Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production

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9 Abstract

10 Plant growth-promoting rhizobacteria (PGPR) are free-living bacteria which actively colonize 11 plant roots, exerting beneficial effects on plant development. The PGPR may (i) promote the 12 plant growth either by using their own metabolism (solubilising phosphates, producing 13 hormones or fixing nitrogen) or directly affecting the plant metabolism (increasing the uptake 14 of water and minerals), enhancing root development, increasing the enzymatic activity of the 15 plant or "helping" other beneficial microorganisms to enhance their action on the plants; (ii) 16 or may promote the plant growth by suppressing plant pathogens. These abilities are of great 17 agriculture importance in terms of improving soil fertility and crop yield, thus reducing the 18 negative impact of chemical fertilizers on the environment. The progress in the last decade in 19 using PGPR in a variety of plants (maize, rice, wheat, soybean and bean) along with their 20 mechanism of action are summarized and discussed here.

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22 **Keywords**: PGPR; wheat; rice; maize; soybean; bean.

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1 1. Introduction.

2 During the past century, industrialization of agriculture has provoked a significant and essential productivity increase, which has led to a greater amount of food available to the 3 4 general population. Along with this abundance the appearance of serious environmental and 5 social problems came with the package: problems that must be faced and solved in the not too 6 distant future. Nowadays, it is urgent to maintain that high productivity, but it is becoming 7 urgent to alter as little as possible the environment. Clearly we must then head for a more 8 environmentally sustainable agriculture while maintaining ecosystems and biodiversity. One 9 potential way to decrease negative environmental impact resulting from continued use of 10 chemical fertilizers, herbicides and pesticides is the use of Plant Growth-Promoting 11 Rhizobacteria (PGPR). This term was first defined by Kloepper and Schroth (1978) to 12 describe soil bacteria that colonize the rhizosphere of plants, growing in, on or around plant 13 tissues that stimulate plant growth by several mechanisms. Since that time, research activities 14 aimed at understanding how these bacteria perform their positive (or negative) effect have 15 steadily increased and many reports have been published on these microorganisms. Firstly, 16 screening of rhizobacteria for *in vitro* production of phytohormones (Cassán et al., 2009; 17 Hoyos-Carvajal et al., 2009; Rashedul et al.; 2009; Abbasi et al., 2011) such as auxins (Khalid 18 et al., 2004); siderophores (Filippi et al., 2011; Yu et al., 2011); phosphorous solubilisation 19 (Yasmin et al., 2004; Tajini et al., 2012; Krey et al. 2013), or nitrogen-fixing (Peix et al., 20 2001, Riggs et al., 2001, Fischer et al., 2007) were used to isolate PGPR from the rhizosphere 21 and to screen for their growth-promoting activity in plants under axenic conditions. Once the 22 PGPR candidates had shown promising performances under controlled conditions, they were 23 then used as inoculants for plants cultivated under natural conditions in pots and/or field 24 trials. Application of PGPR in legumes has been mainly restricted to rhizobia manipulation 25 for studies on increase in legume growth and development, specifically by means of nodulation and nitrogen fixation. Obviously, the main reason for that is because a broad range 26

of soil-borne rhizobia species can establish symbiosis with legumes (Cooper, 2008).
 Therefore, rhizobia can be considered the best known beneficial plant associated bacteria and
 the most important biofertilizer.

4 Benefits to plants from host-PGPR interactions (Fig. 1) have been shown to include 5 plant health and growth, suppress disease-causing microbes and accelerate nutrient 6 availability and assimilation (Mantelin and Touraine, 2004; Yang et al., 2009). These 7 beneficial effects on plants can be achieved by the direct interaction between PGPR and their 8 host plant and are also indirectly due to their antagonistic activity against plant pathogens. 9 Direct stimulation includes several mechanisms such as: producing 1-aminocyclopropane-1-10 carboxylate (ACC)-deaminase to reduce ethylene levels in the roots of developing plants; producing plant growth regulators like auxins, gibberellins, cytokins and certain volatiles; 11 12 symbiotic nitrogen fixation; solubilising mineral like phosphorus and other nutrients, etc. 13 Indirect stimulation is related to biocontrol, by mean of antagonistic activity against 14 phytopathogenic microorganisms inducing plant systemic resistance responses, interfering in 15 the bacterial Quorum Sensing (QS) systems, etc.

16 Some reports show PGPR may use more than one of these mechanisms for 17 accomplishing plant growth enhancement (Bashan and Holguin, 1997; Ahmad et al., 2008). 18 An excellent review about these bacteria has been published recently (Bhattacharyya and Jha, 19 2012).

Different PGPR can be administered to crops in some formulations that are commercially available (Lucy et al., 2004) and, recently, the popularity of microbial inoculants has substantially increased, facilitated by extensive and systematic research that has enhanced effectiveness and consistency (Berg, 2009; Thakore, 2006). Microbial inoculants include three major groups: (1) arbuscular mycorrhiza fungi (AMF), (2) PGPR, and (3) symbiotic-nitrogen-fixing rhizobia (**Fig. 2**). The beneficial capacity of each group has been studied separately (Dobbelaere et al., 2001; Barea et al., 2002; Murray, 2011, Verma et al., 2010). Moreover, numerous studies are being conducted to evaluate plant growth effects
by applying different microbial combinations or consortia (Table 1), such as AMF-PGPR,
symbiotic-nitrogen-fixing rhizobia-PGPR or different PGPR (Singh and Kapoor, 1999;
Swarnalakshmi et al., 2013). However, understanding the mechanisms of plant growth
promotion is important in order to decide what type of microorganism is better to use with
which plant in a given situation.

7 Rhizosphere is the zone of soil surrounding a plant root where the biology and 8 chemistry of the soil are influenced by the roots (Lugtenberg and Kamilova, 2009). Root 9 exudates include amino acids, organic acids, carbohydrates, sugars, mucilage and proteins. 10 The ability of rhizobacteria to use organic acids as carbon sources correlates with rhizosphere 11 competence. Structure of the rhizobacterial community is determined by the plant species just 12 as differences in the composition and amounts of root exudates most likely affect the 13 microbial populations. Understanding how plant roots select soil microbes to form the 14 microbial community of the rhizosphere is an important scientific issue when considering the 15 use of rhizobacteria as plant growth promoters (Drogue et al., 2012).

In this review, our focus is mainly on PGPR. We begin with a description of various mechanisms used by these bacteria to enhance the plant growth and to increase agronomic parameters. Then, the current progress of using PGPR on the most important worldwide cereal crops such as maize, rice, and wheat, along with the ubiquitous legumes, principally soybean and dry bean varieties, is summarized in the review and discussed.

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22 2. Mechanisms for the Plant Growth Promotion.

23 2.1. Biofertilization.

24 Rhizobacteria that promote plant growth by improving the nutrient uptake of the plants 25 are termed biofertilizers. These bacteria have a role of improving the nutrient status of host 26 plants by means of nitrogen fixation, increasing the availability of nutrients in the

3 Plants can assimilate nitrogen (N), which is one of the principal plant nutrients, from soil as nitrite, nitrate or ammonia. These forms of nitrogen are not abundant in most soils and 4 5 the chemical nitrogen fertilization employed in agriculture is frequently lost during rainfall or 6 by mineral leaching of these fertilizers. Atmospheric N₂-fixing bacteria such as *Rhizobium* 7 and Bradyrhizobium can establish symbiosis forming nodules on roots of leguminous plants 8 such as soybean, pea, peanut and alfalfa, in which they convert N₂ into ammonia, which can 9 be used by the plant as a nitrogen source (Murray, 2011). However, this process is practically 10 limited to legume crops. On the other hand, several non-symbiotic bacteria have been 11 identified as free-living N₂-fixers (Azospirillum, Azoarcus, Azotobacter, Bacillus polymyxa, 12 Burkholderia, Gluconoacetobacter or Herbaspirillum). These potential PGPR can fertilize 13 several important agronomic plants such as wheat (Boddev et al., 1986), sorghum (Stein et al., 1997), maize (Garcia de Salamone et al., 1996), rice (Malik et al., 1997) or sugarcane 14 15 (Boddey et al., 2001). Inoculation of these PGPR species usually increases plant's dry weight, flowering and grain production. However, the yield increase caused by inoculation of these 16 17 PGPR could often be attributed to an increase in root development, which allows better rates 18 of water and mineral uptake (Okon et al., 1998).

Another essential nutrient in plants is phosphorus (P). Although the large reserve of P is in soils, most of it is not soluble, which cannot then be absorbed by plants, therefore limiting the plant growth. Certain PGPR are able to solubilise P through acidification (Richardson et al., 2009), chelation or enzymatically (Hameeda et al., 2008). Bacteria such as *Azospirillum, Bacillus, Burkholderia, Erwinia, Pseudomonas, Rhizobium* or *Serratia* are reported as phosphate solubilising bacteria (Sudhakar et al., 2000; Mehnaz and Lazarovits, 2006). Furthermore, inoculation of PGPR can increase plant uptake of several other nutrients such as Ca, K, Fe, Cu, Mn and Zn. This uptake usually occurs during acidification of the soil rhizosphere via organic acid production or via stimulation of proton pump ATPase (Mantelin and Touraine, 2004). In any case, the soil pH decrease improves solubilisation of these nutrients.

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2.2. Rhizoremediation and Stress Control

8 Numerous reports on potential PGPR that degrade soil pollutants have been published 9 (Fig. 3). The contribution of the rhizomicrobial population to degrading pollutants allows 10 plants to emerge as natural vegetation at a contaminated site. Studies focused on degradation of compounds such as herbicides, pesticides and hazardous organic compounds have been 11 12 carried out, although those reports have provided little information on the microbial 13 population. A key step during rhizoremediation consists of the selection of pollutant-14 degrading rhizobacteria that live in the rhizosphere and use the root exudates as an energy 15 source (Kuiper et al., 2001). These bacteria, besides degrading the pollutant compounds, often 16 directly assist rhizoremediation by producing hormones, fixing atmospheric nitrogen, 17 solubilising P or secreting siderophores (Denton, 2007). In the same way, consortia of 18 bacteria (Table 1) are found to be efficient since each partner can accomplish different parts 19 of the catabolic degradation route (Rahman et al., 2002).

When plants are exposed to stress conditions they respond increasing ethylene levels that lead to an increase in cell and plant damage (Argueso et al., 2007). A high concentration of ethylene can be harmful because it induces defoliation and other cellular processes that may affect crop development (Desbrosses et al., 2009). Many PGPR destroy 1aminocyclopropane-1- carboxylate (ACC), a precursor of the ethylene, via production of the enzyme ACC deaminase, wich in turn facilitates plant growth and development by decreasing plant ethylene levels. In addition, several forms of stress are relieved by ACC deaminase producers, such as effects of phytopathogenic bacteria, and resistance to stress from
 polyaromatic hydrocarbons, from Ca²⁺ and Ni²⁺, and from salt and draught (Glick et al.,
 2007).

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5 2.3. *Phytostimulation*.

Diverse PGPR can alter root architecture and promote plant development due to their
ability to synthesize and exudates plant hormones like indole-3-acetic acid (IAA), gibberellins
(GAs), cytokinins and certain volatiles, hence they are termed phytostimulators (Bloemberg
and Lugtenberg, 2001), this capacity being bacterial strain specific (Boiero et al., 2007).

10 The PGPR stimulatory effect comes from a manipulation of the complex and balanced 11 network of plant hormones that directly are involved in growth or stimulation of the root 12 formation. For instance, the biosynthesis of IAA by various PGPR has been demonstrated to 13 enhance root proliferation (Dobbelaere et al., 1999; Khalid et al., 2004). Bacteria use this phytohormone to interact with plants as part of their colonization strategy, including 14 15 phytostimulation and avoidance of basal plant defence mechanisms. Moreover, it has recently 16 been indicated that IAA can also be a bacterial signalling molecule and therefore can have a 17 direct effect on bacterial physiology (Spaepen et al., 2007).

In bacteria there is no known role for GAs, rather they seem to be secondary metabolites that may play a role as signalling factors towards the host plant. In this way, there are many studies where GA production by *Azospirillum* or *Bacillus sp.* induces growth promotion in plants (Bottini et al., 2004; Piccoli et al., 1997; Gutiérrez-Mañero et al., 2001).

Involvement of PGPR cytokinins were observed in root initiation, cell division, cell enlargement and increase in root surface area of crop plants through enhanced formation of lateral and adventitious roots (Salamone et al., 2005; Werner et al. 2003). Some strains of *Azotobacter* spp., *Rhizobium* spp., *Pantoea agglomerans, Rhodospirillum rubrum*, *Pseudomonas fluorescens, B. subtilis*, and *Paenibacillus polymyxa* are reported to produce cytokinins (Glick , 2012; Salamone et al., 2001). However, a detailed understanding of the
 role of PGPR-synthesized cytokinins and how their production is regulated is not currently
 available.

4 It has recently been reported that some rhizobacteria promote plant growth by 5 releasing volatile signals (Ping and Boland, 2004). The discovery of rhizobacterial-produced 6 volatile organic compounds (VOCs), like 2, 3-butanediol, acetoin, terpenes, jasmonates, etc., 7 constitutes an important mechanism for the elicitation of plant growth by rhizobacteria. The 8 synthesis of bioactive VOCs seems to be a strain-specific phenomenon. The VOCs produced 9 by the rhizobacteria can act as signalling molecule to mediate plant-microbe interactions as 10 volatiles produced by PGPR colonizing roots are generated at sufficient concentrations to trigger the plant responses (Ryu et al. 2003). However, more investigations into the volatile 11 12 components in plant-rhizobacteria system should follow.

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14 2.4. Biocontrol.

Plant growth promotion can be achieved indirectly through biocontrol activity against
plant pathogens. Several ways of controlling bacterial pathogens have been described in
PGPR.

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19 2.4.1 Antagonism.

20 Members of the bacterial genera *Bacillus*, *Pseudomonas*, *Serratia*, *Stenotrophomonas*, 21 and *Streptomyces* and the fungal genera *Ampelomyces*, *Coniothyrium*, and *Trichoderma* are 22 well-studied microorganisms with proven microbial influence on plant health. When testing 23 microbial isolates from plant-associated habitats, between 1 and 35% showed antagonistic 24 capacity to inhibit the growth of pathogens *in vitro* (Berg, 2009).

25 Mechanisms responsible for antagonistic activity include inhibition of the pathogen by 26 antibiotics, toxins and surface-active compounds (biosurfactants); competition for minerals, nutrients, and colonisation sites; and a mechanism that develops production of extracellular cell wall degrading enzymes such as chitinase and β -1,3-glucanase (Fig. 4) (Whipps, 2001; Compant et al., 2005; Haas and Défago, 2005). Successful biological control on the basis of plant-associated antagonists not only requires better knowledge of the complex regulation of disease suppression by antagonists in response to biotic and abiotic factors, but also knowledge of the dynamics and composition of plant-associated bacterial communities and what triggers plant colonisation (Normander and Prosser, 2000).

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9 2.4.2. Systemic Response Induction.

10 Induced systemic resistance (ISR) and systemic acquired resistance (SAR), which are 11 part of plants systemic resistance responses, are activated by certain microorganism molecules 12 referred to as elicitors. The ISR is the phenomenon in which the interaction of some bacteria 13 with the plant root results in plant resistance to some pathogenic bacteria, viruses and fungi 14 (Lugtenberg and Kamilova, 2009). ISR is triggered by non-pathogenic microorganisms and 15 starts in the root, extending to the shoot, (Fig. 4) (Ramos-Solano et al., 2008). This defence 16 response is dependent on ethylene and jasmonic acid signalling in the plant (van Loon, 2007). 17 In contrast, SAR is typically activated by necrotic pathogenic bacteria and the molecule that 18 plays a key role is salicylic acid (SA). However, both ISR and SAR can overlap in some cases. In fact, López-Baena et al. (2009) showed that the absence of "nodulation outer 19 20 proteins" from Sinorhizobium fredii HH103, secreted across the type III secretion system, 21 provoked a higher induction of SA dependent PR1 gene with respect to the wild type despite 22 this microorganism being a soybean symbiotic bacterium.

Elicitors are the molecules that induce the ISR defence responses. Cell wall polysaccharides are the most described biotic elicitors, along with flagella, salicylic acid, cyclic lipopeptides, siderophores, antibiotics, the signal molecule AHL or volatile compounds (Shuhegge et al., 2006; van Loon, 2007; Ramos Solano et al., 2008; Berg, 2009).

1 ISR has been reported as one of the mechanisms by which PGPR reduces plant disease 2 modulating the physical and biochemical properties of host plants (Pieterse et al., 2002). The first studies about this process were carried out by van Peer et al. (1991). They inoculated 3 4 non- pathogenic *Pseudomonas* spp. on roots and observed the trigger of a plant-mediated 5 resistance response in above-ground plant parts. Since then, the ISR elicitation by PGPR as a 6 biocontrol method has been studied in many plant species such as bean, tomato, tobacco, radish, cucumber and carnation (van Loon et al., 1998). Obviously, the easy handling of the 7 8 Arabidopsis thaliana plant is being the main model for PGPR-elicited ISR studies (Ruy et al., 9 2004). ISR is characterized by a specificity relationship between plant and PGPR species. In 10 fact, a PGPR that produces ISR in one plant species may not do it in another. Several strains 11 from *Pseudomonas*, *Bacillus* and *Azospirillum* genera are the mayor group of PGPR that have 12 been described eliciting ISR response. There are other species included in the symbiotic group 13 of rhizobacteria that are used in coinoculations with different PGPR and can be involved in 14 ISR (Elbadry et al., 2006). However, the metabolic pathway involved in this process is poorly 15 studied (Ramos Solano et al., 2008).

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17 2.4.3. Interference with Quorum Sensing System.

18 Many bacteria regulate their gene expression in response to changes in their 19 population density in a process called QS, which involves communication between cells 20 mediated by small diffusible signal molecules termed autoinducers (Fuqua et al., 1994). N-21 acyl-homoserine-lactones (AHLs) are the most common autoinducer molecules; they regulate 22 the expression of genes implied in the production of the virulence factor or biofilm formation 23 in several plant pathogens (Quiñones et al., 2005). Many plants are able to produce molecules 24 which specifically interfere in the OS systems of plant associated bacteria and, in any case, 25 depending on the bacterium being detected as a pathogen or as a beneficial microorganism the molecule enhances or inhibits the phenotypes regulated by QS (Fig. 4) (Pérez-Montaño et al., 26

2013). Furthermore, several bacteria produce acylase (*Ralstonia*) or lactonase (*Bacillus*)
enzymes that degrade the AHL molecules (Fig. 4) (Dong et al., 2002; Lin et al., 2003). For all
these reasons, bacteria able to interfere in the QS systems may be potentially used against
bacterial pathogens. In fact, the virulence of *Erwinia carotovora*, whose virulence factors are
regulated by QS, is attenuated in the presence of the lactonase enzyme produced by *Bacillus*(Dong et al., 2002).

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2.4.4. Competence for iron and heavy metals.

9 Iron is an essential nutrient for virtually all forms of life. However, in most aerobic microbial habitats, Fe^{2+} is oxidized to Fe^{3+} , forming insoluble compounds that are unavailable 10 11 to microorganisms. In those circumstances, some bacteria and mycorrhizal fungi produce 12 low-molecular mass iron chelators with high affinity for iron termed siderophores (Miethke 13 and Marahiel, 2007; Machuca et al., 2007). These molecules act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation. Siderophores, 14 generally form 1:1 complexes with Fe^{3+} , which are then taken up by the cell membrane of 15 bacteria, where the Fe^{3+} is reduced to Fe^{2+} and released from the siderophore into the cell 16 17 (Boukhalfa and Crumbliss, 2002).

18 PGPR have been demonstrated as enhancing the plant-growth producing very efficient extracellular siderophores which allow control of several plant diseases by depriving the 19 20 pathogen of iron nutrition, thus resulting in increased crop yield (O'Sullivan and O'Gara, 21 1992). In addition to iron, siderophores can also form stable complexes with other metals that 22 are of environmental concern, such as Al, Cd, Cu, Ga, In, Pb and Zn (Schalk et al., 2011). 23 Braud et al. (2009) have shown that the presence of heavy metals induces bacterial 24 siderophore production. Paradoxically, plants grown in metal-contaminated soils are often 25 iron deficient and the bacteria may help plants to obtain sufficient iron (Burd et al., 2000). Microbial siderophores are used as metal chelating agents that regulate the availability of iron 26

1 in plant rhizosphere. This in turn helps plants to alleviate the toxicity of metals as reported for 2 arsenic uptake by several plants (Wang et al. 2007). Siderophores producing microbes that inhabit the rhizosphere soils are believed to play an important role in heavy metal 3 phytoextraction. Recent studies also suggested that rhizosphere/seed inoculation with 4 5 beneficial microbes helps plants to alleviate heavy metal stress through enhancing the 6 activities of antioxidant enzymes. Mycorrhizal fungi can also affect physiological and 7 biochemical basis of plant tolerance to heavy metals by changing the antioxidant enzyme 8 activities. So, to understand how plant-associated siderophore producing microbes influence 9 heavy metal mobilization and its uptake by plants in multi metal-polluted soils is critical 10 information (Rajkumar et al., 2012).

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12 **3. Use of PGPR on Plants**

13 According to the FAO (http://faostat3.fao.org), after sugarcane the next three first crops in terms of production (million tonnes) in the world are the cereal maize (Zea mays), 14 15 rice (Oryza sativa) and wheat (Triticum aestivum L.). The global production of maize in the year 2011 was more than 883 MT. In 2050, it is estimated that the demand for maize in the 16 developing countries will double (Rosegrant et al., 2009). In 2011, global production of rice 17 18 was almost 723 MT. Rice exhibits wide adaptability to different environments, which makes it the most widespread crop in the world. It can grow in drought conditions or in shallow 19 20 water (up to 50 cm of water), and in a wide range of latitudes and up to 3000 m altitude. For 21 this reason, it is considered a strategic crop for food security in the world by the FAO. With 22 regards to wheat, global production of this cereal was almost 704 MT in 2011. Wheat represents a major renewable resource for food, feed, and industrial raw material and it is the 23 24 most widely grown worldwide crop. Although during the last century, wheat has undergone a 25 spectacular yield increase, annual yield increase began to slow down in 1995 and is now stagnating in nearly every country (Reynolds et al., 2009). A clear link has been established 26

between this stagnation and the increasing frequency of interfering climatic factors such as spring drought during stem elongation, heat stress around flowering time and during grain filling out since 1995 (Brisson et al., 2010, Lobell et al., 2011). During that same time period world population increased from 5.8 billion to 6.6 billion and is expected to surpass 9 billion by 2050. The demand for wheat in the developing world is projected to increase 60% by 2050 while climate change is expected to affect production negatively by 29% in the same areas (Dixon et al., 2009).

8 Soybean represents the most important legume included in human diet in many 9 countries, especially in developing nations. In fact, soybean and soy-products are the only 10 food available to people in some regions of the world (Bouchenak and Lamri-Senhadji, 2013). 11 Soybean seeds are a concentrated source of isoflavones that makes soybean a singular among 12 legumes. Moreover, soybeans are an excellent high quality protein source and are low in both saturated fats and high in dietary fibre. Along the same lines, beans, in general, are especially 13 14 important for human diets mainly in developing countries because they provide an important 15 source of proteins, vitamins (folate), and minerals (Ca, Cu, Fe, Mg, Mn, Zn) (Broughton et al., 2003). In first-world countries, the nutritional benefit of beans and their contribution to a 16 17 healthy human diet that combats diseases like cancer, diabetes, and heart disease, are 18 recognized by non-profit organizations that target human aliments (Hangen and Bennink, 19 2003). Annual production, including both dry and snap bean, exceeds 21 million (MT), which 20 represents more than half of the world's total legume food production.

Having these data in mind, it is clear there would be worthwhile benefits from the use of PGPR as inoculants, which would constitute a biological alternative for sustainable production of these crops. Table 1 shows some examples of the studies accomplished since the year 2000 on PGPR applied individually or on consortia and the plants that are being dealt with in this review. An exhaustive Table showing the majority of studies carried out on those plants and the beneficial effects obtained on them are compiled in Supplementary data (Table
 S1).

3

4 *3.1. Maize.*

5 Biofertilization is one of the uses of PGPR in maize. Many bacteria act like free-living 6 nitrogen-fixing PGPR such as Burkholderia sp., Azospirillum sp., Azotobacter sp., H. 7 seropedicae, Pseudomonas sp. and Bacillus sp. (Riggs et al., 2001; Shaharoona et al., 2006; 8 Table S1). A strong increase in total plant and grain dry weight was obtained when maize 9 plants were inoculated with Burkholderia cepacia, A. brasilense and H. seropedicae in individual experiments, in comparison to plants grown in soils without nitrogen (Riggs et al., 10 11 2001). Krey et al., (2013) studied the effect of PGPR on phosphorus nutrition and they have 12 seen that field application of P. fluorescens DR54 on maize increased plant growth and soil P 13 pools. Since these effects were observed primarily during the P-deficient treatment, the 14 authors suggested the use of P. fluorescens DR54 on P poor soils and concluded that P 15 fertilizers and PGPR should be applied separately. Rosas et al., (2009) studied the promotion 16 effect of *P. aurantiaca* SR1 on maize and wheat in field treatments that included phosphorus 17 and nitrogen fertilization Both crops, when inoculated with the SR1 strain, presented 18 significant promoting effect in growth parameters and higher yields with lower fertilization doses than conventionally applied. 19

Several reports suggest the role of the genera *Azospirillum, Achromobacter*, *Burkholderia*, and *Arthrobacter* as phytostimulator (Cassán et al., 2009). The positive effects of these strains on shoot and root weight and nutrient uptake of maize plants show the beneficial role of these PGPR, which might be attributed to phytohormone production, e.g. IAA, and other activities like phosphorus solubilisation, or even other non-evaluated PGPR traits that stimulate plant growth.

1 The bioprotective role of PGPR on maize crops has also been studied. The toxigenic 2 fungus Fusarium is one of the major genera associated with maize. Some PGPR such as B. amyloliquefaciens and Microbacterium oleovorans were able to protect maize against F. 3 4 verticillioides when applied in the form of seed coatings (Pereira et al., 2011). Interestingly, 5 some PGPR species have appeared to promote plant growth by acting as both biofertilizer and 6 as biocontrol agents. For instances, strains of *B. cepacia* have been observed with biocontrol 7 characteristics against *Fusarium* spp., while they can also stimulate growth of maize under 8 iron-poor conditions via siderophore production (Bevivino et al., 1998).

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10 *3.2. Rice.*

11 The main limiting nutrient for rice crops is nitrogen (Ladha and Reddy, 2003), and 12 only one-third of the N applied as chemical fertilizer is directly used by rice plants (Buresh et 13 al., 2008). Thus, it becomes important to find alternatives to reduce and optimize the use of N 14 fertilizers applied to rice crops; numerous reports describe the use of diazotrophic bacteria due 15 to their ability to fix atmospheric nitrogen (Araújo et al., 2013; Divan-Baldani et al., 2000; 16 García de Salomone et al., 1996), in adition to their ablility to solubilise inorganic phosphate 17 or to produce IAA. Most of these diazotrophic bacteria belong to the genus Azospirillum, 18 although the genera Pseudomonas, Burkholderia or Herbaspirillum are also frequently used 19 as biofertilizers. In most cases, the application of these PGPR in field experiments showed a 20 statistically significant increase in several crop production parameters like grain production, 21 root length, leaf length or plant weight (Araújo et al., 2013).

Many PGPR reports consider that phytostimulation is mainly due to phytohormone production by the bacteria. Thus, the genus *Burkholderia* has shown to be the most widespread rice growth promoting bacteria able to produce plant hormones. Moreover, others genera such as *Azospirillum, Bacillus, Paenibacillus, Brevundimonas, Serratia, Herbaspirillum, Xanthomonas*, etc. enhance rice growth by phytostimulation. Most of them synthesize IAA, gibberellin and ethylene. Interestingly, other PGPR abilities like nitrogen fixation, phosphate solubilisation or siderophores production are usually detected. The application of these PGPR in greenhouse and/or field experiments showed, in most cases, a statistically significant increase in seed germination, weight and length of the plant, which means a better grain production efficiency (Rashedul et al., 2009).

6 More than 70 diseases affecting rice crops have been reported as causing estimated vield losses of 5 to 30%, depending on the year, zone, rice cultivar, pathogen, etc. Resistant 7 8 cultivars and application of pesticides have been used to avoid these yield losses; however, it 9 should be mentioned that using resistant varieties has the limitation of being particularly specific for a determined pathogen and the use of pesticides is both economically and 10 11 environmentally costly. The three main rice pathogens X. oryzae pv. oryzae, Rhizoctonia 12 solani and Magnaporthe oryzae are responsible for bacterial leaf blight, shealt blight and blast on rice plants, respectively. Most of the studies on the use of PGPR in rice biocontrol are 13 14 focused on treatment and prevention of these diseases (Han et al., 2005). Bacillus and 15 Pseudomonas are the predominant PGPR genera used against those pathogens, due to their 16 antagonism against growth of several fungal and bacterial microorganisms. These PGPR 17 usually produce siderophores, antibiotics, guitinases and proteases, which could be 18 responsible for the antagonism against pathogens. The yield of disease control in greenhouse 19 and field experiments is satisfactory, reducing the severity of diseases up to 90 % depending 20 on the PGPR, pathogen and rice cultivar (Filipi et al., 2011).

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22 *3.3. Wheat.*

As it is been mentioned on maize and rice, the use of PGPR for improving crop production, thus reducing the need for chemical fertilizers, is becoming a frequent strategy for sustainable agriculture. For example, inoculation of the wheat seed with ACC-deaminase producer *P. fluorescens* strains allowed the diminishing of N, P and K fertilizer rates (Shaharoona et al., 2008) and, in general, crops presented higher grain yields, harvest index
and protein content with lower fertilizer doses, along with PGPR, than those conventionally
applied (Rosas et al., 2009).

4 Significantly enhanced yields of wheat have been obtained when consortia of PGPR 5 and AMF were applied, particularly if they exhibit different and complementary abilities. 6 Singh and Kapoor (1999) studied the effect of inoculation with the vesicular-arbuscular mycorrhizal fungus *Glomus* sp.88 and two phosphate (PO_4^{3-}) -solubilising miroorganisms 7 8 (PSM), B. circulans and Cladosporium herbarum, in the presence or absence of rock 9 phosphate in a natural P-deficient sandy soil on wheat crops. The significant increase in grain 10 and straw yields due to inoculation with the consortia could be attributed to a high absorption 11 of nutrients. The effects of the application of the consortia AMF and PGPR on wheat crops 12 were investigated in a two-year experiments in different agro-climate zones of India at seven 13 locations extending from the Himalayan foothills to the Indo-Gangetic and it was seen that 14 dual inoculation of this cereal increased crop yield, grain and soil quality and the nutrient 15 uptake of wheat. I addition, it was observed that yield responses to inoculants were highest at 16 locations with previous low yields (Mäder et al., 2011).

17 Different wheat pathogens play a direct role in the destruction of natural resources in 18 agriculture. Traditional use of chemical pesticides to suppress these pathogens is currently 19 under revision due to public concern about the impact on human health and on the 20 environment. For this reason, the interest in biological control has increased recently. Diverse 21 PGPR produce anti-fungal metabolites such as DAPG (Landa et al., 2006), siderophores and 22 secretion of lytic enzymes that may reduce the growth of phytopathogens present in the rhizosphere (Compant et al., 2005). Mavrodi et al. (2012) have isolated new strains of 23 24 *Pseudomonas* from agricultural soils, river silt, and soils from herbarium specimens that show 25 the ability to reduce disease symptoms of both R. solani and Pythium ultimum, two wheat

soilborne fungal pathogens, correlated with growth promotion of wheat seedlings at the same
 time.

Salinity is considered one of the major limitations on crop productivity and quality in the world. Around 10% of world's cropland and as much as 27% of irrigated land may already be affected by salinity. Upadhyay et al. (2012) investigated the effects of two salttolerant PGPR (*B. subtilis* and *Arthobacter* sp.) on wheat plants under different salinity regimes and the results obtained demonstrated alleviation of the salinity stress effects on plants treated with bacteria, above all when a combined inoculation of both PGPR was used.

9 Wheat rhizobacterial community structure is highly dynamic and influenced by different factors such as wheat cultivar lineages, plant's age, soil characteristics and agronomic 10 11 practises (Roesti et al., 2006). These factors most likely determine changes both in the 12 rhizobacterial community and in the bacteria used as bio-inoculants. For instance, Roesti et al., (2006) employed a consortia formed by a PGPR Pseudomonas spp and an indigenous 13 14 AMF to study their effect on the bacterial community structure and wheat growth. The PGPR-15 AMF consortia significantly modified the bacterial community structure, but the loss of 16 certain bacterial species may not change the functioning of the system because other bacterial 17 species can carry out the same function, a phenomenon defined as bacterial redundancy. 18 These authors also found that changes in soils due to invading species were more buffered in 19 soils rich in microorganisms than in poor soils.

20

21 *3.4. Soybean.*

Co-inoculation studies with rhizobia and PGPR are becoming a frequent practice in the development of sustainable agriculture. These experiments are focused on the improvement of soybean yield production by increasing the nitrogen fixed by rhizobia. PGPR tested as co-inoculants with rhizobia include *B. subtilis*, *B. thurigiensis*, *A. brasiliense*, *S. proteomaculans*, *S. liquefaciens* and *P. aureofaciens*, and the rhizobia strains always used have been *B. japonicum*. From these reports the plant-growth promoting capacities of those PGPR are not described, and, in fact, the mechanism in which the non-rhizobial-PGPR is involved is poorly understood. In contrast, Cassán et al., (2009) use *A.brasiliense*, which produces IAA, GA₃ and zeatin, is a clear example of phytostimulation. Some authors support the idea that the role of the non-rhizobial-PGPR on co-inoculant experiments is to emphasize the infectivity and increase competitiveness of the rhizobial strains (Bai et al., 2002a)

7 Other studies have only used non-rhizobial PGPR species to inoculate soybean, most 8 of them endophytes isolated from roots nodules, leaves and stems and are ubiquitous in plant 9 tissues. In spite of the importance of biofertilization, most of those works are focused on 10 biocontrol. Some genera, like Bacillus, Paenibacillus and Pseudomonas are actively being used for this purpose against plant pathogens like R. solani, R. bataticola and Colletotrichum 11 12 (Senthilkumar et al., 2009). However, it has been argued whether these biocontrol agents act 13 as antagonists in the process, or they act as producers of ISR in the soybean. Furthermore, 14 arbuscular mycorrhizal fungi such as G. etunicatum and G. macrocarpum are used for rhizoremediation for metals like manganese (Mn), which in excess in the soil is toxic to 15 16 soybean crops (Nogueira et al., 2007).

17

As it has been addressed on this review, one of the main problems in the crop-field is the lack of nitrogen. The symbiotic association legume-*Rhizobium* is known to be the most efficient system for biological N₂-fixation, and there are experiments where the co-inoculation with other bacteria can increase the nodule number and improve the dry weight of roots, leaves and shoots. Bacteria such as *Pseudomonas* (Grimes and Mount, 1984) or *Azospirillum* clearly enhanced nodulation and N₂ fixation of beans during symbiosis with a rhizobial strain (Okon and Kapulnik, 1986; Tajini et al., 2012).

¹⁸ *3.5. Bean.*

1 Concerning plant pathogens control, the fungus Trichoderma can carry out biocontrol by reducing Fe^{3+} through siderophores, and the plants can take up chelated iron by reductases 2 3 on the plasma membrane (Altomare et al., 1999), in addition to binding Fe, these siderophores 4 can also bind Pb, Cr, Al and actinide ions (Renshaw et al., 2002). Moreover, there are PGPR 5 of the genera Bacillus or Pseudomonas with an important role in biocontrol of bean diseases 6 such as bacterial wilt caused by Curtobacterium flaccumfaciens pv. flaccumfaciens, or root-7 rot caused by R. solani respectively (Martins et al., 2013; Neeraj, 2011). Some PGPR show 8 different abilities, for instance biocontrol and biofertilization, at the same time (Table S1). 9 Thus, inoculation with B. cepacia SAOCV2 promotes the growth of common beans by 10 several mechanisms such as P mobilisation, increasing 44% the plant P content; and 11 promoting also antagonism towards the pathogenic species of *Fusarium*. Moreover, this result 12 is correlated with a larger number of nodules leading to an increase in N₂ fixation, and 13 indicates that the inhibition of fungal growth enhances the bacterial community in the plant 14 rhizosphere, including rhizobia (Peix et al., 2001).

15

16 **4. Conclusions and perspective.**

17 This review has focused on a heterogeneous group of microorganisms that can be 18 found in the rhizosphere. They live in association with roots and stimulate the plant growth 19 and/or reduce the incidence of plant disease. Among the numerous PGP bacteria and fungus described up to now, the bacteria Azospirillum, Bacillus, and Pseudomonas, and the fungus 20 21 *Glomus* are the genera more frequently mentioned on research reports. The important role that 22 PGPR play in agriculture can be clearly deduced from the extensive research published until 23 now. The exact mechanism by which PGPR accomplish the benefits on plants is not fully 24 understood, though it is becoming clear that all or some of the PGPR traits allow them to 25 greater or lesser extent to perform their effect(s). In addition to these traits, PGPR must be rhizospheric competent, i.e. able to survive in the rhizospheric soil where the microbial 26

communities can be affected by a wide range of factors, such as soil characteristics, plant type or agronomic practices which determine the presence or predominance of determined types of bacteria. It would be very useful to match correctly the appropriate PGPR with the right plant and environmental condition to achieve the best results on plant growth. In this sense, more effort should be done on the development of good inoculant delivery systems that facilitate the environmental persistence of the PGPR and this fact would allow diminishing the amount of cheminal fertilizers and pesticides used for enhance soil fertility and crop productivity.

8

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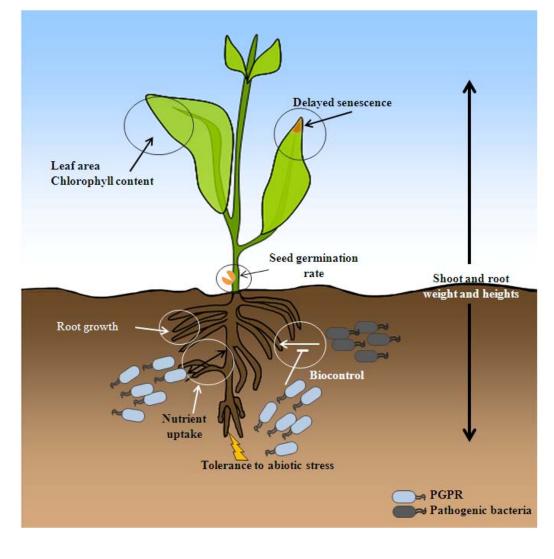
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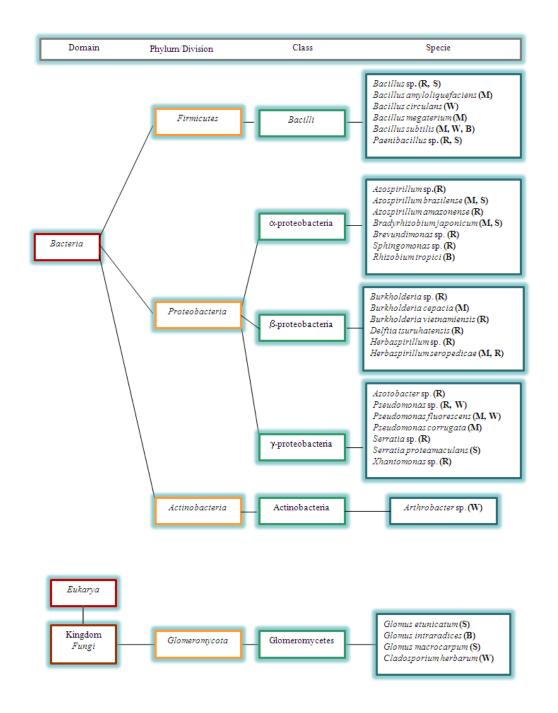
1 Legends to figures

- 2 Figure 1. Benefits to plants from host-PGPR interactions. These benefits have been shown
- 3 to include increase in seed germination rate, root growth, yield, leaf area, chlorophyll content,
- 4 nutrient uptake, protein content, hydraulic activity, tolerance to abiotic stress, shoot and root
- 5 weights and heights, bio-control, and delayed senescence.





8 Figure 2. PGPR most frequently studied, grouping according their phylogenetic
9 classification.



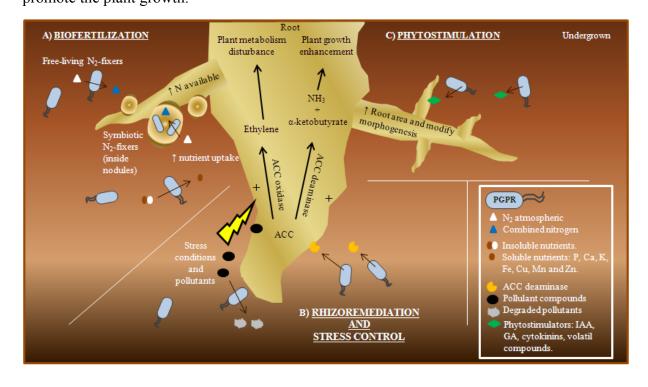
1

Plants where these microorganisms have been studied: M: maize, R: rice, W: wheat, S: soybean, B: bean

2

3 Figure 3. Some forms of plant growth promotion. A) BIOFERTILIZATION, 4 Rhizobacteria are able to promote plant growth by improving the nutrient uptake of plants. 5 Free-living and symbiotic atmospheric N2-fixing bacteria are able to convert by means of 6 nitrogenase enzyme N₂ into ammonia, which can be used by the plant as a nitrogen source. B) 7 RHIZOREMEDIATION AND STRESS CONTROL, Plants exposed to stress conditions and 8 pollutants show an increase in the ethylene levels that lead to a metabolism disturbance and 9 plant damage. PGPR that contain the enzyme ACC-deaminase enhances plant growth and development by decreasing plant ethylene levels. On the other hand, some PGPR are able to 10

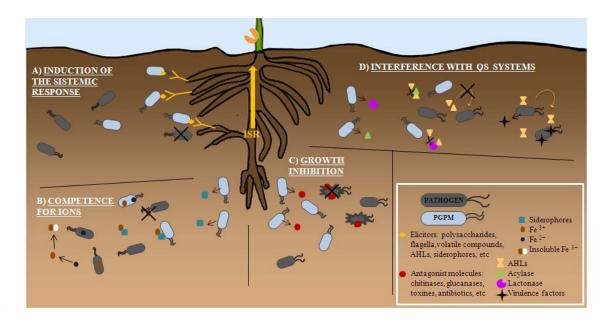
degrade or remove pollutans, avoiding the plant damage. C) PHYTOSTIMULATION,
 Production of substances like IAA, gibberellins, cytokinins and certain volatiles alter the root
 morphogenesis, proliferation and enhance plant mineral uptake and root exudation, which
 promote the plant growth.



5 6

7 Figure 4. Some forms of PGPR antagonism against plant pathogens. A) INDUCTION OF 8 THE SISTEMIC RESPONSE, ISR is triggered by PGPR by means of molecules termed 9 elicitors. This defence response is dependent on ethylene and jasmonic acid signalling in the 10 plant and reduces plant disease modulating the physical and biochemical properties of host plants. B) COMPETENCE FOR IONS. In aerobic microbial habitats, Fe^{2+} is oxidized to Fe^{3+} . 11 which form insoluble compounds, making it unavailable to microorganisms. PGPR produce 12 siderophores which form complexes with Fe^{3+} preventing insolubilization. In the membrane, 13 this ion is reduced to Fe^{2+} and released into the cell. Therefore, cell pathogens would lack 14 Fe²⁺. C) GROWTH INHIBITION, Mechanisms responsible for antagonistic activity include 15 inhibition of the pathogen by antibiotics, toxins, biosurfactants and by the production of 16 17 extracellular cell wall degrading enzymes such as chitinase and β -1,3-glucanase. D) INTERFERENCE WITH QS SYSTEMS, QS is defined as a regulation of bacterial gene 18 19 expression in response to changes in their population density, which is mediated by small 20 diffusible signal molecules like AHLs. This system activates the synthesis of virulence factors 21 such as biofilm formation, toxin and exopolysaccharide production, plasmid transfer and 22 motility. Several bacteria produce acylase or lactonase, enzymes that degrade the AHL

- 1 molecules, thus avoiding the production of virulence factors, which are essential for the
- 2 successful establishment of a pathogenic relationship with the eukaryotic hosts
- 3



1 Table 1. Examples of cooperative interactions between consortia (different PGPR or bacteria and AM Fungi) tested on maize, rice, wheat, soybean and bean

2 crops.

Plant Maize	Bacteria/Consortia ⁽¹⁾	PGPR abilities	Conditions	Results on plants	Reference
Maile	Azospirillum brasilense Az39 Brayrhizobium japonicum E109. (Individual and consortia experiments)	Phytostimulation	Growth chamber		Cassán et al., 2009
	Serratia liqufaciens, Bacillus sp. Pseudomonas sp. (Individual and consortia experiments)	Biofertilization (N) Biocontrol (Several root pathogens)	Greenhouse	Increase up to 14 % maize yield (dry weight) when they are inoculated as consortium respect to controls. In individual experiments, <i>S. liqufaciens</i> increase the dry weight of maize respect to controls more than 10%, <i>Bacillus sp.</i> more that 7% and <i>Pseudomonas sp.</i> more than 10 %.	Lalande et al., 1989
Rice					,
	<i>P. fluorescens</i> Aur6 <i>Chryseobacterium balustinum</i> Aur9 (Individual and consortia experiments)	Biocontrol (<i>Magnaporte</i> grisea) Salinity	Field	Three field experiments in different years. Each strain individually protects rice against rice blast, but the combination of both strains was the most effective treatment (reaching 50% of protection against disease). A relation between protection and increase in rice productivity and quality was found.	Lucas et al., 2009
Wheat					
	<i>Glomus</i> sp. 88, <i>B. circulans</i> and <i>Cladosporium</i> <i>herbarum</i> , (Individual and consortia experiments)	Biofertilization (P)	Pot experiment in greenhouse	The population of PO_4^{3-} solubilizing microorganisms in the rhizosphere of wheat was larger in the treatments that were inoculated with the AMF fungus and Mussoorie rock phosphate.	Singh and Kapoor, 1999
	Arthrobacter sp. and B. subtilis (Individual and consortia experiments)	Stress controller (Salinity)	Pot experiment in greenhouse	Increases dry weight up to 26% when co-inoculated at 2 dS m^{-1} of salinity level and 40% when co-inoculated at 6 dS m^{-1} of salinity level.	

	A natural AMF <i>P. jessenii, P. synxantha</i> (Individual and consortia experiments)	Biofertilization	Field	Dual inoculation of wheat with PGPR and AMF increased grain yield by 41% as compared to un-inoculated controls. Yield responses to the inoculants were highest at locations with previously low yields.	Mäder et al., 2011
	<i>Providencia</i> sp. 2 strains of <i>Anabaena</i> sp. <i>Calothrix</i> sp. (consortia experiment)	Biofertilization	Growth chamber, greenhouse and field	Enhancement 18.6% protein content.	Rana et al., 2012
Soybean					
	<i>S. proteamaculans</i> 1-102 <i>B. japonicum</i> 532C (Consortia experiments)	Biofertilization enhanced	Pot and pouch experiment in greenhouse	This assay is based on an inducible activator like a lipo- chitooligosaccharide (LCO) analogue which stimulates root nodule formation. Thus, addition of PGPR supernatant to <i>B. japonicum</i> inoculant increased nodule weight by $53,7\%$ and plant weight by 31,2% under 25°C root zone temperature, that is at essentially the same level with co-inoculation of <i>B. japonicum</i> with the <i>S. proteamaculans</i> 1-102 culture.	Bai et al., 2002a
	S. proteamaculans 1-102, B. japonicum (Consortia experiments) S. liquefaciens 2-68 B. japonicum (Consortia experiments)	Biofertilization enhanced	Field and greenhouse	The co-inoculation of PGPR at their optimal dose increased nodule number, plant dry weight and fixed nitrogen.	Bai et al., 2002b
	Bacillus subtillis NEB4 and NEB5, Bradyrhizobium japonicum 532c (Consortia experiments) Bacillus thuringiensis NEB17, Bradyrhizobium japonicum 532c (Consortia experiments)	Biofertilization enhanced	Growth pouch experiment in greenhouse	Plants co-inoculated with these strains had significantly higher nodule and plants weights, and NEB5 and NEB17 seemed to increase nodule number per plant.	Bai et al., 2002c
	A. brasiliense Az39 B. japonicum E109 (Consortia experiments)	Phytostimulation	Pot experiment in growth chamber	<i>A. brasiliense</i> Az39 and <i>B. japonicum</i> E109, singly or in combination, promoted seed germination, nodule formation, and early development of soybean seedlings.	Cassán et al., 2009

Paenibacillus rhizosphaerae TGX5E, Glomus intraradices (Consortia experiments) P. favisporus TG1R2, G. intraradices (Consortia experiments) P. rhizosphaerae TGX5E, P. favisporus TG1R2 G. intraradices (Consortia experiments)	Phytostimulation	Pot experiment in greenhouse	The frequency of mycorrhizal colonization and the extraradical mycelium increased significantly with time in plants inoculated with <i>G. intraradices</i> and both <i>Paenibacillus</i> strains. The highest dry biomass was found in soybean plants treated with <i>P. favisporus</i> , when this species was inoculated separately or in combination with <i>G. intraradices</i> .	Bidondo et al.,
Bean				

Rhizobium tropici CIAT899 A. brasilense Cd (ATTCC 29729) (Consortia experiments) R. etli TAL182 A. brasilense Cd (ATTCC 29729) (Consortia experiments)	Biofertilization enhanced (N)	Growth chamber	Different results were obtained changing the concentration of <i>Rhizobium</i> and <i>Azospirillum</i> to inoculate the plants. Finally an increase in nodule number, nodule weight, root weight and shoot weight were observed when the concentration of both bacteria was 10^5 and 10^6 cfu/ml respectively.	
<i>R. tropici</i> CIAT899 <i>G. intraradices</i> (Consortia experiments)	Biofertilization enhanced (N and P)	Pot experiments in greenhouse	Plant growth parameters are enhanced as a consequence of improvement of 30-40% in the amount of P and 29-42% in N in the soil. An increase of 63-70% of nodule number, 40-43% in nodule mass, an enhancement of 23-24% shoot dry weight and 39-48% in root growth.	
G. sinuosum Gs Gigaspora albida Ga P. fluorescens (Consortia experiments)	Biofertilization (P and N) Biocontrol (<i>Rhizoctonia</i> <i>solani</i>)	Field	Coinoculation <i>G. sinuosum</i> + <i>P. fluorescens</i> + Mustard oil cake (MOC) and <i>G. albida</i> + <i>P. fluorescens</i> + Mustard oil cake (MOC) had an incidence disease of 12.35 and 12.72% respectively. Phosphor content was 2.26 and 2.23 mg/g dry tissue and nitrogen content was 3.42 and 3.26 mg/g dry tissue. Furthermore an increase of dry weight shoot, leaves and roots, and length of shoots and roots were observed.	Neeraj, 2011

- 1 ⁽¹⁾ Individual experiments mean that each bacterium has been tested separately and Consortia experiments mean that the microorganisms shown on
- 2 the table cells have been tested together.