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Review Article

Co-inoculation of Rhizobia and Other Plant Growth-Promoting Rhizobacteria as Biofertilizers: A Biotechnological Overview

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ABSTRACT

The use of rhizobia as inoculants in leguminous plants is a traditional technique for nitrogen bioavailability for these plants. In Brazil, this inoculation is capable of supplying all the nutritional needs of soybeans. However, as the world's population and food need increases, it is important to prospect techniques to increase the productivity of crops in an efficient and sustainable way. Co-inoculation of rhizobia with other plant growth-promoting rhizobacteria (PGPR) is promising in this area. Actinobacteria are PGPR with immense biotechnological potential and, in the soil, they perform several functions, such as the production of phytohormones, antibiotics, and exoenzymes that help plant growth. Furthermore, it has been documented that actinobacteria can act as growth promoters of rhizobia. The ecological combinations and the successor plants in the soil are determinants in the soil communities, and all these concepts and perspectives are proposed in this review.

Keywords: bacterial ecological interactions; bioinoculant; co-inoculation; cross-feeding; diazotrophic bacteria; *Streptomyces*;

INTRODUCTION

Cowpea is the oldest crop known to man and has been planted for over 4,000 years [1]. This grain is the source of much of the protein ingested in several African countries due to its low cost and growth in dry and nutrient-poor soils. This plant was the fourth largest source of dry grains worldwide among legumes between 2008 and 2017 [2].

Cowpea, or *Vigna unguiculata*, belongs to the Fabacea family and grows mainly in dry tropical soils in Latin America, Africa and South Asia [3].

Like many other plants, this legume has its roots associated with rhizobia, mainly from the genera *Bradyrhizobium* and *Rhizobium* [4]. Rhizobia are bacteria that are known to infect the roots of legumes to form root nodules. These bacteria can also infect the stems of legumes, but in all cases, they fix atmospheric nitrogen in forms that plants can assimilate. Nitrogen is one of the essential elements for maintaining life, being present in amino acids, nucleic acids, ATP, and NAD in all living cells, in addition to being an important component of chlorophyll [5]. Nitrogen supplementation is expensive and brings major pollution problems, such as eutrophication of aquifer bodies and the release of greenhouse gases [6].

Rhizobia are natural inoculants, and their use in crops is an alternative to the use of chemical fertilizers, due to their ability to transform nitrogen naturally available to plants through a process called biological nitrogen fixation (BNF). BNF is responsible for supplying between 50 and 70 million tons of bioavailable nitrogen per year worldwide [7]. Since 2020, Brazil is the main soybean producer in the world and inoculation of this legume with strains of *Bradyrhizobium* meets the plant's nitrogen needs and generates savings of US$ 15 billion per year that would be spent on nitrogen fertilization [8].

When talking about rhizobacteria, the focus on the symbiosis between rhizobia-plant and the consequent nitrogen fixation can make a very important point go unnoticed: the competition with the local microbiota in rhizospheric environment implies in a need of rhizobacteria to outstand for successfully establishsymbiosis with the host plant[9].

Regarding another important soil bacteria, the bacterial phylum Actinobacteria is one of the most abundant in soil with a relative abundance of approximately 22% of total soil microbiota and reaching up to 62% in desertified soils. In consequence, it interacts directly with rhizobia in the rhizosphere. These bacteria can increase absorption of moisture in soil and stimulate microbial growth [10]. Despite this stimulus, actinobacteria produce a variety of antimicrobial compounds. About two thirds of known antibiotics are produced by actinobacteria, where the genus *Streptomyces* is prominent [11]. Therefore, the compatibility between rhizobia and actinobacteria is important for the establishment of symbiosis between rhizobia-plant and for growth and development of said plant. Based on these definitions, we can assume that the nitrogen fixing capacity of rhizobia combined with the variety of secondary metabolites produced by actinobacteria can result in a promising bioinoculant for plant growth and development.

Bioinoculants are essential microorganisms that are subjected to biotic and abiotic stresses in plants. Also called plant growth-promoting rhizobacteria (PGPR), these microorganisms are able to perform activities, such as hydrolysis of exudates in order to avoid osmotic stress, production of deaminases, indoleacetic acid, siderophores, phosphate solubilizing enzymes and microbiocidal/ biostatic enzymes [12]. Biofertilizers are ‘products that contain live or dormant microorganisms (bacteria, actinobacteria, fungi, algae), alone or in combination, that help fix atmospheric nitrogen or solubilize soil nutrients’. PGPR also benefit the plant through the production and release of growth-promoting substances capable of increasing plant productivity [13]. Some bioinoculants may have biofertilizing and antimicrobial activity, such as some species of *Pseudomonas* and *Bacillus*, which stimulate plant growth while antagonizing pathogens and stimulating plant defenses [14].

These microorganisms must be compatible with each other for a microbial consortium to be able to stimulate plant growth. The interactions between actinobacteria and rhizobia are discussed further in this review, emphasizing the potential of this consortium for the development of a bioinoculant capable of stimulating plant growth.

ECOLOGICAL BACTERIAL INTERACTIONS

Living beings interact when they need to share the same space, and microorganisms are no different. As a colony grows, bacteria can specialize in different tasks, sharing, and optimizing resources. The constant exchange of different metabolites between cells is the result of a greater metabolic efficiency of the colony, which increases its chances of being successful [15].

Bacteria have up to 42% of their genes encoding traits involved in ecological relationships [16]. They often compete for numerous limiting factors in nature, such as better habitats, minerals, and diverse nutrients. Therefore, bacteria developed numerous strategies to allow growth and reproduction under harsh conditions, such as the secretion of toxins and antibiotics. The production of these metabolites confers an advantage on the growth of the bacteria that produced them, to the detriment of the other local microbiota [17].

In general, ecological antagonistic relationships tend to overlap neutral relationships, which in turn overlap positive relationships [18]. These antagonistic interbacterial relationships usually occur through secretion of diffusible small molecules (secondary metabolites) and antibacterial protein toxins (antimicrobial peptides). It is quite common for bacteria to compete for nutrients in order to grow when present in the same medium [19]. However, more complex interactions can occur, where the product of the metabolism of one strain can be used for the growth of another. This positive ecological relationship is called cross-feeding [20].

Bacteria are subject to the loss or degradation of these molecules or even consumption by third parties by transferring molecules via diffusion. Thus, some bacteria have evolved to possess some methods to deliver the intended molecules directly to the recipient. Methods such as vesicles in the outer membrane, channels, nanotubes, *pili*, or even membrane fusion with cytoplasm exchange can be used to enable this exchange more precisely [21]. However, these methods require bacteria to maintain close physical contact. As this proximity is not always possible, it is necessary to adopt other methods so the bacteria can cooperate.

Smith *et al.* (2019) devoted a good part of their literature review on bacterial cross-feeding to classify and describe various forms of metabolic cooperation between bacteria. The authors define four general classes: metabolite cross-feeding, substrate cross-feeding, mutual cross-feeding and augmented cross-feeding. The first one refers to when a bacterium takes advantage of the by-products of the metabolism of another bacterium, which feeds on complex carbon sources. Substrate cross-feeding occurs when bacteria release extracellular enzymes to degrade a particular substrate. The products of this degradation can be used both by exoenzyme-producing bacteria and by the opportunists. Mutual cross-feeding is nothing more than one of the previous examples, but when both bacteria feed and are fed by the other. Augmented cross-feeding is a type of mutual cross-feeding, where bacterium A intensifies the metabolic pathways responsible of synthesizing products used by bacterium B, receiving byproducts of B's metabolism in return [20].

CROSS-FEEDING ON SOIL

The soil is a heterogeneous environment in which biotic and abiotic factors often interact and influence each other. Dynamics of nutrients such as carbon and nitrogen, as well as climate, have a direct effect on the diversity and ecology of soil microorganisms [22]. The abundance and diversity of edaphic bacteria and fungi is such that their metabolic activities have direct impacts on biogeochemical cycles at the biosphere level [23].

There is great diversity and complexity of carbohydrates in soil, as there is a high metabolic cost to produce and excrete enzymes to hydrolyze these carbon sources. Sometimes, it is necessary for different species of microorganisms to cooperate in order to metabolize them. Given the variety of sugars in soil, cooperative systems are common. There are also one-way paths, where 'cheater microorganisms' take advantage of the product of exoenzyme-catalyzed reactions produced by other microorganisms, as these small molecules are able to diffuse away from the enzyme producer [24].

An example of cross-feeding on soil is the formation of multi-species biofilms. The heterogeneity of the soil allows the formation of several structures suitable for the establishment of biofilms, and those preferred by bacteria are carbon-rich surfaces such as roots, fungal hyphae, and decaying organic matter. Biofilms in soil are nothing more than an aggregate of different species of microorganisms that produce a matrix of extracellular polymeric substances (EPS). The physical proximity between microorganisms provided by the biofilm favors the exchange of metabolites, but this structure is better known to protect bacteria against environmental stresses, predation, dehydration, antibiotics, and improving the availability of nutrients and oxygen [25].

From an evolutionary point of view, the sessile lifestyle provided by biofilms gives the different species of microorganisms that form it advantages over free-living organisms. Better growth during periods of dehydration and more opportunities for horizontal gene transfer are some examples. Although the positive points of biofilms are well known and studied, some characteristics such as ecological and biological determinants of their formation, their influence on microbial metabolic activity, and the structure of the communities formed are still unknown [26].

Rhizobacteria are capable of forming biofilms, with the genera *Acetobacter, Alcaligenes, Bacillus, Pseudomonas, Rhizobium, Rhodococcus, Serratia* and *Streptomyces* being some examples of bacteria widely found in the rhizosphere with this ability. In general, rhizobacteria biofilms are the same as ordinary biofilms, but have some ecological dissemblances. Rhizobacteria coexist naturally in a consortium interacting with each other, but also influence plant metabolism. Multispecies rhizobacterial biofilms colonize roots and promote plant growth in addition to preventing pathogen attacks, thus being of fundamental importance for sustainable agriculture [27].

ACTINOBACTERIA AND THEIR BIOTECHNOLOGICAL POTENTIAL

The Actinobacteria phylum is one of the oldest in the Bacteria kingdom and is present mainly in soil, but also in oceans, extreme environments, plant tissues, animal excreta, algae and lichens [28]. These bacteria played a vital role in colonizing the terrestrial environment [29]. Actinobacteria are very diverse among themselves and among other bacteria [30], which has long led them to be called 'actinomycetes' due to their morphological similarity to filamentous fungi. For a long time, actinobacteria were considered a transition between bacteria and fungi due to the common characteristics between these two classes of microorganisms. However, with the advancement of biochemistry and DNA sequencing technologies, it was observed that their morphologywas much more similar to Prokaryotes than Eukaryotes, as thin cells, chromosome stored in a nucleoid, peptidoglycan cell wall and susceptibility to antibacterial compounds [31].

Unlike most bacteria, actinobacteria are capable of forming aerial and vegetative (or substrate) mycelium and reproducing by sporulation (by fragmentation and segmentation or by conidia formation) [32]. The main criteria for the phenotypic differentiation between these microorganisms are the presence or absence of aerial and vegetative mycelium, the color of the mycelium, diffusion of pigments in the medium and the morphology of their spore chains [31]. Actinobacteria are Gram-positive and generally have high levels of guanine and cytosine (G + C) in their single chromosome, reaching up to 70%, and often have long linear plasmids. Some genera such as *Streptomyces, Actinomyces, Amycolatopsis, Actinoplanes, Streptoverticillium,* and *Micromonospora* can also have a linear chromosome [30, 33].

Actinobacteria have a relative abundance of 22% in soil, which is one of their main habitats. Desertified soils' microbiota is constituted of up to 62% of these microorganisms, where they play vital ecological roles such as hydrolyzing complex and recalcitrant polymers (such as lignocellulose, keratin and chitin), stabilizing clay particles and organic matter, alleviating biotic and abiotic stress of plants, fixing atmospheric nitrogen, solubilizing phosphorus sources, decomposing plant residues and solubilizing the cell wall of fungi and plants, insect cuticles, and crustacean shells [10, 34, 35]. In extreme environments such as the Semi-arid, where it is common to observe soils in desertification, the survival of plants relies on the community of microorganisms associated with it, either in the rhizosphere or in symbiosis. In this water-poor soil, microorganisms are vital to modify its structure in order to optimize biological activity, for example, retaining water [34]. The characteristic smell of wet dirt also comes from actinobacteria, which produce the terpene responsible for this odor, called geosmin [36].

Some actinobacteria even act as endophytes, such as in medicinal plants in rainforests. This symbiosis is set when the host plants release exudates by their roots which directly impact the microbiota present in the rhizosphere. When in symbiosis with plants (most commonly in roots), actinobacteria act by producing phytohormones or other growth factors, increasing vegetal resistance to abiotic and biotic stresses such as insects, pests, and pathogens. In exchange, they obtain nutrients and shelter from the host plant. Actinobacteria are also capable of suppressing competitors by synthesizing antibiotics [28, 37]. This diverse production of secondary metabolites justifies about 45% of the 22,500 currently known compounds with biological activity being extracted from actinobacteria. Furthermore, these microorganisms produce 80% of the known antibiotics, especially the genera *Streptomyces* and *Micromonospora* [3].

The biotechnological value of the phylum Actinobacteria is undeniable. These microorganisms are famous for synthesizing and excreting secondary metabolites of high industrial interest, such as immunosuppressants, anticancer, antiviral, antifungal, antiparasitic, anthelmintic and, as already mentioned, antibacterial compounds [38-40]. Actinobacteria also synthesize extracellular enzymes such as amylases, cellulases and xylanases [41, 42].

Amylases are enzymes with potential application in various fields, such as medical, textile, and ethanol production, as well as in the treatment of fruits such as bananas, mangoes and citrus fruits, the washing of bioreactors in the food industry, starch and fermentation and distillation [43]. Cellulases hydrolyze cellulose into glucose due to their exoglucanase, endoglucanase and beta-glucosidase subunits. This ability of cellulase makes it relevant in textile and food industrial processes, animal feed improvement, detergent production, pulp and paper industry, extraction of green tea components and production of bioethanol from lignocellulosic biomass [44, 45]. Lignocellulose, the largest source of plant biomass in the world, is formed mainly by cellulose (35-50%) and hemicellulose (20-35%). Most of hemicellulose is formed by xylan, which is the second most abundant component after cellulose [46]. Xylanase is responsible for converting xylan into xylose, thus degrading hemicellulose, an essential component of plant cell walls, and making these nutrients available to the microorganisms present in vegetal sources [47]. The degradation of lignocellulose from plant residues is the main industrial application of xylanases, where they are used in the production of biofuels, to reduce the viscosity of juices and animal feeds, and in the paper industry, as a bleaching agent for wood pulp for the production of better-quality paper. [48].

The genus *Streptomyces* stands out in the production of these exoenzymes [49-51]. This genus is the most abundant among actinobacteria and is also the source of streptomycin, gentamicin, rifamycin, chloramphenicol and erythromycin which are popular antibiotics [52]. The genus *Streptomyces* has 1134 species and 71 subspecies [53]. Its importance and abundanceare such that actinobacteria belonging to other genera are called ‘rare actinobacteria’ [54]. However, this term seems to be inappropriate. Some authors suggest classifying these microorganisms as ‘non-Streptomyces actinobacteria’, which better defines less abundant actinobacteria, but still highlights the importance of the dominant genus [55].

Streptomyces have a multicellular life cycle where they undergo several morphological and physiological changes. After the spore germinates, the bacteria grow by tip extension and form a network of hyphae known as the vegetative or substrate mycelium. Colony maturation leads to the differentiation of an aerial mycelium from cell division and the generation of monoploid spores. It is at this stage of differentiation that the famous secondary metabolites are produced, in order to provide the bacteria with better chances of survival even in unfavorable environments [56].

Actinobacteria have the necessary metabolic flexibility to become promising targets of metabolic engineering for the production of secondary metabolites of high profitability, mainly due to containing several biosynthetic gene clusters (BGCs) [52]. BGCs are genomic regions that contain three or more non-homologous genes that encode biosynthetic enzymes. The genes present in BGCs usually encode a specific biosynthetic pathway and are often expressed simultaneously [57].

RHIZOBIA

Another group of bacteria present in soil, more specifically in the roots of leguminous plants, are rhizobia. The term ‘rhizobium’ is a term that defines bacteria from eight families, seven of which are α-Proteobacteria (*Rhizobiaceae, Phyllobacteriaceae, Nitrobacteriaceae, Methylobacteriaceae, Brucellaceae, Hyphomicrobiaceae*and*Xanthobacteriaceae*), which are the most common, and one β-Proteobacteria (*Burkholderiaceae*). Currently, there are 226 species of rhizobia known, which are distributed in 19 genera: *Agrobacterium, Allorhizobium, Ensifer (Sinorhizobium), Neorhizobium, Pararhizobium, Rhizobium, Shinella, Aminobacter, Phyllobacterium, Mesorhizobium, Bradyrhizobium, Microviga, Methylobacterium, Brucella (Ochrobactrum), Devosia, Azorhizobium, Cupriavidus, Paraburkholderia*and*Trinickia*[58].

Rhizobia are Gram-negative, motile (have one to six peritrichous flagella), rod-shaped, non-spore-forming aerobic bacteria with mucous and convex colonies ranging from white to beige, and from shiny to opaque [59]. In general, rhizobia are classified according to their growth time (fast or slow) and effect on the pH of YMA (Yeast Extract-Mannitol Agar) culture medium. Fast-growing species tend to acidify the medium, while slow-growing species tend to keep its pH unchanged [60].

The importance of these microorganisms is given by their ability to establish a symbiotic relationship with the roots of legumes and induce the formation of nodules, where they will perform biological nitrogen fixation (BNF). Although nitrogen is the most abundant gas in the atmosphere, it is not bioavailable to plants. In search of nitrogen, legumes release flavonoids by their roots, attracting rhizobia to the rhizosphere, where they will secrete nodulation factors [61]. The rhizobia attach to the roots, then form an infection pocket and secrete cellulases in order to penetrate the roots. Thus, the plant will give rise to a nodular meristem, which will develop and attract bacterial cells to its interior. Finally, bacteria are trapped in organelle-like structures called symbiosomes, where they will differentiate into bacterioids and complete the maturation of the nodules. Within the microaerophilic environment of these mature nodules, rhizobia are ready to reduce atmospheric nitrogen (N_2) to ammonia through nitrogenase enzymes [9].

The nitrogenase enzyme complex is responsible for nitrogen fixation in all known diazotrophic organisms. Rhizobia have the *nif* genes, which are responsible for the synthesis of this enzymatic complex and several regulatory enzymes for nitrogen fixation, which can also be encoded by the *fix* genes [5]. The nitrogenase complex is composed by two proteins: one, dinitrogenase reductase (Fe protein), has a Fe_4S_4 cluster and the other, dinitrogenase (MoFe protein), has Fe_8S_7 clusters and FeMo cofactors ($MoFe_7S_9C$ homocitrate). Therefore, nitrogenase synthesis and consequently nitrogen fixation is sulfur dependent, and its presence in the soil is directly linked to the rhizobia-plant symbiotic relationship [62].

The final form of the nitrogen fixed by the rhizobia will depend on the legume plant it is associated to. In plants in temperate environments, ammonia is converted to glutamine and asparagine, and in tropical environments to ureides, such as allantoin and allantoic acid. Nitrogen is bioavailable to plants through the xylem, which in turn provides sources of carbon and energy in one of the most famous symbiotic relationships [63]. The final form of nitrogen fixed by SNF is as diverse as the leguminous plants colonized by rhizobia, which consists of 750 plant genera [64].

Nodulation is mainly controlled by the plant, which can even prevent the permanence of nodules colonized by inefficient rhizobia. Therefore, some rhizobia may develop mechanisms and strategies to improve their nodulation and nitrogen fixation capacity in a given plant [65]. BNF is the most efficient mean of supplying nitrogen to plants. In addition to saving the billions of dollars annually spent on industrialized nitrogen fertilizers, which are polluting and originated from (nonrenewable) fossil sources, the use of rhizobia as nitrogen providers for plants is an affordable alternative for subsistence agriculture [66].

BIOINOCULANTS

The Green Revolution consisted of a set of initiatives adopted worldwide to improve agricultural production, mainly in developing countries. This was achieved by improving irrigation, using large amounts of chemical fertilizers and planting high-yielding crops. However, these strategies caused serious environmental problems, such as decreased soil biological and physical-chemical health, loss of biodiversity, genetic erosion, ecological imbalance, decreased stress tolerance of plants, among other consequences [67]. A strategy to reduce the use of synthetic agrochemicals and combat soil deterioration would be to take advantage of the genetic and biological potential of crops and microorganisms associated with plants [68].

Endophytic microorganisms are those that colonize plant tissues without causing diseases and can establish a beneficial relationship with their host, producing phytohormones, enzymes, antagonizing pathogens, stimulating phytoremediation and bioavailability of nutrients. These beneficial rhizobacteria include rhizobia, some actinobacteria, mycorrhizal fungi and free-living bacteria [69, 70].

Bacteria that live in the rhizosphere and stimulate plant growth through one or more mechanisms, even in the presence of competitors, are called 'plant growth-promoting rhizobacteria' (PGPR) and represent 2 to 5% of the rhizospheric microbiota. PGPR can be extracellular (ePGPR), living in the rhizosphere, in the rhizoplane or in the extracellular spaces in the plant cortex. Some examples of ePGPR are bacteria of the genus *Agrobacterium, Arthrobacter, Bacillus, Burkholderia, Erwinia, Micrococcus, Pseudomonas* and *Serratia*. Intracellular PGPR (iPGPR) usually colonizes specialized structures (nodules) in the roots and are usually of the genera *Allorhizobium*, *Bradyrhizobium, Mesorhizobium*, and *Rhizobium*, among others [71].

Actinobacteria are able to act as PGPR both directly and indirectly. The direct method includes the production of siderophores and phytochromes, phosphate solubilization, and nitrogen fixation, while the indirect method includes the synthesis of lytic exoenzymes, antibiotics, volatile compounds, and competing with pathogens for nutrients [72]. Actinobacteria of the genera *Streptomyces, Thermobifida, Frankia, Nocardia, Kitasatospora, Micromonospora, Actinomadura, Streptosporangium, Actinoplanes* have been reported as PGPR with the most varied applications [73].

All the microorganisms mentioned so far in this review can be considered examples of bioinoculants. By definition, bioinoculants are microorganisms that can stimulate plant growth. In general, thesemicroorganisms act in the absorption of nutrients and in the protection against diseases, but they can also be applied in the bioremediation of pollutants. The effects of bioinoculants on plant health are also related to the mechanism of induced systemic resistance (ISR), which is activated by the JA/ET (jasmonic acid/ethylene) and salicylic acid pathways [74]. It is for all these benefits that it becomes evident that PGPR-based formulations are a promising alternative to minimize the use of synthetic fertilizers and agrochemicals in agriculture, which in the long term can cause soil acidification and reduced nutrient uptake by the roots [75].

The negative side of using bioinoculants in agriculture regards that PGPR inoculation can affect the native microbiota. These interactions can be positive or negative and will depend on the physical and chemical characteristics of the soil and other abiotic conditions. Changes in the population of microorganisms in a given area can affect the quality and fertility of the soil. It is therefore in the interest of industry to better understand the interactions between bioinoculants and native microorganisms to enhance their effectiveness [76].

The semantics of the terms bioinoculant, biofertilizer, and biopesticide vary widely in the literature, often being taken as synonyms [77]. However, bioinoculants are a general term to address beneficial microorganisms for plants, and they can be classified according to their action as biofertilizers, if they improve the bioavailability of nutrients in the soil, and as biopesticides, if they antagonize phytopathogens. In Table 1 it is possible to observe some bioinoculants known in agriculture.

The use of rhizobia as biofertilizers for legume crops is a well-established technology. These bacteria reduce the need for nitrogen fertilization as a result of the increase in nitrogen uptake by plants promoted by SNF. The symbiotic relationship between rhizobia and plants is already well studied, from quorum-sensing for the formation of nodules, to the directed evolution of rhizobia towards mutualism with the plant. In general, plant growth stimulated by PGPR does not occur directly by fixing nitrogen, but by producing phytohormones [79].

Legumes have been known for centuries to have a positive effect on soil fertility, and it was only later discovered that this effect was actually the result of the biofertilizing action of rhizobia. The advancement of studies in this area led to, in 1896, the deposit of the first patent for a *Rhizobium*-based biofertilizer, which took the name of Nitragin [80]. The use of mixed rhizobia bioinoculants with other PGPR is considered the 'supreme inoculant', due to its potential for the development of new commercial products. However, there is still some hesitation for the use of bioinoculants by farmers. This is because commercial formulations are often established without much scientific rigor, leading to products with little impact on soil fertility and crop yields. Despite this, there is a consensus in the scientific community that biofertilizers are cheap, sustainable and effective products that remedy the negative impacts caused by chemical fertilization [81].

Biofertilizers		
Function	**Type**	**Name**
Nitrogen fixers	Bacteria	*Rhizobium, Azotobacter, Azospirillum, Frankia*
Zinc solubilizers		*Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Mycobacterium* sp.
Phosphate solubilizers		*Bacillus megaterium, Pseudomonas* sp., *Rhodococcus, Serratia, Micrococcus*
Phosphate solubilizers	Fungi	Arbuscularmycorrhizae, *Penicillium, Piriformospora indica*
Micronutrient mobilizers		Arbuscular mycorrhizae
Biopesticides		
Name	**Type**	**Target**
Bacillus sp.	Bacteria	*Fusarium, Verticillium, Ascochyta, Alternaria, Xanthomonas, Erwinia*
Bacillus thuringiensis		Caterpillars, weevils, leafhoppers, insects
Serratia sp.		*Sclerotium*
Pseudomonas sp.		*Penicillium, Botrytis cinerea, Mucor, Pythium*
Gliocladium sp.	Fungi	*Penicillium, Aspergillus, Fusarium*
Trichoderma sp.		*Fusarium, Macrophomina, Ascochyta, Cercospora*
Verticillium lecanii		Thrips, whiteflies, aphids, mealybugs
Nuclear Polyhedrosis Virus (NPV)	Virus	Caterpillars, earthworms, moths, corn borer

TABLE 1 - List of some known bioinoculants and their respective applications;
Source: KUMAR (2018) [78]

Successful co-inoculations between rhizobia and other PGPR are well documented. *Bradyrhizobium*, for example, show positive results in plant growth and development when co-inoculated with *Pseudomonas oryzihabitans, Pseudomonas putida,* *Bacillus megaterium, Bacillus pumillus*, and mycorrhizae (*Glomus clarum, Glomus mosseae, Gigaspora margarita*) [82-85], among others.

Actinobacteria have a positive effect on nodulation and growth of legumes, such as the co-inoculation of soybean with *Bradyrhizobium japonicum*, *Streptomyces* sp. and *Nocardia* sp. Another example is co-inoculation of alfalfa with *Sinorhizobium meliloti* and *Micromonospora* spp. (or *Frankia*, which stimulates nodulation even in soils with high levels of nitrogen, a condition that usually inhibits nodulation) [86].

The prospection of bioinoculants based on actinobacteria and rhizobia, more specifically between the genera *Streptomyces* and *Bradyrhizobium*, is well studied and seems promising [87-90]. Thus, it is evident that these two genera stand out when it comes to prospecting new PGPR with bio inoculating potential. The variety of plants and their respective edaphic microbiota found around the world makes it necessary to use the most suitable bioinoculants for each location and climatic conditions. For example, microorganisms isolated from semiarid zones are more suitable for biotechnological application in cultivars used in this climate, so it is necessary to study the co-inoculation of microorganisms at the local level.

CONCLUSION

Rhizobia have the ability to supply the legume's need for nitrogen and this inoculation saves billions of dollars annually, as well as being more sustainable. The association of rhizobia with other PGPRs is well documented in the literature and presents great potential to improve the growth and productivity of cultivars. The biotechnological potential of actinobacteria, such as their production of secondary metabolites and exoenzymes, makes them strong candidates for such co-inoculation. Actinobacteria and rhizobia are PGPRs that, in coculture, can become promising biofertilizers for agriculture. This co-inoculation must be prospected at local level, once this increases the chance of compatibility between the rhizobia and the actinobacteria.

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