Chapter 12
Biotechnology of Beneficial Bacteria and Fungi Useful in Agriculture

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12.1 Introduction

The majority of plant species in terrestrial ecosystems establish more or less close relationships with rhizospheric microorganisms that somehow make it easier for them to live in normal or stressful environments. The numerous microorganisms that inhabit the rhizosphere include symbiotic nitrogen-fixing bacteria, mycorrhizae and plant-growth-promoting rhizobacteria (Prasad et al. 2015). However, the microorganisms natural role have been marginalized due to modifications induced by tillage and the excessive use of inorganic fertilizers, herbicides and pesticides. Current methods of crop production have created a series of environmental and human health problems. Nowadays, the increase in the appearance of emerging, pre-emergent and endemic pathogens and weeds challenges our ability to protect the growth and health of crops (Miller et al. 2009). That is why, among other

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reasons, there is a growing demand for more ecological strategies in agriculture. Plant biotechnology has contributed to the development of new crop varieties tolerant or resistant to diseases, drought and salinity, and that are of greater nutritional value (Garg and Chandel 2010).

For about 150 years, it has been shown that bacteria and fungi have an intimate relationship with plants; some are pathogenic, others are neutral, while many of them are beneficial. The rhizosphere of plants is highly colonized by microorganisms; of all of them, between 1 and 35% of the arable crops, show antagonism against pathogens, while two-thirds promote plant growth (Singh et al. 2011). The latter can provide both macro- and micronutrients, release phosphorus from organic compounds, modify the pH of the soil, especially that surrounding the root, thereby increasing the availability of phosphorus and other elements (Berg 2009).

These days sustainable agriculture has gained more attention, because it guarantees productivity of plants and animals using their natural adaptive potentials, with a minimal disturbance to the environment (Noble and Ruaysoongnern 2010). To accomplish this goal, it is necessary to reduce the use of harmful agrochemicals (mineral fertilizers, pesticides) and to use more environment-friendly preparations of symbiotic microorganisms, which could improve the nutrition of crops and cattle, as well as their protection from biotic (pathogens, pests) and abiotic (salinity, drought) stresses (Yang et al. 2009). Consequently, agricultural microbiology is a great research field to transfer and apply knowledge to the agricultural biotechnologies (Mohammed et al. 2008).

12.2 Beneficial Fungi in Agriculture

Biotechnology is in use for more than thousand years in the production of beer, bread, wine, through the fermentation of sugar and starch. In the twentieth and twenty-first centuries, biotechnology has evolved and is being used in the synthesis of many useful molecules and has become a very productive industry (Show et al. 2015); for example, the estimated market volume for plant-degrading enzymes from filamentous fungi in 2016 was €4.7 billion, and it is expected to reach up to €10 billion within the next decade (Meyer et al. 2016).

Several microorganisms are found in agricultural soils, and they can have different applications which tend to improve plant development, such as biofertilizers and biopesticides (Prasad et al. 2020). These microorganisms that live in the soil can help plants in nutrients uptake and a symbiotic relationship is established where plants provide their waste by-products for the microbes as food and microbes help the plant to “take up” essential energy sources (Mosttafiz et al. 2012).

Fungi are eukaryotic organism that in agriculture behave as pathogens of many crops *(Magnaporthe oryzae*, *Botrytis cinerea*, *Puccinia* spp, *Fusarium graminearum*, *Fusarium oxysporum*, *Blumeria graminis*, *Colletotrichum* spp, *Ustilago maydis*, and some others) (Dean et al. 2012) and entomopathogens [*Verticillium lecanii*, *Beauveria bassiana, Metarhizium anisopliae* (Li and Sheng 2007), *Paecilomyces fumosoroseus* (Chan-Cupul et al. 2010), *Trichoderma* spp (Zeilinger and Omann 2007)]. Insect populations are regulated mostly by entomopathogenic fungi and the first study was about the silkworm industry (Steinhaus 1975). According to Steinhaus (1975), Bassi in 1835, demonstrated the germ theory using silkworms and muscardine fungus, which was later named *Beauveria bassiana* in his honour.

12.2.1 Mycoinsecticides

The increasing soil and environmental contamination, and the use of chemical pesticides, has increased pest resistance; the need of controlling pests efficiently with non-harming techniques has led to the improvement of friendly insect control methods, such as the use of entomopathogenic fungi that are biological control with a wide host range. These fungi are distributed in a group of over 90 reported genera with approximately 750 species from different insects, and they do not inflict any damage to the environment (Rai et al. 2014).

Fermentation is the process commonly used to produce fungi massively, spores are stored and packed for further field application. The fungi spores contain enzymes that break down the outer surface of the insects' bodies inducing death after they grow; this allow that fugi become into a useful strategy for long-term insect control. These bioinsecticides act in several ways at the same time, which makes the insects almost impossible develop resistance (Zarafi and Dauda 2019).

Bioinsecticides do not persist long in the environment and have shorter shelf-lives; they are effective in small quantities, safer to humans and animals compared to synthetic insecticides; they are very specific, often affecting only a single species of insect and have complicated modes of action; they are slow in action and the timing of their application is relatively critical. Use of fungi as insecticides has been utilized effectively to control devastating insect pests (Zarafi and Dauda 2019). Some examples of fungi controlling insects are as follows:

- The pathogenicity and virulence of fungi ranging from *Metarhizium anisopliae* to *Blissus antillus* (Hemiptera: Lygaeidae) eggs under field conditions were determined and verified that those formulated in mineral oil and in Tween 80 generated 63.5% and 27.1% of mortality, respectively (Samuels et al. 2002).
- The effectiveness of three entomopathogenic fungi (*Beauveria bassiana*, *M. anisopliae* and *Paecilomyces fumosoroseus*) for the control of pests in vegetable crops was evaluated. The fungi were emulsified in diatomaceous earth in proportion 1:10 and was applied in a concentration of 1.2×10^{12} spores ha^{-1} generating mortality higher than 80% after 72 hours of application (García-Gutiérrez and González-Maldonado 2010).
- The effectiveness of *B. bassiana* production in liquid medium for the control of the coffee berry borer (*Hypothenemus hampei*) was evaluated, finding that the culture medium consisting of sugar, yeast extract and peptone is where the best

growth of the fungus occurs on the fourth day, without being affected by the initial pH, nor the temperature of 28 °C; also, it generates mortality of 86.7% (Mata and Barquero 2010).

- Fifteen strains of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* were evaluated on adult one-day-old fruit fly *Anastrepha obliqua* and no significant differences were found in mortality. Mortality of 34–48% during the first 120 hours of evaluation was obtained by applying *B. bassiana* and *M. anisopliae* in a targeted manner to young adults under the canopy of trees (Osorio-Fajardo and Canal 2011).
- Autochthonous isolates of *Beauveria* spp. controlled the white worm (*Premnotrypes vorax* Hustache) in a 77%; this insect causes considerable losses in the cultivation of potatoes, which can reach up to 100% depending on the level of infestation and crop management (Villamil et al. 2016).
- *Beauveria bassiana* and *Metarhizium anisopliae* were used to control the Red Palm Weevils (*Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), a major/main palm pest in the Mediterranean Basin (Yasin et al. 2017).
- Another entomopathogenic fungi that is a dimorphic hyphomycete that can cause epizootic death in various insects is *Nomuraea rileyi*. Several insect species belonging to Lepidoptera including *Spodoptera litura* and some belonging to Coleoptera are susceptible to *N. rileyi* (Ignoffo 1981). Also, several insects are hosts of *N. rileyi* such as *Trichoplusiani*, *Heliothis zea*, *Plathypena scabra*, *Bombyx mori*, *Pseudoplusia includes* and *Anticarsia gemmatalis*.

These days it is necessary to understand entomopathogenic fungi ecology outside of the insect host, specifically fungi strategies and their role in the ecosystem. Some discoveries suggesting that the way to control insect with entomopathogenic fungi must be reviewed. As an example, *M. anisopliae* strain compete for the rhizosphere and this depends on the plant community and not on the insect host presence (Hu and St. Leger 2002), whereas *B. bassiana* strains exist as endophytes in several crops and have the potential for insect and plant disease suppression (Vega 2008).

12.2.2 Mycoherbicides

Weeds are unwanted plants when they grow around crops. The intensive uses of herbicides to their elimination and the resistance that weeds develop against these products has created the necessity to look for new and friendly strategies. The application of fungi to control weeds opens a new field to get this goal because the use of microorganisms is friendly with the environment, they are more specific to the target and less expensive that traditional herbicides. The fungi genera that have been used effectively are *Colletotrichum*, *Phoma* and *Sclerotinia* (Harding and Raizada 2015).

Bioherbicides as definition are products made of phytopathogenic microorganisms or microbial phytotoxins useful for weed control, and they are used in similar way to conventional herbicides (Boyetchko et al. 2002; Boyetchko and Peng 2004). The active ingredient in a bioherbicide is, however, a living microorganism that usually is a fungus, so the term mycoherbicide is frequently used in these cases (Auld and McRae 1997).

The majority of the weeds control in North America has been based on fungi formulations, but just a few of these products were successful in the long term. Here is a list of several examples: a formulation called BioMal that was made with *Colletotrichum gloeosporioides* f.sp. *malvae*, introduced for the control of round leaf mallow (*Malva pusilla* L.) (Mortensen 1988; PMRA 2006); another formulation, Sarritor, includes *Sclerotinia minor* for the control of dandelion (*Taraxacum officinale* (L.) Weber ex F.H. Wigg., Prim. FL. Holsat), white clover (*Trifolium repens* L.) and broadleaf plantain (*Plantago major* L.) in turf (PMRA 2010).

In Brazil, fungi were selected for production of secondary metabolites with herbicidal activity using biological resources of the Brazilian Pampa biome; for this purpose, phytopathogenic fungi were isolated from infected tissues of weeds and the phytotoxicity of fungal metabolites was evaluated using a biological test with *Cucumis sativus* L. Thirty-nine fungi were isolated, and 28 presented some phytotoxic symptoms against the target plant. The best strain was identified through molecular studies. Fungus VP51 belonging to the genus *Diaporthe* showed the most effective herbicidal activity (Castro de Souza et al. 2017).

Hoagland et al. (2007) studied a strain of *Myrothecium verrucaria*, isolated from sicklepod (*Senna obtusifolia* L.), a plant that has bioherbicidal activity against kudzu (*Pueraria lobata* (Willd.) Ohwil) and some other weeds. Those authors found that *M. verrucaria* caused great reductions of kudzu plant biomass production at 30 °C, compared to 20 °C or 40 °C, under experimental conditions.

In a study carried out in West Africa, *Fusarium oxysporum* (PSM 197) controlled 91.3% of *Striga asiatica* (L.) Kuntze (a hemiparasitic plant in the broomrape family), 81.8% of *S. gesneroides* and 94.3% of *S. hermonthica* (Marley et al. 2005). An isolate from Italy of *M. verrucaria* produced trichothecenes (a very large family of chemically related mycotoxins produced by various species of fungi) that could inhibit seed germination of the parasitic plant *Orobanche ramosa* Delile ex Decne. 1824 (Andolfi et al. 2005). Another study with *M. verrucaria* in the south-eastern United States showed that this fungus is very virulent against *Portulaca oleracea* L., *Sesuvium portulacastrum* L., *Euphorbia maculata* L. and *Euphorbia prostrata* Aiton in cultivated tomato (*Lycopersicon esculentum* L.) (Boyette et al. 2007).

Microsphaeropsis amaranthi and a mixture of *Microsphaeropsis amaranthi* and *Phomopsis amaranthicola* were used to control eight *Amaranthus* species, and as a result, severe disease ratings were showed 15 days after treatment (DAT), and mortality ranged from 74% to 100% (Ortiz-Ribbing and Williams 2006).

Microsphaeropsis amaranthi and *P. amaranthicola* have been used as bioherbicide for the control of water hemp [*Amaranthus rudis* (Moq.) J. D. Sauer] and pigweeds (*Amaranthus* spp.); these are weeds that affect many crops and have become resistant to several herbicides. Results showed significant reductions in weed biomass when one or both of the fungal organisms were used; nevertheless, it is necessary to control leaf surface moisture and air temperatures following application because inconsistencies in field results may occur (Ortiz-Ribbing et al. 2011).

According to Hetherington et al. (2002), bioherbicides can improve seedlings growth through the infection and delay of the growth of weed.

12.2.3 *Fungal Symbiosis*

Since plants first colonized terrestrial ecosystems developed several strategies to survive biotic and/or abiotic stresses; among these strategies are symbiosis that they can establish through root systems with microorganisms (Gianinazzi-Pearson 1984; Varma et al. 2020). In this relationship, both plant and microorganisms get something necessary for their growth and development (Fig. 12.1).

The majority of crops are capable of forming symbiosis associations with soil fungi; to facilitate or improve such association, the crops or the fungi can be genetically modified, so it is necessary to identify the genes involved in this relationship (Behie and Bidochka 2013). One study made in *Medicago truncatula* Gaertn. showed that 29 genes were upregulated during mycorrhizal association, 11 of which were not upregulated in plants during bacterial colonization, suggesting that only certain genes play a role in plant-fungal interactions (Weidmann et al. 2004). In this sense, some genes have been identified such as MtScp1, a gene that encodes a carboxypeptidase-related transmission of fungal specific signals; mad229 and myc control the regulation of molecules secreted from the fungus prior to association that stimulate root development and expression of plant genes required for intercellular fungal interaction (Bucher et al. 2009).

Mycorrhizae are fungi that establish a symbiotic relationship with the roots of terrestrial plants and seven associations can be identified: (1) Ectoendomycorrhizae:

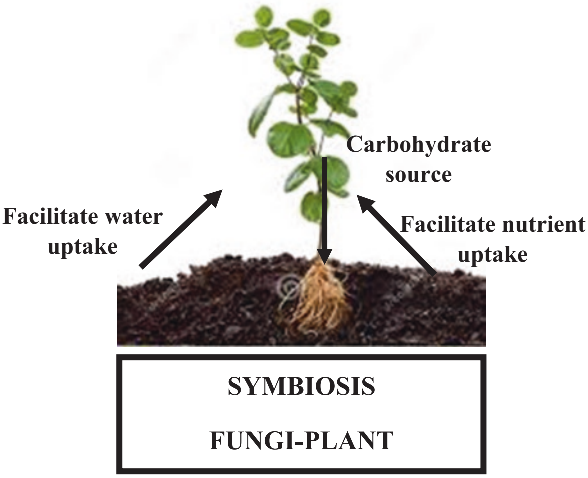

Fig. 12.1 Symbiotic relationship between plant and soil fungi

association of Ascomycetes and the genera *Pinus* and *Larix* of Coniferae (Yu et al. 2001); (2) Ericoid: they are unique mycorrhizae to the families of order Ericales (Cairney and Ashford 2002); (3) Arbutoid: typical arbutoid mycorrhizae are formed with two genera of Ericaceae family (*Arbutus* and *Arctostaphylos*) and several genera of the family Pyrolaceae (Molina and Trappe 1982); (4) Monotropoid: plants that have this kind of mycorrhizae are non-photosynthetic, but this fungi can associate with neighbouring trees that are photosynthetically active to get their photosynthates (Björkman 1960); (5) Orchid: they only exist in the Orchidaceae family (Smith and Read 1997); (6): Ectomycorrhiza has three characteristics that are typical of these mycorrhizae: (a) the formation of a hyphae mantle on portions of the laterals roots, (b) the formation of the Hartig net between the roots cells and, (c) hyphae that emanates from the mantle and grow in the soil (Peterson et al. 2004); (7) Arbuscular: association between most of vascular plants roots and fungi from a new phylum named *Glomeromycota* (Schübler et al. 2001). The last two mycorrhizae described are the most abundant in earth.

Mycorrhizae can protect plants against root pathogens and toxic stresses, and another important role that these fungi could play is the restoration and the improvement of revegetation in soils mined, even when this practice is not well implemented in many parts of the world (Prasad et al. 2017; Varma et al. 2017). One of the main results of soil damage is the destruction of mycorrhizal fungal network, so the restoration of these fungi is essential for the soil habitat (Quoreshi 2008). In vitro culture is an important tool to achieve this result because with this technique, it is possible to obtain a great volume of inoculum and to transport it cheaply (Ceballos et al. 2013).

Ceballos et al. (2013) evaluated the in vitro production of *Rhizophagus irregularis* (mycorrhizal fungus) and its effect on cassava yield; though good production was obtained, no greater return on investment than conventional cultivation was achieved.

The inoculation effect of nine consortiums of arbuscular mycorrhizal fungi (AMF) in coffee seedlings of *Coffea Arabica* (Caturra variety) was compared with a control without inoculation during seven months under greenhouse conditions; three of the nine consortia studied were more efficient during the growth and development of coffee plants seedlings (Del Aguila et al. 2018).

12.2.4 Fungi and Biodegradation

Biodegradation can be defined as the decomposition of dead plant and animals by microorganisms (Kakde and Jamdhade 2009). Plant biomass contributes with sources of carbon on earth and fungi are efficient degraders of this biomass (Mäkelä et al. 2014). Fungi also can degrade polysaccharides in the environment, and 218 have been sequenced, allowing the identification of genes and proteins implicated in this degradation (Berlemont 2017).

The degradation of polysaccharides such as xylan and cellulose from plants and chitin produced by fungi is very important for several ecosystem processes that include nutrient cycles (like carbon cycle) (Nielsen et al. 2011) and the nutrition of animals (herbivores) (El Kaoutari et al. 2013). Cellulose, xylan and chitin are hydrolyzed mainly by microorganisms such as bacteria and fungi through different ways like enzymes that sometimes can be associated with non-catalytic domain (multi-domain glycoside hydrolases [GHs]) (Hervé et al. 2010; Várnai et al. 2013), multi-activity GHs and synthesis of some multi-protein complexes named cellulosomes (Gefen et al. 2012). Multi-domain GHs and cellulosomes can degrade biopolymers (VanFossen et al. 2011; Talamantes et al. 2016), so it is possible to use them for successful processes like biofuel industries.

There are several enzymes that can degrade plant polymers; such enzymes are produced by fungi and they belong to six groups: the glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), auxiliary activities (AAs) and carbohydrate-binding modules (CBMs) (Aspeborg et al. 2012).

Pesticides, that can persist in soils for many years, could be degraded by microorganisms. This is possible because physical, chemical and biological processes such as accumulation in plants, volatilization and others are associated with several soil characteristics like pH, salt content and presence of organic matter (Boivin et al. 2004).

White rot fungi are widely used for bioremediation processes that use microorganisms to degrade contaminants such as heavy metals and pesticides in soil and water. These fungi (white rot) degrade lignin and others polymers using enzymes (Pointing 2001) that are extracellular oxidases and peroxidases: lactases, manganese peroxidases, lignin peroxidases, among others (Novotný et al. 2004).

Brown rot fungi are also used with the same purpose that white rod. These fungi can degrade cellulose and hemicellulose (Schlosser et al. 2000; Newcombe et al. 2002). One example of bioremediation by these fungi is the degradation of DDT by *Fomitopsis pinicola* and *Daedalea dickinsii*, which can transform DDT to DDE 1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene and DDD (1,1-dichloro-2,2-bis (4-chlorophenyl) ethane) via the Fenton reaction (Purnomo et al. 2010, 2011).

To biodegradate pentachlorophenol, several fungi have been used like *Phanerochaete chrysosporium*, *Berjkandera adusta* and *Pleurotus ostreatus*, getting the highest percentage (96%) with *P. chrysosporium* (Lamar et al. 1990; Ruttimann and Lamar 1997). *Trametes hirsuta*, *Pleurotus eryngii* and *P. chrysosporium* have been used for the degradation of lindane (insecticide) and the best results were obtained with *T. hirsute* (10.6% to 96%) (Singh and Kuhad 1999; Quintero et al. 2007).

Tejomyee and Pravin (2007) studied the biodegradation of the insecticide endosulfan, and they demonstrated that *Aspergillus niger* can eliminate a concentration of 400 ppm endosulfan after 12 days of incubation. According to Kamei et al. (2011) *T. hirsute* is able to remove up to 90% of endosulfan and endosulfan sulfate after 14 days of incubation.

12.3 Beneficial Bacteria in Agriculture

Many microorganisms coexist in soils, and the survival capacities of some of them are being studied with increasing interest, mainly as alternatives for the control of pathogenic fungi. Plant-growth-promoting bacteria (PGPB) are recognized for their bio-stimulating, biofertilizing and stress-regulating capacity in vegetables (Lugtenberg and Kamilova 2009; Prasad et al. 2015, 2020; Basu et al. 2021). These bacteria are able to colonize the rhizosphere of the plant and survive in it (Raaijmakers et al. 2009) through mechanisms that allow them to compete successfully with other microbes. For these reasons, they have been studied as potential antagonists/biological controllers of plant pathogens.

Undoubtedly, the bacterial genus that has generated the most research and applications in terms of biological control is *Bhurkolderia* sp. However, in recent years, interest has developed in other genera of bacteria that also show potential in this regard.

12.3.1 The Genus Burkholderia

Although the bacteria that grow in the rhizosphere are a useful source for the protection of plants against pathogenic fungi, it would be preferable for the resistance to be present inside all plant tissues. Certain bacteria – among which are several of the genus *Bhurkolderia* – are able to grow and develop inside the plant, which is why they are called endophytic bacteria. This characteristic means that they can interact with the plant more effectively than those that live in the rhizosphere. Thus, the biocontrol metabolic products expressed by the endophytic bacteria could act more efficiently in the protection of plants against pathogenic microbes or predators.

The genus *Burkholderia* groups bacteria that usually grow in the rhizosphere of numerous plants; consequently, several of their species have been observed with interest to know how they can compete with other bacteria and mainly with phytopathogenic fungi. The identification of the antagonist mechanisms and the metabolites participating in this competition could help the formulation of biopesticides. But also, several of the species of the genus are able to grow in an endophytic manner. *Burkholderia phytofirmans* PsJN, for example, can migrate to the aerial parts of the grape plants and form a biofilm on the leaf surface that restricts the growth of the *Botrytis cinerea* mycelium (Miotto-Vilanova et al. 2016).

Simonetti et al. (2018) isolated the T16 line of *Burkholderia ambifaria* that grows in the rhizosphere of barley plants (*Hordeum vulgare*). This line uses fusaric acid (the main toxic metabolite produced by *Fusarium* spp.) as the sole source of carbon, nitrogen and energy for its own growth in vitro, and is also able to detoxify fusaric acid in barley seeds. Before, Utsumi et al. (1991) had obtained similar results in vitro for a line of *Burkholderia cepacia*.

Through comparison with the genome of other bacteria, Ali et al. (2014) identified genes putatively responsible for the endophytic behaviour of several *Burkholderia* species. On the other hand, it is known that the different *Burkholderia* lines can live in different environments, because they have a large, complex (4.6–9 Mb) and variable genome, with three chromosomes and large plasmids (Esmaeel et al. 2016). As for the metabolites involved in the antagonistic activity of *Burkholderia*, Esmaeel et al. (2017) cite a group of authors who have detected several substances with different properties; among these, the lipopeptides synthesized by several lines of *B. cepacia*, *B.ambifaria* and *B. contaminans* have specifically antifungal activity.

Several authors (cited by Haidar et al. 2016) have reported the antagonistic activity of endophytic bacterial genera such as *Bacillus*, *Pseudomonas*, *Streptomyces* and *Bhurkolderia*, among others, against *Botrytis cinerea*, a necrophyte fungus that inflicts large losses among the plantations of grapes and strawberries. Among the ways in which this antagonism occurs are the synthesis of various antifungal compounds such as antibiotics and lytic enzymes that destroy the cell walls of fungi, the induction of resistance in the host and competition for nutrients (Koch et al. 2021).

Some of these genera – but not *Bhurkolderia* – have been used in the formulation of biopesticides for the control of *B. cinerea*. However, it has been shown that *Bhurkolderia* produces metabolites capable of controlling various fungal species. Mahenthiralingham et al. (Mahenthiralingam et al. 2011) and Masschelein et al. (2017) report that the various species and lines of the genus synthesize several substances (pyrrolnitrin, occidiofungin, cepafungin and burkholdines) and other compounds such as the cepacines that have a broad spectrum of action. Although the focus of these two studies was mainly on the medical applications of such products, the production of these antifungal metabolites demonstrates the potential of the *Burkholderia* genus as biological control in agriculture.

Rika Fithri et al. (2014) tested the application of several isolates of *Burkholderia* sp. in the attempt to control the root rot in oil palm, caused by the fungus *Ganoderma boninense*. As part of this investigation, they detected the synthesis of pyrrolnitrin in the *Burkholderia* 312 isolate, which led to the best results in the plants. Pyrrolnitrin is a secondary metabolite derived from tryptophan (Kirner et al. 1998) that has strong antibiotic activity on various fungi. Ramli et al. (2016) found that the isolates of three endophytic bacteria, including *Burkholderia cepacia*, were able to control the in vitro development of *G. boninense*, and to delay the onset of disease symptoms in the oil palm when the seeds had been pre-treated with these microorganisms.

Bach et al. (2016) analysed the bacterial properties of *Bacillus mycoides* B38 V, *Burkholderia cepacia* 89 and *Paenibacillus riograndensis* SBR5, microbes of the PGPB type isolated from Brazilian soils. It was observed that the three bacteria produce amylases, catalases, esterases and proteases. Aktuganov et al. (2008) have reported that these extracellular enzymes can affect the cell walls of pathogenic fungi. In addition, in the assays by Bach et al. (2016), *Burkholderia cepacia* 89 showed antagonistic activity against several filamentous fungi; under greenhouse conditions, the combined inoculation of wheat plants with this bacterium and the pathogens *Bipolaris cynodontis*, *Drechslera tritici-repentis* and *Fusarium graminearum* led to dry weight values of roots and stems superior to plants inoculated only with pathogens. These values were also higher than those of the plants treated only with fungicides, possibly due to the growth-promoting effect that the PGPBs also provide. Additionally, *Burkholderia cepacia* 89 produced a metabolite with antifungal activity, which can become an important biological fungicide.

The effectiveness of the line JP2-270 of *Burkholderia cepacia*, isolated from the rhizosphere of rice, in the control of the fungus *Rhizoctonia solani* was demonstrated by Song et al. (2018). The analysis of the genome showed that the gene bysR (DM992_17470) is essential for the antifungal activity of *B. cepacia* JP2-270 against *R. solani*. The nature of this gene, which belongs to the lysR family of transcriptional regulators (Lu et al. 2009), allows to suppose that the antagonist activity is exerted through an affectation to the synthesis processes of secondary metabolites. This gene could then become a potential target for its use in genetic engineering in order to take advantage of the controlling potentials of *B. cepacia* JP2-270 (Song et al. 2018).

Kim et al. (2019) studied the activity of *Burkholderia stabilis*, endophytic bacteria isolated from ginseng (*Panax ginseng*), on several pathogens. Both the bacteria and their extracts were able to control the development of *B. cinerea*, *R. solani*, *A. panax*, *Phytium* sp. and in particular of *Cylindrocarpon destructans*, the main pathogen of ginseng, which causes severe root rot. The separation of *B. stabilis* extracts by column chromatography allowed to collect a fraction that inhibited the growth of the five pathogens; another of the fractions was also able to control *C. destructans*.

Mullins et al. (2019) demonstrated that cepacin A synthesized by *Bhurkolderia ambifaria* is an efficient metabolite in the biological control of *Pythium ultimum*, a pathogenic fungus that causes decay in hundreds of useful plant species such as potatoes, wheat and soybeans. Sandani et al. (2019) identified five isolates of four bacteria (*Pseudomonas aeruginosa*, *Burkholderia arboris*, *Burkholderia gladioli* and *Burkholderia rinojensis*) capable of 100% effective inhibition of germination of the spores of *Colletotrichum truncatum*, a pathogenic fungus responsible for anthracnose in chili pepper. In addition, the metabolites secreted by the isolates controlled the development of the disease to a large extent. These compounds, of diffusible nature, could be of various types, such as antibiotics, hydrolytic enzymes of cell walls or other secondary metabolites (Beneduzi et al. 2012).

What has been reviewed up to here suggests that *Burkholderia* is useful and can be applied as a biological fungal control agent, given the effects demonstrated as an antagonist of various fungi. In fact, in the 1990s, several *Burkholderia* lines began to be used as fungi biocontrol in American agriculture. However, risk studies (derived from their pathogenic potential to animals and people) advised their withdrawal from the market (Eberl and Vandamme 2016). What happens is that the genus *Bhurkolderia* can cause opportunistic infections to the plants, becoming a pathogenic agent. This would limit its generalized application as biological control; however, Bolívar et al. (2016) indicate that the genus is divided into two large groups, the so-called *Bhurkolderia cepacea* complex (BCC) constituted by opportunistic pathogenic species, and another phylogenetically distant group composed of beneficial species, promoters of growth and with biotic activity against known pathogens. Also, Eberl and Vandamme (2016) point out that the genus can be divided into two clades genetically separated from each other: one which contains pathogenic species to plants, animals and humans, and other grouping species that promote plant growth and protection of plants against numerous pathogens. It has even been proposed and accepted to rename this second group as a new genus (*Paraburkholderia*) (Sawana et al. 2014; Oren and Garrity 2015).

Regardless of the potential dangers of using *Bhurkolderia* in agriculture, the possibility of modifying the genome of the genus with useful characteristics opens up new possibilities of employment in plant production. Li et al. (2017) introduced the cry218 gene of *Bacillus thuringiensis* by electroporation into the genome of *Burkholderia pyrrocinia* JKSH007, which lives as endophyte in the poplar. The transgenic bacterium thus obtained was effective in the control of the larvae (second instar) of *Bombyx mori* (silkworm) which is a lepidopteran used as a model in these investigations. Consequently, it could potentially be used for the control of harmful lepidoptera.

12.3.2 Other Bacterial Genera

In addition to *Bhurkolderia* sp., other bacterial genera have been studied with the aim of using them directly as biological controls or of using the metabolites that they synthesize and that have an antagonistic effect with pathogenic microbes. The main approaches have been directed towards the genera *Pseudomonas* sp. and *Bacillus* sp.

Several species of the genus *Pseudomonas* exhibit antifungal activity, and have been used for the control of various pathogens in beet, tobacco, cucumber, cotton, wheat, rice, eucalyptus and other species (several authors, cited by Sindhu et al. 2016). *Pseudomonas aeruginosa* and *Pseudomonas viridiflava* were useful in the control of *Lasiodiplodia theobromae*, the main causal agent of crown rot in banana (Thangavelu et al. 2007). Other species are able to act as antagonists only under certain conditions; for example *Pseudomonas fluorescens* controls *Rhizoctonia solani* and *Pythium aphanidermatum* when the culture medium is rich in nitrogen, but not when it is rich in carbon (Michelsen and Stougaard 2012).

From wheat leaves, Müller et al. (2015) isolated 20 lines of *Pseudomonas fluorescens* and *Pseudomonas gessardii*, carriers of the gene phlD, which codes for the synthesis of the antibiotic 2,4-diacetylphloroglucinol, and are able to suppress in vitro *Fusarium* and *Alternaria*, important pathogens of this and other crops. The role of antibiotics such as pyrrolnitrin is decisive in the control of other microbes by *P. fluorescens*, as in the case of the prevention of damage caused by *R. solani* in cotton (Hill et al. 1994) or phenazine in the control of *F. oxysporum* and *G. graminis* (Chin-A-Woeng et al. 2003). The production of phenazine by species of the genus *Pseudomonas* is the control route of several fungi (Suryadi et al. 2014; Parvin et al.

2016; Irma et al. 2018). The MP12 line of *Pseudomonas protegens*, isolated from the soil and identified by Andreolli et al. (2019), carries phlD, pltB and prnC genes, which encode the synthesis of 2,4-diacetylphloroglucinol, pyoluteorin and pyrrolnitrin, respectively. This bacterium inhibits the in vitro growth of several phytopathogenic fungi of the vine: *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*, and these are responsible for the esca disease, not controllable by the methods available in agriculture.

The ability of *Pseudomonas* to colonize different organs of the plant, its versatility in terms of the use of organic substrates exuded by seeds and roots, the diversity of metabolites that they synthesize and their compatibility with other biological control agents and chemical pesticides make this genus a powerful candidate for its use in the control of damage caused by pathogens (Sindhu et al. 2016).

Within the genus *Bacillus*, both those who live in the rhizosphere and in an endophytic form have been studied for purposes of biological control. In wheat, three endophytic isolates of *Bacillus subtillis* and one of *Bacillus megaterium* inhibited the in vitro growth of *Fusarium graminearum*; the *B. megaterium* isolate is the most effective in field conditions (Pan et al. 2015). In corn, Figueroa-López et al. (2016) found three rhizospheric isolates of species of the genus (*B. megaterium*, *B. cereus* sensu lato and *Bacillus* sp.) that reduce the damage caused by *Fusarium verticillioides*, apparently thanks to the synthesis of glucanases, proteases, chitinases and substances that stimulate growth, such as siderophores and auxins.

Two endophytic isolates, one from *Bacillus cereus* and the other from *Bacillus mojavensis*, inhibit the development of *F. proliferum*, *F. verticillioides* and *F. fujikuroi*, rice pathogens (Etesami and Alikhani 2017). Melnick et al. (2008) were successful in controlling *Phytophthora capsici* in cocoa by applying *B. cereus* isolated from tomatoes and potatoes, and also *Bacillus* sp. from the cocoa plants themselves; equivalent results in the control of *Moniliophthora roreri* with *Bacillus* sp. in cocoa were obtained by Villamil et al. (2015). The genus *Bacillus* is able to synthesize lytic enzymes that, by destroying the cell walls of pathogens, impedes their growth (Tirado-Gallego et al. 2016).

Finally, the known toxicity of certain proteins of *Bacillus thuringiensis* on insects is another promising route (Malathi et al. 2006; Sujatha et al. 2009), taking advantage in this case of the facilities of genetic engineering. However, genetic engineering processes to control insects with *Bacillus thuringiensis* must be carried out with great foresight, since Bt toxins can be dangerous for useful insects such as the silkworm (Kumar et al. 2016). Although it is feared that insects may develop resistance to *B. thuringiensis* toxins, Badran et al. (2016) have discovered mechanisms to obtain new Bt toxins that do not adhere to their traditional receptors but to new adhesion sites in *Trichoplusia ni*. In this way, the resistance to the Bt toxins that already begins to appear in the field could be overcome.

The potentialities of the genus *Bacillus* as a biological control agent are given not only by its antagonistic capacity, but because it produces stable endospores that are able to withstand high temperatures and desiccation (Sindhu et al. 2016).

The production of antibiotics and hydrolytic enzymes are not the only mechanism important in the biological control of diseases that some bacteria exert. In addition to these, other mechanisms are known, such as the production of phytoalexins, the induction of systemic resistance, the synthesis of secondary metabolites of various types and the production of siderophores (Sindhu et al. 2016). The genera *Arthrobacte*r, *Curtobacterium*, *Enterobacte*r, *Microbacterium*, *Stenotrophomonas* and even *Pseudomonas*, which are able to control the damage caused by *Xanthomonas axonopodis* pv. *passiflorae*, do it through competition for iron and nitrogen compounds (Halfeld-Vieira et al. 2014).

Indirectly, in addition, the protection of the plants can be carried out in ways that improve their constitution and nutritional status, which makes them more resistant to pathogenic infections. The genus *Rhizobium* form nodules in the roots of Fabaceae (Fig. 12.2), reducing atmospheric N_2, which is very stable and relatively inert, to ammonium ions (NH_4^+) easily assimilated by most plant species (Marquina et al. 2011). This association between bacteria and plants from Fabaceae family is an efficient process in the biological fixation of atmospheric nitrogen (BFAN). According to Ángeles-Núñez and Cruz-Acosta (2015), nitrogen fixation could vary from 24 to 584 kg ha^{-1} and may supply up to 90% of the needs of the plant. Also, BFAN can reduce drastically the application of nitrogen fertilizers, which brings less contamination of soil and water, also reducing production costs (Yadegari and Rahmani 2010; Granda et al. 2014). The final result is a vigorous and healthy plant, more able to defend itself from pathogenic infections.

Rhizobium characterization studies have been carried out in order to know their growth and nodulation properties with a view to their use in agriculture. Morphological and biochemical traits from several *Rhizobium* strains (9 of them from wild common bean roots and 11 from domesticated bean roots from Western Mexico) were characterized by López-Alcocer et al. (2017). Results from the morphological characterization showed that all strains had a rapid growth (2–3 days),

Fig. 12.2 Nodules of *Rhizobium* (www.farmersjournal.ie)

white colour and smooth border; 14 had a convex shape, and 12 were translucent. With respect to biochemical characterization, all strains grew at a pH of 6.0 or higher, and when a pH from 4.0 to 5.5 was fixed, four strains did not grow. A great variability between strains was found in this study showing generally rapid growth, tolerance to acid pH values, tolerance to moderate concentrations of sodium chloride, susceptibility to heavy metals and resistance to antibiotics, which is consistent with bacteria of the genus *Rhizobium* (López-Alcocer et al. 2017).

Gómez-Padilla et al. (2017) characterized six bacteria isolated from roots of *Vigna unguiculata*; they were subjected to different salt concentrations (0.17–6.6 dSm^{-1} of NaCl), pH levels (4.5–9.0) and temperatures (28–45 °C). The variation of 16S rRNA gene was examined by amplified 16S rDNA restriction analysis (ARDRA) and direct sequencing to show genetic diversity. Three isolates (VIBA-1, VIBA-2 and VIBA-6) achieved similar results as the control with 2.6 and 3.4 dSm^{-1} of NaCl. All of the isolates could grow at pH 7 and 9 and could grow until 40 °C, meanwhile only two of them (VIBA-4 and VIBA-5) grew at 45 °C. VIBA-1 was closely related to *Bradyrhizobium liaoningense*, VIBA-4 to *Rhizobium radiobacter* and the remaining to *Bradyrhizobium yuanmingense.* All of them, with the exception of VIBA-4, were able to nodulate in the plants when they were inoculated.

Bacteria producing organic acids such as lactic acid and acetic acid are used in the biopreservation of plant products (Trias et al. 2008a) mainly because the low pH prevents the growth of fungi that rot the edible fruits and leaves. *Enterococcu*s, *Lactobacillus*, *Leuconostoc*, *Lactococcus* and *Pediococcus* produce various antifungal compounds, among which are protein molecules, peptides, fatty acids, organic acids and reuterin, a metabolite resulting from the degradation of glycerol. Although the use of these bacterial genera as biological controls of fungi has not been widely studied, their antagonist activity has been reported in some cases (Sathe et al. 2007; Rouse et al. 2008; Trias et al. 2008b; Lan et al. 2012, and others) which allows considering them as potential candidates for this purpose. In addition, unlike other microorganisms such as *Bhurkolderia*, there are no reports of toxicity to plants, animals or humans related to these bacterial genera, and they are easy to isolate from different environments, including the aerial parts of plants (Gajbhiye and Kapadnis 2016).

An important and recent application of the properties of bacteria is the control of weeds. Four main reports were pioneers in this topic: a limited effect of *P. fluorescens* strain D7 on *Bromus tectorum* (Kennedy et al. 1991), the control of *Poa annua* and *Poa attenuata* by *Xanthomonas campestris* pv. *poae* JT-P482 (Imaizumi et al. 1997), the antagonist activity of *P. fluorescens* strain BRG100 on *Setaria viridis* (Quail et al. 2002) and the inhibition of 29 species between monocotyledonous and dicotyledonous plants by *P. fluorescens* strain WH6 (Banowetz et al. 2008). In recent years, several reports have appeared on the herbicidal activity of other genera (Patil 2014; Sayed et al. 2014; Juan et al. 2015; Boyette and Hoagland 2013, 2015). Recently, *P. fluorescens* strain BRG100 has been used successfully in the formulation of a bioherbicide (Agriculture and Agri-Food Canada 2019).

The use of bacteria for the control of insects and other invertebrates has also been limited to some genera (Lacey et al. 2015). First, there are the subspecies of *Bacillus* *thuringiensis*, which in addition to their well-known success in the suppression of lepidoptera have achieved success in nematodes (Carneiro et al. 1998; Wei et al. 2003; Khan et al. 2010), coleoptera (Suzuki et al. 1992) and hymenoptera (Porcar et al. 2008). In 2014, only four biopesticides (three based on B. *thuringiensis* and one based on *B. firmus*) were registered in Europe for use in the greenhouse (Gwynn 2014); the subspecies *israelensis*, *japonensis* and *galleriae* (all of *B. thuringiensis*) began to be used experimentally for the control of insects in peanuts, vegetables, grass and turf (Kergunteuil et al. 2016). However, future employment prospects are broad, since 150 proteins of *B. thuringiensis* toxic to insects have been isolated (Crickmore et al. 2018). The toxins of *B. thuringiensis* have been the main base for the creation of transgenic crops resistant to lepidoptera, although their biosecurity for other insects and humans has been questioned; they also have the fact that they generate resistance in the target insects (Lacey et al. 2015). However, the above-mentioned results of Badran et al. (2016) promise substantial improvements in this last direction.

A promising prospect – at least for greenhouse plants – seems to be the combined use of bacterial biopesticides with the natural enemies of insects, in particular using the former as correction tools in cases where the latter do not work at all to the extent to which it is needed (Gonzalez et al. 2016).

12.4 Conclusions and Future Outlook

Fungi and bacteria can play an important role in agriculture on the basis of their properties that help commercial crops to acquire nutrients and water through symbiotic associations, stimulating their growth and development and/or protecting them against infections of other microbes, competition with undesirable vegetation and attacks from other predatory organisms.

Biotechnology has been useful in the identification and characterization of useful fungi and bacteria and their metabolites, as well as in the formulation of bioinsecticides, biofungicides and bioherbicides that begin to be used in a larger or smaller scale. The possibilities opened by the use of genetic engineering in the transformation of beneficial microorganisms make it a useful tool for the more exact and targeted application of these microbes and the products obtained from them.

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