



## Bacterial Endophytes Associated to Crops: Novel Practices for Sustainable Agriculture

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### Abstract

For many decades, rhizosphere bacteria have been studied for their potential to promote crop growth and control certain pathogens. Compared to the studies conducted, there are relatively few examples of microbial inoculants based on rhizosphere bacteria that have had commercial success, mainly due to the strong competition present in the rhizosphere. In the last twenty years, studies on endophytes have multiplied, also and above all as a possible alternative to rhizobacteria, for the development of microbial inoculants capable of replacing some agrochemicals and reducing the environmental impact of agronomic management of crops. This minireview summarizes the most important characteristics and qualities of endophytic bacteria and describes the path that can be followed to identify and deepen the knowledge of candidates suitable for the development of microbial inoculants. The nature of endophytes requires careful isolation methods; the growth promotion potential can be identified by evaluating some traits-related phenotypes and metabolic capacities; relations with the host plant and capacity of colonization, the influence of the external environment and sensitivity to agronomic practices are pivotal factors in establishing the endophytic population in the different parts of the plant. All of this must be taken into consideration in selecting the most promising endophytes for field trials.

**Keywords:** Bacterial endophytes; Microbial inoculants; Plant-bacteria interactions; Plant growth promoting bacteria

### Introduction to plant bacterial endophytes

Rising global population increases demand for food and drives the need for improved crop productivity over the next 30 years. This requires an increase in the current ability of agriculture to produce sufficient food to meet demand of providing food security and feeding the expanding population using efficient and sustainable crops management practices. The yield improvements need to be achieved without significant costs increases and still maintaining acceptable yields. Thus, crop enhanced productivity is needed without significant increases in land, water, or fertilizer use as these are all becoming limited and pose additional threats to the environment. In this regard, the soil microbial diversity and plant-microbe associations is considered one of the most researched areas to exploit and develop sustainable agriculture production systems.

In the last ten years many studies have been published regarding the endophytic bacterial populations living associated with crops such as soybean, maize, sugarcane, wheat and many others. The aim of these studies was to identify the bacteria associated to crops which were not pathogenic and could have been beneficial, such as the Plant Growth Promoting Bacteria (PGPB) and which were living inside the plant tissues, such as the endophytes, with the purpose of assessing the mechanisms of plant-bacteria interaction and the potential beneficial effects of these bacteria on growth and health of hosting crops. Among the microorganisms living in close associations with plants, the Plant Growth Promoting Bacterial Endophytes (PGPBEs) could be more successful in plant growth promotion through the combined action of mechanisms enhancing the plants growth and protecting them from diseases and abiotic stresses [1-4]. Besides the genotypic characterization, the main and most important metabolic activities within the plant were also investigated, with the purpose of assessing the mechanisms

of plant-bacteria interaction and the potential beneficial effects of these bacteria on growth and health of hosting crops. In this respect the definition of endophytes becomes relevant. The most commonly accepted definition for endophytic bacteria, as well as fungi, is “Bacteria that are detected from inside surface-disinfected plants or extracted from inside plants and have no visibly harmful effects on the plants” [5]. The purpose of this minireview is to highlight the potential of these endophytes as plant growth promoting bacteria to be used to improve crop production in a sustainable agronomic management system.

### Isolation and biodiversity of bacterial endophytes

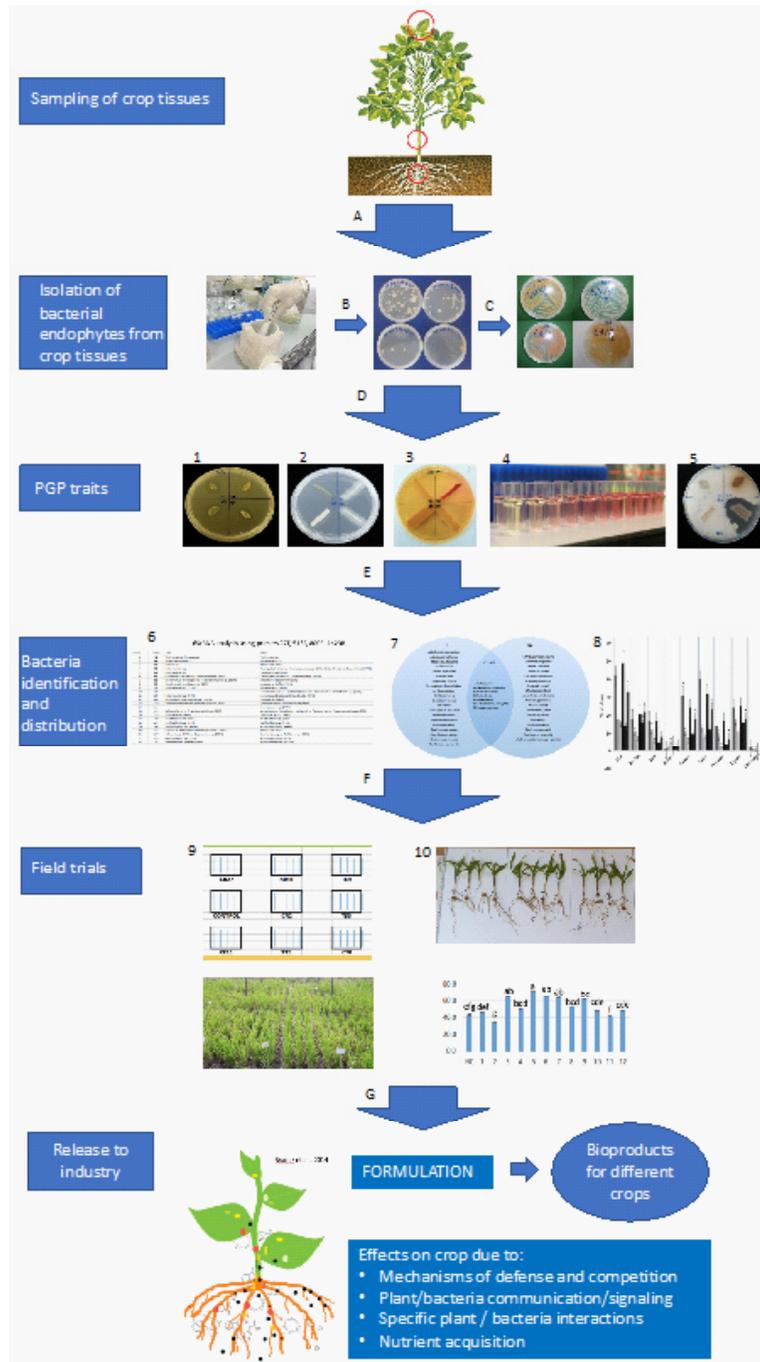
Although the list of isolated endophytes from crop plants is considerable, the most studied and most predominant endophytes belong to the three major phyla (Actinobacteria, Proteobacteria and Firmicutes) and the most commonly found genera are *Azoarcus* [6], *Acetobacter* (renamed as *Gluconobacter*) [7], *Bacillus* [8], *Enterobacter* [9], *Burkholderia* [10], *Herbaspirillum* [11], *Pseudomonas* and *Serratia* [9], *Stenotrophomonas*, *Micrococcus*, *Pantoea*, *Microbacterium* and *Streptomyces* [12]. Species of these genera are ubiquitous in soil/rhizosphere and this is not a coincidence since soil and rhizosphere represent the main sources of endophytic colonizers [5,13-18]. All of these genera, described as bacterial endophytes, are also common inhabitants of the rhizosphere. Therefore, it has been suggested that the endophyte microbiome may be a subpopulation of the rhizosphere inhabiting bacteria [17,19,20].

PGPBs have extended colonization on the host plant organs and tissues which reflects the ability of bacteria to selectively adapt to these specific ecological niches and consequently, intimate associations between bacteria and host plants can be formed [21]. The plant colonization by endophytes may occur mainly through the roots if the bacteria can hydrolyse the epidermal, hypodermal, endodermal and other cortical cells barriers [22]. Once inside, the colonization can be either local in specific plant tissues such as cortex, or systemic or transported across or through the conductor apoplastic elements [23]. Plant colonization by bacterial endophytes is preferably intercellular [24], with only a few reports

of intracellular colonization, such as in the case of banana [25] or Arabidopsis [26]. It is also suggested that the endophytic bacteria are evolutionarily intermediate between saprophytic and pathogenic bacteria, speculating on the possibility that endophytic bacteria are more evolved than pathogenic, being able to take nutrients from the host without killing it [5]. According to Azevedo, et al. [27], the difference between endophytes, epiphytes and pathogens is purely didactic since there is a gradient between them that makes complex to discriminate each category. Endophytic bacteria may, depending on the conditions and the genotype of the host, become a pathogen; and may, depending on the phase of the lifecycle, live in harmony with the host [28].

Based on the original definition of bacterial endophytes given by Hallmann, et al. [5] and broadly accepted, the importance of methods and criteria of endophytes isolation results clear. As the character of endophytic bacteria is attributed to bacteria isolated exclusively from plant tissues superficially sterilized, surface disinfection processes have to be well defined to retain the concept and limit the endophytic habitat. Errors may arise as result of either incomplete disinfection of the surface or adsorption of bacteria through plant cell structure or penetration of sterilant within the tissues of the plant cells, resulting in loss of endophytism [5].

There are two main methods for identification and characterization of endophytic bacterial populations, depending on the possibility of culturing the bacteria. Culture dependent methods for identification of culturable endophytes involve the isolation and growth of the bacteria from surface-sterilized root, stem and leaves sections [29-33]. This step is usually followed by the phenotypic characterization of these bacteria focused mainly on properties and metabolic activities such as the secretion of hydrolytic enzymes, production of indole acetic acid and siderophores for iron uptake, phosphate solubilization, biofilm formation and exopolysaccharides production, nitrogen fixation, antimicrobial activity, different types of motility as well as other characteristics related to competitiveness, plant growth promotion, antagonism and capacity of plant colonization (Figure 1).



**Figure 1:** Flow chart summarizing method for isolation and characterization of bacterial endophytes, development of bacterial inoculants and benefits to agricultural crops. Sampling and sterilization of tissues (A), maceration and growth of bacteria on solid media (B), and isolation of pure cultures (C). Characterization of isolates in relation to Plant Growth Promoting (PGP) traits (D) such as phosphates solubilization (1), production of exopolysaccharides (2), siderophores (3) and indolactic acid (4), and antimicrobial activity (5). Characterization of the endophytic bacterial population (E) by amplification and sequence analysis of the 16S rRNA gene (6), composition of bacterial population in different agronomic managements (7) and localization of bacteria in different tissues (8). Field trials (9) to establish colonization and growth promotion capabilities (10). Release to industry (G), followed by development of proper formulations and different crop-specific bioproducts.

On the other hand, some endophytes are obligate because they are unable to proliferate outside of plants and require the plant for survival, thus are often nonculturable [34]. Obligate endophytes can be studied by means of culture-independent techniques [35] based on molecular tools as Polymerase Chain Reaction (PCR) for amplification of variable regions of DNA, often from the 16S rRNA gene. This step is followed by downstream methods to analyze the endophyte community composition, which may include cloning or other community fingerprinting techniques. Considering the implications in identifying and measuring endophyte diversity and community structure, the complementarity of both culture-dependent and independent methodologies has received a broad consensus [31-33].

The Next Generation Sequencing (NGS) technology and bioinformatic tools allow the characterization of many endophyte communities from a broad variety of plant species, including their structure and dynamics based on information regarding endophyte genomes, proteomes, and transcriptomes. The complete genomes of *Gluconacetobacter diazotrophicus* [9], *Bacillus subtilis* [8], *Burkholderia* sp. [36], *Kosakonia oryzae* [37], *Enterobacter* sp. [9], *Burkholderia phytofirmans* [10], *Azospirillum* sp. [38], *Pseudomonas stutzeri* [39], *Herbaspirillum seropedicae* [11], *Azoarcus* sp [6], among others, have been sequenced and analysed. Bioinformatic analysis of genomes provides additional information on the role of these bacteria and their effects inside the plant, and represents an important tool for further studies such as the regulation of gene expression also in relation to the mechanisms of plant-microbes interaction [40,41]. In addition, the comparisons between the endophytic bacterial genomes and the genomes of rhizospheric plant growth-promoting bacteria could shed light on potential genetic factors specifically responsible of the endophytic lifestyle, therefore contributing to a better understanding of the functioning of complex interactions between bacterial endophytes and plants [20].

### Mechanisms of plant growth promotion

Plant growth promotion by bacterial endophytes is usually the result of direct or indirect mechanisms. Direct promotion occurs either by increased acquisition of essential nutrients which involve nitrogen, phosphorus and iron or by modulation of hormone levels synthesizing auxin, cytokinin or gibberellins. In addition, some endophytes can lower levels of the phytohormone ethylene by synthesizing an enzyme, 1-Aminocyclopropane-1-Carboxylate (ACC) deaminase that cleaves the compound ACC, the immediate pre-cursor of ethylene in all higher plants. Indirect mechanisms of growth promotion occur by production of substances that inhibit bacterial or fungal pathogens and by indirect effects such as antagonism against pathogens and competition for iron [20,42-46]. For example, siderophores such as pyochelin from *Pseudomonas* sp., chelate iron and can indirectly contribute to suppression

and control of disease by competing with phytopathogens for trace metals [47]. Endophytes also contribute to the control of phytopathogens primarily for their ability to induce plant defense reactions through a mechanism called Induced Systemic Resistance (ISR), that leads to a higher tolerance of pathogens [48]. Systemic resistance develops when plants successfully activate their defense mechanism in response to primary infection by a pathogen, notably when the latter induces a hypersensitive reaction through which it becomes limited in a local necrotic lesion of brown, desiccated tissue. Bacterial strains of the genera *Pseudomonas* and *Bacillus* can be considered the most common groups inducing ISR [49], although induction of resistance is not exclusive to these groups and to this mechanism. For example, the shoot endophyte *Methylobacterium* sp. strain IMBG290 induced resistance against the pathogen *Pectobacterium atrosepticum* in potato, in an inoculum-density-dependent manner. The observed resistance was accompanied by changes in the structure of the innate endophytic community. Endophytic community changes were shown to correlate with disease resistance, indicating that the endophytic community as a whole, or just fractions, can play a role in disease suppression [50]. Bacterial factors responsible for ISR induction were identified to include flagella, antibiotics, N-acylhomoserine lactones, salicylic acid, jasmonic acid, siderophores, volatiles (e.g., acetoin), and lipopolysaccharides [51].

The potential of bacterial endophytes to Promote Plant Growth (PGP) by means of abiotic stress tolerance and disease protection is associated with their ability to produce different compounds such as secondary metabolites that are involved in mechanisms of signaling, defense and genetic regulation of the establishment of symbiosis. It has been observed that plant inoculation with endophytic bacteria leads to accumulation of “protective” compounds, such as proline, carbohydrates and antioxidants, in addition to antibiotics and fungal cell-wall lytic enzymes, which can inhibit growth of plant pathogens. Plants acclimate to environmental stresses by altering their physiology to be able to overcome stress factors such as dehydration, mechanical injury, nutrient deficiency, high solar radiation, or biotic/abiotic factors. Proline accumulation stimulating effect by endophytic strains of the actinobacteria *Arthrobacter* sp. and the Firmicutes *Bacillus* spp. were reported in pepper (*Capsicum annuum*) plants *in vitro* where their synthesis was related to osmotic stress responses. In addition, plants inoculated with bacterial endophytes could tolerate abiotic stresses by increasing enzymatic activity. *B. cereus* CSR-B-1, *B. marisflavi* CSR-G-4, *B. pumilus* CSR-B-2, *B. saffensis* CSR-G-5, *B. subtilis* CSR-G-1 and *B. thuringiensis* CSR-B-3, induced increment of superoxide dismutase, phenylalanine lyase, catalase, and peroxidase enzymes activity in gladiolus plants under high sodium concentration conditions [19]. Tolerance to low temperatures and growth promotion by bacterial endophytes activity has also been reported by Verma, et al. [52] that found

psychrotolerant *Bacillus* and *Bacillus* derived genera as wheat (*Triticum aestivum*) endophytes, among others. Phosphate and iron are major essential nutrients, but soluble phosphate and iron concentrations in soil for plant intake are usually very low; their acquisition can be facilitated by plant-associated bacteria [20].

The benefits of plant growth promotion mechanisms by bacterial endophytes colonization was investigated for the main crops by several authors [6,7,10-13,23,31-34,36-39,53-85]. Table 1 illustrates examples of PGPBEs in plants and crops such as Banana, Canola, Citrus, Coffee, Common Bean, Grapevine, Maize, Potato, Rice, Soybean, Sugar beet, Sugar cane, Tomato and Wheat.

**Table 1:** Some crop-associated bacterial endophytes and their plant-growth promoting traits.

| Host plant                              | Endophyte species   | Plant growth- promoting traits   | Reference |
|---|---|--|-----------|
| Potato                                  | <i>Bacillus</i> spp.  | ACC deaminase activity, phosphate solubilization, siderophore production.  | [53]      |
|   | <i>Streptomyces</i> spp.  | PGP and biological control   | [12]      |
|   | <i>Pseudomonas putida</i> and <i>Serratia plymuthica</i>  | Production of the antibiotic 2,4-diacetyl-phloroglucinol ( <i>Pseudomonas</i> ) and antagonism ( <i>Serratia</i> )     | [13]      |
|   | <i>Burkholderia phytofirmans</i> PsJN   | ACC deaminase activity and production of indole acetic acid (IAA)  | [10]      |
| Rice                                    | <i>Streptomyces</i> sp. strain A20  | Production of three antibiotics: streptothricins D, E and F; production of siderophores and IAA, and P solubilization. | [54]      |
|   | <i>Bacillus</i> sp.   | multiple PGP and antagonistic activity   | [56]      |
|   | <i>Azoarcus</i> sp. BH72  | N-fixation   | [6]       |
|   | <i>Azospirillum</i> sp.   | Production of IAA and ACC deaminase  | [118, 38] |
|   | <i>Pseudomonas stutzeri</i>   | N-fixation   | [39]      |
|   | <i>Burkholderia</i> sp.,  | Antifungal activity  | [36]      |
|   | <i>Herbaspirillum seropedicae</i>   | N-fixation   | [11]      |
|   | <i>Kosakonia oryzae</i>   | Siderophore production, auxin biosynthesis and N-fixation  | [37]      |
|   | <i>Azospirillum</i> sp.   | Promotion of rice growth   |           |
|   | <i>Rhizobium</i> spp.   | Growth promotion   | [119]     |
|   | <i>Herbaspirillum</i> , <i>Pseudomonas</i> ,  | IAA, N-fixing, P solubilization, ACC deaminase, etc  | [23]      |
|   | <i>Pantoea</i> , <i>Methylobacterium</i> ,  |  | [55]      |
|   | <i>Kosakonia</i> , <i>Burkholderia</i> ,  |  |           |
| <i>Rhodococcus</i> , <i>Ralstonia</i> , |   |  |           |
| <i>Brevibacillus</i> , <i>Bacillus</i>  |   |  |           |
| Soybean                                 | <i>B. subtilis</i> and <i>B. thuringiensis</i> ;  | Production of siderophores, IAA synthesis and ACC-deaminase  | [57]      |
|   | <i>Pseudomonas</i> , <i>Ralstonia</i> , <i>Enterobacter</i> , <i>Pantoea</i> and <i>Acinetobacter</i>   | Antifungal activity; phytases; N-fixation; phosphate solubilization;   | [58]      |
|   | <i>Bacillus</i> spp.  | Cellulase, pectinase and motility  | [59]      |
|   | <i>Agrobacterium</i> , <i>Enterobacter</i> , <i>Kosakonia</i> , <i>Pantoea</i> , <i>Pseudomonas</i> , <i>Ralstonia</i> , <i>Serratia</i> , <i>Rhizobium</i> , <i>Stenotrophomonas</i> , etc | Production of IAA and exopolysaccharidies, P solubilization, etc.  | [31, 33]  |
|   | <i>Enterobacter</i> sp., <i>Bacillus</i> sp., <i>Variovorax</i> sp., <i>Serratia</i> sp., <i>Burkholderia</i> sp., <i>Pantoea</i> sp., <i>Kosakonia</i> sp.                                 | Antimicrobial activity   | [32]      |

|             |   |  |          |
|-------------|---|--|----------|
| Wheat       | <i>Bacillus subtilis</i>  | Antifungal activity against Puccinia   | [60]     |
|             | <i>Bacillus cereus</i>  | Biofilm formation, colonization and biocontrol   | [61]     |
|             | <i>Bacillus thuringiensis</i>   | Biocontrol   | [62]     |
|             | <i>Azospirillum</i> sp.   | Phytormone synthesis: IAA, GA, ABA; phosphate solubilization   | [38]     |
|             | <i>Arthrobacter</i> sp.   | Siderophore-production and Zn solubilization   | [63]     |
|             | <i>Burkholderia cepacia</i>   | Plant growth promotion   | [64]     |
| Sugar Beet  | <i>Bacillus pumilus</i> , <i>Chryseobacterium indologene</i> , <i>Acinetobacter johnsonii</i>   | increased concentration of carbohydrates and growth photosynthetic efficiency  | [65]     |
| Sugar Cane  | <i>Gluconacetobacter diazotrophicus</i>   | N-fixation, plant growth promotion, secretion of organic acids, synthesis of auxin and bacteriocins                    | [7]      |
|             | <i>Azospirillum amazonense</i> , <i>Burkholderia tropica</i> , <i>Herbaspirillum seropedicae</i> , <i>H. rubrisubalbicans</i> , <i>Gluconoacetobacter diazotrophicus</i>  | acceleration of budding; increase in biomass; N-fixation; production of siderophores and IAA; phosphate solubilization | [66, 67] |
| Tomato      | <i>Bacillus subtilis</i>  | Control of <i>Alternaria solani</i> and <i>Phytophthora infestans</i>  | [68]     |
|             | <i>Burkholderia phytofirmans</i> PsJN   | IAA synthesis, ACC deaminase   | [10]     |
|             | <i>Sphingomonas</i> sp.   | Production of gibberellins and IAA   | [69]     |
| Common Bean | <i>Microbacterium testaceum</i>   | Inhibition of bacterial pathogens and quorum sensing   | [70]     |
|             | <i>Rhizobium endophyticum</i>   | Solubilization of phytate  | [71]     |
|             | <i>Bacillus</i> , <i>Delftia</i> , <i>Methylobacterium</i> , <i>Microbacterium</i> , <i>Paenibacillus</i> , <i>Staphylococcus</i> and <i>Stenotrophomonas</i>   | To be determined   | [72]     |
| Citrus      | <i>Bacillus</i> sp.   | IAA production and P solubilization  | [73]     |
| Maize       | <i>Bacillus</i> spp.  | Production of lipopeptides active against <i>Fusarium moniliforme</i>  | [74]     |
|             | <i>Azospirillum brasilense</i>  | Plant growth promotion   | [75]     |
|             | <i>Enterobacter</i> sp.   | Improved photochemical efficiency and flowering anticipation; N-fixation   | [76]     |
|             | <i>Paenibacillus polymyxa</i>   | N-fixation and growth promotion  | [77]     |
|             | <i>Pseudomonas</i> spp., <i>Enterobacter asburiae</i> , <i>Sinorhizobium meliloti</i>   | PGP traits and antifungal activity   | [78]     |
| Canola      | <i>Bacillus subtilis</i>  | Antibacterial and antifungal activity  | [79]     |
|             | <i>Burkholderia phytofirmans</i>  | ACC deaminase activity and production of IAA   | [10]     |
| Coffee      | <i>Escherichia fergusonii</i> , <i>Acinetobacter calcoaceticus</i> , <i>Salmonella enterica</i> , <i>Brevibacillus choshinensis</i> , <i>Pectobacterium carotovorum</i> , <i>Bacillus megaterium</i> , <i>Microbacterium testaceum</i> , <i>Cedecea davisae</i> | Production of phosphatase and indol acetic acid; control of coffee leaf rust, <i>Hemileia vastatrix</i>                | [80]     |
| Grapevine   | <i>Bacillus pumilus</i> , <i>Paenibacillus</i> sp.  | Biocontrol of fungal pathogen <i>Phaeoconiella chlamyospore</i>  | [81]     |
|             | <i>Bacillus subtilis</i> , <i>Curtobacterium</i> sp.  | Biocontrol of <i>Agrobacterium vitis</i> crown gall disease  | [82]     |

|             |   |   |      |
|-------------|---|---|------|
|             | <i>Bacillus</i> , <i>Staphylococcus</i> , <i>Microbacterium</i> , <i>Paenibacillus</i> ,<br><i>Curtobacterium</i> , <i>Stenotrophomonas</i> , <i>Variovorax</i> , <i>Micrococcus</i> ,<br><i>Agrococcus</i> | To be determined  | [83] |
| Banana      | <i>Pseudomonas aeruginosa</i>   | Antagonism against pathogen   | [34] |
|             | <i>Serratia</i> sp.   | Plant growth promotion  | [84] |
| Poplar tree | <i>Enterobacter</i> sp., <i>Stenotrophomonas maltophilia</i> , <i>Pseudomonas putida</i> , <i>Serratia proteamaculans</i>   | Acetoin and 2,3-butanediol synthesis;<br>production of IAA and ACC deaminase; | [85] |

### Importance of endophytes and potential use as inoculants

The potential of PGPBEs to improve plant health has led to a great number of studies examining their application as inoculants, primarily in agricultural crops [67,86-88]. Development and use of microbial inoculants can lead to a reduction of the need and use of agrochemicals such as pesticides and fertilizers [89] and help to promote sustainable agricultural practices. Several studies are being conducted in order to identify possible candidates for crops inoculation [58,59,90]. However, plant growth promotion and protection remain priorities in studying bacteria associated to crops, and the development of new inoculants could provide a sustainable biotechnological approach as demonstrated by those already on the market [91]. In this respect, *Azospirillum brasiliense* as well as *Bradyrhizobium japonicum* are examples of efficient plant growth promoting bacteria widely used in Brasil in soybean and maize [86]. However, it happens that many PGPB with good properties in the laboratory or greenhouse conditions, poorly behave when delivered in the field, mainly due to the high competitions for and complex mechanisms involved in colonization of the rhizosphere [92].

Endophytic bacteria are not subject to competition for nutrients that typically occurs in the rhizosphere, and can have higher efficiency than the rhizosphere colonizing bacteria in promoting growth, water absorption and the elimination of harmful microorganisms which are in the inside of the root system [93,94].

Endophytes, which are protected by the host against environmental stresses and competition with other organisms, are normally found in low densities compared to pathogenic bacteria or rhizobacteria [5,16]. Therefore, the potential of PGPBEs can be a valid alternative to the PGP rhizobacteria, since the competition inside the plant is lower and, once the plant is colonized by means of mechanisms involving motility, attachment, plant-polymer degradation, iron uptake and evasion of plant defenses [20,24,95], these bacteria could exploit their PGP functions in a more efficient manner.

Despite the fact that endophytes are protected inside the plants, there are several factors that can affect the type and density of the endophytic bacterial population in the interior of the host

such as plant genotype and the type of farming, the tissue where they are located and the stage of plant development; in addition, also environmental factors such as variations in temperature and humidity, agronomic management adopted and application of agrochemicals can have significant effects. However, there are probably other limiting factors that may be relevant during the establishment of populations in the plant tissues. Thus, the establishment and maintenance of the bacterial population could be limited and influenced by the same factors that affect the health of the plant [5]. Currently it is unclear whether plants benefit more from the colonization by endophytic bacteria or rhizobacteria. However, both can promote plant growth. Therefore, there is an increasing interest in studying these bacteria and in the development of biofertilizers to increase crop yields [96].

### Plant growth promoting bacterial endophytes and crop management

Plant genotype can have a direct influence on plant-associated bacteria, as this can change the exudates released by the roots and made available to bacterial groups, affecting colonization by rhizobacteria [97] and, consequently, entry into the plant and colonization by endophytes. Besides the genotype, the stage of development of the host may also influence the bacterial population. In endophytic bacteria associated with soybean the population increases with the development of the plant and start to decrease at the beginning of the reproductive stage [98]. However, a major influence is determined by environmental factors such as variations in temperature (heat or cold depending on the season in which the samples were collected) and the observed tissue (roots, stems or leaves) [99]. Furthermore, the soil was found to be the main factor in determining the population variance of *Burkholderia cepacia* associated with maize roots [100].

Crop agronomic management is a factor of great importance and impact on the microbial community, mainly due to the use of agrochemicals. The bacterial community and the density of bacteria populations in transgenic and non-transgenic sugarcane plants were found to be affected by crop management; the density of bacterial population associated with the rhizosphere of transgenic sugarcane plants treated with the herbicide Imazapyr was found reduced if compared with the non-transgenic one [101].

The most important herbicide used in herbicide-resistant soybean and maize fields is glyphosate. Glyphosate is a systemic herbicide with wide spectrum of activity, recommended for post-emergence control of a wide range of plants, and not selective in its action on mono and dicotyledons since it inhibits the enzyme 5-Enolpyruvylshikimate-3-Phosphate Synthase (EPSPS) involved in the synthesis of aromatic amino acids, therefore causing developmental delay, imbalance of amino acids and eventual death of the plants [102]. The use of herbicide-tolerant soybean yielded several benefits to farmers, including a decreased use of more toxic herbicides, and the ease of management. According to Cerdeira and Duke [103], glyphosate would have a much smaller residual effect in the soil if compared with the replaced active ingredients used for broad and narrow leaf plant control, therefore representing an option with less impact on soil, water and subsequent cultures in the area.

Despite its advantages, the use of glyphosate has some impacts on crops and also on the soil biota. It is reported that the application of glyphosate produces a shift in the microbial community [104,105]. In addition, glyphosate may be toxic to certain organisms or, in others, be used as a source of energy and nutrients [106]. de Almeida Lopes, et al. [31] found that the bacterial endophytic population associated to conventional and glyphosate-tolerant soybean in Brazil is significantly different both in term of composition and density, suggesting the importance of weed control strategy in determining the crop-bacterial endophyte association. Dallmann, et al. [107] found that the use of herbicides in herbicide-tolerant soybeans reduced the fungal flora of the soil and did not significantly alter the bacterial count. However, Arantes, et al. [108] found that glyphosate reduced microbial activity in two types of soil (Psament and Oxisol), whether or not the use of liming. A similar result was reported by Andrea, et al. [109], which found that the biomineralization of glyphosate decreased with increasing applications, suggesting an effect of the herbicide on soil microbial activity. Studying fungi and soil bacteria, Busse, et al. [106] found that there was toxic effect on population growth and metabolic diversity for continuous use (9-13 years) of glyphosate in the same area.

Regarding the endophytic populations associated to crops, herbicides has a significant influence on their composition [110] and contribute to select those endophytes that are able to withstand the adopted weed control strategy or those able to metabolize the used herbicide. This observation represents a pivotal starting point for the isolation of endophytes that can degrade herbicides with the aim of using them to inoculate crops and confer herbicide tolerance as an alternative approach to genetic modification [111].

The promotion of plant growth through protection from phytopathogens is known as biocontrol. Several mechanisms may be involved [112], including the production of antibiotics or

secondary metabolites, the acquisition of iron by siderophores, and the secretion of enzymes such as chitinases and proteases. Crops are exposed to many microbial diseases whose agents come in contact with the endophytic population during the infection of inner parts of plant tissues. The development of competitive factors in endophytes is therefore highly probable in such conditions as a necessary step to be competitive and survive. Therefore, the isolation and characterization of these bacteria could lead to the identification of suitable candidates to be used for the development of biological control agents [113]. In the work of de Almeida Lopes, et al. [32] bacteria of genera *Bacillus* and *Burkholderia* isolated from soybean in Brazil were found to have antimicrobial properties against several soybean bacterial and fungal pathogens, and the molecules responsible for this activity produced by these bacteria were secondary metabolites, peptides, lipopeptides and bacteriocins. Among the bacterial endophytes reported to have antimicrobial activity, the endophyte *Enterobacter* sp. strain 638 produces antibiotic substances, including 2-phenylethanol and 4-hydroxybenzoate [9]. Endophytic *Streptomyces* spp. are known producers of antimicrobial compounds such as kakadumycins [114], coronamycin [115] and multicyclic indolosesquiterpenes [116]. Recently, *Streptomyces* sp. was found able to inhibit the growth of *Burkholderia glumae*, a bacterial rice pathogen, and other bacterial and fungal pathogens. Interestingly, this strain produced three small antibiotics, streptotricins D, E and F, in addition to colonization of rice plants and growth promotion [54]. These and other similar reports suggest further investigation to assess the ability of antimicrobial compounds-producing bacterial endophytes to efficiently colonize the plant from where they have been isolated and the ability to control the pathogens sensitive to their antimicrobial molecules from inside the plant.

As discussed previously, many bacterial endophytes have been found to have PGP properties and are potential candidates for the development of biofertilizers. However, they are sensitive to the agronomic management, including soil and plant fertilization. Therefore, the study of compatibility with mineral and organic fertilizers is advisable to optimize their use in combination and reduce the use of chemical fertilizers and the loss of fertilization potential of both components [117-119].

## Conclusion and future perspectives

In conclusion, the current knowledge about the actual role of endophytes associated with crops, their beneficial effects and the influence of the environment as well as the agronomic management on the bacterial population suggest additional effort to explore the potential of endophytes as new biological tools for a range of applications in agriculture mainly as biofertilizers and biocontrol agents. More investigations on the effects of agronomical practices on endophytes and the differences between the populations associated to transgenic and conventional crops

could also help in this direction. Biotechnology can be applied to further improve strains that have prized qualities. All these aspects should be further investigated and put together to the development of commercially viable PGPBE inoculant strains to be utilized in agriculture, able not to lose PGP qualities under the environment variation, as sustainable strategies to promote growth and to protect crops.

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