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Review

Role of bacterial biofertilizers in agriculture and forestry

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Abstract: Many rhizospheric bacterial strains possess plant growth-promoting mechanisms. These bacteria can be applied as biofertilizers in agriculture and forestry, enhancing crop yields. Bacterial biofertilizers can improve plant growth through several different mechanisms: (i) the synthesis of plant nutrients or phytohormones, which can be absorbed by plants, (ii) the mobilization of soil compounds, making them available for the plant to be used as nutrients, (iii) the protection of plants under stressful conditions, thereby counteracting the negative impacts of stress, or (iv) defense against plant pathogens, reducing plant diseases or death. Several plant growth-promoting rhizobacteria (PGPR) have been used worldwide for many years as biofertilizers, contributing to increasing crop yields and soil fertility and hence having the potential to contribute to more sustainable agriculture and forestry. The technologies for the production and application of bacterial inocula are under constant development and improvement and the bacterial-based biofertilizer market is growing steadily. Nevertheless, the production and application of these products is heterogeneous among the different countries in the world. This review summarizes the main bacterial mechanisms for improving crop yields, reviews the existing technologies for the manufacture and application of beneficial bacteria in the field, and recapitulates the status of the microbe-based inoculants in World Markets.

Keywords: plant growth-promoting Rhizobacteria; sustainable agriculture; biofertilizers; nitrogen fixation; phytohormones; bacterial inoculants; plant stress resistance; nutrient solubilization

1. Introduction

Plant growth-promoting rhizobacteria (PGPR) are naturally-occurring soil bacteria able to benefit plants by improving their productivity and immunity. These bacteria are associated with the rhizosphere, the part of soil under the influence of plant roots and their exudates. According to their interactions with plants, PGPR can be divided into symbiotic bacteria, which live inside plants and exchange metabolites with them directly, and free-living rhizobacteria, which live outside plant cells [1]. Most symbiotic bacteria live in the intercellular spaces of the host plant, but there are some bacteria able to form truly mutualistic interactions with their hosts and penetrate plant cells. Moreover, some of them are able to integrate their physiology with the plant, resulting in the formation of specialized structures. The best known mutualistic symbiotic bacteria are the rhizobia, which establish symbiotic associations with leguminous crop plants, fixing atmospheric nitrogen for the plant in certain root structures known as nodules. Other examples of mutualistic bacteria associated with plants are *Frankia,* which induces the formation of nodules in actinorrhizic plants, such as *Alnus*trees, where bacterial nitrogen fixation takes place.

Several PGPR have been used worldwide as biofertilizers, contributing to increasing crop yields and soil fertility and hence with the potential to contribute to more sustainable agriculture and forestry [2]. According to Malusá and Vassilev [3], a biofertilizer is “the formulated product containing one or more microorganisms that enhance the nutrient status (the growth and yield) of the plants by either replacing soil nutrients and/or by making nutrients more available to plants and/or by increasing plant access to nutrients”. This definition of the term biofertilizers *sensu stricto* restricts the concept to only those bacteria able to facilitate nutrients to the plant. Nonetheless, there are other bacteria able to promote plant growth through other mechanisms, such as the production of phytohormones, environmental stress relief, or the prevention of plant diseases. In this review, we shall address biofertilizers in a broader manner, including all those products containing bacteria that benefit plant growth, and therefore crop yields.

This review summarizes the main mechanisms of the bacteria able to improve crops yields, and refers to some of the latest studies that have evaluated the potential of the application of bacterial isolates indifferent plants. Furthermore, we recapitulate the technologies developed to apply beneficial bacteria in the field and the status of microbe-based inoculants in the world markets. Finally, we add some concluding remarks and suggest future perspectives concerning the role of bacterial biofertilizers in agriculture and forestry.

2. Mechanisms of Plant Growth-Promoting Rhizobacteria

Rhizobacteria can promote plant growth through a broad variety of mechanisms (Table 1), which can be grouped according to their mode of action in: (i) the synthesis of substances that can be assimilated directly by plants, (ii) the mobilization of nutrients, (iii) the induction of plant stress resistance and (iv) the prevention of plant diseases (Figure 1).

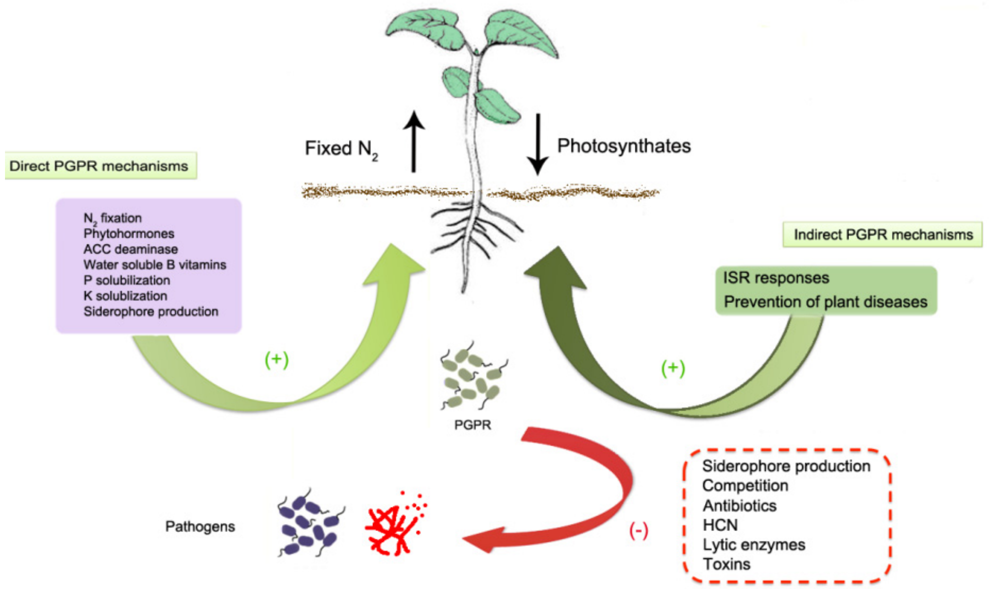

Figure 1. Mechanisms of Plant Growth-Promoting Rhizobacteria.

Table 1. Plant-growth promotion mechanisms by rhizobacteria.

PGP Rhizobacteria	**PGP Mechanisms**	**Crops**	**Reference**
Azoarcus	Nitrogen fixation	Rice	[20]
Azobacter	Cytokinin synthesis	Cucumber	[52]
Azorhizobium	Nitrogen fixation	Wheat,	[23]
Azospirillum	Nitrogen fixation	Cereals, rice, sugar cane	[6,7,9,13]
Azotobacter	Nitrogen fixation	Wheat, barley, oats, rice, sunflowers, maize, line, beetroot, tobacco, tea, coffee and coconuts	[11]
Bacillus	Auxin synthesis	Potato	[46]
Bacillus	Cytokinin synthesis	Cucumber, oriental thuja	[53,55]
Bacillus	Gibberelin synthesis	Pepper	[57]
Bacillus	Potassium solubilization	Wheat, Sudan grass, eggplants, pepper, cucumber	[81,83–85]
Bacillus	Induction of plant stress resistance	Maize, peanuts	[92,93]
Bacillus	Antibiotic production	Alfalfa	[98]
Bacillus	Siderophoreproduction	Maize, pepper	[105]

Beijerinckia	Nitrogen fixation	Sugar cane	[6,10]
Burkholderia	Nitrogen fixation	Rice	[21,22]
Chryseobacterium	Siderophore production	Tomato	[108]
Frankia	Nitrogen fixation	*Alnus*	[40]
Gluconacetobacter	Nitrogen fixation	Sugar cane	[12]
Herbaspirillum	Nitrogen fixation	Sugar cane, bean, rice, sorghum	[15,17,19]
Mycobacterium	Induction of plant stress resistance	Maize	[92]
Paenibacillus	Indole acetic acid synthesis	Lodgepole pine	[49]
Paenibacillus	Potassium solubilization	Black pepper	[82]
Phyllobacterium	Phosphate solubilization	Strawberries	[79]
Phyllobacterium	Siderophore production	Strawberries	[79]
Pseudomonas	Chitinase and β-glucanases production	Several crops	[102]
Pseudomonas	ACC deaminase synthesis	Mung beans, wheat	[63,67]
Pseudomonas	Induction of plant stress resistance	Cotton, Maize	[91,92]
Pseudomonas	Antibiotic production	Wheat	[96]
Pseudomonas	Chitinase and β-glucanases production	Pigeon pea	[101]
Pseudomonas	Siderophore production	Potato, maize	[105]
Rhizobia	Nitrogen fixation	Legumes	[29–36,39]
Rhizobia	Induction of plant stress resistance	Peanuts	[93]
Rhizobia	Hydrogen Cyanide production	Legumes	[100]
Rhizobium	Nitrogen fixation	Rice	[25]
Rhizobium	Indole acetic acid synthesis	Pepper, tomato, lettuce, carrot	[50,51]
Rhizobium	ACC deaminase synthesis	Pepper, tomato mung beans,	[50,63]
Rhizobium	Phosphate solubilization	Carrot, lettuce, Tomato, pepper	[50,51]
Rhizobium	Siderophore production	Carrot, lettuce, Tomato, pepper	[50,51]
Sinorhizobium	Chitinase and β-glucanases production	Pigeon pea	[101]
Sphingomonas	Gibberelin synthesis	Tomato	[58]
Streptomyces	Indole acetic acid synthesis	Indian lilac	[48]
Streptomyces	Siderophore production	Indian lilac	[48]

2.1. Synthesis of substances that can be assimilated directly by plants

Nitrogen, required for the formation of aminoacids and proteins, is the most limiting nutrient for plants. The process by which atmospheric nitrogen is combined into organic forms that can be assimilated by plants is exclusive to prokaryotes [4,5]. Some examples of free-living nitrogen-fixing organisms are *Azospirillum*, commonly associated with cereals in temperate zones [6,7] and also reported to be able to improve rice crop yields [8,9]; *Beijerinckia*, which seems to be associated with sugar cane plantations in tropical zones [6,10], and *Azotobacter*, which plays an important role in nitrogen fixation in rice crops [8] and is used as a biofertilizer for wheat, barley, oat, rice, sunflowers, maize, line, beetroot, tobacco, tea, coffee and coconuts [11]. Some species belonging to the genera *Gluconacetobacter* [12], *Azospirillum* [13] and *Herbaspirillum* [14] are sugarcane endophytes and contribute to its nitrogen fertilization. *Herbaspirillum* has also been isolated from bean and rice [15–18]. Some studies report how *Azoarcus*, *Azospirillum* and *Burkholderia* strains enter rice roots and increase the amount of nitrogen compounds in the crop [19–22]. Nitrogen-fixing *Azorhizobium* strains have been isolated from wheat roots [23], and *Rhizobium* and *Bradyrhizobium* in rice roots [24,25]. Moreover, certain diazotrophic bacteria establish truly mutualistic symbiosis with some plants through the formation of root nodules. These symbioses are found between rhizobia and legumes [26–36] and *Frankia* and actinorhizal plants [37–41].

Plant hormones are organic molecules involved in several plant growth and development processes. Phytohormone biosynthesis by some microorganisms is involved in pathogenesis in plants [42], but a wide spectrum of beneficial bacteria produce phytohormones that are involved in plant-growth promotion [43]. Auxins act as key molecules, regulating most plant processes directly or indirectly [44]. Several bacteria secrete auxins, which seem to act as signaling molecules for bacterial communication in order to coordinate activities [45]. Auxin-producing *Bacillus spp.* have been reported to exert a positive effect in *Solanuntuberosum* development [46]. Indole-3-acetic acid (IAA) is the best known and most active auxin in plants [47]. Verma et al. [48] reported that endophytic *Streptomyces* isolated from *Azadirachtaindica* produce IAA and are potential plant-growth promoters. Bent et al. [49] have reported elevated root IAA level in lodgepole pine plantlets inoculated with *Paenibacilluspolymyxa*. Rhizobial strains producing IAA improve the growth of several crops such as *Capsicum annuum*, *Solanumlycopersicum*, *Lactuca sativa* and *Daucuscarota* [50,51]. Cytokinins promote cytokinesis, vascular cambium sensitivity, vascular differentiation and root apical dominance [52]. *Azotobacterchroococcum* and *Bacillus megaterium* strains were found to produce cytokinins and promote cucumber growth [53]. Ortiz-Castro et al. [54] also described that *Bacillus megaterium* promotes plant growth by cytokinin synthesis. Liu et al. [55] reported that oriental thuja seedlings inoculated with cytokinin-producing *Bacillus subtilis* strains were more resistant to drought stress. Gibberellins are involved in seed germination and emergence, stem and leaf growth, floral induction and flower and fruit development [56]. The growth of red pepper plants was enhanced by treatment with a *Bacillus cereus* strain producing gibberellins [57]. Tomato plants inoculated with the gibberellin-producing *Sphingomonas* sp. LK11 strain showed a significant increment in several growth attributes [58]. Ethylene is a plant hormone known to regulate several processes such as the ripening of fruits, the opening of flowers or the abscission of leaves [59]. However, it also promotes seed germination, secondary root formation and root-hair elongation [60]. *Phyllobacterium brassicacearum* STM196 emits ethylene and contributes to root-hair elongation in *Arabidopsis thaliana* [61].

High levels of ethylene, produced under stressed conditions, can inhibit certain processes such as root elongation or nitrogen fixation in legumes [62] and contribute to premature senescence [63]. Some bacteria produce the enzyme, 1-aminocyclopropane-1-carboxylate, to hydrolyze ACC, the precursor molecule of ethylene in plants [64], to obtain ammonia and α-ketobutyrate, which can be used as nitrogen and carbon sources [65]. Therefore, these bacteria lower ethylene levels in plants and hence prevent some of the negative effects produced by high ethylene concentrations [66]. Ahmad et al. [63] report that *Rhizobium* and *Pseudomonas* ACC-deaminase-producing strains improve the growth, physiology and quality of mung beans under salt-affected conditions. Shaharoona et al. [67] reported that two ACC-deaminase-containing *Pseudomonas* strains improved the growth and yield of wheat crops, with varying levels of NPK nutrients. Also, *Rhizobium leguminosarum* strains producing ACC-deaminase promoted pepper and tomato plant growth [50].

Microbial vitamin production promotes crop yields, affecting plant growth at different levels [68,69], enhancing plant-rhizobial symbiosis and plant mycorrhization [70,71]. Plant growth-promoting strains of *Azotobacter* have been described to be able to produce B-group vitamins [72].

2.2. Nutrient mobilization

After nitrogen, phosphorous (P) is the second essential nutrient in terms of necessary uptake amounts in plants [73]. This element is fairly insoluble in soils [74] and accordingly, traditional agriculture has been based on the application of chemical P fertilizers. Nevertheless, when applied as fertilizer to fields P passes rapidly to become insoluble and hence unavailable to plants [75,76]. Accordingly, the use of P-solubilizing bacteria represents a green substitute for chemical P fertilizers. *Micrococcus, Pseudomonas*, *Bacillus* and *Flavobacterium* have been reported to be efficient phosphate solubilizers [77,78]. Phosphate-solubilizing rhizobial strains promote *Daucuscarota* and *Latuca sativa* growth [51] and a *Phyllobacterium* strain able to solubilize phosphates improves the quality of strawberries [79]. *Rhizobium leguminosarum*strainPETP01 and *R. leguminosarum* strainTPV08 solubilize phosphate and are PGPR for pepper and tomato plants [50].

Potassium (K) is the third essential nutrient necessary for plant growth. Some rhizobacteria are able to solubilize insoluble potassium forms [80]. *Bacillus edaphicus* has been reported to increase potassium uptake in wheat [81] and *Paenibacillusglucanolyticus* was found to increase the dry weight of black pepper [82]. Sudan grass inoculated with the potassium-solubilizingbacterium *Bacillus mucilaginosus*had higher biomass yields [83]. Also, *Bacillus mucilaginosus* in co-inoculation with the phosphate-solubilizing *Bacillus megaterium* promoted the growth of eggplant, pepper and cucumber [84,85].

Siderophores are organic compounds whose main function is to chelate the ferric iron (Fe (III)) from the environment. Microbial siderophores also provide plants with Fe, enhancing their growth when Fe is limiting [86], but the exact mechanisms of Fe supply to the plant are not well understood [87]. Siderophores from endophytic *Streptomyces* promote *Azadirachtaindica* plant growth [48]. Rhizobial strains able to produce siderophores have been reported to be potential biofertilizers, improving the production of carrots, lettuce, peppers and tomatoes [50,51]. One siderophore-producing *Phyllobacterium* strain promotes the growth and quality of strawberries [79].

2.3. Induction of plant stress resistance

Abiotic stress in plants, originated in situations such as drought, water logging, extreme temperatures, salinity and oxidative stress, are the primary cause of crop loss worldwide [88]. Liddycoat et al. [89] described *Pseudomonas* strains enhancing asparagus seedling growth and seed germination under water-stress conditions. *Pseudomonas fluorescens*MSP-393 acts as a PGPR for many crops grown in the saline soils of coastal ecosystems [90] and *Pseudomonas putida*Rs-198 promotes cotton seedling grown under salt stress, increasing germination rates and protecting against salt stress by increasing the absorption of Mg^{2+}, K^{+} and Ca^{2+}, decreasing Na^{+} uptake, and improving the production of endogenous indole acetic acid [91]. The inoculation of peanuts cultivated under salt-stress conditions with rhizobial strains showed comparable efficiency to the application of N fertilization in the same crop [92]. El-Akhal et al. [93] described that strains of *Paenibacillusalcaligenes*, *Bacillus polymyxa* and *Mycobacterium phlei* produce calcisol and improved maize growth and nutrient uptake under high temperature conditions as well as under salinity.

2.4. Prevention of plant diseases

The mechanisms of bacterial plant disease prevention may be direct, if pathogens are inhibited as a result from PGPR metabolism, or indirect, when the bacteria compete with the pathogens, reducing their ability to induce disease [94]. Some PGPR synthesize antibiotic substances, that inhibit the growth of some plants pathogens [95]. For instance, *Pseudomonas* sp. produces antibiotics that inhibit *Gaeumannomycesgraminis* var. tritici, the causal agent of take-all of wheat [96]. Most *Bacillus* ssp. produce antibiotics that are active against Gram-positive and Gram-negative bacteria, as well as many pathogenic fungi [97]. *B. cereus* UW85 contributes to the biocontrol of alfalfa damping-off [98]. Cyanogenic compounds are nitrogen-containing compounds that have been shown to repel leaf-chewing herbivores [99]. Rhizobia-legume symbioses have been demonstratedto enhance the resistance of plants to herbivore attack. Presumably, an additional nitrogen provided by the bacterium allows the plant to synthesize cyanogenic defense compounds [100].

Since chitin and β-glucan are the major fungal cell wall components, bacteria producing chitinases and β-glucanases inhibit fungal growth. Kumar et al. [101] have reported that *Sinorhizobiumfredii* KCC5 and *Pseudomonas fluorescens* LPK2 produce chitinase and β-glucanases and control the fusarium wilt produced by *Fusariumudum. Pseudomonas spp.* exhibitschitinase and β-glucanases production and the inhibition of *Rhizoctoniasolani* and *Phytophthoracapsici*, two of the most destructive crop pathogens in the world [102].

Some rhizobacteria are able to synthesize proteins with toxic properties against certain crop insect pests. *B. thuringiensis*subsp. *kurstaki* HD-1 has been widely used in the forest industry for controlling the gypsy moth [103]. Also, bacteria belonging to the genera *Photorhabdus* and *Xenorhabdus*, associated with entomopathogenic nematodes, inhibit harmful insects and there are some nematodal-bacterial formulations used in the field to control damaging insect populations [104].

Apart from the supply of Fe, microbial siderophorescontrol plant pathogens by limiting the Fe available for the phytopathogens [105]. *Pseudomonas*siderophores control the *Fusarium* wilt produced by *Fuxariumoxysporum* in potato [106]. *Pseudomonas* sp. and *Bacillus* sp. strains produce siderophores that inhibit fungal pathogens in maize [107]. Siderophores from the

*Chryseobacterium*sp C138 strain are effective in supplying Fe to iron-starved tomato plants [108]. Yu et al. [109] reported that a siderophore-producing strain identified as *Bacillus subtilis*exertsa biological control effect on *Fusarium* wilt and promotes pepper growth, and Verma et al. [48] reported that endophytic *Streptomyces* isolated from *Azadirachtaindica* produce siderophores with biocontrol potential.

Finally, the presence of PGPR in the rhizosphere and rhizoplane might prevent plant diseases by competing for available nutrients, reducing the contact surface between the pathogen and the plant root or by interfering with the mechanisms leading to plant disease [94].

3. Technology for the Use of PGPR in Agriculture and Forestry

PGPR-based inoculants are formulations containing one or more beneficial bacteria in a carrier material. Carrier materials are used as vehicles for the bacteria in the formulation of the biofertilizer. There are different kinds of substances suitable for use as carriers, i. e. clay, talc, peat, vermiculite, perlite, bentonite, zeolite, diatomaceous earth, rice or wheat bran, rock phosphate pellets, charcoal, soil, sawdust or compost. Usually, the selection of the carrier material is made on the basis of the longer viability of the bacteria transported (not only during storage, but also after the application of the biofertilizer to the soil) and the desired type of application (liquid, powder, granulated or as a seed coating); the price of the material is another important factor affecting choice. Apart from these, other desirable characteristics for a good carrier according to Bashan [110] are: (i) it should allow the addition of bacterial nutrients, (ii) it must have a high water-holding capacity, (iii) it should allow easy sterilization, (iv) it must have a good pH buffering capability, (v) it must be non-pollutant and biodegradable and (vi) it must allow easy handling by the farmer. It is difficult to find a natural product exhibiting all these properties. Nevertheless, new technologies are currently heading towards the development of novel carriers with better characteristics. One example is polymer-based carriers, which encapsulate the bacteria in their matrix and release them gradually in the soil during their degradation process. The best-known are alginate beads. Alginate, a natural polymer of D-mannuronic and L-glucuronic acids derived from macroalgae such as *Macrocystispyrifera* or *Sargassumsinicola*, forms beads when added to a cationic solution. Alginate beads have a diameter of 2–3 mm with a pore size of 0.005–0.2 mm and are frequently used for microbial cell encapsulation [111]. Microalginate beads with a diameter of 100–200 µm have proved to be a good carrier for the immobilization of *Azospirillumbrasilense* (>10^{11}cfu/g inoculant) and the biofertilizer produced enhanced wheat and tomato crops [112]. Another process for the storage and application of bacterial bioproducts uses water-in-oil emulsions [113]. Bacteria in water-in oil emulsions can be applied to the crops through irrigation systems.

The carrier is previously sterilized and then mixed with liquid culture of the bacteria with a high number of viable cells per milliliter, usually between 10^8–10^9 CFU/ml [114]. To produce the bacterial culture, inocula containing pure cultures of the desired PGPR strains plus growth media containing the bacterial nutrients are placed in fermentors. Since the price of a biofertilizer is a key point to ensure its commercialization [115], several organic residues have been proposed as possible means of growth [116,117,118]. In many cases, consortia of diverse bacteria with different plant growth-promotion mechanisms have resulted in higher crop yields because they act synergically. Accordingly, many commercial biofertilizers contain more than one bacterial strain. Sometimes, bacterial and fungal strains are combined, with excellent results [119,120].

Bacteria can also be lyophilized and stored with no carrier, although always after the application of cryoprotectants such as mannitol or microcrystalline cellulose. The addition of carbon sources or cell protectants could increase the shelf-life and efficiency of biofertilizers. Glucose, sucrose, maltose, trehalose, molasses, and glycerol are some of the nutrients/cell protectants frequently used [121].

Biofertilizers formulated with solid carriers have an average life of six months. Nevertheless, when the biofertilizer formulation is liquid, nutrients and cell protectants can be added readily so that the shelf-life of the final product will be long-lasting and the product will tolerate higher temperatures; as a result, liquid biofertilizers can survive temperatures of up to 55°C and last for even two years. The drawback of liquid formulations is their higher price [122,123].

Formulated biofertilizers are packed and each package should contain at least the following information: (i) the name of the product, (ii) the bacterium or bacterial consortia contained in it, (iii) crops for which it is appropriate, (iv) the date of manufacture, (v) the expiry date and (vi) instructions and directions for application.

The whole biofertilizer production process must be subjected to quality control. The quality of a bacterial biofertilizer demands the presence of the right type of bacteria in active form and in the desired numbers [124]. Quality is one of the most important factors influencing the success and acceptance of the product by farmers. The key steps in the production process that require quality controls are: (i) the fermentation process, (ii) carrier preparation, (iii) the mixing of bacterial broth and carrier, (iv) packing, and (v) storage. In the fermentation process, the identity and density of the strains must be controlled, as must the absence of contaminant strains. The carrier must be sterilized and both the mixing of bacterial strains with the carrier and the packaging process must be performed aseptically so that the absence of microbial contaminants can be guaranteed. Finally, storage should take place under specific temperature and humidity conditions to ensure the viability of the bacteria during the expected lifetime of the biofertilizer. For the final product, several parameters should be checked in order to guarantee its quality (Figure 2). The identity of the strains is usually performed through phenotype tests and genomic identification. The viability of the bacteria in the final product can be checked by plate counting or qPCR using a propidium monoazide treatment during the DNA extraction process to differentiate between dead and viable cells. In the case of biofertilizers formulated as bacterial consortia, each strain is usually grown in an independent fermenter and the identity and density of each bacterial broth is checked prior to their mix.

Application of bacterial fertilizers on fields depends on the type of formulation. Ideally, it should be feasible to apply the biofertilizers with the farmer's own infrastructures/machinery. Liquid formulations can be added to the soil using irrigation systems or mist-sprayers. Powdered or encapsulated products can be spread over the fields with spreader centrifuges. Inoculation can be also done by coating the seeds and in the case of trees, the roots of seedlings can be dipped in a liquid formulation.

In some cases, biofertilizers are based on genetically engineered bacteria. In these bacteria, one or more genes have been modified or introduced *de novo* using recombinant DNA technology. Likewise, genetically modified bacteria can accomplish certain functions that their wild-type parental strains can never achieve, or they can enhance properties that the natural strains already possess. For well-known for their potential to increase legumes and non-legume plant yields notably, at least under laboratory conditions, but several of these strains fail to increase productivity under certain soil conditions. Some authors have suggested that the main reason for this is the weak ability of rhizobial-based biofertilizers to compete with indigenous soil microorganisms to obtain the soil nutrients necessary for their metabolism. In this regard, an iron-increasing gene isolated from *Bradyrhizobiumjaponicum* 61A152 was introduced into *Mesorhizobium* sp., with good results [127]. Thus, genetic manipulation is another way to enhance the stability of biofertilizers and contribute to the goal of a more sustainable agriculture. Accordingly, much attention is currently being devoted to the development of efficient biofertilizers that will be compatible with a wide range of soils and plants by molecular and genetic engineering. Nevertheless, the production, importation and release into the environment of biofertilizers containing genetically modified microorganisms have different levels of allowance, depending on the corresponding biosafety laws in the different countries.

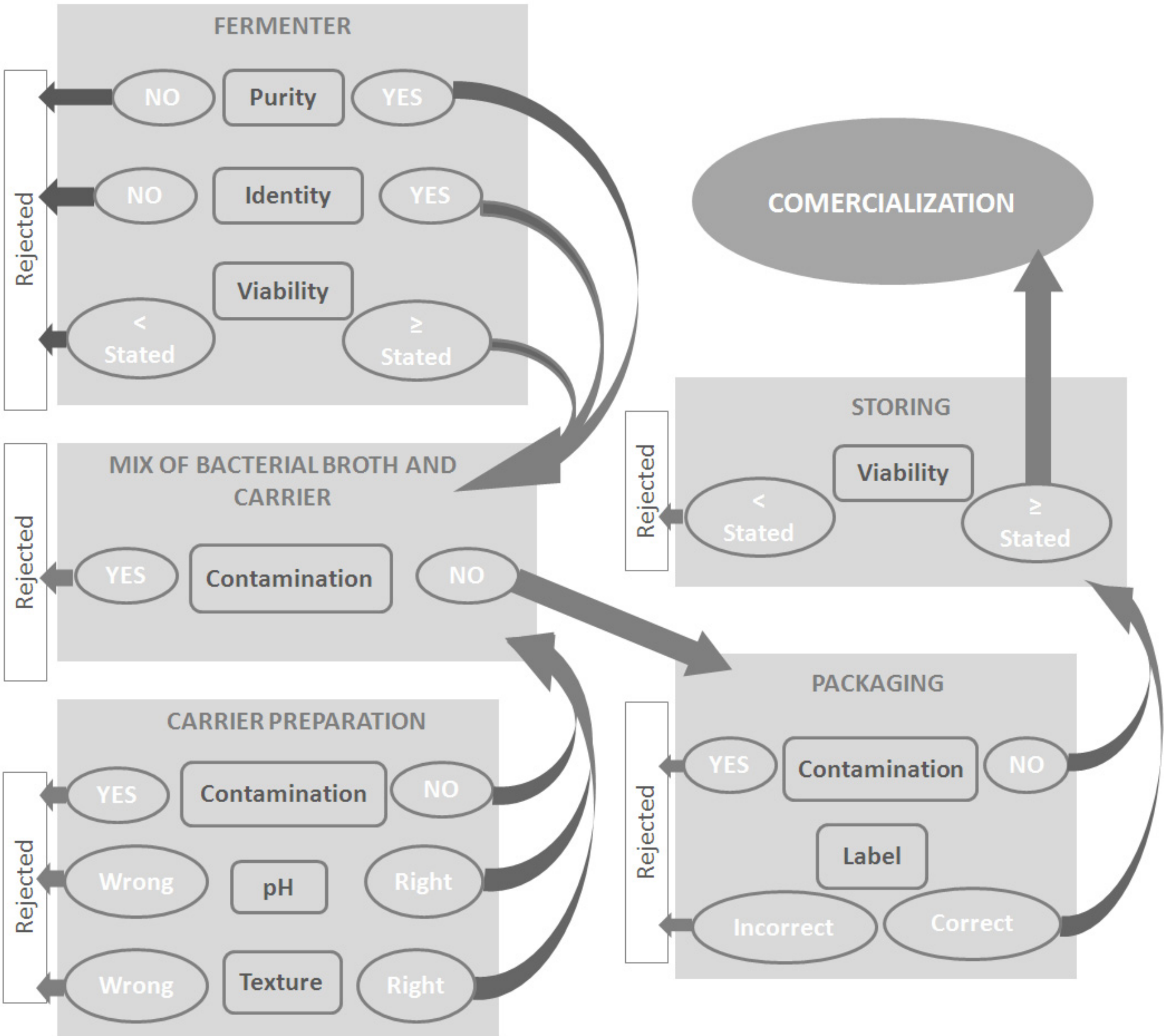

Figure 2. Quality control in the biofertilizer manufacturing process.

4. Situation of PGPR Inoculants Worldwide

The application of PGPR inoculants in agriculture can be traced back to the beginning of the past century, when a *Rhizobium* based product named "Nitragin" was patented (Nobbe and Hiltner 1896, cited in [110]). Currently, the demand for microbial biofertilizers is increasing worldwide owing to a higher degree of environmental awareness, the increasing number of laws protecting the environment, and the ever-expanding demand for ecological products. For the time being, these products are not sold as widely as chemical ones, for several reasons. On the one hand, the application of microbe-based biofertilizers raises a certain amount of distrust owing to the variability of the conclusions drawn from the different studies and to the inconsistency of the results from laboratory and field experimentation [115]. Moreover, in most countries PGPR inoculants require long-lasting and expensive registration procedures. Accordingly, PGPR-inoculated crops are currently used on only a small fraction of agricultural lands worldwide. Nevertheless, the PGPR-based biofertilizer market is increasing yearly and an increment of nearly 14% is anticipated by the end of the present decade (for an overlook of some currently commercialized products, see Table 2).

There are some multinational companies that sell biofertilizers all around the world. For instance, Rizobacter is an important company engaged in research and innovation that was established in 1977 in Argentina and that distributes rhizobial-based biofertilizers for legume crops in Argentina, Brazil, Bolivia, Paraguay, Uruguay, the U.S.A, Europe and Africa. Also, the Novozymes Company produces and distributes throughout Asia, Australia, Brazil, Canada, Europe and the United States and manufactures several bacterial-based biofertilizers that solubilize phosphates and/or fix nitrogen. Some of their most popular solutions are Cell-Tech® or Nitragin Gold®, containing rhizobial strains able to nodulate legumes, and TagTeam®, which combines rhizobial strains with the fungal species *Penicilliumbilaii*. These products are commercialized in peat, granular and liquid formulations. Recently, Novozymes established a partnership with Monsanto, named the BioAgAlliance. The combination of Novozymes and Monsanto capabilities establishes one of the industry's most advanced microbials platform. The alliance aims to screen hundreds of strains in thousands of yield plots to select for microbes that provide a consistent crop benefit, identifying synergies, streamlining handoffs, and eliminating redundancies prior to new products development (F. Bjørndal, press officer in Novozymes, personal communication).

In the U.S.A., most of the agricultural surface is dedicated to wheat, corn, soybean, cotton and forage crops. These crops are relatively low-value products and farmers avoid the use of biofertilizers. The exception is the application of rhizobial strains in legume crops. Nevertheless, there are several companies that are placing some microbial-based fertility products on the markets, which are raising acceptance. For instance, Accomplish®, trademarked by Loveland Products, Inc., is a specifically formulated biochemical fertilizer with viable microorganisms plus enzymes, organic acids and chelators, registered as organic by the Washington State Department of Agriculture (WSDA). Loveland Products, Inc. states that this product improves the availability of nutrients from fertilizers and soil and increases root size and branching, so plants can take up more nutrients and water. Studies carried out by the manufacturer and the University of Minnesota in 2010 indicate increases in corn and soybean production in fields supplied with Accomplish®.

The Canadian Food Inspection Agency is responsible for the legislation of fertilizer supplements, including compounds recently placed on the market (microbial and chemical). It specifies the efficacy requirements for biofertilizers as well as the prerequisites for registering them.

Table 2. Commercial biofertilizer products of plant growth promoting rhizobacteria.

Product	Company	Bacterial strains
Cell-Tech®	Novozymes	rhizobia
Nitragin Gold®	Novozymes	rhizobia
TagTeam®	Novozymes	rhizobia + *Penicillium bilaii*
Accomplish®	Loveland Products, Inc	PGPR + enzymes + organic acids + chelators
Nodulator®	BASF Canada Inc.	*Bradyrhizobium japonicum*
Nodulator® N/T	BASF Canada Inc.	*Bacillus subtilis MBI 600 + Bradyrhizobium japonicum*
Nodulator® PRO	BASF Canada Inc.	*Bacillus subtilis* + *Bradyrhizobium japonicum*
Nodulator® XL	BASF Canada Inc.	*Rhizobium leguminosarum biovar viceae 1435*
Bioboots®	Brett-Young Seeds	*Delftia acidovorans*
Bioboots® (soybean)	Brett-Young Seeds	*Delftia acidovorans* + *Bradyrhizobium* sp.
EVL coating®	EVL Inc.	PGPR consortia
Nitrofix®	Labiofam S. A.	*Azospirillum* sp.
Bioativo®	Instituto de Fosfato Biológico (IFB) Ltda.	PGPR consortia
VitaSoil®	Symborg	PGPR consortia
Azotobacterin®	JSC "Industrial Innovations"	*Azospirillum brasilense* B-4485
Mamezo®	Tokachi Federation of Agricultural Cooperatives (TFAC)	rhizobia (in peat)
R-Processing Seeds®	Tokachi Federation of Agricultural Cooperatives (TFAC)	rhizobia (coated legume seeds)
Hyper Coating Seeds ®	Tokachi Federation of Agricultural Cooperatives (TFAC)	rhizobia (coated grass legume seeds)
Life®	Biomax	PGPR consortia
Biomix®	Biomax	PGPR consortia
Biozink®	Biomax	PGPR consortia
Biodine®	Biomax	PGPR consortia

A list of registered products can be found at the Canadian Food Inspection Agency website (www. inspection. gc. ca). In that list there are more than 150 microbe-based biofertilizers with contents ranging between 10^6 and 10^9 CFU per gram. Most of the products contain rhizobial strains for legume crops. However, BASF Canada Inc. have also products with mixed *Bacillus subtilis* and *Bradyrhizobiumjaponicum* which are manufactured for global markets including North America, Africa, South America, Australia and Europeand Brett-Young Seeds produces Bioboots®, a product containing *Delftiaacidovorans*, with three variants, two for canola crops, one of them using peat as carrier and the other as a liquid product, while the third one combines *D. acidovorans* with *Bradyrhizobium* sp. and is destined for soybean. Moreover, EVL Inc. commercializes a product based on the plant growth-promoting bacterium *Lactobacillus helveticus* and the biostimulant EVL coating®, which includes several microbial strains that act in a synergic way and was developed to be used together with solid chemical fertilizers. EVL Inc. sells this product or licences the technology to fertilizer companies and they apply it to their products following their proprietary procedures (M. Macouzet-Garcia, EVL Scientific Director, personal communication). Finally, Lallemand Inc. is another key company in the Canadian market of biofertilizers, developing products applicable in agriculture, horticulture and forestry.

In Cuba, an essentially agricultural country that implements strategies aimed at the sustainability of the sector, scientific institutions have developed biofertilizers since 1991. Currently, the company Labiofam S. A. produces Nitrofix®, a product containing *Azospirillum* sp. strains, which have been demonstrated to fix nitrogen and produce phytohormones, promoting the growth of sugar cane and other tropical crops.

According to the studies carried out by the International Plant Nutrition Institute, around 60,000–70,000 tons of biofertilizers are used in Brazil every year in crops of beans, maize, rice, sugarcane, soybean, carrots, tomatoes, cotton, forage, citrus and eucalyptus. There are several large companies in Brazil that commercialize biofertilizers used in agricultural crops, such as Embrafós Ltda., the Instituto de FosfatoBiológico (IFB) Ltda., Biofosfatos do Brasil Ltda., and Liderfós Ltda. IBF produces Bioativo®, with the patent number PI-9401724-7 (Brazilian Institute for Intellectual Property), which is described as a product containing organic matter plus macro and micro essential nutrients and a beneficial microbial complex that solubilizes phophates and fixes atmospheric nitrogen.

Reports from the International Plant Nutrition Institute reveal that in the South American cone (Argentina, Paraguay, Bolivia and Uruguay), more than 30 million hectares of soybean crops are sown every year and of those, more than 70% are inoculated with *Bradyrhizobium* sp. Moreover, plantations of wheat and maize are sometimes inoculated with other bacteria, mainly from the genera *Azospirillum* and *Pseudomonas*.

Europe is one of the regions of the planet with the most developed biofertilizer market and government policies in most European countries support its expansion. Economy reports estimate that in Europe the biofertilizer market will reach a value of more than four thousand five hundred million dollars by 2017. One of the biofertilizer-manufacturing companies currently in expansion is Symborg. Symborg commercializes VitaSoil®, a microbial mix of 2.3×10^6 CFU/ml, which is indicated for the promotion of horticultural and floral plants, citrus and other fruit trees, cereal crops, tobacco plantations and vineyards.

In Russia, bacterial fertilizers are also commercialized and applied. Companies such as JSC "Industrial Innovations" commercialize bacterial-based biofertilizers. One of those most frequently used is Azotobacterin®, containing the diazotrophic bacterial strain *Azospirillumbrasilense* B-4485. The application of this biofertilizer produces up to 20% increases in the yield of crops such as wheat, barley, maize, carrot and cabbage, among others.

In Asia, growth of the biofertilizer market is determined by government efforts to promote a more sustainable agriculture. Despite these, however, farmers are reticent about moving on from their traditional techniques. Also, the market depends on the development of the organic food industry. The economic boom in some Asian countries has raised people's purchasing power and this has led to an increase in demand for organic products. According to the Biofertilizer Manual, edited and published by the FNCA (Forum for Nuclear Cooperation in Asia) Biofertilizer Project Group [128] in 2006, the Tokachi Federation of Agricultural Cooperatives (TFAC) was the only organization producing and distributing *Rhizobium*–based biofertilizers in Japan. The factory was manufacturing three products: Mamezo®, which contains rhizobia mixed with peat, R-Processing Seeds®, which are legume seeds inoculated with rhizobia, and Hyper Coating Seeds®, legume grass seeds coated with a capsule of calcium carbonate containing rhizobia.

It has been estimated that In India the money spent on biofertilizers and biopesticides is around USD 1.5 billion. Organic agriculture in the country occupies a surface greater than 100. 000 hectares and is expanding; in organically-grown crops biofertilizers play a key role, so their use is expected to increase. The decrease in chemical products can already be noted. Moreover, there are over 100 biofertilizer producers in the country. One of the key suppliers of biofertilizers worldwide, Biomax, is based in India. Biomax commercializes several products containing microorganisms (i. e. Life®, Biomix®, Biozink®, Biodine®) that are recommended for a broad variety of plants able to fix atmospheric nitrogen, solubilize phosphate, iron, magnesium and zinc and that play an active role in organic matter degradation. Other large biofertilizer manufacturing companies are also present in India: Ajay Biotech Ltd., National Fertilizers Ltd., Madras Fertilizers Ltd., Gujarat State Fertilizers & Chemicals Ltd., T. Stanes & Company Ltd., Camson Bio Technologies Ltd., Rashtriya Chemicals & Fertilizers Ltd. To achieve production objectives, the Indian Government created the National Project on Development and use of Biofertilizers (NPDB) and a National Biofertilizer Development Centre was established, with six regional centers.

In China, the Ministry of Agriculture began regulating the management and registration of biofertilizers in 1996. After 10 years, in 2006 the number of registered products reached 511. Currently, several big companies are producing and selling biofertilizers. Probably the leading manufacturer in the market is probably China Bio-Fertilizer AG ("CBF"), which produces two bacteria able to solubilize phosphorus and potassium. According to the company's studies, by using their products the need for chemical fertilizers is reduced by 30% and crop yields increase by up to 30%, depending on the plant species.

Apart from South Africa and some other small markets, across the rest of the African continent farmers are usually reticent about the application of bacterium-based biofertilizers. It is difficult to convince peasant farmers, with very tight economies, to use these bacterial inoculants when their effects are not as evident as the effects produced by chemical fertilizers [129].

It is noteworthy that in developing countries farmers lack proper education about modern agricultural techniques and they therefore tend to grow their crops using traditional practices, with no knowledge either about the environmental implications of such methods or possible alternatives. Moreover, in several Latin American, African, and Pacific Island countries, there are economic strictures to the importation of large quantities of chemical fertilizers. As a result, their agricultural production remains well below its agronomic potential [130]. With the exception of rhizobial inoculants, which have a long tradition in legume crops and were promoted by several governments during the last century, the application of beneficial microorganisms in these countries is almost unknown. Except for soybean crops, in developing countries inoculants have had only a small effect on increasing legume crop yields. Furthermore, in many developing countries, there are no inoculant industries (e.g. in Central America). Finally, bacterium-based biofertilizers require infrastructures for such products to be stored and transported and these infrastructures are generally lacking. Accordingly, farmers are averse to replacing nitrogen fertilizer with these biofertilizers. Nevertheless, since these countries represent only a small share of the overall biofertilizer market, the particular problems of applying these inoculants in developing countries are not considered [110].

In general, in order to broaden the worldwide commercialization of PGPR-based biofertilizers several issues should be addressed. First of all, there is a need for consistency among the regulatory agencies of the different countries regarding which bacteria can be released into the environment. Providing information to farmers for a better understanding of the advantages and disadvantages of using biofertilizers versus chemical fertilizers would improve their disposition towards these products. Also, efforts to develop more effective means of applying PGPR to crops would boost farmers' approval. Finally, simplifying product registration processes would motivate more companies to commercialize a wider variety of products specific to different crops and adapted to different abiotic conditions, with higher probabilities of success.

5. Conclusions and Future Perspectives

As long as the human population continues to increase the world will have to deal with an escalating demand for food. Cirera and Masset [131] estimate that during the 21st century agricultural crop production should increase by about 100%. Seventy years ago, the Green Revolution increased agricultural production worldwide, saving about one billion people from starvation and undernourishment, and was founded on the development of chemical fertilizers, along with other advances. The synthesis of chemical biofertilizers consumes enormous amounts of energy, around 1% of the total energy consumption of the world, contributing heavily to climate change. However, their application in the field has an efficiency of just 60–70% and it has been shown that they produce negative effects on human health and the environment. Therefore, the development of a more efficient and sustainable agriculture, guaranteeing food supply for an expanding world population and minimizing damage to the environment, is one of the greatest challenges for humankind today. Promotion of the use of PGPR is one possible way to achieve the goal. Most soils are well inoculated with the organisms involved in the general decomposition processes taking place there. However, the inoculation of soils with special-purpose microorganisms can increase plant growth. There is a plethora of studies showing that bacteria can improve crop yields in agriculture and forestry, through many and diverse mechanisms. PGPR bacteria promote plant growth not only by supplying nutrients to the plant, but also by producing phytohormones, inducing stress resistance, or preventing pathogen-induced plant diseases. Thus, the development of the biofertilizer market and the promotion of bacterial inoculations in the field is an environmentally friendly way to meet the worldwide need to raise crops yields.

Consumers demand more and more organic food, and most countries have developing policies to reduce the use of chemical fertilizers. As a result, the commercialization and application of bacterial biofertilizers on agricultural fields or in arboriculture are increasing year by year. Nevertheless, their use is still far from that of chemical fertilizers. Demand from farmers is one of the most critical steps required for the promotion of biofertilizers. Farmers may be undecided as to whether they should adopt new technologies or trust biofertilizer efficiency. Therefore, governmental and international policies promoting this type of farming are needed urgently. Also, coordinated work by bacteriologists, chemists, geneticists, agronomists and farmers could allow the adaptation of bacterium-based biofertilizers to the different agricultural systems by making them more efficient in the field. Consortia of various organisms with different benefits for crops can be integrated to combine different microbial capabilities into one product with several yield-promoting effects. Additionally, advances in new technologies leading to the enhancement of biofertilizer shelf-lives, facilitating their distribution and application, are essential for their use to be extended.

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Conflict of Interest

All authors declare that there are no conflicts of interest.

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