Beneficial Microorganisms: Current Challenge to Increase Crop Performance

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Abstract

The major goal of agricultural microbiology is a comprehensive analysis of beneficial microorganisms. Fundamental knowledge of the ecology and evolution of interactions could enable the development of microbe-based sustainable agriculture. Plant growth-promoting bacteria (PGPB) have gained worldwide importance and acceptance for their agricultural benefits. This is due to the emerging demand to reduce dependence on synthetic chemical products within a holistic vision of developing and focalizing environmental protection. Beneficial microorganisms also help to solubilize mineral phosphates and other nutrients, enhance resistance to stress, stabilize soil aggregates, improve soil structure and organic matter content, and inhibit phytopathogens. Several efforts have been made in research to clarify definitions as well as develop commercial inoculants using these organisms, with a special emphasis on formulations that interact synergistically and are currently being devised. In addition, numerous recent studies indicate increased crop performance with the use of these commercial inoculants. In this chapter, the progress to date in the use of beneficial microbes for agricultural applications is summarized and discussed.

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3.1 Introduction

Agricultural practices are the major factors underlying many environmental problems, and thus, contemporary agriculture faces enormous challenges (Hazell and Wood 2008). All around the world, agriculture is frequently produced using intensive production methods and indiscriminate agrochemicals that increase production costs and contribute to increased environmental pollution that significantly affects human and animal health (Javaid 2010). Therefore, alternatives to chemical-based conventional agriculture that increase a farmer's profit and that are environmentally safe are required (Singh et al. 2011).

Considering that agricultural productivity is strongly related to microbial activity in the soil system (Chaparro et al. 2014), the use of beneficial microorganisms makes a positive contribution to environmentally safe agriculture (Kloepper et al. 1980; Chanway 1998; Vessey 2003; Gray and Smith 2005; Figueiredo et al. 2012). In addition, the presence of these microorganisms in the soil is a known characteristic of pathogen-suppressive soils (Fliessbach et al. 2009). In biological studies, crustacean chitosan is frequently used to increase plant resistance against pathogens (Goy et al. 2009). On the other hand, fungal chitosan has antimicrobial properties and increases the availability of nutrients (Franco et al. 2011; Berger et al. 2013).

The modes of action of the microorganisms and their various benefits to plants, as described in the literature, range from the simple occupation of biological empty spaces to ecological relationships such as antibiosis, competition, predation, and symbiosis, among others (Kilian et al. 2000; Kloepper et al. 2004; Avis et al. 2008). In addition, activities related to the production of hormones and enzymes, that are important for plants, occur in the soil or phylloplane (Araujo et al. 2005; Raaijmakers et al. 2009). The use of selected microorganisms may represent an important biotechnological approach to decrease the deleterious effects of stress in crops (Egamberdieva et al. 2013; Nadeem et al. 2014).

Studies have also shown that the growth-promoting ability of some bacteria may be highly specific to a certain plant species, cultivar, or genotype (Bashan 1998; Figueiredo et al. 2010). Many of these microorganisms synthesize extracellular polysaccharides or exopolysaccharides (EPS) with a commercially significant applicability (Nwodo et al. 2012). The formulation step is a crucial aspect of producing microbial inoculants, and it determines the success of a biological agent (Brahmaprakash and Sahu 2012).

In recent years, the strong potential of biopolymers to be used as inoculants has been studied (Rodrigues 2012; Bashan et al. 2014). Another possibility for the development of new inoculants or biofertilizers is the use of biofilm (Seneviratne et al. 2009). Furthermore, the role of these compounds in stress adaptation may be an important criterion for the selection of inoculant strains to increase plant productivity through biological nitrogen fixation (BNF) under different soil and climatic conditions (Bomfeti et al. 2011; Sharmila et al. 2014).

3.2 Combinations of Beneficial Microorganisms: Keys for Effective Use in Agriculture

The rhizosphere microbiome consists of three types of microbe groups (Figueiredo et al. 2012; Chaparro et al. 2014): microorganisms that are beneficial to plant growth, plant pathogenic microorganisms, and human pathogenic bacteria. Beneficial microorganisms can fix atmospheric nitrogen, decompose organic wastes and residues, detoxify pesticides, suppress plant diseases and soilborne pathogens, improve nutrient cycling, and produce bioactive compounds that stimulate plant growth (Singh et al. 2011). Thus, the use of beneficial microorganisms present in the rhizosphere is crucial for the development and health of plants and is advantageous to soil fertility (Chaparro et al. 2014). The group of microbes that is beneficial to plants consists of a wide variety of microorganisms, such as nitrogen-fixing bacteria, plant growth-promoting bacteria (PGPB), and endo- and ectomycorrhizal fungi (Mendes et al. 2013).

Nitrogen-fixing bacteria constitute a microorganism group that carries out BNF, an important process in ecosystems that supplies nitrogen to plants in a utilizable form (Peix et al. 2015). These microorganisms are also called diazotrophic bacteria and may act as free-living bacteria or form a symbiosis with legumes and establish root nodules where BNF occurs (Bonfante and Anca 2009; Chaparro et al. 2014; Peix et al. 2015). Rhizobia, a group of associative diazotrophic bacteria, are often used in combination with other microbes, either bacteria or fungi, to improve the growth and yield of many plant species (Figueiredo et al. 2008, 2010; Hungria et al. 2013; Rodrigues et al. 2013a).

Various bacteria distributed across different genera, such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Serratia*, and *Variovorax*, are collectively designated as PGPB due to their noticeable positive effects on plant metabolism (Nadeem et al. 2014). PGPB are commonly used as plant biostimulants (Bhattacharyya and Jha 2012) and increase proliferation and elongation of roots, facilitate nutrient acquisition, modulate plant hormone levels, and act as biocontrol bacteria (Ali et al. 2014; Bashan et al. 2014). Considering these positive effects, PGPB are commonly used to improve crop yields and in combination with rhizobia may constitute an alternative to increase crop plant performance under normal and stress conditions (Figueiredo et al. 2010; Bhattacharyya and Jha 2012; Rodrigues et al. 2013b; Bashan et al. 2014).

Under stress situations, plants trigger defense mechanisms that involve gene expression that is linked with hormonal balance between roots and shoots (Munns and Tester 2008). In this sense, PGPB may provide beneficial effects for plant drought tolerance due to modulation stimulated by hormones, mainly abscisic acid, ethylene, and cytokinins (Bhattacharyya and Jha 2012; Ali et al. 2014). The inoculation of wheat with the PGPB *Azospirillum lipoferum* alleviated plant drought stress and increased the growth and yield (Arzanesh et al. 2011). Drought situations may trigger oxidative stress in plants and induce a decline in nitrogen fixation rates (Arzanesh et al. 2011; Rodrigues et al. 2013b; Larrainzar et al. 2014). To cope with detrimental oxidative situations, a triple inoculation with rhizobia, *Paenibacillus graminis* and *P. durus*, was used in cowpea, which improved symbiotic performance and BNF as well as decreased the deleterious effects of the oxidative stress (Rodrigues et al. 2013a).

The use of selected PGPB may represent an important biotechnological approach to decrease the deleterious effects of salt stress in crops (Egamberdieva et al. 2013; Nadeem et al. 2014). Approximately 45 million hectares of irrigated land worldwide are affected by salinity, which represents a limiting factor to plant growth and reduces crop productivity (Munns and Tester 2008). Under salt stress, the PGPB *Bacillus amyloliquefaciens* promotes the growth of rice plants (Nautiyal et al. 2013). Moreover, positive effects on plant biomass were also observed in maize inoculated with the PGPB *Azotobacter chroococcum* when cultivated under saline stress (Rojas-Tapias et al. 2012). Additionally, the combined action of rhizobia and PGPB *Pseudomonas* spp. alleviated deleterious symptoms of salt stress in *Galega officinalis* and improved root and shoot growth (Egamberdieva et al. 2013).

Among PGPB, *Pseudomonas*, *Bacillus*, and *Azospirillum* are the most studied genera, and of these, *Azospirillum* is considered the most important due to the strong improvement in plant growth (Bashan et al. 2014). *Azospirillum* are free-living PGPB that are able to colonize root surfaces and often possess endophytic ability (Okon and Labandera-Gonzalez 1994). Moreover, these PGPB are capable of positively affecting the yield of many plants growing in different soils and regions, and co-inoculation with other microorganisms improves their beneficial effects (Mendes et al. 2013; Bashan et al. 2014). The co-inoculation of *Azospirillum* with phosphate-solubilizing bacteria allows an improvement in absorption of mineral nutrients

(Singh et al. 2011). Additionally, co-inoculation of *Azospirillum* and rhizobia induces positive responses in plant growth (Star et al. 2012; Hungria et al. 2013).

The rhizobia-PGPB symbiosis is often utilized to improve plant nutrition in terms of macro- and micronutrients. The presence of the microorganisms in the soil can efficiently affect the solubility and therefore the availability of several nutrients (Mendes et al. 2013); this is termed biofertilization (Adesemoye and Kloepper 2009). The use of a biofertilizer containing beneficial microbes, i.e., a mix of *A. chroococcum*, *Bacillus megaterium*, and *Bacillus mucilaginosus*, promoted the growth of maize and ameliorated the soil properties (Wu et al. 2005). In common bean (*Phaseolus vulgaris*), dual and triple inoculations with *Rhizobium*, *Bacillus subtilis*, and *B. megaterium* significantly increased the uptake of macro- and micronutrients (Elkoca et al. 2010). The co-inoculation of rhizobia with phosphate-solubilizing microbes, such as *Aspergillus*, *Pseudomonas*, and *Trichoderma*, increased the nodulation and availability of phosphorus to plants (Yadav et al. 2013).

Trichoderma are filamentous fungi that are widely used in agriculture as biocontrol agents due to their antagonistic abilities against phytopathogenic fungi, mainly *Fusarium* sp. and *Rhizoctonia* sp. (Brotman et al. 2013). Inoculation with *Trichoderma* induces systemic resistance to diseases in many crop plants that results in beneficial effects on plant growth (Hermosa et al. 2012). In Brazil, ten products containing *Trichoderma* are registered and commercially available to control various types of crop diseases. *Trichoderma* strains are frequently applied singly to many plants and induce resistance to biotic and abiotic stresses (Brotman et al. 2013; Colla et al. 2014). Nevertheless, the combination of *Trichoderma* with other microbes potentiates their beneficial effects, as shown by Colla et al. (2014) who studied many vegetable crops that were simultaneously co-inoculated with *T. atroviride* and *Glomus intraradices*, an arbuscular mycorrhizal fungi (AMF).

The term "mycorrhiza" (pl. mycorrhizae) is used to indicate nonpathogenic symbiotic associations between soil fungi and plant roots (Bonfante and Genre 2008). Mycorrhizal symbiosis plays important ecological roles that include the ability to increase nutrient uptake and protect plants from numerous stress situations (Figueiredo et al. 2012). Mycorrhizae are divided into ecto- and endomycorrhizae, and in the latter, the hyphae penetrate the root cells and establish an intracellular symbiosis (Bonfante and Anca 2009). Among the endomycorrhizae, AMF have great importance in tropical ecosystems and are the fungi that are most frequently found in the roots of crop plants (Bonfante and Genre 2008). AMF are a group of soil-dwelling fungi that are obligatory symbionts belonging to the *Glomeromycota* phylum; these fungi have a substantial influence on plant productivity (Redecker et al. 2013).

The positive effects of AMF colonization are commonly attributed to an improvement in nutrient uptake, mainly phosphorus, and to an increased pathogen resistance and herbivore tolerance in plants (Bonfante and Genre 2008; Smith and Smith 2012). Other advantageous effects of AMF-plant interactions are the increase in plant tolerance to stressful environments, phytoremediation promotion, and improvement of soil stability (Meier et al. 2012; Smith and Smith 2012; Estrada et al. 2013). Some rhizobacteria favor the formation of mycorrhizae on roots and are commonly referred to as "mycorrhiza helper bacteria" (MHB) (Frey-Klett et al. 2007). These MHB increase the spore germination and mycelial growth of AMF and are involved in the establishment of AMF-plant symbiosis. The MHB group includes nitrogen-fixing and PGPB (Table 3.1).

MHB aid AMF establishment in roots and the associations between AMF and MHB induce various positive aspects of plant development, mainly increases in the tolerance against drought and salt stress in plants (Gamalero et al. 2009; Lingua et al. 2013). For example, the co-inoculation with a mixture of AMF from the genera *Glomus*, *Gigaspora*, and *Acaulospora* and the rhizobia *Sinorhizobium terangae* resulted in a positive osmotic adjustment and an improved tolerance of *Acacia saligna* to saline soil

Table 3.1 Examples of the mycorrhiza helper bacteria (MHB) associated with arbuscular mycorrhizal fungi (AMF) identified in different host plants (Frey-Klett et al. 2007; Soliman et al. 2012)

Genres and groups of the MHB		Species of the AMF	Host plant
Gram-negative *Proteobacteria*	*Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Bradyrhizobium*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Rhizobium*, and *Sinorhizobium*	*Endogone* sp., *Acaulospora* sp., *Gigaspora margarita*, *Glomus caledonium*, *G. clarum*, *G. deserticola*, *G. fasciculatum*, *G. fistulosum*, *G. intraradices*, and *G. mosseae*	*Medicago sativa*, *Morus alba*, *Carica papaya*, *Zea mays*, *Solanum tuberosum*, *Allium cepa*, *Anthyllis cytisoides*, *Hordeum vulgare*, *Triticum aestivum*, *Trifolium* sp., *Acacia saligna*, *Cucumis sativum*, *Pennisetum americanum*, *Ipomea batatas*, *Uniola paniculata*, and *Licopersicum esculetum*
Gram-positive *Firmicutes*	*Bacillus*, *Brevibacillus*, and *Paenibacillus*	*G. etunicatum*, *G. fasciculatum*, *G. mosseae*, and *G. intraradices*	*Sorghum bicolor*
Gram-positive actinomycetes	*Streptomyces*	*Glomus intraradices*	*Sorghum* sp.

(Soliman et al. 2012). In cowpea co-inoculated with rhizobia, AMF *Glomus etunicatum* and the PGPB *Paenibacillus brasiliensis*, it was observed that AMF positively affected the nitrogen acquisition by rhizobia and increased the symbiotic efficiency, which enhanced plant growth (Lima et al. 2011). For all the above mentioned reasons, the use of beneficial microbes must be increased and disseminated considering that this is a sustainable and environmentally friendly agricultural practice.

3.3 Prospective Biocontrol Agents of Plant Diseases

Beneficial microorganisms have been widely used in agriculture to control diseases and pests of different plant species, in addition to other benefits they provide to plants. Within this group, we can highlight the bacteria of the genera *Bacillus*, *Azospirillum*, *Pseudomonas*, and *Rhizobium* and fungi of the genera *Beauveria*, *Gliocladium*, *Metarhizium*, and *Trichoderma*, as well as actinomycetes of the genus *Streptomyces*.

Within the microorganism groups in the soil, rhizobacteria can be highlighted, which develop a close relationship with plants and in most cases occupy the space known as the rhizosphere (Raaijmakers et al. 2009). In this environment, which is rich in sugars, amino acids, flavonoids, proteins, and fatty acids (Badri et al. 2009), there is a higher fungal and bacterial presence because these microorganisms are attracted by these compounds. However, there occurs a greater prevalence of rhizobacteria, which in most cases are able to form a biofilm that protects the root surface against pathogens (Bogino et al. 2013).

The maintenance of these beneficial microorganisms is supported by the host plant; the microorganisms drain carbon-rich compounds (exudates) produced by the roots that are essential for microbial nutrition in the rhizosphere (Vandenkoornhuyse et al. 2007). However, exudates may also act as antimicrobial molecules, inhibiting the growth of some species. This indicates that the host plant can select or attract microorganisms for their rhizosphere colonization (Chaparro et al. 2012), which has led to the occurrence of plant species that respond more to the presence of growth-promoting microorganisms in the soil.

Some practices, such as tillage, organic fertilization, crop rotation, and residue management, can also increase or decrease microbial activity in the rhizosphere (Raaijmakers et al. 2009). Chemical factors, such as pH and organic matter nutrient availability, are considered important factors for maintaining microbial activity (Chaparro et al. 2012).

The presence of beneficial microorganisms in the soil is a known characteristic of pathogen-suppressive soils. These soils can maintain conditions favorable for diverse microfauna, preventing the prevalence of pathogens. It has also been found that soils rich in microbial biomass and diversity have been less responsive to microbial inoculant introduction, indicating that this introduction is more suitable for poor soils in this regard (Fliessbach et al. 2009).

Recent studies indicate that plants that interact with rhizobacteria are able to increase their photosynthetic capacity (Xie et al. 2009), soil salinity tolerance (Dimkpa et al. 2009), disease suppression, and iron absorption efficiency (Zhang et al. 2009). These results confirm the potential use of inoculants containing rhizobacteria in agriculture. Plants play a critical role in influencing the microbial community composition of the rhizosphere. Some species can attract larger amounts of microorganisms by releasing signaling substances in the rhizosphere. It has also been observed that depending on the plant growth stage, the signaling substances can influence the microbial community structure present in the rhizosphere (Chaparro et al. 2014).

The multiple beneficial effects related to rhizosphere microorganism activities are well explained by the diagram presented in Fig. 3.1. In this representation, it can be seen that microorganisms considered to possess biological control activity can play indirect roles in growth promotion, such as increasing the supply of macro- and micronutrients to plants. Moreover, it has been found that nitrogen-fixing species, such as *Rhizobium* sp., can play a role as biological control agents (Avis et al. 2008).

The main plant growth promotion forms are based on three important actions, i.e., phytostimulation, biofertilization, and pathogen control. Some microbial species exhibit good development of these three activities in the soil, indicating good potential for the market. Some of these species and the previously mentioned activities described in the literature are presented in Table 3.2.

In the case of *B. subtilis*, pathogen control is performed by different modes of action,

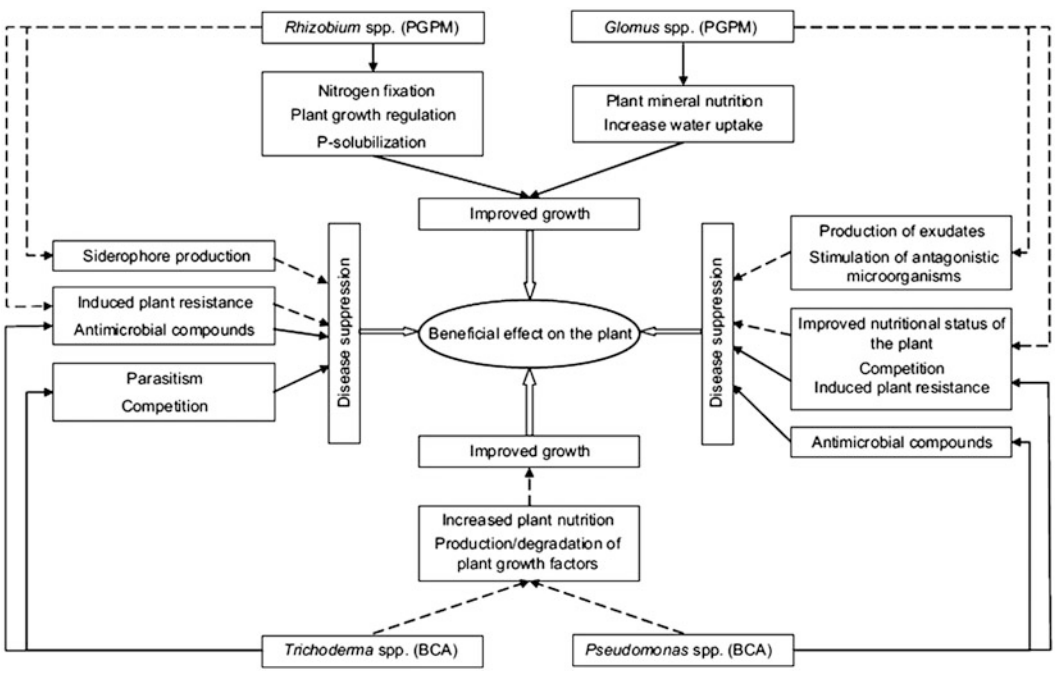

Fig. 3.1 Potential modes of action of plant growth-promoting and biological control agents (Avis et al. 2008)

Table 3.2 Simultaneous actions developed by different species of soil microorganisms within the beneficial activities for plants

Species	Biofertilization	Phytostimulation	Pathogen control
Azospirillum brasilense[a]	Nitrogen fixation	Auxins and gibberellins	Induced systemic resistance (ISR) and siderophores
B. subtilis[b]	Increase in phosphorus and nitrogen	Auxins	Antibiotics
Pseudomonas fluorescens[c]	Increase in phosphorus	Auxins	2,4-diacetyl-phloroglucinol
Streptomyces griseus[d]	Increase in phosphorus	Auxins	Chitinases
Trichoderma harzianum[e]	Increase in phosphorus and nitrogen	Auxins	Mycoparasitism and enzymes

[a]Dobbelare et al. (1999)
[b]Araujo et al. (2005) and Araujo (2008)
[c]Raaijmakers et al. (1997), Fernández et al. (2012), and Pallai et al. (2012)
[d]Hamdali et al. (2008)
[e]Contreras-Cornejo et al. (2009), Molla et al. (2012), and Vos et al. (2014)

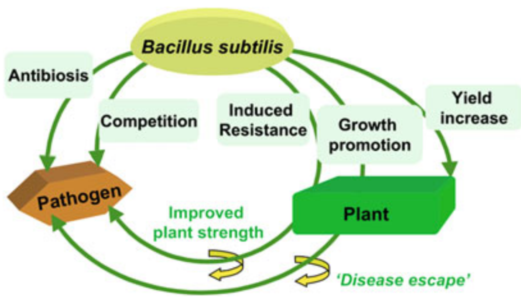

Fig. 3.2 Modes of action of *B. subtilis* and the interaction between *Bacillus*-plant pathogen (Kilian et al. 2000)

especially antibiosis, competition for ecological niches, and resistance induction in the host (Fig. 3.2). These main modes, in addition to some secondary modes, provide effective control of pathogens and reduce the possibility of the pathogens developing resistance. These secondary modes of action reinforce the immense potential that bioinoculants have in agriculture. The applications of different mixtures of rhizobacterial species to the soil can result in systemic resistance induction, which increases the efficiency against various pathogens (Ramamoorthy et al. 2001). Taking into account both the primary and secondary benefits related to the microorganisms mentioned above (Table 3.2), the addition of multiple microorganisms to agricultural production systems appears attractive (Avis et al. 2008). Many of the individual effects of co-inoculated microorganisms have been enhanced, resulting in synergistic effects in many cases (Kohler et al. 2007).

At present, the use of bacterial consortia in bioinoculant formulation has been highlighted with good prospects for the future, which may reflect multiple benefits in crop yield and the biological balance of the soil (Pindi 2012). This line of action of combining different microbial species with multiple functions into one product was recently used for the development of so-called biofilms (Triveni et al. 2012). In this case, there is the possibility of greater microorganism protection and survival with the use of the final product. The formulation of biofilms typically involves the combination of fungi and bacteria in a product developed in the laboratory. However, the analysis of this new technology is limited in recent studies that have examined the use and development of new inoculants.

Currently, more than 78 brands of products with a microbiological origin are being used in agriculture, covering more than 5 million ha. Some of these products are in the registration process or are marketed only to the organic agricultural market. It is also noted that most of these products are related to pest control. Worldwide, several commercial products developed using rhizobacteria are on the market and are mainly indicated for plant disease control (Table 3.3).

Table 3.3 Commercial products developed using different PGPR species

Species	Products	Intended crop
B. subtilis	Epic, Kodiak, Rhizo-Plus, Serenade, and Subtilex	Fruit, cereals, ornamentals, trees, and forage crops
P. fluorescens	BlightBan, Conquer, and Victus	Apple, cherry, peach, potato, strawberry, and tomato
T. harzianum	Custom and RootShield	Turf and ornamentals
A. brasilense	Azo Green	Forage crops
S. griseus	Mycostop	Ornamental and vegetable crops

The prospects for the future market are very promising due to a greater receptivity of organic products by farmers and also due to higher environmental and health restrictions on the registration and authorization of conventional chemicals used for disease control in plants. In this context, organic products are used more often in integrated control strategies.

3.4 Microbial Polysaccharides: Production and Application as a Vehicle for Inoculation

The term "microbial polysaccharides" includes polysaccharides produced by fungi and bacteria, which may be located inside the cell, on the cell wall or yet be released out of the cell (Donot et al. 2012). Polysaccharides are classified by the sugar composition in homopolysaccharides, which enclose a single type of monosaccharide and are usually neutral glucan, and heteropolysaccharides, which are composed of different sugar residues and usually display a regular backbone structure with a linear or branched repetitive unit (Delbarre-Ladrat et al. 2014). Heteropolysaccharides may also contain organic or inorganic substituents, such as phosphate, sulfate, acetate, and pyruvate, which confer polyanionic characteristics to these molecules (Schmid et al. 2011). Collectively, the sugar sequence in the molecule, the presence or absence of substituents, and how the chains intertwine influence the physicochemical features of polysaccharides (Rehm 2010).

Some bacteria and fungi secrete polysaccharides as an evolutionary adaptation to help them adhere to different surfaces or for adaptation to stressful environments (Sharmila et al. 2014). Polysaccharides protect against dehydration, serve as barriers to prevent viruses and antibodies from binding to specific sites on the cell wall, neutralize toxins, act as a carbon source, and also interact with plant cells in specific pathogenic or symbiotic relationships (Badel et al. 2011; Donot et al. 2012). In addition, microbial polysaccharides, especially those released to the external environment, have been reported to protect microbial cells against heavy metals or environmental stresses (Poli et al. 2011). For example, *Pseudomonas* strains survive under stress conditions due to polysaccharides released to the outside of the cell, which protect them from water stress and then regulate the diffusion of carbon sources in the microbial environment (Sandhya et al. 2009).

In addition to singular biological characteristics, polysaccharides exhibit chemical and physical properties, and these peculiar features represent advantages compared with plant polysaccharides for their use at large scales (Mahapatra and Banerjee 2013). Polysaccharides are generally nontoxic, biodegradable compounds (Rehm 2010) and are oftentimes named according to the biopolymers or gums. In contrast to traditional gum, microbial polysaccharide production is advantageous because it is more rapid and requires less space for manufacture (Badel et al. 2011). Among the polysaccharide types, the extracellular polysaccharides or EPS are the most interesting due the possibility of recovering these directly from the environment in which they are secreted, enabling higher productivity. Fungi and bacteria release EPS in extracellular media, and these EPS are useful to promote cell-cell recognition (Donot et al. 2012).

Table 3.4 Some extracellular polysaccharides (EPS) produced by fungi or bacteria with commercial and economic importance (Freitas et al. 2011; Delbarre-Ladrat et al. 2014)

EPS	Microorganism	Application
Homopolysaccharides		
Curdlan	*Agrobacterium*, *Rhizobium*, and *Alcaligenes*	Food additive, applied in pharmaceutical industries, bioremediation
Dextran	*Leuconostoc* and *Streptococcus*	Food industry, biomedical as plasma volume expander, biotechnological support for separation
Pullulan	*Aureobasidium pullulans*	Food, adhesive, and cosmetic additives; flocculant; thickener; viscosity stabilizer in preparation of nontoxic, biodegradable, edible plastic materials; health care
Scleroglucan	Fungi of the genus *Sclerotium*	Oil, ink, cosmetic, and pharmaceutical industries; animal feed; food additive
Heteropolysaccharides		
Alginate	*Pseudomonas aeruginosa* and *Azotobacter vinelandii*	Food hydrocolloid, wound care, drug encapsulating agent
Gellan	*Sphingomonas paucimobilis* and *Pseudomonas elodea*	Gelling in culture medium, food additive
Succinoglycan	*Alcaligenes*, *Agrobacterium*, and *Rhizobium*	Food and pharmaceutical industries, oil recovery
Xanthan	*Xanthomonas campestris*	Viscosifying and texturizing agent in various foods, used for oil recovery in petroleum industry, health care

Many microorganisms synthesize EPS with commercially significant applications (Nwodo et al. 2012). Biopolymers derived from natural resources, such as microbial EPS, have a competitive advantage due their sustainable production, biodegradability, and, often, biocompatibility (Rehm 2010). Due to their ability to alter the rheology of aqueous solutions and their potential use as a biomaterial, EPS possess great economic importance (Table 3.4) and wide applicability in various industry sectors with significant commercial value (Freitas et al. 2011; Finore et al. 2014). EPS show huge production in a short time as well as ease of isolation and purification in relation to intracellular and cell wall polysaccharides (Delbarre-Ladrat et al. 2014). They can function as thickeners, gelling, emulsifiers, stabilizers, lubricants, or as suspending agents and film formers (Freitas et al. 2011; Mahapatra and Banerjee 2013).

Although they exhibit a wide range of biological functions, the production of EPS by fungi remains poorly studied (Giavasis 2014). The EPS produced by fungi are frequently highly hygroscopic β-glucans (Mahapatra and Banerjee 2013; Sharmila et al. 2014) that possess numerous applications in the food and pharmaceutical industries. Basidiomycetes, filamentous fungi, and yeasts from different ecological niches are known as being capable of synthesizing EPS in different production systems (Delbarre-Ladrat et al. 2014). The production of fungal EPS depends on the fungal strain used and the physicochemical conditions applied inside the fermentative broth (Mahapatra and Banerjee 2013). Collectively, the oxygen levels, carbon and nitrogen sources, pH, and temperature influence the production and composition of fungal EPS (Badel et al. 2011; Mahapatra and Banerjee 2013; Zhang et al. 2013; Giavasis 2014).

Scleroglucan, schizophyllan, and pullulan are types of fungal EPS commonly produced at a large scale and that possess singular rheological properties. Scleroglucan is an extracellular glucan excreted by *Sclerotium glucanicum* or *S. rolfsii* that is used frequently in petroleum recovery and has great potential for use in the agricultural, food, and pharmaceutical industries (Ansari et al. 2012). Schizophyllan is an EPS secreted by the basidiomycete *Schizophyllum commune* that is used in many fields, such as the food industry and pharmaceuticals (Zhang et al. 2013). Pullulan is a water-soluble glucan gum commonly used as a food additive and is

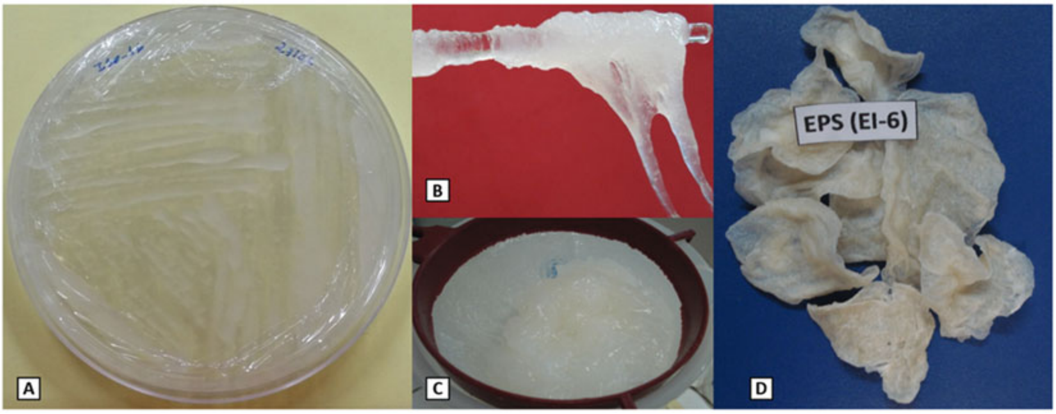

Fig. 3.3 Exopolysaccharides (EPS) produced by the EI-6 strain of *R. tropici*: (**a**) culture medium showing the EPS production, (**b**) EPS recovered from the fermentation broth, (**c**) EPS washed with 70 % alcohol, (**d**) dry EPS after 3 days in the stove at 30 °C (Photos courtesy of Artenisa Cerqueira Rodrigues)

produced by *Aureobasidium pullulans* (Singh et al. 2008). This compound has adhesive properties and the ability to form fibers and thin biodegradable films that are transparent and waterproof to oxygen because of their physical properties and distinctive linkage patterns (Cheng et al. 2011).

Researchers have extensively studied bacterial EPS due their structural variability that offers a large range of physicochemical and biological properties (Badel et al. 2011; Poli et al. 2011). Numerous bacterial EPS were reported in recent decades as biopolymers with industrial importance and significant commercial value, particularly xanthan, which occupies a prominent place in the market by presenting very different and unusual rheological properties (Freitas et al. 2011). Many soil bacteria from different habitats are recognized to produce complex and diverse EPS, such as *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Sinorhizobium* strains (Albareda et al. 2008; Staudt et al. 2012; Castellane et al. 2014). In a biological context, bacterial EPS are necessary to mediate *Rhizobium*-legume symbioses and are indispensable to promote the formation of efficient nodules on different hosts (Bomfeti et al. 2011; Castellane et al. 2014).

The viability of rhizobia in the field is maintained by their production of EPS (Staudt et al. 2012), and EPS can protect nitrogenase enzyme involved in the nitrogen-fixing process from high oxygen concentration in the nodules (Vu et al. 2009). Rhizobia are among the well-known EPS producers and can excrete large amounts of these polysaccharides in the rhizosphere (Albareda et al. 2008; Serrato et al. 2008; Bomfeti et al. 2011; Nwodo et al. 2012; Castellane et al. 2014) or culture medium (Fig. 3.3). Rhizobial inoculants have been used for many years to obtain greater yields in legumes and are often produced with peat, a material with a fossil origin (Rivera et al. 2014). Peat mines are situated in preserved environments where extraction is forbidden, and a lack of natural peat deposits exists in some countries (Albareda et al. 2008). The use of peat as a bacterial carrier, although established, has decreased, and alternative materials are being sought.

Alternative materials for bacterial inoculation can maintain the quality and efficiency of the inoculum and reduce production costs and negative environmental impacts compared with peat inoculants (Fernandes Júnior et al. 2009, 2012; Herrmann and Lesueur 2013; Rivera et al. 2014). For example, the combination of carboxymethylcellulose (CMC) and starch forms a polymeric carrier that adequately maintains rhizobial cell viability and has the same performance as peat inoculants (Fernandes Júnior et al. 2009, 2012). Rivera et al. (2014) tested eight polymers to use

Table 3.5 Number of nodules and amount of nitrogen accumulated in the shoots of cowpea plants inoculated with strain BR 3267 of *Bradyrhizobium* sp. using different inoculants (carriers): peat, a combination of the carboxymethylcellulose (CMC) and starch, and EPS produced by the EI-6 strain of *R. tropici*

Carrier	Number of nodules (nodule plant^{-1})	Nitrogen in shoot dry mater (mg N SDM^{-1})
Peat[a]	78.0	78.7
Combination of CMC and starch[a]	77.0	92.0
EPS from by *R. tropici* (EI-6)[b]	94.0	145.2

[a]Fernandes Júnior et al. (2009)
[b]Rodrigues (2012)

as vehicles for bacterial inoculation, and among them, sodium alginate and hydroxypropyl methylcellulose (HPMC) were able to provide a higher viability for strain G58 of *Rhizobium* sp. in comparison to the control (peat), without affecting the physiological process of nodulation in cowpea roots.

CMC and HPMC are competent bacterial inoculants; however, these compounds are semi-synthetic polymers (Fernandes Júnior et al. 2012; Rivera et al. 2014). Because of their biological nature, EPS synthesized by rhizobia are currently considered as excellent carriers for inoculation (Table 3.5). EPS can encapsulate living bacterial cells and gradually release these to the soil in large quantities, which confirms that EPS are degraded by soil microorganisms (Albareda et al. 2008). *Rhizobium leguminosarum*, *R. meliloti*, and *R. tropici* (SEMIA 4080 and EI-6 strains) are known to produce significant amounts of rhizobial EPS (Rodrigues 2012; Rodrigues et al. 2013a, b; Castellane et al. 2014). The composition of the EPS produced by *R. tropici* SEMIA 4080 shows relatively higher contents of glucose and galactose combined with small amounts of mannose, rhamnose, and glucuronic and galacturonic acids (Castellane et al. 2014).

The EI-6 strain of *R. tropici* was first isolated from root nodules of cowpea (*Vigna unguiculata*) cultivated in an arid environment (Figueiredo et al. 1999; Oliveira et al. 2012) and is known to produce significant amounts of EPS with good quality (Xavier 2009; Santos 2010; Oliveira 2011; Rodrigues 2012). The EPS produced by the EI-6 strain of *R. tropici* (already shown in Fig. 3.3.) are a polysaccharide of glucose and galactose without the presence of uronic acids (Rodrigues 2012). Additionally, the EPS possess acetyl and pyruvate residues and a higher amount of sodium and potassium ions (Xavier 2009; Rodrigues 2012). This feature resulted in the EPS produced by *R. tropici* EI-6 being classified as polyanionic heteropolysaccharides (Rodrigues et al. 2013a). Currently, after intensive studies on *R. tropici*, it is possible to confirm that their EPS have high potential for use in agriculture.

3.5 Biofertilizers: Agricultural Innovations

Soluble fertilizers are of great importance for plant growth and production, but their use by low-income farmers is limited due to the high price. Furthermore, the fertilizers contain high amounts of soluble nutrients and may easily undergo lixiviation in the deeper soil layers and promote plant damage through soil and water contamination (Van Straaten 2007). In modern and sustainable agriculture, the application of slowly soluble fertilizers is required to economically increase food production and soil fertility while minimizing environmental damage (Stamford et al. 2008). Biofertilizers produced from phosphate and potash rocks mixed with elemental sulfur and inoculated with sulfur-oxidative bacteria (*Acidithiobacillus*) may be an alternative for effective and economic fertilization and have considerable influence on the availability of elements derived from rocks through the metabolic effect of sulfuric acid (Stamford et al. 2008), as shown in the equation below:

$$S + H_2O + 1.5\ O_2 \xrightarrow{Acidithiobacillus} H_2SO_4$$

Nitrogen (N) is one of the most important nutrients for plant growth and yield, and due to its role in some chemical compounds such as proteins, nucleic acids, and many other components, it is necessary for all types of life in the world (Berger et al. 2013). However, phosphorus (P) and potassium (K) rock biofertilizers do not contain nitrogen for plants and microbial organisms in the soil, and the production of sulfuric acid may reduce soil pH, which damages and reduces plant growth. Therefore, rock biofertilizers can be mixed with organic matter (e.g., earthworm compound) that has a high pH (pH 7.9) and inoculated with selected free-living diazotrophic bacteria to promote N enrichment through the process of BNF (Lima et al. 2010).

In biological studies, crustacean chitosan is frequently used to increase plant resistance against pathogens. The chitosan displays better chelating properties compared with other polymers and can release nutrients into the environment (Boonlertnirun et al. 2008; Goy et al. 2009). The use of chitosan from fungal biomass has great advantages compared with crustacean chitosan, such as the independence of seasonal factors and the simultaneous extraction of chitin and chitosan (Franco et al. 2004). Mixed biofertilizers (NPKB) provide nutrients for plants, especially when inoculated with fungal chitosan such as *Cunninghamella elegans,* which also increases inorganic phosphate (Franco et al. 2004). Furthermore, the bioprotector (NPKP) has antimicrobial properties, promotes plant protection against pathogens, and increases the availability of nutrients (Berger et al. 2013; Franco et al. 2011).

3.5.1 Production of Biofertilizer (NPKB) and Bioprotector (NPKP)

The biofertilizer (NPKB) was produced from phosphate and potassium rock biofertilizers (PKB) mixed with organic matter (OM) in a 1:3 ratio and incubated for 30 days. The PK rock biofertilizers were produced at the Horticultural Experimental Station of the Federal Agricultural University of Pernambuco (UFRPE). Two furrows (10 m long, 1.0 m wide, 0.5 m deep) were used following the procedure described by Stamford et al. (2007).

The sulfur-oxidative bacteria were grown in Erlenmeyer flasks that contained 1000 mL of culture-specific medium (El Tarabily et al. 2006) and sterilized for 30 min at 120 °C. The Erlenmeyer flasks were shaken (150 rpm) for 5 days at 30 °C. The materials (phosphate and potash rocks plus elemental sulfur) were incubated for 60 days, and the humidity was maintained at a level close to field holding capacity. To avoid excessive humidity due to rain and to increase the efficiency of the sulfur-oxidative bacteria, the furrows were covered with black plastic (Fig. 3.4).

The biofertilizer (NPKB) was processed by mixing PK rock biofertilizers with organic biofertilizer (earthworm compound) enriched in N by inoculation with selected free-living diazotrophic bacteria (NFB 10001), as reported by Lima et al. (2010). The bioprotector (NPKP) represents the biofertilizer (NPKB) inoculated with *C. elegans* (UCP 542), which are fungi that contain chitosan in their cellular wall (Franco et al. 2004). The *C. elegans* fungus was purified in potato dextrose agar medium and grown for 10 days at 28 °C. A monosporic culture of *C. elegans* was obtained by growing the *Mucorales* fungus in potato dextrose medium using Erlenmeyer flasks (containing 1000 mL) maintained under shaking conditions (180 rpm, 96 h, 28 °C). The culture diluted in distilled water (20 L^{-1}) was applied using manual irrigation. The chemical differences between the P and K biofertilizers and the biofertilizer (NPKB) obtained following the procedure of Stamford et al. (2007) are presented in (Table 3.6).

The results of several experiments carried out in Brazilian soils cropped with different economic cultures such as cowpea, yam bean, grapes, melon, sugarcane, green pepper, and tomato showed that the biofertilizer (NPKB) and bioprotector (NPKP) increased crop performance and soil nutrients compared with the mineral soluble fertilizer. The NPKB and NPKP may be alternatives to replace conventional NPK soluble fertilizer and pesticides.

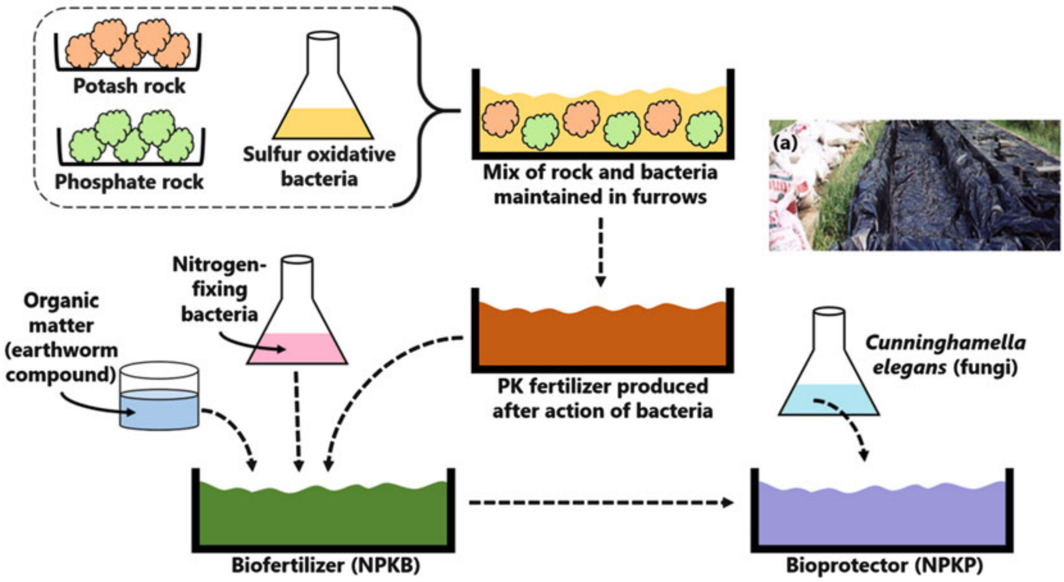

Fig. 3.4 Simplified schematic of the production of the biofertilizer (NPKB) and bioprotector (NPKP). The furrows covered with *black* plastic under field conditions are shown in (**a**)

Table 3.6 Chemical analyses of the P and K biofertilizers produced with sulfur-oxidative bacteria and of the biofertilizer (NPKB) after inoculation with selected free-living bacteria and mixed with organic matter as described in the text

Biofertilizer type	pH	Phosphorus (P)	Potassium (K)	Nitrogen (N)
P biofertilizer	3.8	60.0 g kg^{-1}	–	–
K biofertilizer	3.3	–	10.0 g kg^{-1}	–
PK rock after bacterial inoculation (NPKB)	6.4	21.0 g kg^{-1}	19.0 g kg^{-1}	20.0 g kg^{-1}

3.6 Conclusion

Beneficial microorganisms can be a potential tool for sustainable agriculture as well as a trend for the future. The beneficial effects include biological control of diseases, promotion of plant growth, increases in crop yield, and quality improvement. Knowledge of the complex environment of the rhizosphere, the mechanisms of action, and the practical aspects of inoculant formulation using these microorganisms is important in the search for new products to increase crop performance. New biotechnological methods for crop protection are based on the use of beneficial microorganisms applied as biofertilizers and bioprotectors. This approach represents an important tool for plant disease control and can lead to a substantial reduction of synthetic chemical products, which are important source of environmental pollution. Future studies are needed to identify management conditions that can contribute to the optimization of several mechanisms of the plant-microorganism interrelationship in real-life agricultural systems.

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