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Chapter

Endophytic Microorganisms as an Alternative for the Biocontrol of *Phytophthora* spp.

Hernando José Bolívar-Anillo, Victoria E. González-Rodríguez, Giovanna Reyes Almeida, Inmaculada Izquierdo-Bueno, Javier Moraga, María Carbú, Jesús M. Cantoral and Carlos Garrido

Abstract

The genus *Phytophthora* with more than 100 described species and 58 officially recognized, phylogenetically distributed in ten clades, are important pathogenic oomycete chromists that cause important diseases in agricultural crops, trees and forests worldwide. This genus is known as "The Plant Destroyer" which causes great economic losses with costs between 2 and 7 billion dollars per year in agricultural systems and unquantifiable losses in natural ecosystems. The host plants of the genus *Phytophthora* can vary from a wide range in some species to only one host, however, the host plants of the new species are still being determined and therefore the range continues to expand, that makes control exceedingly difficult. Plant damage can range from alterations in roots, fruits, trunks, stems, foliage and crown to invasive processes in highly susceptible species. Considering the wide range of hosts and organs that can be affected by *Phytophthora*, the use of endophytic microorganisms for the biocontrol of this phytopathogen can be an alternative to avoid losses of both crops and forests worldwide. Endophytes are microorganisms that live inside plant tissues without causing disease under any circumstances. The fact that endophytic microorganisms are able to colonize an ecological niche similar to that of some plant pathogens qualifies them as potential biocontrol agents. This chapter describes the endophytic bacteria and fungi isolated from different plant species that have shown antagonistic activity against different species of *Phytophthora*, as well as the metabolites isolated from these microorganisms that have shown fungicide activity and other biocontrol strategies (enzyme production, siderophores, substrate competition, among others) against *Phytophthora*.

Keywords: biological control agents, biocontrol, inhibitory mechanisms, endophytic fungus, bacteria

1. Introduction

Phytopathogenic microorganisms are one of the main factors to causes losses in yield and quality of the crop along the world (worldwide). The economic losses due to diseases caused by microorganisms during pre- and post-harvest has been estimated to be between 30–40%, reaching almost 40 billion dollars worldwide annually [1, 2].

There is a great biodiversity of microorganisms that can cause diseases in plants. The group formed by phytopathogenic oomycetes (fungal-like organisms), is one of the most important and oldest. They have affected humankind since the beginning of agriculture in early civilisations [3]. During the last few centuries, these pathogens were responsible for the Potato Famine in Ireland, also known as the Great Famine, which caused almost a million deaths and triggered a mass migration in 1840 in that country [4, 5]. Even today, *Phytophthora infestans* is the causal agent of this disease in potatoes, it being the most important biotic limitation for the production of this tuber worldwide [4]. Other species of oomycetes, such as *Phytophthora ramorum*, do not only affect agriculture but also the environment, as they cause several diseases in many species of trees. As a consequence of the loss of forest mass due to infections and dead plants, it has been estimated an indirect impact on the environment that could reach a cumulative loss of 230–580 megatons of dissolved CO₂ during the last century [3]. Currently, these phytopathogens continue to represent a significant danger in agricultural and forestry systems because they have accelerated their evolution. This is caused by the continued use of fungicides, together with dispersal dependent on anthropogenic activities and climate (i.e. natural aerial dispersal and climate change). The use of monocultures as well as the greater use of perennial crops also increase the sexual recombination events of the populations of these oomycetes [4, 6]. This could cause an adaptation and improvement of these pathogens that would allow them to expand the range of hosts [4].

Among the phytopathogenic oomycetes, those of the genus *Phytophthora* are the best studied [1]. The genus *Phytophthora* is presently placed in the kingdom *Straminipila*, phylum *Heterokonta*, sub-phylum *Pernosporemycotina*, class *Pernosporemycetes* (Oomycetes), subclass *Pernosporemycetidae*, order *Pythiales* and family *Pythiaceae* [7]. *Phytophthora* has more than 100 described species and 58 officially recognized, phylogenetically distributed in ten clades, are usually soil-borne plant pathogens that cause important diseases in agricultural crops, trees and forests worldwide [8, 9]. This pathogen can present biotrophic, necrotrophic, or hemibiotrophic lifestyles [1, 3]. They reproduce asexually giving rise to sporangia, which divide into zoospores. When conditions are favourable, zoospores germinate to form mycelia or a specialized infection structure called appressorium. Sporangia can also germinate directly to produce mycelia or form an appressorium. Both sporangia and zoospores are important cells in the dissemination and infection processes [1].

Among the crops that can be infected by the genus *Phytophthora* are potato, tobacco, soybean, avocado, macadamia, cocoa, rice, tomato, pistachio, red pepper, strawberry, raspberry, among others [9–12]. Natural vegetation and ornamentals can also be infected by *Phytophthora* species, i.e. oaks, alder, holm, chestnut, cork oak, beech, rhododendron, viburnum, magnolia, pieris, among others [11–14]. Some species are highly specific to the host (i.e. *P. sojae*) or with a wide range of possible hosts (i.e. *P. cinnamomi*). However, the host plants of the new species are still being determined and, therefore, the range continues to expand, making control exceedingly difficult [7, 11]. Plant damage can range from alterations in roots, fruits, trunks, stems, foliage and crown to invasive processes in highly susceptible species [8, 9].

The control of infections caused by *Phytophthora*, in agriculture, forestry and natural systems is very limited. The fungicides available are usually not efficient against oomycetes since they are not true fungi [11]. Furthermore, the use of chemical fungicides is being increasingly restricted due to the adverse effects they produce on human, animal and environmental health [15]. As an alternative to the use of chemical products, the idea of using antagonistic microorganisms or the metabolites that they produce is proposed for the biocontrol of these oomycetes. The microorganisms used for biocontrol do not have negative effects on human or animal health and are considered friendly with the environment. Biocontrol carried

out by microorganisms offers multiple modes of action, both direct, indirect or mixed, in addition, it prevents the appearance of resistance, which makes them an attractive alternative or complement for the control of phytopathogens [16]. The ability to biocontrol diseases through the use of microorganisms highlights the importance of interactions between the plant, the pathogen, the antagonist, the microbial community associated with the plant and environmental conditions [17, 18]. In this sense, most of the microorganisms used in biological control have been isolated from areas related to plants such as the rhizosphere, endosphere, phyllosphere, spermosphere, among others [19]. Although rhizosphere microorganisms are the most used in biocontrol, in recent decades a considerable number of endophytic microorganisms have been studied for their ability to biocontrol and for being a new source of natural products for use in agriculture [18, 20–22]. Therefore, this chapter describes the endophytic bacteria and fungi isolated from different plant species that have shown antagonistic activity against different species of *Phytophthora*, as well as the metabolites isolated from these microorganisms that have shown fungicide activity and other biocontrol strategies (enzyme production, siderophores, substrate competition, among others) against *Phytophthora*.

2. Endophytic microorganisms as a biocontrol strategy

Endophytes are microorganisms that are found inside plant tissues during at least part of their life cycle. They do not cause disease under any circumstances, and many

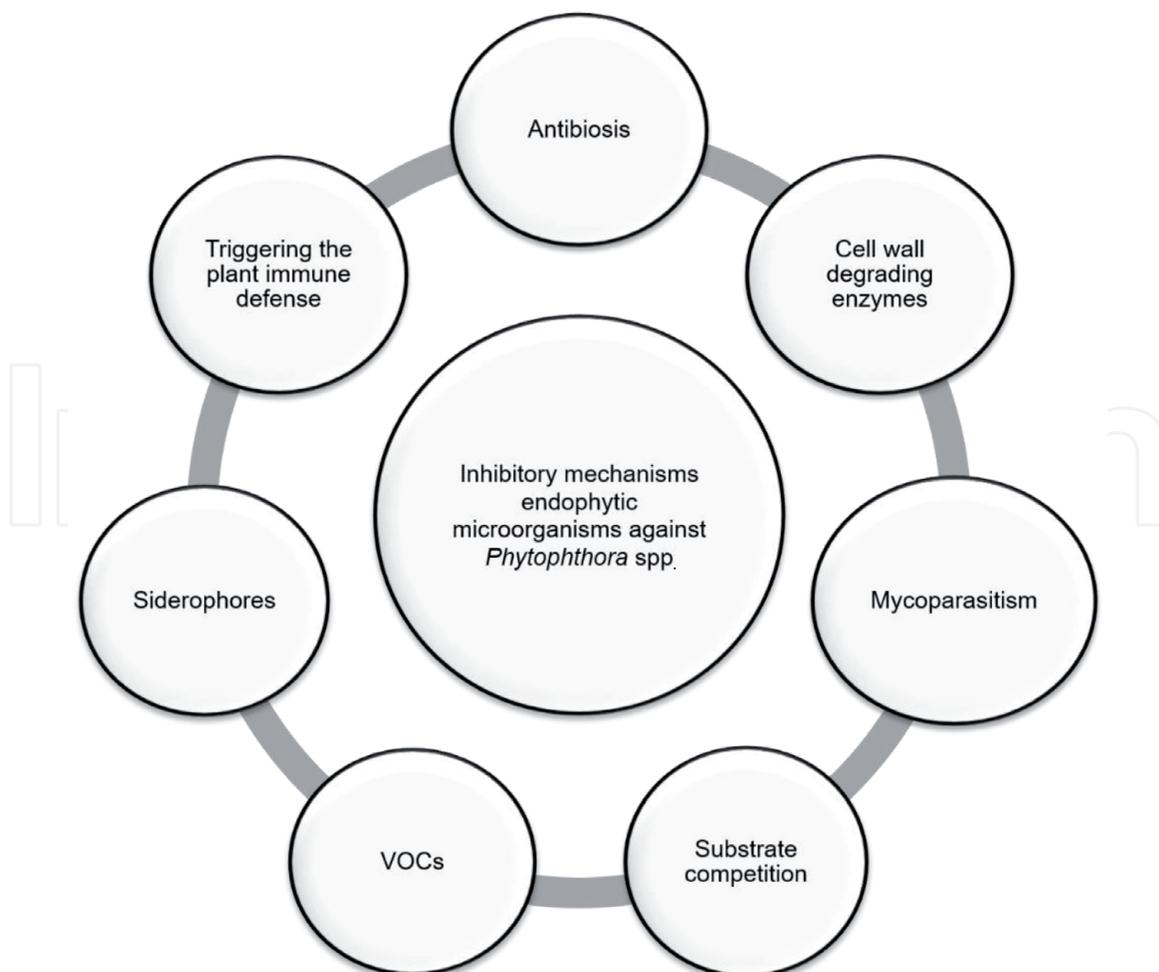


Figure 1.
Mechanisms of biocontrol showed by endophytic microorganisms.

show properties that promote plant growth [23, 24]. Approximately 300,000 species of plants have been described, and it is believed that each may possess different genera and species of endophytic microorganisms. However, it has only been studied the endophytic microbiome of 1–2% of plants. There are many unexplored fields of research on endophytes and their potential as biocontrol agents [25–28]. Although most endophytes are considered commensals, a large number of them establish mutualistic relationships with their host plant, playing a fundamental role in the adaptation of plants to biotic and abiotic factors [29–32]. Their use as biocontrol agents is considered one of the main characteristics to be used in the control of phytopathogens in agriculture. In this way we could reduce or avoid the use of antimicrobial compounds of chemical origin [18]. Endophytes can exert their biocontrol activity through various mechanisms including competition for a niche or substrate, hyperparasitism, predation, allelochemical production (antibiotics, lytic enzymes, siderophores) and by inducing systemic resistance in plants (**Figure 1**) [26, 33]. Now, the efficiency of endophytes as biological control agents depends on factors such as the specificity of the host, the physical structure of the soil, environmental conditions, the growth phase and the physiological state of the plant, among others [18, 34]. The development of a disease in a plant by any phytopathogenic microorganism will depend on three factors: the plant-the microbiota-the pathogen, whose interaction will be influenced by environmental factors. The loss of balance in any of these three factors would therefore lead to the development of an infectious process or not. On the other hand, most endophytic microorganisms originate in the soil (rhizosphere), therefore their recruitment (by the plant) will depend on their existence in soil, which is because they are not always present [35].

3. Biocontrol of *Phytophthora* spp. by endophytic bacteria

The promotion of plant growth by endophytic bacteria can be carried out through direct or indirect mechanisms [26, 36]. Among the indirect mechanisms, there is the biological control of phytopathogens, which is carried out through various strategies such as competition for nutrients and space, antibiosis, production of lytic enzymes, inhibition of toxins and induction of defense mechanisms in plants. All these strategies can be compatible with each other and may co-act simultaneously or synergistically [16, 18, 26, 37]. In this regard, there have been various studies that have evaluated the potential of endophytic bacteria for the biocontrol of different species of *Phytophthora*. These bacteria have been isolated from different plant species, which has led to the identification of microorganisms and the mechanisms used by them to inhibit the growth of this oomycete. **Table 1** shows some endophytic bacteria isolated from different plant species and the possible mechanisms they use for the biocontrol of *Phytophthora* spp.

El-Sayed *et al.*, (2018) [38] isolated forty morphologically distinct bacterial from roots, stems and leaves of *Smilax bona-nox* L. and they belonged to the genera *Burkholderia*, *Pseudomonas*, *Xenophilus*, *Stenotrophomonas*, *Pantoea*, *Enterobacteriaceae*, *Kosakonia*, *Microbacterium*, *Curtobacterium*, *Caulobacter*, *Lysinibacillus* and *Bacillus*. Out of these isolates, the ones that showed the highest *in vitro* growth inhibition capacity of 5 species of *Phytophthora* (*P. parasitica*, *P. cinnamomi*, *P. palmivora*, *P. tropicalis* and *P. capsici*) were two strains of *Pseudomonas fluorescences* (EA6 and EA14). The percentage of inhibition of mycelial growth against different strains of *P. parasitica* was between 47% and 80%. On the other hand, the crude proteins (extracellular hydrolytic enzymes) obtained from *P. fluorescence* EA6 were able to inhibit the mycelial growth of *P. parasitica*. The analysis of these proteins revealed that they were glucanolytic enzymes (β -1,3 and β -1,4 glucanases) which act by

Microorganisms	Plant species	Inhibitory mechanisms	Ref.
<i>Pseudomonas fluorescences</i>	<i>Smilax bona-nox</i> L	Glucanolytic enzymes	[38]
<i>Burkholderia</i> spp.	<i>Huperzia serrata</i>	Siderophores	[39]
<i>Acinetobacter calcoaceticus</i>	<i>Glycine max</i> L.	Siderophores	[40]
<i>Bacillus cereus</i>	<i>Lycopersicon esculentum</i>	Triggering the plant immune defense	[41]
<i>Bacillus</i> <i>Paenibacillus</i> <i>Lactococcus</i> <i>Pediococcus</i> <i>Enterobacteriaceae</i> <i>Cronobacter</i> <i>Pantoea</i>	Seeds Cucurbits	Antibiosis VOCs RNase activity	[42]
<i>Streptomyces Microbispora</i>	<i>Lens esculentus</i> <i>Cicer arietinum</i> L. <i>Pisum sativum</i> <i>Vicia faba</i> <i>Triticum vulgare</i>	Antibiosis Siderophores	[43]
<i>Bacillus thuringiensis</i> <i>B. vallismortis</i> <i>B. amyloliquefaciens</i>	<i>Cornus florida</i> <i>Carica papaya</i>	Antibiosis Triggering the plant immune defense	[44]
<i>Pseudomonas putida</i>	<i>Piper nigrum</i>	VOCs	[45]
<i>Streptomyces deccanensis</i> <i>Bacillus</i> spp. <i>Rhizobium radiobacter</i> <i>Pantoea dispersa</i> <i>Bacillus velezensis</i> <i>Acinetobacter</i> spp.	<i>Piper colubrinum</i>	Competition Antibiosis Triggering the plant immune defense	[46]
<i>Streptomyces alboniger</i> <i>Pseudomonas taiwanensis</i> <i>P. geniculata</i> <i>Enterobacter hormaechei</i> <i>Bacillus tequilensis</i> <i>B. flexus</i> <i>Arthrobacter phenanthrenivorans</i> <i>Delftia lacustris</i>	<i>Dodonaea viscosa</i> <i>Fagonia indica</i> <i>Caralluma tuberculata</i> <i>Calendula arvensis</i>	Antibiosis VOCs Cell wall degrading enzymes Siderophores	[47]
<i>Bacillus megaterium</i>	<i>Piper nigrum</i>	VOCs HCN Hydrolytic activity Siderophore	[48]
<i>Pseudomonas aeruginosa</i> <i>Chryseobacterium proteolyticum</i>	<i>Theobroma cacao</i>	VOCs Hydrolytic activity Siderophore HCN	[49]
<i>Bacillus velezensis</i>	<i>Olea europaea</i>	Antibiosis VOCs Cell wall degrading enzymes Siderophores	[50]
<i>Alcaligenes</i> spp.	<i>Hevea brasiliensis</i>	PCA	[51]
<i>Bacillus siamensis</i> <i>B. amyloliquefaciens</i> <i>B. velezensis</i> <i>B. methylotrophicus</i>	<i>Piper nigrum</i>	Cell wall degrading enzymes Antibiosis	[52]

Table 1.
 Endophytic bacteria able to biologically control Phytophthora spp.

hydrolyzing the cell wall of *Phytophthora*. In addition, the crude glucanolytic extract was shown to have higher activity than the purified β -1,3-glucanase enzyme, which means that these enzymes act synergistically on the cell wall of *Phytophthora*. Want *et al.*, (2010) [39] from *Huperzia serrata*, isolated the endophytic bacteria identified as *Burkholderia* spp. H-6, which was able to inhibit the *in vitro* mycelial growth of *Phytophthora capsici* with a diameter of inhibition zones of 23 mm. Furthermore, in greenhouse pot experiments, the soils treated with *Burkholderia* spp. densities of 10^6 , 10^8 and 10^{10} CFU ml⁻¹ reduced *P. capsici* infection in pepper seedlings by 51.7, 58.7 and 60.2%, respectively. This strain presented the ability to synthesize siderophores, which could be related to its biocontrol capacity. Zhao *et al.*, (2018) [40] isolated a total of 276 endophytic bacteria from *Glycine max* L. nodules, of which 6 had an inhibition capacity greater than 63% against *Phytophthora sojae* and were identified as *Bacillus cereus*, *Acinetobacter calcoaceticus*, *Enterobacter cloacae*, *Bacillus amyloliquefaciens*, *Pseudomonas putida* and *Ochrobactrum haematophilum*. The strain identified as *Acinetobacter calcoaceticus* DD16 was the one that presented the highest inhibition of mycelial growth of *P. sojae* with 71.14%. *A. calcoaceticus* DD16 caused morphological abnormal changes of fungal mycelia (e.g. lysis, formation of a protoplast ball at the end of hyphae, and split ends) that could be related to the production of anti-fungal substances and fungal cell-lysing enzymes. In addition, *A. calcoaceticus* DD16 was the strain that presented the highest capacity to produce siderophores ($54.33 \pm 0.093 \mu\text{g mL}^{-1}$) and was capable of fixing nitrogen and producing indole acetic acid, activities related to the promotion of plant growth. The regression analysis showed a significant positive correlation between siderophore production and inhibition ratio against *P. sojae*. Melnick *et al.*, (2008) [41] isolated from *Lycopersicon esculentum* a strain of endophytic bacteria identified as *Bacillus cereus* BT8, which *in vitro* test did not show the ability to inhibit the mycelial growth of *Phytophthora capsici*. However, this strain exhibited the ability to colonize *Theobroma cacao* seedlings and reduce the severity of *Phytophthora capsici* infection. The suppression of *P. capsici* was only observed in leaves which were not inoculated with the endophytic bacteria after colonization of the plant in other leaves, which suggests that the mechanism of suppression of the disease is through the induction of defense mechanisms in the plants (Induced Systemic Resistance) rather than antagonistic mechanisms. Khalaf *et al.*, (2018) [42] isolated a total of 169 bacterial endophytes from seeds of diverse cultivated cucurbits (*Luffa acutangula*, *Curcubita moschata*, *Curcubita pepo*, *Lagenaria siceraria*, *Citrullus lanatus*, *Cucumis melo* and *Cucumis sativos*), of which 26% (44/169) of isolates showed anti-pathogenic traits *in vitro* against *Phytophthora capsici*, of these 44 isolates, 16 were obtained from *Cucumis melo* seeds. These bacteria with activity against *P. capsici* belonged to the genera *Bacillus*, *Paenibacillus*, *Lactococcus*, *Pediococcus*, *Enterobacteriaceae*, *Cronobacter* and *Pantoea*. Of these microorganisms, those of the genus *Bacillus*, *Paenibacillus*, *Enterobacteriaceae* and *Pantoea* showed acetoin/diacetyl production (volatile organic compounds VOCs) and RNase activity *in vitro*, known to be implicated in triggering the plant immune defense. Therefore, these bacteria may control the phytopathogen directly (antibiosis) and/or indirectly (induction of host defense).

Misk and Franco (2011) [43] isolated thirty-six actinobacterial strains from different plants (root, stem and leaf), lentil (*Lens esculentus*), chickpea (*Cicer arietinum* L.), pea (*Pisum sativum*), faba bean (*Vicia faba*) and wheat (*Triticum vulgare*). Eleven of the isolates had antimicrobial activity against *Phytophthora medicaginis*, where ten of those isolates belonged to *Streptomyces* and one to *Microbispora*. The strains identified as *Streptomyces* spp. WRA1 and BSA25 were the most efficient as they significantly inhibited 100% and 85% *in vitro* of *P. medicaginis*, respectively, which showed a good capacity to produce siderophores. Furthermore, *in vivo* tests both strains (WRA1 and BSA25) significantly inhibited

P. medicaginis root rot compared to infected control. This inhibition capacity against *P. medicaginis* could be related to their antibiotic and siderophores production. Bhusal and Mmbaga (2020) [44] evaluated the biocontrol capacity of three endophytic bacterias *Bacillus thuringiensis* isolated from flowering dogwood stem; *B. vallismortis*; and *B. amyloliquefaciens* isolated from papaya stem against *Phytophthora capsici*. *B. amyloliquefaciens* was the most effective in suppressing *P. capsici* mycelial growth *in vitro* up to 46.62%, followed by *B. vallismortis* 45.95% and *B. thuringiensis* 27.59%. Under the greenhouse environment, *B. amyloliquefaciens* and *B. vallismortis* were most effective in suppressing *P. capsici* symptoms. Agisha *et al.*, (2019) [45] evaluated the antimicrobial capacity on phytopathogens of VOCs produced by the black pepper endophytic bacterium, *Pseudomonas putida*. Of the VOCs produced by *P. putida*, those identified as 2,5-dimethyl pyrazine; 2-methyl pyrazine; dimethyl trisulphide; 2-ethyl 5-methyl pyrazine; and 2-ethyl 3, 6-dimethyl pyrazine showed inhibitory activity (sealed plate method) against *Phytophthora capsici*. Among these VOCs, 2-ethyl-3, 6-dimethyl pyrazine was the most effective with an EC₅₀, EC₉₀ and EC₉₅ of 66.1 $\mu\text{g cm}^{-3}$, 244.8 $\mu\text{g cm}^{-3}$ and 382.1 $\mu\text{g cm}^{-3}$, respectively. In trials to evaluate the effect of VOCs against *Phytophthora* rot on black pepper shoot cuttings, 2, 5 dimethyl pyrazine, 2-ethyl 5-methyl pyrazine and 2-ethyl 3, 6-dimethyl pyrazine displayed reduction of lesion at 21 $\mu\text{g cm}^{-3}$ and, 2-methyl pyrazine at 42 $\mu\text{g cm}^{-3}$ with no signs of toxicity. While in the tests for fumigant activity of volatiles, dimethyl trisulphide demonstrated complete inhibition against *P. capsici* at a concentration of 6.25 $\mu\text{g cm}^{-3}$, which demonstrated that these VOCs can be an alternative for the control of *P. capsici* infections. Kollakkodan *et al.*, (2020) [46] isolated endophytic bacteria from the roots, stem and leaves of *Piper colubrinum*. Seven of these isolates showed *in vitro* inhibition capacity against *Phytophthora capsici* with zones of inhibition between 2.4 and 5.8 mm, which were identified as *Streptomyces deccanensis*, *Bacillus* spp., *Rhizobium radiobacter*, *Pantoea dispersa*, *Bacillus velezensis* (PCSE8), *Bacillus velezensis* (PCSE10) and *Acinetobacter* spp. The maximum inhibition zone was produced by the two strains of *B. velezensis*. In leaf assay (leaves of black pepper), the highest suppression of the disease was presented by the strains identified as *Pantoea dispersa* and *Bacillus velezensis* (PCSE10), with percentages of 74% and 79%, respectively. The mechanisms of these endophytic bacteria which are responsible for the inhibition of *P. capsica* seem to be mainly related to competition, antibiosis and triggering of the plant's immune defence. Iqrar *et al.*, (2021) [47] isolated endophytic bacteria from medicinal plants, *Dodonaea viscosa*, *Fagonia indica*, *Caralluma tuberculata* and *Calendula arvensis*. Bacteria that exhibited biocontrol activity on screening assays (production of cell wall degrading enzymes and siderophores) were identified as *Streptomyces alboniger*, *Pseudomonas taiwanensis*, *Pseudomonas geniculata*, *Enterobacter hormaechei*, *Bacillus pfeustrivo*, *Bacillus flexus* and *Delftiartibacteris*. In the *in vitro* growth inhibition test against *Phytophthora parasitica*, the highest inhibition was presented by the bacterium identified as *P. taiwanensis* with 55%, as well as in the bipartite split-plate growth inhibition assays (VOCs) with an inhibition of 80%. In addition, the crude extracts from the culture of this bacterium presented an inhibition of 92% at a concentration of 400 $\mu\text{g mL}^{-1}$ and the ethyl acetate extract presented an inhibition of 60%. The hyphae of *P. parasitica* subjected to these extracts showed alterations in their structure (convoluted, swollen nodes and abnormal growth of hyphae). The inhibition capacity of these endophytic bacteria on *P. parasitica* seems to be related to multiple mechanisms of action such as antibiosis, VOCs, cell wall degrading enzymes and siderophores. Munjal *et al.*, (2016) [48] isolated an endophytic bacterium identified as *Bacillus megaterium* from the black pepper root that was capable of inhibiting different phytopathogens *in vitro*, including *Phytophthora capsici*. This bacterium exhibited the ability to produce hydrogen cyanide (HCN),

protease, cellulase and siderophore. In VOCs' activity tests, it was observed a growth inhibition of *P. capsica* of 28%. These VOCs were mainly composed of 2,5-dimethyl pyrazine, 2-ethyl-3-methyl pyrazine, 2-ethyl pyrazine and 2-methyl pyrazine and they were able to inhibit individual mycelial growth by more than 60% at a concentration of $336 \mu\text{g mL}^{-1}$. Among these VOCs, the most effective was 2-ethyl-3-methyl pyrazine, which 100% inhibited the mycelial growth of *P. capsici* at a concentration of $168 \mu\text{g mL}^{-1}$. Therefore, the antagonistic activity of this bacterium is related to the ability to produce VOCs, HCN, protease, cellulase and siderophore. Alsultan *et al.*, (2019) [49] isolated 103 endophytic bacteria from cacao plants (leaves, branches and fruits) of which two that showed an 80% *in vitro* inhibition of *P. palmivora* and were identified as *Pseudomonas aeruginosa* and *Chryseobacterium proteolyticum*. While in the culture filtrate test, the inhibition percentages were 100% and 62% to *P. aeruginosa* and *Ch. proteolyticum*, respectively. In the volatile metabolites test, *P. aeruginosa* and *C. proteolyticum* strains showed an inhibition of pathogen growth of 61.88% and 60.94%, respectively. The VOCs produced by *P. aeruginosa* were identified as eicosane, hexatriacontane, tetratetracontane, trans-2-decenoic acid and 1-phenanthrenecarboxylic acid, 1,2,3,4,4 α ,9,10,10 α -octahydro-1,4 α -dimethyl-7-(1-methylethyl), while those produced by *C. proteolyticum* were identified as eicosane, tetratetracontane, heneicosane, hexatriacontane and phenol 2,4-bis(1,1-dimethylethyl). Regarding the hydrolytic activity, these two strains were capable of producing cellulase, protease, pectinase and lipase. Only *P. aeruginosa* was able to produce siderophores and HCN. The inhibition capacity of both strains is related to the capacity to produce hydrolytic enzymes, VOCs, HCN and siderophores that can act individually or synergistically. Cheffi *et al.*, (2019) [50] isolated the endophytic bacterium identified as *Bacillus velezensis* from olive trees, which exhibited an inhibition ranged from 40 to 75% with oomycetes, including *Phytophthora ramorum*, *P. cactorum*, *P. cryptogea*, *P. plurivora* and *P. rosacearum*. Regarding its biocontrol capacity, *B. velezensis* presented the capacity to produce VOCs, among which ethylbenzene, phenylethyl alcohol, E-caryophyllene and cyclo (Leu-Pro) were detected. Through genome analysis, diverse secondary metabolite clusters were uncovered such as bacillomycin, amylocyclin, mersacidin, bacilysin, macrolactin, bacillibactin, bacillaene, surfactin, fengycin, didicin, subtilin and locillomycin. The analysis of the culture extracts by means of LC-MS, detected the production of surfactin B, surfactin C15, plipastatin B1, Fengycin B, IX and XII. Furthermore, this strain was able to produce cell wall degrading enzymes (protease, chitinase and glucanase) and siderophores. All these metabolites could be responsible for the inhibition capacity of *B. velezensis* on these oomycetes. Abraham *et al.*, (2015) [51] isolated the endophytic bacterium identified as *Alcaligenes* spp. from *Hevea brasiliensis*, that presents antagonistic activity against *Phytophthora meadii*. By means of the spectrometric study of the culture supernatant of *Alcaligenes* spp., it was established that the compound identified as phenazine-1-carboxylic acid showed inhibition of *P. meadii* growth. The minimum inhibitory concentration of this compound against *P. meadii* was optimized at $5 \mu\text{g mL}^{-1}$. In addition, this compound presented zoospore-lytic activity, the structure of which was completely altered and lysis of the same occurred. The zoospores were not able to germinate when they were cultured in the presence of this compound. Ngo *et al.*, (2020) [52] isolated endophytic black pepper bacteria, of which six showed the ability to inhibit the growth of *Phytophthora* spp. by more than 60%. These bacteria were identified as *Bacillus siamensis*, *B. amyloliquefaciens*, *B. velezensis* and *B. methylotrophicus*. These strains presented high chitinase and protease activities. In the *in vivo* test, the strains identified as *B. siamensis*, *B. velezensis* and *B. methylotrophicus* (EB.KN13) had the lowest rate of root disease (8.45–11.21%) and lower fatal rate (11.11–15.55%).

4. Biocontrol of *Phytophthora* spp. by endophytic fungi

Like bacteria, endophytic fungi can protect their host plant against both biotic and abiotic stressors; which are considered a rich source of bioactive metabolites [32, 53, 54]. Among the main mechanisms by which endophytic fungi prevent infections by phytopathogens are induced resistance, antibiosis, mycoparasitism, competition and extracellular enzymes [31, 32, 54]. **Table 2** summarizes the species of endophytic fungi with biocontrol capacity against *Phytophthora* spp. and the plant species from which they were isolated, revealing the wide diversity of endophytic fungi that can be used for the biocontrol of this phytopathogen. Hanada *et al.*, (2010) [55] evaluated the antagonistic capacity of endophytic fungi isolated from *Theobroma cacao* and *Theobroma grandiflorum* against *Phytophthora palmivora*.

Microorganisms	Plant species	Inhibitory mechanisms	Ref.
<i>Trichoderma</i> <i>Pestalotiopsis</i> <i>Curvularia</i> <i>Tolypladium</i> <i>Fusarium</i>	<i>Theobroma cacao</i> <i>T. grandiflorum</i>	Antibiosis	[55]
<i>Muscodor crispans</i>	<i>Ananas ananassoides</i>	VOCs	[56]
<i>Trichoderma viride</i> <i>T. pseudokoningii</i>	<i>Piper nigrum</i>	Antibiosis	[57]
<i>Trichoderma ovalisporum</i> <i>T. theobromicola</i> <i>T. hamatum</i> <i>T. stilbohypoxyli</i> <i>T. caribbaeum</i> var. <i>aequatoriale</i> <i>T. theobromicola</i>	<i>Banisteriopsis caapi</i> <i>Theobroma cacao</i> <i>Theobroma</i> <i>gileri</i> <i>Theobroma cacao</i> <i>Theobroma gileri</i> <i>Cola praecuta</i>	Mycoparasitism Antibiosis Systemic induced resistance	[58]
<i>Aureobasidium pullulans</i> <i>Nigrospora oryzae</i> <i>Chaetomium globosum</i> <i>Trichoderma</i> <i>asperellum</i> <i>Penicillium commune</i>	<i>Espeletia</i> spp.	Antibiosis Competition for substrate	[59]
<i>Phialocephala europaea</i>	<i>Picea abies</i>	Antibiosis	[60]
<i>Phoma terrestris</i> <i>Fusarium oxysporum</i> Ascomycete spp.	<i>Panax quinquefolius</i>	Antibiosis Cell wall degrading enzymes	[61]
<i>Cryptosporiopsis</i> spp. <i>Phialocephala</i> <i>sphaerooides</i>	<i>Picea abies</i>	Antibiosis	[62]
<i>Ceriporia lacerate</i> <i>Phomopsis</i> spp. <i>Diaporthe</i> spp. <i>Daldinia eschscholtzii</i> <i>Annulohypoxyton nitens</i> <i>Fusarium</i> spp.	<i>Piper nigrum</i>	Competition Antibiosis Mycoparasitism VOCs	[63]
<i>Purpureocillium lilacinum</i>	<i>Solanum lycopersicum</i>	Antibiosis Cell wall degrading enzymes	[64]
<i>Xylaria</i> spp.	<i>Haematoxylon brasiletto</i> Karst	Antibiosis VOCs	[65]
<i>Hypoxyton anthochroum</i>	<i>Gliricidia sepium</i>	Antibiosis	[66]

Table 2.
 Endophytic fungi with biocontrol capacity against *Phytophthora* spp.

A total of 103 endophytic fungi were isolated of which ~70% showed some degree of reduction in the disease severity in three cacao pods. Eight isolates from genera *Trichoderma*, *Pestalotiopsis*, *Curvularia*, *Tohyopcladium* and *Fusarium* showed the highest level of activity against the pathogen. The possible responsible mechanisms for the ability to inhibit *P. palmivora* were related to the production of bioactive compounds. Mitchell *et al.*, (2010) [56] evaluated the ability of the VOCs of the endophytic fungus *Muscodor crispans* isolated from *Ananas ananassoides* to inhibit the growth of phytopathogens, among which there were *Phytophthora cinnamomi* and *P. palmivora*. The VOCs produced by *M. crispans* that were composed mainly of propanoic acid, 2-methyl; propanoic acid, 2-methyl-; 1-butanol, 3-methyl-; 1-butanol, 3-methyl-, acetate; propanoic acid, 2-methyl-, 2-methylbutyl ester; and ethanol and were able to inhibit the growth of *Phytophthora cinnamomi* and *P. palmivora* by 100% with an IC_{50} ($\mu\text{L mL}^{-1}$) of 0.056 and < 0.02 , respectively. Mathew *et al.*, (2011) [57] isolated two endophytic fungi identified as *Trichoderma viride* and *T. pseudokoningii* from black pepper plants which showed *in vitro* inhibition capacity against *Phytophthora capsici* with an inhibition percentage of 64.4% and 65.6%, respectively. In the *in vivo* study, the lowest percentage in the incidence and severity of the disease caused by *P. capsici* was presented by the strain identified as *T. viride*. Bae *et al.*, (2011) [58] evaluated the antagonism capacity against *Phytophthora capsici* of six species of *Trichoderma* (*T. ovalisporum*, *T. theobromicola*, *T. hamatum*, *T. stilbohypoxyli*, *T. caribbaeum* var. *aequatoriale* and *T. theobromicola*) isolated from *Banisteriopsis caapi*, *Theobroma cacao*, *Theobroma gileri*, and *Cola praecuta*. All strains except for *T. caribbaeum* var. *aequatoriale* showed the ability to parasitize the mycelium of *P. capsici*. However, the culture filters of *T. caribbaeum* var. *aequatoriale* completely prevented growth of *P. capsici*, while *T. stilbohypoxyli* and *T. ovalisporum* presented inhibition percentages of 56.5% and 30.7, respectively. In addition, it was shown that the inoculation of *Trichoderma* strains in pepper seedlings activated genes associated with responsive to stress. *In vivo* tests, the strain identified as *T. theobromicola* delayed the onset of disease symptoms for more than 3 days and between 26 and 60% of the pepper seedlings remained asymptomatic. Miles *et al.*, (2012) [59] studied the biocontrol potential of 100 fungal endophytes isolated from *Espeletia* spp. Among the phytopathogens used to measure this potential was *Phytophthora infestans*. The growth of *P. infestans* *in vitro* was completely inhibited by eight endophytes which were identified as *Aureobasidium pullulans*, *Nigrospora oryzae*, *Chaetomium globosum*, *Trichoderma asperellum* and *Penicillium commune*. The crude extract of the culture of *A. pullulans* and *P. commune* also showed the ability to inhibit 100% the growth of *P. infestans*. Tellenbach *et al.*, (2013) [60] evaluated the ability of *Phialocephala europaea* isolated from *Picea abies* to inhibit the growth of *Phytophthora citricola* s.l. The strain of *P. europaea* was able to reduce the growth of *P. citricola* *in vitro*. The four compounds isolated from this microorganism were identified as sclerin, sclerolide, sclerotinin A and sclerotinin B. Sclerin and sclerotinin A were the main compounds produced, which *in vitro* significantly reduced the growth of *P. citricola* at a concentration of 30 mg mL⁻¹. Park *et al.*, (2015) [61] isolated the endophytic fungi identified as *Phoma terrestris*, *Fusarium oxysporum* and *Ascomycete* spp. from *Panax quinquefolius*, which inhibited the growth of *Phytophthora cactorum* with percentages between 64% to 82% and from 71% to 80% in the disk diffusion tests and fermentation broth tests, respectively. The main metabolites produced by *P. terrestris*, *F. oxysporum* and *Ascomycete* spp., were identified as N-amino-3-hydroxy-6-methoxyphthalimide, 3-methylthiobenzothiophene, phthalic acid, erucylamide and 2H-1-benzopyran-2-1, 3,4,5,6,7,8-hexahydro-4,7-dimethyl-. In the enzyme assays, the endophytic fungus identified as *P. terrestris* showed activity for the cellulase, xylanase, β -glucanase, pectinase and chitinase enzymes that could play a role in the inhibition of phytopathogens.

Terhonen *et al.*, (2016) [62] isolated the endophytic fungi identified as *Cryptosporiopsis* spp. and *Phialocephala sphareoides* from *Picea abies* which were able to inhibit the growth of *Phytophthora pini* *in vitro*. In addition, a decrease in the growth of *P. pini* was observed when the crude extract of the culture medium of *Cryptosporiopsis* spp. were tested. Subsequently, the analysis of the crude extract by UPLC-QTOF/MS was able to establish that the main metabolites produced by *Cryptosporiopsis* spp. had the following chemical formula $C_{19}H_{30}O_6$, $C_{20}H_{28}O_8$, $C_{20}H_{30}O_7$ and $C_{18}H_{28}O_6$. Sreeja *et al.*, (2016) [63] isolated 125 endophytic fungi from *Piper nigrum* which were evaluated to measure the ability to inhibit *Phytophthora capsici* *in vitro*. Of the 125 isolated fungi, 23 presented this capacity in more than 50%. The fungi with the highest inhibition capacity (78%) were identified as *Ceriporia lacerate*, *Phomopsis* spp. and *Diaporthe* spp. Other strains identified as *Daldinia eschscholtzii*, *Annulohyphoxylon nitens* and *Fusarium* spp. presented inhibition capacity between 74% to 75%. Competition, VOCs antibiosis and mycoparasitism were reported to be among the biocontrol strategies for these fungi against *P. capsica*. Wang *et al.*, (2016) established by genome mining the biocontrol capacity of two strains of *Purpureocillium lilacinum* (PLBJ-1 and PLFJ-1) isolated from *Solanum lycopersicum*. Among the genes detected that may be useful in biocontrol were those that code for CAZymes, protease, glycoside hydrolases, and carbohydrate esterase. Regarding the production of secondary metabolites, genes coding for polyketide synthase, non-ribosomal peptide synthetase, terpene synthase and dimethylallyl tryptophan synthase were detected. Among these genes, those responsible for the synthesis of leucinostatin A and B were detected, which was confirmed by the production of mutants incapable of producing this compound. *In vitro* tests with the wild type and the mutant strain showed that the synthesis of leucinostatin A and B is closely related to the ability of these strains to inhibit the growth of *Phytophthora infestans* and *P. capsici*. Sanchez-Ortiz *et al.*, (2016) [65] evaluated the biocontrol capacity and VOCs of the endophytic fungus of *Haematoxylon brasiletto* Karst identified as *Xylaria* spp. PB3f3. The endophytic fungus was able to inhibit *Phytophthora capsici* by 48.3% *in vitro* and it was able to produce forty VOCs composed mainly of 3-methyl-1-butanol and thujopsene. Sánchez-Fernández *et al.* (2020) [66] studied antifungal and antioomycete activities of the compounds synthesized by the endophytic fungus *Hypoxylon anthochroum* isolated from *Gliricidia sepium*. The chemical study of the culture medium and the organic extracts of mycelium of the endophytic fungus led to the isolation of three isobenzofuranones: 7-hydroxy-4,6-dimethyl-3H-isobenzofuran-1-one (1), 7-methoxy-4, 6-dimethyl-3H-isobenzofuran-1-one (2), 6-formyl-4-methyl-7-methoxy-3H-isobenzofuran-1-one (3) and one compound was isolated for the first time as a natural product, 7-methoxy-4-methyl-3H-isobenzofuran-1-one (4) and another obtained by chemical synthesis 7-methoxy-6-methyl-3H-isobenzofuran-1-one (5), which showed the ability to inhibit the radial growth of *Phytophthora capsici* with an IC_{50} mM of 0.76, 0.62, > 0.97, > 1.12 and 2.12 respectively. Regarding the ability to alter the permeability of the *P. capsici* membrane, compounds 1, 2 and 5 presented an IC_{50} mM of <1.40, 0.55 and 2.03, respectively. In addition, these compounds were able to inhibit the respiration of *P. capsici*, being 2 the most efficient with an IC_{50} mM of 0.34.

5. Conclusions

Currently, the control of infections caused by *Phytophthora* spp. is very complicated, mainly due to the fact that many of the fungicides available on the market are not effective against this oomycete and also many of them are associated with

environmental and health damage. Therefore, the use of biocontrol agents as an alternative opens the possibility of using endophytic microorganisms, associated with the plant environment, which show great potential against this oomycete. Endophytic microorganisms isolated from different plant species have shown the ability to inhibit the growth of different *Phytophthora* species through various mechanisms such as antibiosis, VOCs, enzyme production, competition, among others. Therefore, the isolation of endophytic microorganisms and the study of their antagonistic capacity allows us to find new biocontrol agents, or their bioactive molecules, that allow controlling the enormous economic losses caused by *Phytophthora* spp.

Author details

Hernando José Bolívar-Anillo¹, Victoria E. González-Rodríguez²,
Giovanna Reyes Almeida¹, Inmaculada Izquierdo-Bueno², Javier Moraga²,
María Carbú², Jesús M. Cantoral² and Carlos Garrido^{2*}

¹ Programa de Microbiología, Facultad de Ciencias Básicas y Biomédicas,
Universidad Simón Bolívar, Barranquilla, Colombia

² Facultad de Ciencias del Mar y Ambientales, Laboratorio de Microbiología,
Departamento de Biomedicina, Biotecnología y Salud Pública, Universidad de
Cádiz, Puerto Real, España

*Address all correspondence to: carlos.garrido@uca.es

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