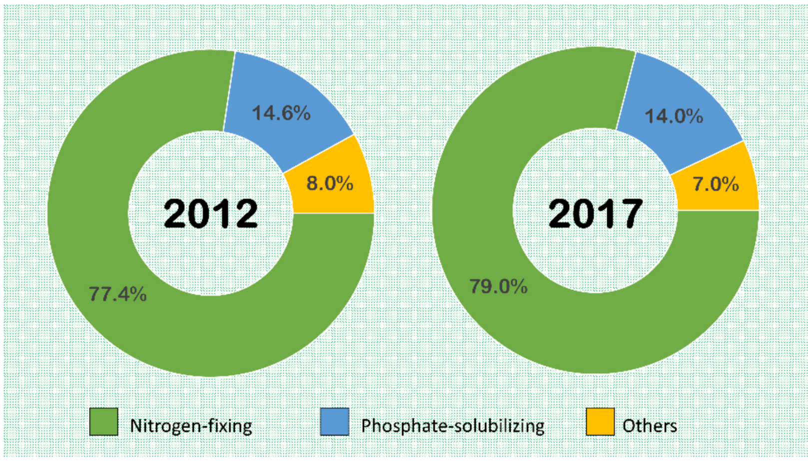

Figure 2. Global biofertilizer market share by product typology (nitrogen-fixing and phosphate-solubilizing microbe-based biofertilizers and others). Market data of 2012 (left panel) and 2017 (right panel) respectively compiled from Timmusk et al. [107] and Soumare et al. [108].

Geographically, the global biofertilizer market canopies several regions of the world, such as North America, Europe, Asia-Pacific, Latin America, Middle East, and Africa (Figure 3). In terms of revenues generated from biofertilizer production, North America (USA, Canada, and Mexico) dominates the global biofertilizer market, followed by Europe (Germany, UK, Spain, Italy, Hungary, and France) and the Asia-Pacific region (China, Japan, India, Australia, New Zealand, and the rest of Asia). As of 2017, the biofertilizer markets were valued at USD 495 million in North America, USD 450 million in Europe, USD 284 million in Asia-Pacific, USD 240 million in South America, and USD 44 million in Africa [108]. It is estimated that the global biofertilizer market would reach USD 3.5 billion by 2025. Some of the commonly used PGPR-based biofertilizer products commercially available across the globe are represented in Table 2.

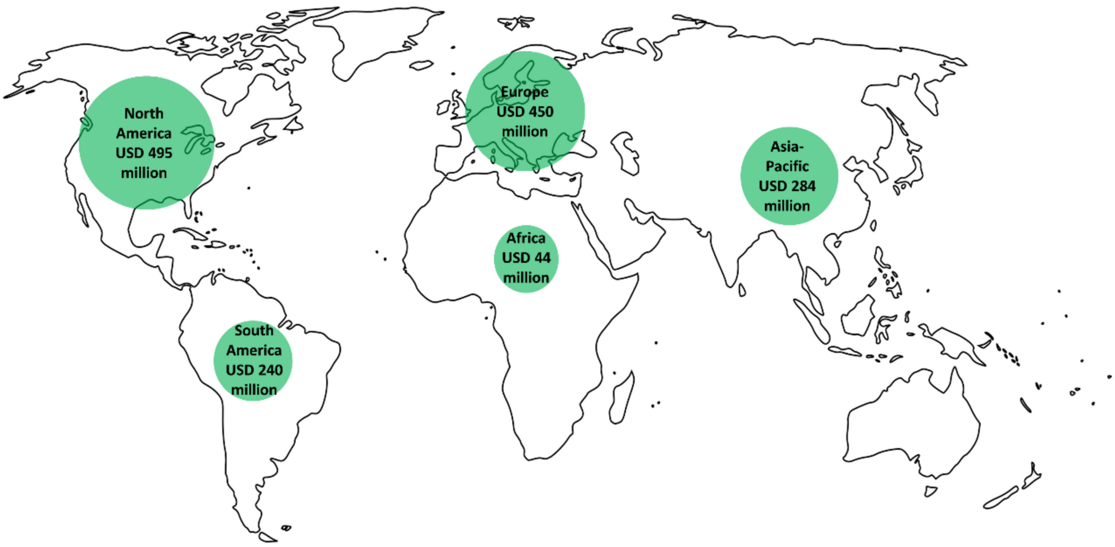

Figure 3. Size and distribution of the global biofertilizer market in USD million per region. The area of each circle is proportional to the size of the biofertilizer market (in USD million) in the specific region. Data compiled from Soumare et al. [108].

Table 2. An overview of globally available PGPR-based biofertilizer products.

Type of Biofertilizer	Name of Biofertilizer	PGPR Strain(s)	Manufacturer's Country	Market Region	Reference(s)
Nitrogen fixer	Nitragin Gold®	Rhizobia	USA	North America	[110]
	Cell-Tech®	Rhizobia	USA	North America	[110]
	TagTeam®	Rhizobia, *Penicillium bilaii*	USA	North America	[110]
	Custom N2	*Paenibacillus polymyxa*	USA	North America	[110]
	Nodulator®	*Bradyrhizobium japonicum*	Canada	North America	[110]
	Nodulator® PRO	*Bacillus subtilis, Bradyrhizobium japonicum*	Canada	North America	[110]
	Bioboots®	*Delftia acidovorans, Bradyrhizobium* sp.	Canada	North America	[105,110]
	Azofer®	*Azospirillum brasilense*	Mexico	North America	[110]
	Rhizofer®	*Rhizobium etli*	Mexico	North America	[110]
	Nitrofix®	*Azospirillum* sp.	Cuba	North America	[105,110]
	Rhizosum N®	*Azotobacter vinelandii, Rhizophagus irregularis*	Spain	Europe	[110,111]
	Rhizosum Aqua	*Azospirillum* sp.	Spain	Europe	[105,110]
	Legume Fix	*Rhizobium* sp., *Bradyrhizobium japonicum*	UK	Europe	[112,113]
	BactoFil® A10	*Azospirillum brasilense, Azotobacter vinelandii, Bacllius megaterium*	Hungary	Europe	[112]
	BactoFil® Soya	*Bradyrhizobium japonicum*	Hungary	Europe	[114]
	Phylazonit M	*Azotobacter chroococcum, Bacillus megaterium*	Hungary	Europe	[115]
	Azotobacterin®	*Azospirillum brasilense* B-4485	Russia	Europe	[105,110]
	Azoter	*Azotobacter chroococcum, Azospirillum brasilense, Bacillus megaterium*	Slovakia	Europe	[116]
	TwinN®	*Azorhizobium* sp., *Azoarcus* sp., *Azospirillum* sp.	Australia	Asia-Pacific	[113]
	TripleN®	*Azorhizobium* spp., *Azoarcus* spp., *Azospirillum* spp.	Australia	Asia-Pacific	[111]

Table 2. *Cont.*

Type of Biofertilizer	Name of Biofertilizer	PGPR Strain(s)	Manufacturer's Country	Market Region	Reference(s)
Nitrogen fixer	Bio-N	*Azospirillum* spp.	Philippines, Australia	Asia-Pacific	[112,117]
	BioGro®	*Pseudomonas fluorescens / putida, Klebsiella pneumoniae, Citrobacter freundii*	Vietnam	Asia-Pacific	[117]
	Mamezo®	Rhizobia	Japan	Asia-Pacific	[105,110]
	Agrilife Nitrofix	*Azotobacter chroococcum, A. vinelandii, Acetobacter diazotrophicus, Azospirillum lipoferum, Rhizobium japonicum*	India	Asia-Pacific	[118]
	Ajay Azospirillum	*Azospirillum* sp.	India	Asia-Pacific	[112]
	Symbion N	*Azospirillum* sp., *Rhizobium* sp., *Acetobacter* sp., *Azotobacter* sp.	India	Asia-Pacific	[115]
	Zadspirillum	*Azospirillum brasilense*	Argentina	South America	[112]
	Rizo-Liq	*Bradyrhizobium* sp., *Mesorhizobium ciceri, Rhizobium* spp.	Argentina	South America	[112,113]
	Nodulest 10	*Bradyrhizobium japonicum*	Argentina	South America	[118]
	Rizo-Liq Top	*Bradyrhizobium japonicum*	Argentina	South America	[113]
	BiAgro 10®	*Bradyrhizobium japonicum*	Argentina, Brazil, Bolivia	South America	[117]
	Dimargon®	*Azotobacter chroococcum*	Colombia	South America	[117]
	Nitrasec	*Rhizobium* sp.	Uruguay	South America	[112]
	Biofix	Rhizobia	Kenya	Africa	[112,113]
	Nodumax	*Bradyrhizobium* spp.	Nigeria	Africa	[112,113]
	Azo-N	*Azospirillum brasilense, A. lipoferum*	South Africa	Africa	[113]
	Azo-N Plus	*Azospirillum brasilense, A. lipoferum, Azotobacter chroococcum*	South Africa	Africa	[113]

Table 2. *Cont.*

Type of Biofertilizer	Name of Biofertilizer	PGPR Strain(s)	Manufacturer's Country	Market Region	Reference(s)
Phosphate solubilizer	Fosforina®	*Pseudomonas fluorescens*	Cuba	North America	[117]
	Rhizosum PK®	*Bacillus megaterium*, *Frateuria aurantia*, *Rhizophagus irregularis*	Spain	Europe	[110,111]
	Phosphobacterin	*Bacillus megaterium* var. *phosphaticum*	Russia	Europe	[31]
	CataPult	*Bacillus* spp., *Glomus intraradices*	Australia	Asia-Pacific	[118]
	Symbion van Plus	*Bacillus megaterium*	India	Asia-Pacific	[112]
	P Sol B	*Pseudomonas striata*, *Bacillus polymyxa*, *B. megaterium*	India	Asia-Pacific	[115,118]
	CBF	*Bacillus mucilaginosus*, *B. subtilis*	China	Asia-Pacific	[117]
	Bio Phos®	*Bacillus megaterium*	Sri Lanka	Asia-Pacific	[115,118]
Potassium solubilizer	Rhizosum K	*Frateuria aurantia*	Spain	Europe	[105,110]
	K Sol B	*Frateuria aurantia*	India	Asia-Pacific	[118]
Zinc solubilizer	Biozink®	PGPR consortia	India	Asia-Pacific	[110]
	Zn Sol B	*Thiobacillus thiooxidans*	India	Asia-Pacific	[118]
Phytostimulator	EVL Coating®	PGPR consortia	Canada	North America	[105]
	Amase®	*Pseudomonas azotoformans*	Sweden	Europe	[114,118]
	Bio Gold	*Azotobacter chroococcum*, *Pseudomonas fluorescens*	Sri Lanka	Asia-Pacific	[115,118]
	Bioativo	PGPR consortia	Brazil	South America	[112]
Biocontrol	Cedomon®	*Pseudomonas chlororaphis*	Sweden	Europe	[114]
	Cedress®	*Pseudomonas chlororaphis*	Sweden	Europe	[114]
	Cerall®	*Pseudomonas chlororaphis*	Sweden	Europe	[114]
	Biotilis	*Bacillus subtilis*	India	Asia-Pacific	[118]
	Soilfix	*Brevibacillus laterosporus*, *Paenibacillus chitinolyticus*	South Africa	Africa	[112]

5. Challenges and Constraints with PGPR-Based Biofertilizers

Presently, there is an escalating interest in the use of microbial-based products as bioinoculants. Still, their use is associated with several challenges moving from the lab to the field. The preliminary use of these bioinoculants has been made on crop plants such as legumes and cereals [119]. For developing a new PGPR strain as an effective bioinoculant, an initial laboratory screening is required, which depends on specific direct and indirect mechanisms of plant growth promotion by PGPRs. Mere primary screening of axenic culture isolates for PGPR traits does not guarantee efficacious plant growth promotion under field conditions. Parallelly, those pure culture isolates that exhibit less in vitro growth promoting activities might possess different plant growth promotion strategies. Because these mechanisms are not fully understood, such isolates exhibit difficulty in screening under standard conditions. Henceforth, sometimes such useful strains exhibiting these mechanisms get discarded due to their poor in vitro performance [120]. The large-scale utilization and application of PGPRs necessitate addressing several important issues and overcoming quite a few challenges and constraints (Figure 4).

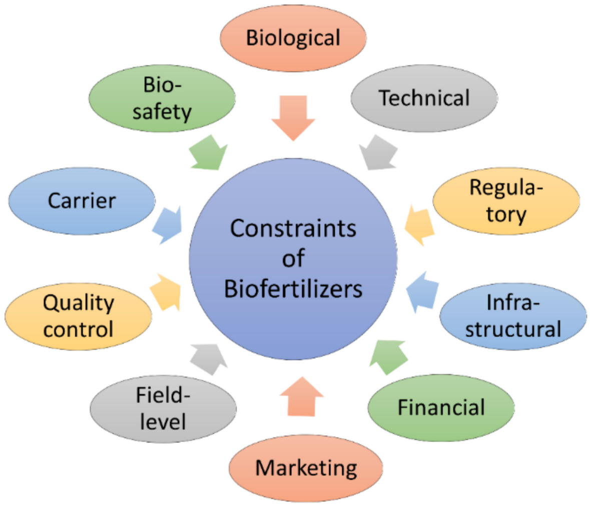

Figure 4. Constraints in the utilization, production, and commercialization of PGPR-based biofertilizers.

5.1. Biological Constraints

Selection of specific PGPR strain(s) for biofertilizer development is a challenge in itself. The strain(s) should not be selective or highly targeted (to specific crops) in nature, and it should exhibit a broad host range. One of the main limiting issues is their selectivity. Conventional agrochemicals tend to impact the entire resident microbiota, whereas PGPRs remain highly targeted and specific. Nevertheless, the quality and efficacy of these PGPRs under field conditions invariably changes due to the presence of several other microorganisms. Potential isolates should be selected based on their performance under field conditions with a wide range of crops across diverse soil types and environmental conditions [32]. The strains must be effective in replacing the native inefficient strains and should not antagonize with other beneficial microbes in the rhizosphere [31].

As biofertilizers, PGPRs should be able to sufficiently colonize host plant roots, create a proper rhizosphere for plant growth, and increase the bioavailability of N, P, K, and antagonistic properties [16,45]. PGPRs should possess specific characteristics for their utilization as an efficient and successful bioinoculant. It should be able to survive in soil, compatible with the crop on which it is inoculated, and interact with indigenous microflora in soil and abiotic factors. Necessary measures should be taken to avoid any non-target effect of the bioinoculant and stabilize them in soil systems. These measures will guarantee the durability of the plant growth effect and the good performance of introduced PGPRs as bioinoculants.

An important factor in PGPR colonization is PGPR dynamics, which mainly changes with the host crop, the midterm and long-term effects, the crop-rotation impact, and site variation. Another challenge using PGPRs is their diverse mode of action, as all the rhizobacteria do not possess the same mechanisms of action for plant growth promotion [121]. Several Gram-negative rhizobacteria are known to exhibit biocontrol potential. The constraint arises in their formulation, as they are difficult to formulate because of their inability to produce spores. In addition to this, their formulations lack a longer shelf life, and the bacteria are prone to get killed upon desiccation [51,122,123].

5.2. Technical Constraints

One of the significant challenges encountered during the development of a biofertilizer and the commercialization of an effective PGPR strain is its shelf life [22,124]. Biofertilizers with a short shelf life carry the risk of recycling if they are not used or sold before expiry resulting in a net monetary loss to the marketing agency. Since biofertilizers contain live microbial cells, their storage and transportation require extra care and precaution. The technical constraints involve the risk of deterioration of the product due to shorter shelf life or spontaneous mutations arising during fermentation or storage [31]. The mutations result in a net reduction in bioinoculant effectiveness and lead to a severe problem that raises the cost of production and quality of the bioinoculant. Inadequate availability of soil-specific strains region-wise considerably limits the widespread use of bioinoculants.

5.3. Regulatory Constraints

Regulatory constraints include the challenges in product registration and patent filing. The rules often vary between different regions and nations and are not consistent. In addition, the regulatory processes are quite complex, and the fees, though variable, are mostly on the higher side [32,107]. The documentation procedures for product registration are equally extensive and complicated. The absence of a standardized legal and regulatory definition for "plant biostimulants" is the primary reason behind the lack of a globally coordinated uniform regulatory policy [30,125].

The process of registering the biocontrol agent within a country is normally in two phases and is quite lengthy and complicated [32,107]. Generally, in any country, the active ingredient present within a biofertilizer must get an authorization certificate from the Directorate-General for Health and Consumer Affairs, and after that, the formulated product has to be nationally approved. The Food Safety Authority and the National Commission of any country will critically analyze and give relevant comments followed by several rounds of review by experts, sometimes taking an additional two to three years. Thus, the entire process starting from registration to commercializing a potential biofertilizer is lengthy and might stretch to several years. The countries have their own guidelines and norms to respond in their specific language, and the registering agency can also require even additional data.

5.4. Infrastructural Constraints

Manufacturing and quality control of biofertilizers involve sophisticated technology and qualified and trained human resources. Lack of sophisticated technology, necessary technical support and proper equipment, trained workforce, and skilled technical personnel are the major infrastructural constraints [31].

5.5. Financial Constraints

Lack of sufficient financial resources in the large-scale production of biofertilizers is a significant drawback [124]. Once the biofertilizer is manufactured, small producers do not have enough funds to distribute on their own. Because of this delay in distribution, lowering of the quality of the product occurs, deteriorating its biocontrol potential [31].

5.6. Marketing Constraints

One of the major limitations for developing the product in the market is the unavailability of proper transportation services along with storage facilities. Farmers possess little or inadequate knowledge regarding the advantages of biofertilizers over hazardous agrochemicals for sustainable agriculture. Thus, the demand for such eco-friendly products is reduced. The establishment of extension centers does not help in creating awareness among farmers due to the lack of well-qualified technical staff [31].

The biofertilizer developers face a significant problem because the agricultural crops are grown under various physicochemical and environmental conditions, including diverse ranges of temperature, rainfall, soil type, and crop variety. These conditions tend to change from farm to farm or even within a single field. Therefore, such variations cause a discrepancy in the efficacy of PGPR-based biofertilizers [122,126].

There is a general strategy followed in any state within a country before any microbial products attain the stage of commercialization. The ministry/department of agriculture gives a green signal for placing orders mostly from their own production units. From here, biofertilizer packets are transported to several districts. A chain of extension workers gets involved in the next step before these packets reach the field. During this course, the microorganisms present as bioinoculants get exposed to high temperatures (above 40 °C), which might lead to either their inactivation or death, thus rendering them low- or poor-quality biofertilizers. Henceforth, these low-quality packets will be disadvantageous for the farmers, as well as for the entire crop yield.

5.7. Field-Level Constraints

The response of crops toward the applied biofertilizers is very slow and sometimes futile since the inoculum will take time to build its concentration and root colonization. This results in a low level of acceptance of biofertilizers by the farmers. The purity of inoculants, along with inoculation techniques, play a vital role in field application. The effectiveness of biofertilizers gets reduced because of the harmful residual effects of synthetic chemicals and existing unfavorable abiotic conditions [31,127]. Environmental stresses such as salt and drought in certain areas play another important role in reducing biological activity. The inoculants are under biotic and abiotic stresses [124]. In addition to these factors, several other factors that holistically result in poor performance of the bioinoculants include acidity and alkalinity of the soil and application of pesticides and high concentrations of nitrate in the soil, limiting the N-fixing ability of the bioinoculants. Many soils possess toxic concentrations of heavy metals like Cd, Hg, Cr, etc., and a deficiency of other important nutrients like P, Cu, Mo, and Co that reduce the biological potential of the PGPR-based fertilizers [23,128].

PGPRs function through a series of mechanisms. The foremost step in plant growth promotion is the colonization of plant roots by the microbe, which is an intricate process requiring the ability of bacteria to compete in the rhizosphere soil for a suitable niche to bring about a positive plant-microbe interaction [129]. In addition to this, the abiotic factors, viz. soil type, temperature, pH, radiation, oxygen concentration, nutrient availability, and the degree of interaction with the native soil microbiota, too drastically affect the plant-microbe interaction, affecting their existence and survivability within the host plant. Thus, the success of the field application of PGPRs depends upon the climatic factors required for a particular variety of cultivated crops [21]. Identification of region-specific microbial strains is highly recommended to exhibit maximum effectiveness by the employed PGPR strain. Quite often, PGPRs are directly used as an inoculum for host plants without mixing them with an appropriate carrier. In addition to this, their quantities are insufficient to allow efficient rhizosphere colonization existing in a field because of the competition with the already existing soil micro- and macro-biota [130].

Broad-spectrum biocidal fumigants are generally used to fumigate soils associated with high-value crops. These fumigants result in altering the microbial community of such soils. As a consequence of long-term fumigation, soil microbial community, and their beneficial interactions that help host plants obtain nutrients and mobilization, get largely affected [131]. This leads to decreased rhizosphere colonization by the PGPR inoculant.

5.8. Quality Control Constraints

The most important parameter which the farmers look for in any biofertilizer is quality control. Being natural products, living microorganisms possess a very short shelf life [32]. The failure of any microbial-based product in fields can be due to the supply of low- or spurious-quality products. Presently, there is the unavailability of any quality check for biofertilizers. Henceforth, in order to prove the plant growth promoting efficacy in the fields, setting up quality control standards for biofertilizers is quite essential [31].

5.9. Biofertilizer Carrier

A suitable carrier is required for field application of biofertilizer because of the short shelf life of the bioinoculant agent. Thus, the unavailability of an appropriate carrier proves to be one of the major constraints for its large-scale use in fields. Ideal carriers used in biofertilizer production are peat, charcoal, lignite, etc. These carriers again pose technical constraints because most of them are unavailable in developing countries like India. There is a lack of sufficient quantities and a desirable quality of these carriers. Only charcoal is readily available in the Indian market, and therefore it can be used as a formulating agent [31]. Peat is recognized as the most suitable carrier among the available carriers, but the challenge is its shorter shelf life, which is less than six months. Due to its ability to improve soil and plant health, biochar can be used as a suitable carrier for biofertilizers [14,30]. In order to prove itself as an efficient and potential carrier, the bioinoculant should possess several other characteristics. It should be of low cost, the organic matter content and water-holding capacity should be high, and the organism-retention capacity should be longer. It should be nearly sterile, with zero moisture content, and it should be non-polluting, non-toxic, and with nearly neutral pH so that the biofertilizer is of good quality [132].

5.10. Biosafety of PGPRs

PGPRs are considered to be practical candidates for sustainable agriculture. An essential characteristic of PGPRs and other biofertilizer agents is that these microbes should not elicit any harmful effects on the environment or humans. According to the guidelines on biosafety in microbiological and biomedical laboratories, published by the U.S. Department of Health and Human Services in 1999 and World Health Organization guidelines on the usage of microorganisms, biosafety levels (BSLs) were made to categorize the usable microorganisms in a range of biosafety classes, based on the different categories of risk posed by them [32]. The communicable agents were classified into four risk groups (BSL-1–4) based on their pathogenicity to human health, mode of transmission, and available treatments. These levels have to be strictly followed in handling these microorganisms. The microbial strains selected for biofertilizer development should preferably belong to the low-risk group of non-pathogenic BSL-1 microorganisms.

6. Guidelines and Precautions for Using PGPRs as Biofertilizers

The major safety measures and guidelines [31] essential for using PGPRs as biofertilizers are:

(1) It is essential that the supplied biofertilizer to be used in fields is of good quality, contains 10^7 viable cells per gram as an inoculum, and is purchased from a reputed manufacturer only.
(2) Since the biofertilizer exhibits specificity, it should only be used for the crop(s) specified on the commercially available product packet.
(3) The culture bag should have a tag of the name of the crop for which it has to be used.
(4) While inoculating, excess culture should be inoculated, or any remnants/residual culture should be immediately put in grooves of the field so that inoculum microorganisms start interacting with other microbiota in the rhizosphere and begin colonizing the rhizosphere.

(5) Since the biofertilizers are microbial products, for achieving better shelf life, before their application in fields, they should be stored in cool and shady places, preferably at room temperature (25–28 °C).
(6) During storage or application, direct contact of the biofertilizers with agrochemicals (herbicides/weedicides/pesticides) should be strictly avoided.
(7) Generally speaking, 200g biofertilizer can be effectively used to treat 10 kg of seeds.
(8) In the case of unfavorable soil conditions, especially where the soil is strongly acidic, soil amendments such as lime or rock phosphate, are usually preferred.

7. Roadmap to the Commercialization of PGPR-Based Biofertilizers

Using PGPRs as biofertilizers for promoting plant growth and crop yield, improving soil fertility, and biocontrol of phytopathogens promotes sustainable agriculture by offering eco-friendly alternatives to synthetic agrochemicals like chemical fertilizers and pesticides. The development and commercialization of PGPR-based biofertilizers generally follow the following roadmap (Figure 5) [30,108].

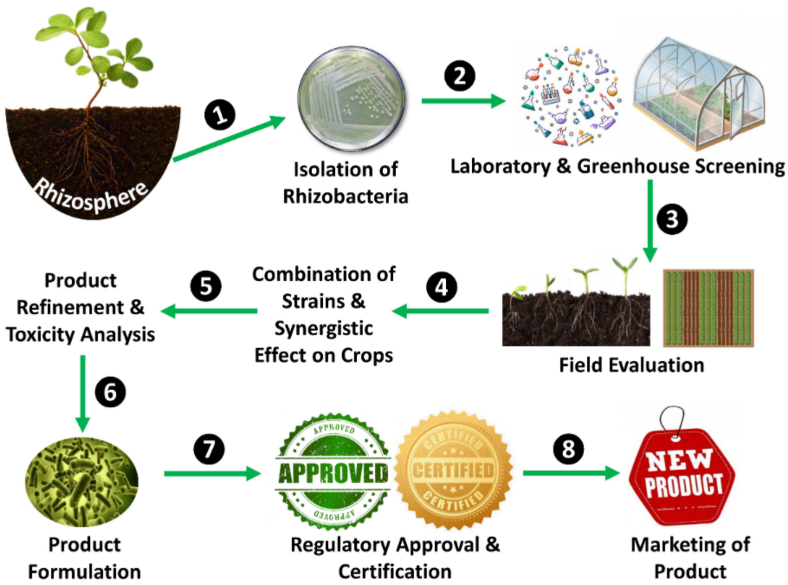

Figure 5. A roadmap for commercializing PGPR-based biofertilizers.

8. Conclusions and Future Perspectives

Among various industries present within a nation, the agriculture industry not only plays a pivotal role in survival but also facilitates meeting the demands of the growing population and economic exports. Post Green Revolution, the agroindustry has witnessed several scientific advances that resulted in better crop productivity but with environmental complications. Chemical fertilizers prove detrimental to soil and environmental health, while biofertilizers are natural products and do not pose threats to the ecosystem. Thus, to manage long-term soil fertility and sustain crop productivity, natural-products-based fertilizers prove to be an integral and vital component of sustainable agriculture. The last decade has inevitably seen a revolution because of the increased use of biological inoculants instead of agrochemicals for sustainable agriculture globally. The triad of interactions existing between the bioinoculant microorganism(s), resident soil microbiota, and host plant(s) is vital not only for the overall growth and higher productivity of the crop plants but also for maintaining the integrity of our planet's health and proper biogeochemical cycling.

A growing apprehension concerning food safety and the rising need for controlling food production quality to cater to the changing consumer demand is expected to shift farmers' attention toward organic farming and adopt sustainable agricultural practices. Thus, while seeking eco-friendly alternatives to toxic chemicals, there is a need to consider the three crucial "Ps", which include the people, prosperity, and the planet. Before its complete implementation, however, this microbial product-based technology needs to be researched profoundly and improved to elicit desired results and gain the trust of the farmers, the real stakeholders of agriculture. The thrust areas that need to be further focused on for research include quantifying commercial production, strain improvement, and authentication. Governments and federal agencies should promote the use of biofertilizers as eco-friendly alternatives for crop improvement. Entrepreneurs should invest more in the biofertilizer industry and provide financial assistance for start-ups. In addition to this, mass public awareness is required to educate the farmers and consumers alike on the advantages of using microbe-based biofertilizers for ensuring a greener tomorrow.

Author Contributions: Conceptualization, A.B. and S.K.; writing—original draft preparation, A.B., P.P., S.N.D., and S.K.; writing—review and editing, S.K., R.Z.S., M.S.R., and H.E.E. All authors have read and agreed to the published version of the manuscript.

Funding: H.E.E. would like to thank Universiti Teknologi Malaysia (UTM) for financial support through project No. QJ130000.3609.02M43 and All Cosmos Industries Sdn. Bhd. for financial support with project No. R.J130000.7344.4B200.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: A.B. and P.P. acknowledge University Grants Commission (UGC), New Delhi, India, for research fellowships. A.B. acknowledges his mentor, Appa Rao Podile, Vice-Chancellor, University of Hyderabad, for his constant support and encouragement. S.N.D. acknowledges the UGC start-up research grant for financial support. S.K. acknowledges Principal Sister Amrutha, St. Ann's College for Women, Hyderabad, Telangana, India, for her incessant support and Department of Biotechnology (DBT), New Delhi, India, for infrastructural support to the department under the DBT-STAR College Scheme.

Conflicts of Interest: The authors declare no conflict of interest.

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