

Plant growth-promoting rhizobacteria: a biofertilization alternative for sustainable agriculture.

ABSTRACT

Modern agriculture faces new challenges, integrating ecological and molecular approaches, to achieve higher crop yields and minimize environmental impacts. In order to generate higher yields, synthetic fertilizer doses per unit area have been significantly increased, which can cause pollution, damage to health and loss of soil fertility, becoming one of the most important concerns in agricultural production. To improve production without the use of synthetic fertilizers, research has been directed towards the development of new biotechnologies, leading to a growing interest in beneficial soil microorganisms that can promote plant growth and, in some cases, prevent infections of plant tissue by pathogens. The interactions of plant growth-promoting rhizobacteria (PGPR) with the biotic environment - plants and microorganisms - are very complex and use different mechanisms of action to promote plant growth. These mechanisms are grouped into: 1) Biofertilization; 2) Phytostimulation; and 3) Biocontrol. Inoculation of crops with PGRP substantially reduces the use of synthetic fertilizers and negative impacts on the soil, increases crop yields, and contributes to the producer's economy and the population's food supply. This review describes the basic aspects inherent to the interaction between the CVPGRs and plant species, focusing on the benefits that CVPGRs bring to agricultural activity.

Keywords: biotic activity, biocontrol, inoculation, microorganisms, rhizosphere.

INTRODUCTION

Agriculture is the main sector of economic growth in developing countries (Nehra, Saharan and Choudhary, 2016), including Mexico. However, in contemporary production systems, most crops are very demanding with respect to fertilizer demand (Naqqash, Hameed, Imran, Hanif, Majeed et al., 2016). In relation to this demand, it has been estimated that by 2018 there will be a global consumption of 200.5x10⁶ tons of N-P₂O₅-K₂O (FAO, 2015). On the other hand, every day there is more evidence that the continuous application of nitrogen fertilizers can cause negative impacts on agro-ecosystems, such as nitrate leaching, contamination of water resources, and gaseous emissions, causing irreparable damage to the environment (Zahid, Abbasi, Hameed, & Rahim, 2015) and with potential risk to humanity (Vejan, Abdullah, Khadiran, Ismail, Nasrulhaq-Boyce, 2016). The above, makes it evident that there are several

problems that demand ongoing attention from researchers. Specifically, a promising method to reduce the use of synthetic fertilizers in agriculture is the application of PVGRs as microbial inoculants (Nehra et al., 2016). The use of PVGRs as biofertilizers are a sustainable option to promote the availability of nutrients, plant growth and yields (Zahid et al., 2015). In view of the above, we describe the elements inherent to the interaction between the PVGRs and plant species, focusing on the effects they cause to agricultural crops, through their root system.

Plant Growth Promoting Rhizobacteria

The expression Plant Growth Promoting Rhizobacteria (PGPR) was coined by J. W. Kloepper and M. N. Schroth in 1978, to describe bacteria that inhabit the rhizosphere and positively affect plant development (Labra-Cardón, Guerrero-Zúñiga, Rodríguez-Tovae,

Montes-Villafán, Pérez-Jiménez et al., 2012). -These bacteria have the ability to -actively- colonize -the root system to promote and/or improve its growth and yield (Berendsen, Pieterse and Bakker, 2012). RPCVs account for about 2 to 5 % of rhizospheric bacteria (Jha and Saraf, 2015). The acronym RPCVs refers to all bacteria that are capable of enhancing plant growth through one or more mechanisms. The following genera -of bacteria have been reported as RPCV: Agrobacterium, Arthrobacter, Azoarcus, Azospirillum, Azoto- bacter, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Enterobacter, Erwinia, Flavobacterium, Klebsiella, Micrococcus, Pantoea, Pseudomonas, Rhizobium and Serratia (Ahemad and Kibret, 2013).

RPCVs exert beneficial effects on plants through direct and indirect mechanisms, or a -combination of both (Aguado-Santacruz, Moreno- Gómez, Jiménez-Francisco, García-Moya, & Preciado- Ortiz, 2012; Parray, Jan, Kamili, Qadri, Egamberdieva et al., 2016). Direct mechanisms occur when bacteria synthesize metabolites that facilitate -plants, or when plants increase the availability of different nutritive elements, required for their metabolism and to improve their nutrition process (Gómez-Luna, Hernández-Morales, Herrera-Méndez, Arroyo-Figueroa, Vargas-Rodríguez et al., 2012). Among the direct mechanisms, the following stand out: nitrogen -(N) fixation-; -the synthesis of phytohormones, vitamins and enzymes-, the solubilization of inorganic phosphorus (P) and the mineralization of organic phosphate, the oxidation of sulfides, the increase in root permeability, the production of nitrites, the accumulation of nitrates, reduction of heavy metal toxicity and ACC deaminase enzyme activity, secretion of siderophores, reduction of ethylene levels in soils, and increased root permeability (Esquivel- Cote, Gavilanes-Ruiz, Cruz-Ortega and Huante, 2013).

Indirect mechanisms are characterized by -the reduction or -elimination of phytopathogenic microorganisms, either through the production of antimicrobial substances or antibiotics, lytic enzymes or a combination of these; by competition for nutrients

or space in the ecological niche, as well as by stimulation of the plant's natural defenses through biocontrol mechanisms; the induction of systemic resistance (IRS) to a broad spectrum of pathogenic organisms and the production of siderophores, as a mechanism to sequester available Fe in soils and thus -limit the development and presence of these phytopathogens; production of antibiotics and hydrogen cyanides that impact on phytopathogens; hydrolysis of molecules such as fusaric acid generated by them to release 1-3-glucanase, which inhibits the development of the fungal wall of fungi such as *Phytophthora ultimum* and *Rhizoctonia solani* (Esquivel-Cote et al., 2013).

One of the limitations of PVGRs is that the -beneficial effect -they promote on a given plant species is not the same for other plants. In this regard, Xu, Jeffries, Pautasso and Jeger (2011) point out that the combined use of biocontrol agents should generally not be recommended in practice without a clear understanding of their main control mechanisms and their relative competitiveness. To avoid the above, to date there are a number of -reports that clarify the type of RPCV, their effect and the crops where they can be applied, see -examples in -Table 1.

Root colonization

An essential step for RPCVs to efficiently carry out biological control and promote plant -growth is undoubtedly the colonization of their root system (Noumavo, Agbodjato, Baba- Moussa, Adjanohoun and Baba-Moussa, 2016). -Key elements for efficient colonization include, among others, the ability of microorganisms -to: a) survive after inoculation; b) grow in the spermosphere (region surrounding the seed) in response to the production of exudates by the seed-; c) attach to the surface of the first roots; and d) colonize the entire root system (Nihorimbere, Onge- na, Smargiassi, and Thonart, 2011; Noumavo et al., 2016). For example, Labra-Cardón et al. (2012) point out that colonization in the spermosphere of *Cyperus elegans* (Cyperaceae) and *Echinochloa polystachya* (Poaceae) seeds was an important event to promote the growth of

the seedlings obtained.

Root colonization by endophytic microorganisms includes four stages: 1) attraction; 2) -recognition; 3) adhesion; and 4) invasion, which are affected by biotic and abiotic factors (Nihorimbere et al., 2011). In addition, seed colonization is the -first step in the process itself. Microorganisms that settle on seeds during germination -can grow and colonize the roots to their full -extent. Seed colonization during the impregnation or immersion phase has a significant effect on plant growth (Noumavo et al., 2016).

Colonization ability is a key factor in the prevention and treatment of fungal diseases because host plants are closely related to biofilm formation; -strong colonization leads to proper biofilm formation (Zhou, Luo, Fang, Xiang, Wang et al., 2016). Better crop results depend on proper colonization of bacteria in the rhizosphere; applying the correct inoculation technique to -seeds will be reflected in higher germination percentage -as well as crop productivity in -addition to increasing its resistance to stress (Mahmood, Turgay, Farooq, & Hayat, 2016).

Table 1. Plant growth-promoting rhizobacteria, effects and crops where they have been evaluated.

RPCV	Effect	Crops
Azospirillum spp, Azotobacter spp, Bacillus spp, Burkholderia spp, Gluconacetobacter spp, Herbaspirillum spp.	Biofertilization Fixed N ₂	Corn, rice, wheat, sorghum, sugar cane.
Bacillus spp, Pseudomonas spp, Streptomyces spp, Paenibacillus spp, Enterobacter spp, Azospirillum spp.	Biocontrol (diseases, pathogens and insects)	Tomato, tobacco, cucumber, bell pepper, peanut, alfalfa, chickpea, bean, plum.
Methylobacterium spp, Bacillus spp, Alcaligenes spp, Pseudomonas spp, Variovorax spp, Enterobacter spp, Azospirillum spp, Rhizobium spp, Klebsiella spp.	Elongation, growth	Turnip, carnation, canola, soybeans, beans, corn, beans, peas.
Aeromonas spp., Agrobacterium spp, Alcaligenes spp, Azospirillum spp, Bradyrhizobium spp, Comamonas spp, Enterobacter spp, Rhizobium spp, Paenibacillus spp, Pseudomonas spp, Bacillus spp.	Phytohormone producers [3-indole-acetic acid (IAA), cytokinins, gibberellins].	Rice, lettuce, wheat, soybean, radish, rapeseed, alder.

Source: Parray et al. (2016)

Adriano, Jarquín, Hernández, Salvador and Monreal (2011) point out that when biofertilizers are applied to seeds, plant surfaces or -soils, they colonize the rhizosphere or the interior of -plants and favor their growth, they also promote the development of plant defense mechanisms and generate adverse environments against pathogenic organisms-. -For example, *P. fluorescens*, a natural soil inhabitant, is predominantly numerous in the -rhizosphere microflora -of many plant species and are the first to colonize young roots. Many of these organisms suppress diseases in -plants, protecting roots and seeds from infection by pathogens present in the soil (Guerra, Betan- courth and Salazar, 2011).

Mechanisms used by the RPCV that affect plant species

The following sections describe the mechanisms of action of the CVERs, through which they favor an increase in agricultural productivity in different regions, from a sustainable approach.

Biofertilization

Among the processes that affect the development and production of plant species, nutrition is considered essential. This is because crops are demanding with respect to appropriate mineral nutrition levels, a requirement that is due to their production volumes per unit area (Chailleux, Mohl, Teixeira, Messelink and Desneux, 2014). In this sense, soil fertility improvement has been one of the strategies commonly used to increase agricultural production. However, over time, it has become evident that the use of synthetic fertilizers has not proved to be the expected panacea, since of the total fertilizers applied only 10 to 40 % is assimilated by plants (Bhardwaj, Ansari, Sahoo and Tuteja, 2014) and furthermore, because the loss of soil fertility, in intensive systems, has forced producers to increase the use of these fertilizers to maintain their production, at the cost of increased production costs and environmental impacts (Cotler, Martinez and Etchevers., 2016). On the other hand, as a consequence of the rising cost of synthetic fertilizers, the scarce natural reserves of some minerals, as well as the large energy consumption for their production, the use of biological alternatives has been promoted, not only as a necessity in agricultural production, but also in scientific agriculture today and in the future, without affecting the environment in addition to economic feasibility (Barroso, Abad, Rodríguez and Jerez, 2015). Thus, in the early 1990s, new concepts and practices inherent to plant nutrition were developed, conditioned by the growing concern for the environment worldwide. Research in this field of knowledge was oriented towards the use of nutrition that included fertilization alternatives with less dependence on polluting inputs (Armenta-Bojórquez García-Gutierrez and Camacho-Báez, 2010). In other words, new technologies should be focused on maintaining and preserving the sustainability of the production system through the rational exploitation of natural resources and the application of relevant measures to preserve the environment (Grageda-Cabrera, Díaz-Franco, Peña-Cabriales and Vera-Nuñez, 2012). In this sense, the inoculation and agronomic management of

microorganisms with biofertilizing properties became rational technologies and emerged as innovative and promising practices for agricultural activity (Sánchez et al., 2011).

Biofertilizers are understood as all those products that contain live microorganisms, with the capacity to colonize the rhizosphere or the interior of plants, which when applied to the soil and/or plants, through inoculation, can live associated or in symbiosis with the plant species and help them in their nutrition and protection, with the aim of partially or totally replacing the application of synthetic fertilizers, and reducing their polluting effect (Mishra and Dash, 2014). Unlike synthetic and organic fertilizers, biofertilizers do not directly supply any nutrients to crops and these are bioproducts, mainly made from bacteria. Armenta-Bojórquez et al. (2010), describe that the microorganisms used in biofertilizers are grouped into those that: (a) have the capacity to synthesize substances that promote plant growth, through various processes such as atmospheric N₂ fixation, solubilization of inorganic Fe and P, increase tolerance to drought stress, salinity, toxic metals and excess pesticides; b) are able to diminish or prevent the effects of pathogenic organisms; and c) fulfill both functions - promote growth and inhibit the effects of pathogens, e.g. *Bacillus subtilis* produces auxins to promote tomato growth and induces systemic resistance against *F. oxysporum*, responsible for root wilt and rot.

Inoculation of biofertilizers containing rhizospheric bacteria has led to significant increases in the productivity of agricultural crops (Armenta-Bojórquez et al., 2010). This is due to the fact that bacteria associated with plant species have the capacity to produce or generate growth regulators and approximately 80% of these are auxin producers. In quantitative terms, the most important auxin is indole acetic acid (IAA), which is responsible for increasing both the root system and the absorption of nutrients (Grageda-Cabrera et al., 2012). As a complement, and with respect to the role played by bacteria, and of which there are myriad research

reports, Mishra and Dash (2014) elaborated a list of advantages and disadvantages inherent to the use of biofertilizers, which are described below:

Advantages

- The supply of nutrients is more balanced, and helps maintain plant health.
- They help to increase the biological activity of the soil, thus improving the mobilization of nutrients and the decomposition of toxic substances.
- Increase soil structure, favoring better root growth.
- They increase the organic matter (OM) content of the soil, thereby improving cation exchange capacity, increasing moisture retention, promoting aggregate formation and buffering sudden changes against acidity, alkalinity-, salinity, pesticides and toxic heavy metals.
- They gradually or slowly release nutrients and contribute to the soil's residual organic N and P reserve, reducing N losses by leaching and P fixation and can also supply micro nutrients.
- They favor the growth of earthworms and beneficial microorganisms.
- They help suppress diseases and parasites transmitted by native soil organisms.

Disadvantages

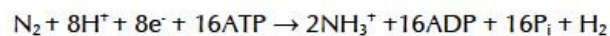
- Compared to synthetic fertilizers, they have a reduced content of nutrients, which requires the use of large volumes to cover the nutrient demand during crop growth.
- The rate of release of nutrients is too slow to meet plant requirements, so nutrient deficiencies may occur in plants.
- Primary macro elements may not be in sufficient quantities in organic fertilizers to support maximum crop growth.

An additional disadvantage is that in several countries,

preferably in rural areas, the use of biofertilizers has been hindered or delayed, largely due to the idiosyncrasies of their inhabitants, since the basic reluctance to use bacteria as beneficial microorganisms is due to the fact that these, in these regions, are still associated with human and animal diseases (Grageda-Cabrera et al., 2012).

Atmospheric nitrogen fixation

Biological nitrogen fixation (BNF) is limited to prokaryotes that possess - unlike the plant - a nitrogenase enzyme complex, which consists of two proteins dinitrogenase and dinitrogenase reductase: both contain Fe in their structure, furthermore dinitrogenase contains Mo (Acuña, Pucci and Pucci, 2010) which catalyzes the reduction of atmospheric N into ammonia (Venieraki, Dimou, Pergalis, Kefalogianni, Chatzipavlidis et al., 2011) process that is explained, in general terms-, by equation 1. Lopez and Boronat (2016) describe this equation noting that, microbiological activation of atmospheric N₂ generates ammonia-, which ionizes to the ammonium cation (NH₄⁺), via ATP hydrolysis and the transfer of reducing power coupled to an electronic transport chain. Bacteria that fix atmospheric N into biologically usable ammonium are called diazotrophs. Diazotrophic organisms include a wide range of Archaea and bacteria that colonize diverse plant species in a wide variety of ecosystems (Venieraki et al., 2011).



(Equation 1)

Among the symbiotic rhizospheric N-fixing prokaryotic rhizobial bacteria in association with legumes are the rhizobia group, e.g. Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium and Allorhizobium, and Frankia strains, belonging to the genus Streptomicetaceae, filamentous sporulating bacteria associated with actinorrhizal plants, especially genera Alnus and Casuarina (Noumavo et al., 2016; Venieraki et al.,

2011). For more than a century, symbiosis between bacteria of the genus *Rhizobium* and legumes has been considered the most efficient way to fix atmospheric N, making it available to plants. However, in recent decades, the study of GNF by associative, free-living or asymbiotic bacteria, a process discovered in 1901 by Martinus Willem Beijerinck, has gained more attention by researchers, with the purpose of finding alternatives to the growing demand for synthetic fertilizers (Pazos, Hernández, Paneque and Santander, 2000).

Under normal conditions, N-fixing microorganisms benefit from this element without excreting nitrogen compounds. But upon their death and after their decomposition, N will be available to plants, generating an average of 25 kg N ha⁻¹ year⁻¹ on the continents. This process is then sufficient to maintain the reserves and to recover the losses of these compounds in the ecosystem (Noumavo et al., 2016). Finally, due to the importance of the FBN process, regardless of whether it is by symbiosis or asymbiotically, Neyra, Terrones, Toro, Zárate and Soriano (2013) describe that the incorporation of this element promotes the stimulation of microbial processes during the decomposition of OM and the recycling of essential nutritional elements within productive agricultural systems, as well as in those considered as sustainable agro-ecosystems. They also mention that it is necessary to continue with the study of the growth promoting capacity of *Rhizobium* because sustainable agriculture demands improving the efficiency of the FBN process through the use of competitive bacteria capable of extending the advantage of symbiosis to other non-leguminous plant species.

Phosphate solubilization

P is the most important mineral element after N; its deficiency crucially limits plant growth (Beltran, 2014). As for N, it can nowadays be asserted that some PGR, specifically phosphate solubilizing bacteria (PSB), solubilize insoluble phosphates in soils and make them available to plants (Goswami, Thakker, Dhandhukia and Tejada-Moral, 2016) and these, in

turn, provide them with carbon compounds that are metabolized for microbial growth, while root exudates and plant detritus provide the energetic substrate that favors their solubilizing activity. BSF constitute from 1 to 50 % (Table 2) of the total microbial population in soils (Beltran, 2014). It is also necessary to highlight that some microorganisms that solubilize soil phosphates can also present other plant growth promoting activities such as the production of AIA, gibberellic acid, hydrogen cyanide (HCN), cytokinins, ethylene, asymbiotic N fixation and resistance to soil pathogenic organisms (Beltrán, 2014).

The bacterial genera presented in Table 2 belong to the BSF group and they solubilize phosphates due to their capacity to produce organic acids, among which the following acids stand out: acetic, adipic, citric, formic, fumaric, glycolic, gluconic, indolacetic, lactic, malic, malonic, oxalic, propionic, succinic and 2-ketogluconic (Beltrán, 2014; (Goswami et al., 2016). The most frequently reported as phosphate solubilizers are gluconic and 2-ketogluconic (Paredes-Mendoza and Espinosa-Victoria, 2010).

Most of the organic acids produced by BSPs are aliphatic, i.e., they are non-aromatic acids (Paredes-Mendoza and Espinosa-Victoria, 2010). These acids modify the pH causing the dissolution of insoluble phosphates from soils (Goswami et al., 2016). In addition to having a direct action on acidification, chelation, precipitation and oxide-reduction reactions in the rhizosphere, they form complexes with metals, solubilize metals and participate in their transport. The solubilization of minerals may be due to these acids lowering the pH and, moreover, to the formation of stable complexes with cations such as Ca⁺², Mg⁺², Fe⁺³ and Al⁺³. The effect of organic acids also affects phosphates incorporated into the soil through synthetic fertilizers, reducing the precipitation of these phosphates by Fe and Al (Paredes-Mendoza and Espinosa-Victoria, 2010).

In soils, the solubilization of phosphate by organic acids depends on the pH and mineralogy of the soil, this process is carried out by two mechanisms: (a) an acid exchange, for example, H⁺ ions from citrate are

exchanged for P bound to the surface of $\text{Al}(\text{OH})_3$ or $\text{Fe}(\text{OH})_3$ crystals, reducing them and releasing P; and (b) the other which depends on the concentration of organic acids produced by BSF; the amount and type of organic acids released depend on the type of microorganisms, the quantum effect of these acids on solubilization consists in that through their hydroxyl and carboxyl groups they sequester the cations attached to the phosphate converting it to soluble forms (Beltran, 2014; Bhardwaj et al., 2014).

Table 2. Main phosphate solubilizing bacterial genera.

BSF		
Achromobacter	Erwinia	Rahnella
Acinetobacter	Flavobacterium	Ralstonia
Aereobacter	Gordonia	Rhodobacter
Agrobacterium	Kitasatospora	Rhodococcus
Arthrobacter	Klebsiella	Serratia
Bacillus	Mesorhizobium	Sinorhizobium
Bradyrhizobium	Micrococcus	Streptomyces
Burkholderia	Mycobacterium	Streptosporangium
Chryseobacterium	Pantoea	Thiobacillus
Delftia	Phyllobacterium	Yarrowia
Enterobacter	Pseudomonas	

BSF = Phosphate solubilizing bacteria; Source: Beltran (2014).

BSF are also capable of mineralizing insoluble organic phosphate through the excretion of extracellular enzymes such as phosphatases - catalysts of phosphate ester hydrolysis - phytases and C-P lyases. It should be noted that solubilization and mineralization mechanisms can coexist within the same BSF. Inoculation with BSF increases P availability in the rhizosphere and its uptake by the plant (Faria, Dias, Melo and de Carvalho-Costa, 2013). A 50% reduction in P fertilizer application could be -achieved by the -combined use of P-solubilizing microorganisms and RPCV without affecting yield (Tahir and Aqeel, 2013). On the -other hand, the introduction of overexpressed genes in rhizosphere bacteria to solubilize phosphates, and improve the ability of microorganisms as inoculants, has attracted the -attention of researchers. To date, the bases of the genetics of mineral phosphate solubilization are not clearly established, because although it is known that the generation of organic acids is the main mechanism to carry out this process, it could be assumed that any gene involved in the synthesis of organic acids could have some effect on this characteristic: called solubilizing phenotype. In relation -to the above, it has been evidenced that the -ability of some Gram-negative bacteria to dissolve calcium phosphates - solubilizing phenotype - is the -result of the periplasmic oxidation of glucose to

gluconic acid by the glucose dehydrogenase quinoprotein (G-DH) pathway, a component of the -direct glucose -oxidation pathway (-Beltran, 2014).

Finally, Beltrán (2014) highlights that, although -there are efforts to understand P solubilization at the molecular scale, the mechanism used by -different BSF requires further research. In -addition to the above, despite the advantages that genetic modification of microorganisms can bring-, their release into the environment remains -controversial; some nations encourage the management of genetically modified organisms, while others prohibit it and require labeling of products containing genetically modified ingredients-; however, and despite this controversy, there is scientific evidence that following the appropriate regulations, -genetically -modified microorganisms -could, in the near future, be used without risk in agricultural activities.

Production of siderophores: iron chelation

Siderophores are small molecules produced by microorganisms under Fe-limiting conditions that increase their entry into the microbial cell (Saha, Saha, Donofrio and Bestervelt, 2013). Commonly-, bacteria

acquire Fe by secreting low molecular weight Fe chelates, called siderophores, which are in constant association with Fe complexes (de los Santos-Villalobos, Barrera-Galicia, Miranda-Salcedo and Pena-Cabriales, 2012; Parray et al., 2016). In addition to their low molecular weight, these compounds are soluble in aqueous solutions at neutral pH and can be fluorescent or not (Tejera-Hernández, Rojas-Badía and Heydrich-Pérez, 2011).

Various organisms synthesize small molecules, non-ribosomal peptides, with high affinity for Fe⁺³ that act specifically as chelating agents to sequester Fe in the presence of other metals, and cause its reduction to Fe⁺² which is a much more soluble and usable form for the nutrition of these organisms (Aguado-Santacruz et al., 2012). The sequestration of Fe by microorganisms, under scarce conditions, develops as follows: once Fe is sequestered from the surrounding medium, the siderophore-Fe complex is recognized by specific receptors of the microbial membrane and once inside the cells, it is deposited in a specific site by a process involving a ligand exchange that may or may not be preceded by Fe reduction or hydrolysis of the siderophore (Rives, Acebo and Hernández, 2007). The presence of siderophores would represent a great advantage for microorganisms, since they could acquire Fe from the surrounding medium more easily than the rest of their competitors (Tejera-Hernández et al., 2011).

Siderophores are divided into three main families depending on the characteristic functional group: hydroxamates, catecholates and carboxylates (Ahmed and Holmstrom, 2014). To date, more than 500 siderophores are known and the chemical structures of 270 of them have already been determined (Aguado-Santacruz et al., 2012). Siderophores produced by heavy metal-resistant isolates have been implicated in the biological control of diseases, such as vascular wilt caused by *Fusarium oxysporum* and pea nut stem rot caused by *Rhizoctonia solani* (Sayyed and Patel, 2011).

RPCV have developed several strategies, both to survive and adapt to their environment, and to provide

the plant with Fe, one of these strategies is the production of siderophores (de los Santos-Villalobos et al., 2012). For example, several species of the genera *Pseudomonas*, *Bacillus* and *Enterobacter* have been evaluated and reported as biocontrol agents against plant pathogens, under Fe stress conditions, these strains produce siderophores that chelate available Fe and deprive the respective phytopathogens of this element, thus restricting their proliferation and root colonization (Solanki, Singh, Srivastava, Kumar, Kashyap et al., 2013).

Extracellular polysaccharide production

The ability to produce, secrete or exude polysaccharides is another of the multiple benefits that RPCVs possess (Jha and Saraf, 2015). These exudates include polysaccharides: structural, intracellular and extracellular or exopolysaccharides (EPS) (Noumavo et al., 2016), which are mainly composed of carbohydrates, forming homo- or heteropolymers and may contain organic and inorganic substituents. These products offer advantages such as the great versatility of microorganisms to synthesize neutral or negatively charged polysaccharides, from renewable sources, under controlled conditions and the possibility of genetic manipulation, with which it may be possible to obtain products with better functional properties and even higher quality (Fuentes, Carreño and Llanos, 2013). The main contribution of rhizospheric microorganisms to soil stability is associated with the production of EPS. These products are found in the form of hydrated gels around the cells and constitute the interface between the microorganisms and their immediate environment (Noumavo et al., 2016).

The production of high levels of EPS, by certain bacteria, allows plants to better withstand adverse environments (Nadeem, Ahmad, Naveed, Imran, Imran, Zahir et al., 2016). In the rhizosphere EPS produced by rhizobacteria enter the soil aggregates and alter their porosity. Therefore, porosity, which is directly

related to the transfer of water from the soil to the roots, is partially controlled by bacterial activity. They also help to: maintain the water film, required for photosynthetic activity and plant growth, improve the soil aeration process and infiltration, and cover and protect roots against attack by phytopathogens (Noumavo et al., 2016). Under salt stress conditions, EPSs make cations available in the root zone, thus contributing to reduce the salinity of the rhizosphere. Bacterial EPS under soil water stress conditions can limit or postpone the desiccation of the medium. Conversely, in case of excess water (rain, flooding), EPS, contribute to prevent the dispersion of clay soils (Noumavo et al., 2016).

Production of plant growth promoting substances

These substances are signaling molecules that act as chemical messengers that influence the ability of plants to respond to their environment (Tahir and Aqeel, 2013). They regulate the expression of genes involved in plant growth and development, which are synthesized in different plant structures and their action varies depending on environmental changes that modify the organism's gene expression (Molina-Romero, Bustillos-Cristales, Rodriguez-andrade and Elizabeth, 2015) and are generally effective in small concentrations (Tahir and Aqeel, 2013). It has been established that they are also involved in catabolic pathways and regulation of biofilm formation. Moreover, these molecules exhibit specific effects on plant physiology, such as increasing root volume, increasing the rate of respiration of the host plant root and the flow of protons in the root membrane; consequently, the absorption of soluble mineral elements is favored (Molina-Romero et al., 2015). However, although it is known that phytohormones regulate plant development and physiology, as well as immunity, their production by microorganisms has not been considered as a biological control mechanism; for example, it has been identified that *P. fluorescens* G20-18 the ability

to efficiently control *P. syringae* infection in the Arabidopsis model, allowing the maintenance of tissue integrity and, ultimately, biomass yield (Großkinsky, Tafner, Moreno, Stenglein, Garcia-de-Salamone et al, 2016).

Molina-Romero et al. (2015), have established that RPCVs have the capacity to produce more than one type of growth-promoting substances, within these five groups stand out: 1) auxins (AIA); 2) gibberellins; 3) ethylene; 4) cytokinins; and 5) abscisic acid (ABA) and of these, the first four are involved in phytostimulation by rhizobacteria (Tahir and Aqeel, 2013). Sarma and Saikia (2014) emphasize that phytostimulation is considered to be the most studied mechanism of RPCVs. The phenomenon of phytostimulation is particularly given by the manipulation of the complex and balanced network of hormones or similar compounds that directly influence plant growth or stimulate plant root formation. For example, many *Azospirillum* species produce auxins, cytokinins and gibberellins, which stimulate root development, leading to significant increases in agricultural yields (Rives et al., 2007).

Biocontrol or antagonism mechanisms

The deleterious microbes that inhabit the rhizosphere cause diseases and sometimes lead to the complete loss of crops (Raza, Wang, Wu, Ling, Wei et al., 2016). Different microorganisms have been used for the biological control of these diseases: various RPCVs as biocontrol agents naturally eliminate phytopathogens by producing secondary metabolites, which, in addition to being excreted locally or near the plant surface, are biodegradable molecules and are not required in high quantities, unlike pesticides that are resistant to degradation and are applied in large quantities to maintain plant health (Molina-Romero et al., 2015).

Some VSRs have the ability to prevent the development of soil-borne diseases of plants by keeping the level of deleterious microbes below the threshold (Raza et al., 2016). These RPCVs produce

antibiotics, antimicrobial volatile organic compounds (VOCs) and hydrolytic enzymes, siderophores or bacteriocins, on the one hand they can stimulate resistance, systemic or induced, of plants against different phytopathogens or on the other hand eliminate such organisms (Raza et al., 2016). Specifically, several molecules produced by Gram-negative antagonistic bacteria capable of exerting biocontrol against pathogens causing root diseases have been characterized, among which are: hydrocyanic and phenazine-1-carboxylic acids, pyoluteorin, pyrrolnitrin, cyclic lipopeptides and diacetylphloroglucinol (Molina-Romero et al., 2015).

Deleterious rhizobacteria are known to be a group of saprophytic, non-parasitic pathogens that excrete exopolysaccharides and chemicals in the form of cyanide-, phytohormones, siderophores and phytotoxins that can negatively affect plant metabolism, recently this group of microorganisms has been used as a biological weed control agent (Lakshmi, Kumari, Singh, & Prabha, 2015). As an example, de los Santos-Villalobos et al. (2012), highlight that siderophores produced by *Burkholderia cepacia* XXVI, isolated from mango orchards, proved to be an alternative biocontrol of the fungus *Colletotrichum gloeosporioides* causing anthracnose in this crop.

The efficacy of bacterial biocontrol agents is strictly associated with their ability to actively colonize the ecological niches occupied by plant pathogens; to reach these niches, bacteria move in the environment thanks to external appendages-, such as flagella (Tomada, Puopolo, Perazzolli, Musetti, Loi et al., 2016). On the other hand, some aerobic spore-forming bacteria possess advantages that make them suitable candidates for use as biocontrol agents, e. g., species of the genus *Bacillus* are able to produce spores that allow them to resist adverse environmental conditions, in addition to favoring easy formulation and storage of commercial products (Raza et al., 2016). Biocontrol activity, should be considered as a mode of behavior, dependent on the prevailing conditions, rather than as an inherent property of a bacterial strain (Cray,

Connor, Stevenson, Houghton, Rangel et al., 2016). Recently, the development of molecular techniques has enabled the construction of genetic recognition tools to analyze and study bacterial behavior within the soil microbial community (Kong, Kim, Lee, & Lee, 2016). For example, Gopalakrishnan, Upadhyaya, Vadlamudi, Humayun, Vidya et al. (2012), obtained seven bacterial isolates (SRI-156, SRI-158, SRI-178, SRI-211, SRI-229, SRI-305 and SRI-360), from the rhizosphere of an intensive rice (*Oryza sativa*) system with potential for both plant growth promotion and biocontrol of charcoal rot of sorghum, caused by *Macrophomina phaseolina*, and concluded that the selected bacterial strains produced siderophores, indoleacetic acid (except SRI-305), hydrogen cyanide (except SRI-158 and SRI-305) and phosphate solubilization (except SRI-360).

Antibiotic production

Antibiosis is probably the best known and perhaps the most important mechanism used by RPCVs to limit pathogen invasion in plants. It consists of inhibiting the development of phytopathogenic microorganisms through the production of secondary metabolites (Saraf, Pandya and Thakkar, 2014), or the secretion of broad-spectrum molecules (Molina-Romero et al., 2015). The latter authors highlight that these metabolites act through mechanisms of action such as: a) inhibition of cell wall synthesis; b) structural destabilization of the cell membrane; and c) inhibition of the formation of the translation initiation complex of phyto-pathogenic organisms. In addition, Raaijmakers and Mazzola (2012) note that antibiotics comprise a chemically heterogeneous group of low molecular weight organic compounds produced by microorganisms that are detrimental to the growth or metabolic activities of other microorganisms, generally acting on several vital processes of these, including cell wall biosynthesis and DNA, RNA and protein synthesis. Examples of antibiotic-producing PCVRs include the genera *Burkholderia* and *Streptomyces* (Molina-Romero et al., 2015).

Bacteria that reduce the incidence or severity of plant

diseases are referred to as biological control agents, while those that exhibit antagonistic activity towards a pathogen are defined as antagonists. The following rhizospheric environment and antagonistic activities can be highlighted: 1) synthesis of hydrolytic enzymes, such as chitinases, glucanases, proteases and lipases, which can lyse pathogenic fungal cells; 2) competition for nutritive elements and adequate colonization of niches on the root surface; 3) regulation of plant ethylene levels through the enzyme ACC-desaminase, which can act to modulate the ethylene level in a plant in response to stress imposed by infection; and 4) production of siderophores and antibiotics (Beneduzi, Ambrosini and Passaglia, 2012). RPCVs as biocontrol agents are isolated and introduced in an optimal amount into the rhizosphere to control the development of plant diseases (Raza et al., 2016). For example, *P. fluorescens* generate phenazines and pyrrolnitrin which are broad-spectrum antibiotics (Upadhyay and Srivastava, 2011).

Production of lytic enzymes

A great variety of microorganisms produce different enzymes that act against other microorganisms present in their habitat, which can act as phytopathogens, causing economic losses in crops such as rice, wheat, soybean, among others (Tejera-Hernández et al., 2011). Due to this characteristic, various RPCV strains have the ability to degrade the cell walls of certain microorganisms, through the production of hydrolytic enzymes, such as β -glucanases, cellulases, dehydrogenases, exo- and endo-polygalacturonases, phosphatases, hydrolases, lipases, pectinolyases, proteases and chitinases, which act primarily against fungi (Molina-Romero et al., 2015; Noumavo et al., 2016). Tejera-Hernández et al. (2011), point out that *B. subtilis* produces these metabolites with the capacity to counteract the effects of the fungus *F. oxysporum*.

Among the rhizobacteria that produce these enzymes are *B. altitudinis*, *B. amyloliquefaciens*, *B. cereus*, *B. subtilis* (Molina-Romero et al., 2015). These *Bacillus* utilize various mechanisms that can inhibit fungal

pathogens, including competition for nutrients, production of antifungal lipopeptides, or production of lytic enzymes such as chitinases, which can degrade the fungal cell wall, as a means to prevent the spread of fungal hyphae (Figuerola-Lopez, Cordero-Ramirez, Martinez-Alvarez, Lopez-Meyer, Lizarraga-Sanchez et al., 2016). The antifungal activity of microorganisms is due to their ability to generate lipopeptides and glycopeptides: examples of these are rhamnolipids and surfactin produced by *P. aeruginosa* and *B. subtilis*, respectively, which allow them to solubilize the main components of microbial cell membranes, in addition to giving them a better chance of survival in habitats with high competition for nutrients (Jiménez, Medina and Gracida, 2010). This antifungal activity allows RPCVs to protect the plant against biotic stress by eliminating pathogens (Mahmood et al., 2016).

Synthesis of hydrogen cyanide and volatile compounds

The antagonistic activity of RPCVs also works through the production of volatile compounds (Lakshmi et al., 2015). These compounds play a primary role in pathogen control, in contrast to antibiotics, which can only prevent pathogens from infecting plants, additionally, these compounds can spread over long distances and create a bacteriostatic microenvironment around antagonistic communities (Raza et al., 2016). The best known volatile compound is hydrogen cyanide (HCC). The main group of RPCVs used as biological control agents is the genus *Pseudomonas*, which are considered the most common producers of cyanide, in addition to having a wide application in biotechnological processes, of great importance for agroindustries (Lakshmi et al., 2015).

As complementary examples, the bacteria *P. fluorescens* strain F113rif (F113) is a biocontrol agent isolated from the rhizosphere of sugar beet (*Beta vulgaris* var. *altissima*) capable of suppressing the disease produced by the oomycete *Pythium ultimum*, the biocontrol capacity of this strain has been related

to the production of secondary metabolites, among which stand out: siderophores, diacetyl-floro-glucinol (DAPG), CNH and an extracellular protein (Barahona, Navazo, Martinez, Zea, Perez et al, 2011). For their part, *B. methylotrophicus* bacteria presented the greatest antagonistic effects against phytopathogens, due to their ability to produce antibiotics and/or volatile organic compounds such as CNH, which inhibits the growth of phytopathogenic fungi and exerts deleterious effects on their growth in vitro (Rios, Caro, Berlanga, Ornelas, Salas et al., 2016). Another significant effect of the CNH-producing CVRs is that their incorporation into the rhizosphere of the weed reduces its growth parameters, with little or no effect on plant species of economic interest; this practice turns out to be a low-cost and environmentally friendly alternative for weed biocontrol, against the application of herbicides and synthetic compounds, which are harmful to the environment. In addition, the incorporation of these rhizobacteria offers several advantages, such as a shift in the balance of competition between weed and crop in favor of the crop and against the weed; greater selectivity, lower resistance and the introduction of agricultural practices that are sustainable (Lakshmi et al., 2015). Regardless of the benefits already described, the role of cyanide production is contradictory as it may be associated with deleterious rhizobacteria as well as beneficial bacteria (Saharan and Nehra, 2011).

Induced and acquired systemic resistance

Like all living beings, plants possess genes that encode to generate various chemical weapons -- small exogenous molecules, called inducers, which are extremely efficient, constituting defense mechanisms that protect them against the attack of pathogenic organisms, either by diminishing or preventing such attack. This biological phenomenon has been called resistance (Gómez and Reis, 2011). Systemic resistance is a physiological state that enhances defensive capacity, elicited by specific environmental

stimuli. Innate plant defenses are enhanced toward subsequent biotic challenges. This enhanced state of resistance is effective against a wide range of pathogens, plant parasites and insect herbivores (Canchignia, Cruz, Barrera, Morante, Cancignia et al., 2015). The mechanism of induced resistance involves two phenomena: induced systemic resistance (ISR) and acquired resistance (ASR), which, although distinct, are phenotypically similar (Canchignia et al., 2015). The ISR is associated with the ability of the PVGRs to promote plant growth and protect against pathogen attack, and the ASR is associated with the responses of plant species to the presence or attack of pathogens (Diaz-Puentes, 2012). The similarity of both resistances is based on the fact that plants, after being exposed to an inducing agent, activate defense mechanisms both at the point of infection and in other regions (systemic resistance), in a more or less generalized way, and their difference lies in the nature of the elicitor molecule present in the inducer and the signaling pathways. When the latter are triggered by a biotic agent they can depend on both salicylic acid (AS), associated with the accumulation of pathogenesis-related proteins (PRP), and jasmonic acid (jasmonate) and ethylene, is not associated, in this case, with the accumulation of PRP, is known as RSA, while if the inducer is of abiotic type only follows the pathway of jasmonic acid and ethylene, then it corresponds to RSI (Gomez and Reis, 2011; Cancignia et al., 2015).

RSI can be induced by a wide variety of microorganisms including Gram-positive bacteria such as *B. altitudinis*, *B. amyloliquefaciens*, *B. cereus*, *B. mycoide*, *B. pasteurii*, *B. pumilus*, *B. sphaericus*, or Gram-negative bacteria belonging to the genus *Pseudomonas* e.g., *P. fluorescens*, *P. putida*, *P. aeruginosa* and enterobacteria such as *Serratia* e.g., *S. marcescens*, *S. plymuthica*, as well as *Pantoea* agglomerans, through the generation of diverse metabolites, among which stand out: AS, lipopolysaccharides (LPS), siderophores, cyclic lipo-peptides, 2,4-diacetylphloroglucinol, homoserine lactones and volatile compounds such as acetoin and 2,3-butanediol (Molina-Romero et al., 2015).

Saharan and Nehra (2011) note that, triggered by local infection, plants respond with an AS-dependent signaling cascade that leads to systemic expression of broad-spectrum and long-lasting disease resistance that is effective against fungi, bacteria, and viruses. After infection, endogenous AS levels increase locally and systemically, and AS levels increase in the phloem before RSI occurs. They further state that, AS is synthesized in response to infection, both locally and systemically: consequently, new AS production in uninfected plant parts may contribute accordingly to the systemic expression of RSI. On the other hand, Camarena-Gutiérrez and de la Torre-Almaráz (2007) point out that if plants survive an initial attack by pathogenic organisms or if protection is generated after: a) an attack by herbivorous arthropods or b) mechanical damage or contact with some synthetic chemicals, they can protect themselves against subsequent attacks by homologous pathogens even when the plants do not possess genes that determine cultivar-specific resistance, thus becoming immune. This ability of cells to repel subsequent attacks, which is dispersed throughout the plant, corresponds to the ASR. The ASR has four peculiar characteristics: 1) it is effective against a broad spectrum of pathogenic organisms, depending on the plant species treated; 2) it is long-lasting; 3) it is dispersed in plants, mainly in the apical direction; and 4) it moves to the grafted buds: these last two characteristics strongly suggest that the signals established by the ASR are translocated throughout the plant. The timing and degree of ASR protection depends on the plant species and the inducer, because some effectors induce ASR in some species and not in others (Diaz-Puentes, 2012). Additionally, over time, its strength and stability can be affected by climatic conditions and nutrition (Camarena-Gutiérrez and de la Torre-Almaraz, 2007).

An essential aspect of ASR is that the first infection caused by a pathogen generates a necrotic lesion, which can be the result of programmed cell death after pathogen recognition in an incompatible interaction where a hypersensitive response was generated or of cell death originated by the action of the pathogen in a compatible interaction (Camarena-Gutiérrez and de la

Torre-Almaráz, 2007). The sequence of events that favor ASR starts locally: that is, in cells adjacent to the hypersensitive response, cell wall thickening is observed by incorporation of structural proteins or lignin, deposition of callose and induction of phytoalexin synthesis (Camarena-Gutiérrez and de la Torre-Almaráz, 2007).

ASR has a very interesting practical aspect. In agriculture, it can be induced by infecting the plant to be protected, using first an avirulent or virulent strain, whose response to infection should produce a large necrosis. Alternatively, plants can be sprayed with either Gram-positive or Gram-negative bacterial culture filtrates or, even better, with one of the identified signal products, such as AS. Since these substances are biologically decomposed and the spectrum of pathogens that can be repelled is very broad, their application in systemic response release has good potential in plant protection. Intensive research on ASR, in particular its molecular genetics, will soon show that it can be successfully applied, perhaps in combination with other protection measures (Camarena-Gutiérrez and de la Torre-Almaraz, 2007).

Areas of opportunity inherent to the RPCVs

Based on the review, analysis and interpretation of the publications cited in this document, it is suggested that the research activities related to the CVS should focus on the following topics, since it is estimated that these will provide more and better knowledge on the development of agricultural crops and their production in an environmentally friendly manner:

1. Since a large part of the deterioration of agroecosystems is due to the irrational use of agrochemicals to control pests, diseases and weeds, there is a continuous demand for alternatives that promote the use of products of ecological origin that minimize their impact and reduce environmental contamination. From a sustainable

point of view, it is necessary to study the potential of PGR in the improvement of economically and socially important crops, with the consequent preservation of the environment.

2. Soil microorganisms are of great importance for sustaining life on Earth. The study of their diversity is vital and transcendent, since they are part of numerous complex and dynamic communities, and to understand their function, as well as the changes that occur in these communities, in response to different factors and environmental perturbations, in specific niches, it is essential to identify and quantify each of the members of these communities.
3. One of the permanent challenges, due to the myriad of microorganisms present in the microbial rhizosphere, of which it is estimated that a large part remains to be identified and characterized, and given the relevance that the development of agricultural production with a sustainable approach has acquired, is to continue to determine the functional role of this population in the diverse ecosystems of the Earth. This will make it possible to answer questions such as: Which species of microorganisms not yet identified could have biotechnological relevance?
4. There is a need to continue gathering information to understand in greater detail the mechanisms of plant resistance and their relationship with the application of PVPRs.
5. Due to the limitations of synthetic fertilizers, both for their use of non-renewable natural resources for their production and for their residual effect on the environment, and organic fertilizers or manures, for their reduced content of nutrients, the study of the RPCV, with a broad spectrum of colonization of plant species, will help these microorganisms to be used as effective biofertilizers or phytostimulants in the agricultural sector, seeking to improve the processes of nutrition and crop resistance to drought, salinity and high temperatures, in order to increase yields, given the

need for satisfiers demanded by the growing world population in the primary production sector.

CONCLUSION

Because several experts point out that it is necessary to further elucidate the mechanisms, described in this document, through which the CPGRs increase the growth and yields of plant species. Strengthening the integral knowledge of the rhizosphere has been, is and will continue to be essential for the understanding of a myriad of aspects, processes and phenomena that are pillars for agricultural and environmental sustainability. In addition, having a **more complete** description of soil microbial diversity will allow us to broaden our knowledge of its role in the biogeochemical cycles of the elements essential for plant development.

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