Chapter

Plant Beneficial Microbes Controlling Late Blight Pathogen, *Phytophthora infestans*

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Abstract

Potato (*Solanum tuberosum*) as a food source and culinary ingredient varies is the fourth most produced noncereal crop in the world. Among multiple biotic stresses, late blight caused by *Phytophthora infestans* is the most destructive disease. Control of this pathogen is usually by the synthetic fungicides which have been fueled by the public concern about toxicity and environmental impact and development of pathogens resistance. Biological control agents (BCAs) seems the potentially alternative to these pesticides, biological disease control is now recognized and constitute an important tool in integrated pest management. BCAs strains should be able to protect the host plant from pathogens and fulfill the requirement for strong colonization. Bacteria such as Bacillus, Pseudomonas and Streptomyces and fungi such as Trichoderma and *Penicillium* were the most reported as a BCA against *P. infestans* using different direct antagonistic mode on the pathogen (via e.g. parasitism, antibiosis, or competition) or via exerting their biocontrol activity indirectly by induction in the plant of an induced systemic resistance to the pathogen. In this study, we present an overview and discussion of the use of beneficial microbes (bacteria and fungi) as novel BCAs for biocontrol of *P. infestans*.

Keywords: *Solanum tuberosum*, *Phytophthora infestans*, biological control agents, beneficial microbes

1. Introduction

Plant diseases need a good control strategies in order to maintain the quality and abundance of food around the world. Especially, human population growth has been the source of two major concerns: providing sufficient food for humanity and minimizing worldwide environmental pollution. Several approaches may be used to protect or control plant diseases. Beyond good cultural practices, harvest and post-harvest approaches in reduction of pathogen growth, growers often rely heavily on chemical fertilizers and pesticides. However, many countries have reported alarming residues of agricultural chemicals in soil, water, air, agricultural products, and even in human blood and adipose tissue [1, 2]. Additionally, research suggests that the massive use of inorganic fertilizers world-wide is associated with the accumulation of in agricultural soils [3]. Researchers and Policy makers recognize that the excessive and unsystematic application of agrichemical inputs poses a threat to the

environment and humans alike. Consequently, several biologist have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases [4]. Among these alternatives those referred to as biological control by using one or more beneficial microbes to suppress the damaging activities of soil-borne pathogens.

Plant growth-promoting microbes (PGPM) are free-living microorganisms of beneficial agricultural importance. The PGPM present important beneficial effects on plant health and growth, suppress disease-causing microbes and improve nutrient. PGPM exist in the rhizosphere and this is defined as the region around the root. PGPM compensate for the reduction in plant growth caused by weed infestation [5], drought stress [6], heavy metals [7], salt stress [8, 9] and some other unfavorable environmental conditions. Beneficial microbes are also the microorganisms that produce hormones, vitamins and growth factors that improve plant growth and increase crop production. Many research reported the ability of this microorganisms to produce indole acetic acid (IAA), gibberellic acid, and cytokinins [10] and production of important metabolites such as siderophores, HCN, and antibiotics that have immense potentiality in enhancing the root surface area, altering root architecture and promoting plant growth. Among the numerous plant growth-promoting microbes (PGPM) are the most commonly applied in the biological control strategies. PGPM may affect plant performance through multiple defense mechanisms against several pathogens, operating directly by the production of specific substances that are able to promote plant growth, increase the availability and uptake of plant nutrients under biotic stress and induce the defense response of plants attacked or indirectly through the suppression of plant pathogen [11, 12].

For the biological control of late blight which is Late blight disease, caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most serious threats to potato production worldwide [13], Applications of different beneficial microbes as a biocontrol bacteria, fungi, algae or their metabolites, have been tested their ability to inhibit potato late blight, and when used as part of an integrated pest management system, they have had varying degrees of success [14–16]. Bacteria with antagonistic activity toward *P. infestans* are found mainly in the genera *Pseudomonas* and *Bacillus*. Although some fungal antagonists such as *Trichoderma atroviride* and *Muscodor albus* showed effective inhibition [17–20]. The objective of this chapter is to review the ability of beneficial microbes used to control late blight of potato caused by *P. infestans*, building on recent detailed reviews and research articles on microorganisms antagonistic to late blight of potato and their management approaches.

2. The methods of isolation of *P. infestans*

P. infestans causes potato and tomato late blight, economically the most important disease of these plant species. The oomycete pathogen is frequently sampled, isolated to pure cultures and stored. Efforts were made to develop isolation and culturing techniques based on tomato and potato. There are two major steps of isolating *P. infestans*, Field collection and isolation of *P. infestans* from infected tissue [21]. Petri dish method makes easier the collection of largest number of diseased samples in the field because is based on selective medium. Leaves, stems, petioles and even slices of diseased tubers can be collected. It allows the transfer of samples from the field to the laboratory in good condition and in turn stimulating sporulation of the lesions for easy isolation. The petri dishes were prepared with 1.5-% water agar, the sample with only one lesion were chosen and placed on the plate lid with the abaxial side up, in such a way so that the agar is on the sample but never in contact. The plates must be

sealed with Parafilm paper and placed in a cooler. In the laboratory, samples should be incubated at 15–18° C for 3–7 days with light and dark periods of 12 hours and grown hyphal tip of *P. infestans* transferred on a selective medium. Previous reports mentioned the use of some effectiveness selective medium for the isolation of *P. infestans*. The application of fungicides against *P. infestans* can affect the establishement of the oomycetes and their isolation. It is recommended to use tubers from fields where systemic fungicides against *P. infestans* have not been applied. Gamboa et al. [22] reported a method named sandwich method, tuber aseptically were cut in half and quickly place an infected leaflet between both halves. The both halves were attached with adhesive tape and wrap the tuber with paper towels, then place it in a paper or plastic bag for transfer to the laboratory. In the laboratory, the tuber were cuted in slices from the place of contact between the infected leaf and the tuber then put them in a wet chamber and incubate for 7 days to induce pathogen development. Incubation temperature should be 15–18° C with light and dark periods of 12 hours [22]. In the laboratory the isolation *P. infestans* from infected plant tissue can be using different infected tissues from potato or tomato plants. Sporulating lesion on potato/tomato leaves taken from field are washed in fresh water and placed in a humid chamber (inverted petri dish with water agar) with the leaf's abaxial side up and incubated at 15–18°C for 1 day or until fresh sporulation appears. Small pieces of infected tissue from the sporulating border of the lesion are cut and placed under potato/tomato slices in an empty petri dish. Dishes are incubated at 15–18°C for 1 week, until there is abundant sporulation on the upper side of the slice. To re-inoculate leaves, pick sporangia from the top of the tuber and place them in a drop of water on a potato leaf or another tuber slice. If isolating from infected tubers, slice the tuber where infection has occurred and place in a moist chamber until sporulation occurs. When clean inoculum appears on the upper side of an infected tuber slice or leaf, the sporangia are harvested in a flow chamber, by picking them up with an inoculating needle and placing the sporangia on selective medium [23, 24]. Tumwine et al. [25] reported that *P. infestans* grew successfully and well on Rye A agar without the need of antibiotics is one of the recommended medium for the isolation of *P. infestans*. The Rye A agar was described for the first time in 1968 by Caten when Rye B agar were used for the sporulation. However, V8 juice agar (V8A) which is blend of 8 vegetable juices, which supplies the trace ingredients to stimulate the growth of fungi. The acidic pH of the medium favors fungal growth and suppresses bacterial growth. V8A has been one of the most popular and commonly used medium for growth and reproduction of *Phytophthora* species [26]. In 2020, [23] were studied five different media in order to select the optimal culture conditions of *P. infestans*. Modifiations were made to use ingredients available in local markets on the following media: lime bean agar (LBA), Tree tomato or tree tomato agar (TA), carrot agar (AZ), Rye A modifid agar and 32% non-clarifid V8 agar. The findings results showed that as was described before media such as Rye A favored the ability of *P. infestans* to grow effiently.

3. Bacteria

The use of biological agents to control or suppress *Phytophthora infestans* provides an economic and environmentally friendly approach. As a biopesticides bacteria are the most common and cheaper form of microbial pesticides. The potential of a range of bacterial strains as biocontrol agents of plant pathogens has been reviewed by many scientific reports [27–29]. *Streptomyces*, *Pseudomonas* and *Bacillus* were the most tree bacteria reviewed to control *P. infestans* [30, 31]. *Actinomycetes* were isolated from in general from soil. Samples were diluted to go on serial dilution and plate on humic acid vitamin agar as described by [32] supplemented with an antifungal and antibacterial

Gram- such as nalidixic acid. The isolation plates were incubated at 35 ± 2°C for 7 days. The colonies had been transferred to International Streptomyces Project (ISP) medium No. 2 agar [33, 34] plates for purity check. This isolation method can be improved using same modifications. In the other hand, The isolation methods used to collect *bacillus* and *Pseudomonas* from soil as an endophytic or epiphytic strains were routinely grown on Luria-Bertani (LB) medium and incubated in the dark at 30°C [31, 35].

3.1 Bacillus

Bacillus and its products have been known for application as biological control agent against a range of plant pathogen. The success of *Bacillus* species as biocontrol agent could be ascribed to a wide array of peptide antibiotics produced such as iturin A, mycobacillin, subtilin and bacilysin as well as 25 different basic chemical structures with proven antifungal secondary metabolites [36, 37]. Lamsal et al. [38] found after a dual culture inhibition assay was conducted on V8-PDA in plastic petri plates (8.5 cm diameter) that seven bacterial isolates (AB05, AB11-AB15 and AB17) qualified previously as beneficial microbes of tomato plants, inhibit efficiently *P. infestans* affecting tomatoes in Korea by more than 60% in vitro. However, AB15 was the most effective, inhibiting mycelial growth of the pathogen by more than 80% in vitro. For greenhouse evaluation, targeted plants were left to dry for 2 days, and then 100 ml of bacterial spore solution (10^7 spores/ml) was added to each pot 7 days before infection so that only soil, but no above-ground parts, received any bacterial spores. The results showed that AB15 was the most effective suppressing disease by 74% compared with control plants under greenhouse conditions. According to 16S rDNA sequencing, a majority of the isolates are members of *Bacillus*, and a single isolate belongs to *Paenibacillus*. In India, for *Bacillus subtilis* strains were tested for their biocontrol activity against *P. infestans* in presence of the fungicide (Mancozeb) M45 (CURZATE®) as positive control. Before the sowing of potato seeds in blocks, all blocks were drenched with different bacterial cultures at the concentration $2x10^6$ CFU/ml, with the exception of chemical fungicide and control blocks. The potato seed tubers were treated with 0.2–0.3% of M 45 (Mancozeb) fungicide before ten days of planting. Results revealed that, bacterial treatments signifiantly reduced disease incidence of late blight compared with the control. Bacterial treatments increased the plant vegetative parameters like plant height, sprouting, number of leaves, fresh weight and dry weight of plants. In addition, treatments also showed the clear difference between commercial and non-commercial tuber yield/hectare. In a view of this results they suggest that the mode of the action were the ability of bacillus subtilis strains to produce mycotoxins which can inhibit *P. infestans* growth and the capacity of bacillus to induce the peroxidase activity [39]. Elliott et al. [40] have been reported that Companion® and Serenade® are two *Bacillus subtilis* commercial biocontrol products which reported to suppress *P. infestans*. However, resistance to this bioproducts develops and some isolates of *P. ramorum* from North American and European population have been shown to be resistant [41]. Bacillus strains could control *P. infestans* directly by inhibiting the mycelial growth, germination of the cysts or the swimming of the motile zoospore by producing many antifungal compounds which suppress the pathogen or indirectly mechanisms by inducing the inhabitation of the activity of ribosome or stimulate active oxygen burst, NO production, callose deposition, and lignification [42–44].

3.2 *Pseudomonas*

Among biocontrol agents of interest, Pseudomonas spp., are known for their production of antibiotics involved in biocontrol, such as

2,4-diacetylphluoroglucinol and phenazines [45–47], which have been widely studied in various plant-pathogen systems. Phenazine-1-carboxylic acid (PCA)-producing Pseudomonas spp., have been found effective against numerous phytopathogens, including bacteria, fungi, and oomycetes, such as the causal agent of bacterial blight of rice, *Xanthomonas oryzae* pv. oryzae [48], *Gaeumannomyces graminis* var. tritici [49] and the oomycetes *Phytophthora* spp., and Pythium spp., [50, 51]. PCA has been linked to biofilm formation, favoring attachment of PCA-producing Pseudomonas spp., to plant roots which facilitate the role of this beneficial microbes as biocontrol agents [52]. The mechanisms involved to control *P. infestans* by Pseudomonas were recently investigated, a previous study by [53] reported that the biocontrol of the pathogen could be by inhibiting sporangia and zoospore germination which suggesting the presence of many yet unknown anti-oomycete determinants. However, [54] suggests that Phenazine-1-carboxylic PCA produced by *Pseudomonas spp*., is involved in *P. infestans* growth repression and led to important transcriptomic changes by both up and down regulating gene expression in *P. infestans* over time. Different metabolic functions were altered and many effectors were found to be upregulated after the application of PCA, suggesting their implication in biocontrol. The cyclic lipopeptide surfactant massetolide A is a metabolite with versatile functions in the ecology of *Pseudomonas fluorescens* SS101 [55]. To study the effects of *P. fluorescens* SS101 and massetolide A on late blight of tomato, two leaves located on the second branch from the stem base of 5-week-old tomato plants were immersed in bacterial suspension (10^9 CFU ml^{-1}) for 1 min or in a solution of massetolide A in sterile demineralized water (pH 8). Leaves immersed in sterile demineralized water (pH 8) for 1 min served as a control. Treated tomato plants were transferred to trays covered with transparent lids. After incubation for 1 d in a growth chamber at 15°C, the lower side of each treated tomato leaf was inoculated with 3 μl droplets of a *P. infestans* zoospore suspension (3–4 × 10^3 swimming zoospores ml^{-1}) or 3 μl droplets of sterile demineralized water (pathogen-free control). *P. fluorescens* SS101 was effective in preventing infection of tomato (*Lycopersicon esculentum*) leaves by *P. infestans* and significantly reduced the expansion of existing late blight lesions. Massetolide A was an important component of the activity of *P. fluorescens* SS101, since the massA-mutant was significantly less effective in biocontrol, and purified massetolide A provided significant control of *P. infestans*, both locally and systemically via induced resistance [56]. Additionally, Biosurfactants (Rhamnolipids) produced by *fluorescent Pseudomonas* have zoospore lysis activity and biosurfactant-producing strain *Pseudomonas koreensis* 2.74 has potential to induce resistance in potato plant against late blight disease. High sensitivity of *P. infestans* zoospores to biosurfactants suggest that they can be used to dampen the spread of potato late blight once infection has been detected in the field [57, 58].

3.3 *Actinomycetes*

Actinomycetes are Gram+ bacteria that represent a high proportion of the soil microbial biomass and have the ability to produce a wide variety of antibiotics and of extracellular enzymes. Several strains of actinomycetes have been found to control plant diseases [59–61]. Recently, [62] were identifed β-rubromycin as a *P. infestans* cyst germination inhibitor by screening compounds produced by *Streptomyces* isolated from soil. For that, an acetone extract was prepared from *Streptomyces* cultures grown for 5 days in liquid medium A at 30°C by adding an equal volume of acetone followed by mixing. 20-μL aliquots were mixed with 1 × 10^3 *P. infestans* sporangia in total 70 μL (14.2% acetone solution), incubated at 10°C for 18 h, and examined using an inverted microscope. As a control, it is

confirmed that 15% acetone had no effect on morphological change in *P. infestans*. The isolation of the cyst germination inhibitor enabled to identify β-Rubromycin which can inhibit *P. infestans* cyst germination and hyphal elongation from sporangia, while not affecting zoospore release, cyst formation, or appressorium formation. Chemical genetic analyses using β-rubromycin identifed a RIO kinase-like gene, PITG-04584, as a critical contributor to zoosporogenesis, cyst germination, and the formation of appressoria in *P. infestans*. The Lubimin is a vetispirane sesquiterpenoid that consists of (2R,5S,6S,8S,10R)-8-hydroxy-10-methyl-2-(prop-1-en-2-yl)spiro[4.5]decane bearing a formyl substituent at position 6. It has a role as an antifungal agent and a phytoalexin. The synthesis of this biocompounds in noninoculated potato tuber slices have been elicited after using culture filtrates of *Streptomyces* isolates which induce the resistance of potato plants against late blight caused by *P. infestans* [63]. In this sense, the reliance on actinomycetes as promising biocontrol strategies are very useful in controlling *P. infestans*. Several actnimoycetes most of which were *Streptomyces* strains have been demonstrated to be effective [64–67].

From the gastrointestinal tract of a fish dredged near the South Orkney Islands in Antarctica, [68] isolated the psychrotolerant bacterial *Vibrio splendidus* T262. Investigation of this strain led to the isolation of a rare series of 15 bis- and trisindole derivatives. Among them, six new indole alkaloids. Using the agar diffusion method, at 10 μg/paper disk, some of the isolated compounds showed activity against both gram-positive and gram-negative bacteria when trisindolal was active against the *P. infestans* and a number of other plant-pathogenic fungi.

Independently of the mode of action of biological control agents, the successful application of rhizobacteria to suppress late blight was confirmed by several research using a range of bacteria such as *Micrococcus luteus*, *Paenibacillus* spp., *Flexibacteraceae bacterium*, and *Enterobacter cloacae* [35, 40, 69, 70]. However, there is a lack of research that highlight the effectiveness of the combination assays of one or more bacteria to control *P. infestans*. Whereas, the combinations have potential for extensive colonization of the rhizosphere, more consistent expression of beneficial traits under a broad range of soil conditions, and antagonism to a larger number of pathogens than strains applied individually.

4. Fungi

The beneficial fungi have gained immense attention as biofertilizers due to their role in maintaining plant quality and quantity and their environment-friendly relationship. Nowadays, use of this microorganisms as biocontrol agent (BCA) is considered to be a rapidly developing natural phenomenon in research area. Fungal biocontrol agents (BCAs) do not cause any harm to the environment, and they generally do not develop resistance in various types of pathogens due to their complex mode of action. They have been proved to be an alternative against the undesirable use of chemical products [71–73]. Previous reports have detailed the importance of various fungi species as effectiveness biocontrol agents against *P. infestans* [74–76]. For beneficial fungi isolation, the same method was adopted for years ago based on PDA medium and it can have same small modifications. PDA with chloramphenicol 0,016% (PDAc) and Rose Bengal Agar (RBA) (dextrose 10 $g.l^{-1}$, meat peptone 10 $g.l^{-1}$, K2HPO4 1 $g.l^{-1}$, MgSO4.7H2O 0.5 $g.l^{-1}$, Rose Bengal 30 mg/l, Agar 20 g/l) media were used. Petri dishes were incubated for 4 days for bacterial isolation and 7 days for fungal isolation at 25°C in the dark [59, 77].

4.1 *Trichoderma*

In the thick of various beneficial microbes have been investigated by several scientists, Trichoderma genera is a well-known biocontrol fungi that has been used since the 1930s to help plants acquire nutrients and control the plant pathogens [78]. Several Trichoderma species have been developed commercially as biofungicides and biofertilizers.

Fungi in the genus *Trichoderma* and bacteria such as *Bacillus amyloliquefaciens* have shown in vitro potentiality to reduce the mycelial growth of *Phytophthora infetsans*, *P. quercina*, *P. capsici*, *P. cactorum* and P. plurivora attacking *Quercus robur*, *Fagus sylvatica* and *Capsicum annuum* [35, 79, 80]. The biocontrol roles of *Trichoderma* against *P. infestans* could be attributed to the Trichoderma's rhizosphere competence and competitive ability [81], via the use of many mycoparasitic strategies which are a direct mechanism for biological control that works by parasitizing, detecting, growing, and colonizing pathogen involving the detection of pathogens through chemotropism; lysis of the pathogen's cell wall, pathogen's hyphal penetration by appresorial formation; production of cell wall-degrading enzymes (CWDEs) and peptaibols and parasitizing pathogen's cell wall contents [82], antibiosis or by activating a defense response as well as increased plant growth [83]. Many studies have shown the biocontrol activity of Trichoderma against *P. infestans*. Khan et al. [84] reported for the first time the elucidation and production of viridiofungin A (VFA) from *T. harzianum* isolate T23 cultures and the antifungal potential of VFA against *P. infestans* by suppressing zoosporangia germination and exhibiting a high activity on germ-tube growth. In the assay, 0.3 ml PDB/V8 medium in 0.6 ml Eppendorf tubes containing VFA concentrations from 50 to 200 μg ml^{-1} and sporangial suspensions of the pathogen were prepared. Control medium contained 2% acetone. Cultures were incubated on a shaker at 100 rpm at 25°C in the dark for 24 h. Subsequently, aliquots were taken from the cultures. Germination rates of sporangia and germ tube elongations were determined. Moreover, [85] highlighted the ability of 14 strains of *Trichoderma* to emit volatile compounds that decreased or stopped the growth of *P. infestans*. The experiments were performed in Petri plates divided into two compartments. The first compartment, containing V8 agar, was inoculated in the center with a 5 mm diameter mycelial disk of *P. infestans*. The second compartment, containing PDA, was inoculated with 5 mm mycelial disk of actively growing mycelia from one of the 14 *Trichoderma*. The plate-dividing wall prevented any physical contact between the *Trichoderma* strains and *P. infestans* but allowed the free exchange of VOCs. After inoculation, the plates were sealed with two layers of Parafilm and incubated at 21°C for 6 d, at which point the growth diameters were recorded. Volatile organic compounds (VOCs) emitted from *Trichoderma* strains inhibited the mycelial growth of *P. infestans* grown on a laboratory medium by 80% and on potato tubers by 93.1%. Using GC–MS analysis showed that the most abundant compounds were 3-methyl-1-butanol, 6-pentyl-2-pyrone, 2-methyl-1-propanol, and acetoin. Electron microscopy of the hyphae treated with *T. atroviride* VOCs revealed serious morphological and ultrastructural damages, including cell deformation, collapse, and degradation of cytoplasmic organelles.

4.2 *Penicillium*

Large number of reports mentioned that *Penicillium* spp., interact positively with plants roots. Some *Penicillium* species have shown an antagonistic activity against plant pathogens by producing antibiotics which is a primary mechanism of disease suppression by *Penicillium* also induce resistance in plants by activating

defense signals [86, 87]. The adaptability to different environments and tolerance to various abiotic stresses gives theses fungi species an advanced ranking to suppress many plant pathogens [87]. Previous reports have demonstrated that *Penicillium* species show efficacy as biocontrol agent against *P. infestans*. Based on the study conducted by [77] reported that *Penicillium chrysogenum* induce resistance against *P. infestans* in tomato plants. Dry *Peni. chrysogenum* mycelium extract was prepared using a detailed protocol described by [77] the extract was diluted with distilled water to a total carbohydrate content of 1.5 g l^{-1}. The tomato plants were treated two times with about 25 mL extract per plant as foliar spray. Leaf discs (diam. 18 mm) of plants treated were laid onto moist filter paper. Leaf discs were inoculated with 10 L droplets of zoospore suspension. The inoculated leaf material was kept at 23°C in the dark with a relative humidity at 100%. Three days later biochemical assays for the peroxidase activity and isoenzyme analysis were conducted. The application of the water extract of killed *Peni. chrysogenum* has shown no direct antifungal activity against the pathogen, however the protective effect of the extract was shown under controlled conditions after application on the whole plants and on leaf disk. The findings suggest that control resulted from the induction of defense mechanisms in the tomato plants. According to this many reports have been shown that the ability of *Penicillium* species to induce systemic expressions of defense-related genes [peroxidases (POX) and phenylalanine ammonia lyase (PAL) and PR-1 genes] is the key used by *Penicillium* to induce plant defense systems as well protects plants from pathogens [85, 86]. Otherwise, antagonistic activity of endophytic fungi associated with *Artemisia nilagirica* was studied against the pathogen *P. infestans* by the presence or absence of inhibition zone observed in dual cultures by using dual culture methods. The study has shown that among the endophytic fungal tested *Penicillium atrovenetum* and *Trichoderma viride* showed direct inhibition activity of pathogen mycelia growth [87]. Additionally, [88] reported that *P. striatisporum* Pst10 isolated from the rhizosphere of chili peppers showed very high antagonistic effects on mycelium growth and sporangia/spore formation or germination of *Phytophthora* spp., The analysis of the Pst10 organic solvent extract by thin-layer chromatography (TLC) and the antagonistic activity tests highlight the existence of three different antifungal compounds produced by *P. striatisporum* Pst10. To study the Pst10 antifungal spectrum of Pst10 the dual culture assays were used. In the other hand, To determine the effect of Pst10 sterilized liquid culture filtrates (SLCF) on sporangium and spore germination, 100 μl of sporangium or spore suspensions of *P. capsici* were spread on 20 ml PDA agar containing 1 ml Pst10 SLCF. PDA plates were incubated at 28 C for 24, 72, and 120 h. After each incubation time, 100 sporangia or spores were counted and germination rate was calculated under a light microscope.

Using fungi as biological agents to control or suppress the growth of *P. infestans* is not just limited to *Trichoderma* and *Penicillium* even they were the most fungi reported. In 2020 [75] Isolated *Aspergillus flavipes* from agricultural soils as a strong inhibitor for growth of various species of *Phytophthora*. As well as, the crude extracellular extract of broth cultures of *A. flavipes* displayed a significant growth inhibition of various *Phytophthora spp*., The putative compounds from *A. flavipes* were chemically verified as 3-hydroxy-2′,4,4′,6′-tetramethoxychalocone, 7,3,4,5′-tetramethoxyflavanone, isovitexin and amodiaquine. The non-activity of this compounds on several pathogens while their noticeable drastic effect on *Phytophtora* zoospores germination, mycelial anastomosis, sporangial formation and causing enlarged hyphal tips, dwarfness to the hyphal length. This results suggest that *A. flavipes* compounds are considered potentially as antiphytophthoral. Moreover, [89] described an antifungal metabolite, oosporein, which was isolated from the liquid culture of *Verticillium psalliotae* that produced the antagonistic effects on *P. infestans*. Oosporein exhibited a significant growth-inhibitory effect

on *P. infestans* in comparison with other phytopathogenic fungi. De Vries et al. [90] found that Out of an analysis of 12 fungal endophytes, *Phoma eupatorii* isolate 8082 and *Monosporascus* spp., inhibited the growth of *P. infestans* in co-culture using the agar diffusion assays, co-inoculation in planta and anthocyanin, presumably through the secretion of secondary metabolites, particularly since their culture extracts were also active. Furthermore, the study reported that the two of the endophytes exhibited global inhibition of nine European *P. infestans* isolates. These examples indicate that many fungi species as a beneficial microbes are also characterized with high potential to control *P. infestans* directly by antagonistic activity which inhibit the mycelia growth and the zoospore/zoosporangia germination via the production of a range of biocompounds and by the induction of defense mechanisms. Nonetheless, the use of beneficial fungi as a potential candidate to be more studied and tested as a novel biocontrol agent in the field providing an alternative to resistance gene breeding and application of agrochemicals.

5. Mode of action

As mentioned early, previous investigations highlight the importance of fungi and bacteria as biological control against *P. infestans*. Thus, gaining insight into mechanisms is of high importance for disease control. It is reported that microorganisms engage several antagonistic mechanisms against plant pathogens, including antibiosis, mycoparasitism, competition for nutrients and space, promotion of plant growth, induced plant defense mechanisms, and modification of environmental conditions. Among those mechanisms, the antibiosis refers to interaction lethal between microorganisms through secondary metabolites, which is of high importance to identify target cell, protein or enzyme, in concrete, implicated in the mechanism. Moreover, identification of chemical substance responsible on inhibiting of plant pathogens is a task challenge, due to volatility of compounds and their synergetic effects. Until now, fewer compounds from microorganisms were shown to effectively affect *P. infetans*. These include Phenazine-carboxylic acid [91], Oosporein [92], β-Rubromycin [93], Iturin A [94], Fenngycin A' [95, 96], Thiobutacin [97], Bikaverin [98], Fusaric acid [98], 2,5-diketopiperazine [99] and Xenocumacine 1 [100], listed in **Figures 1** and **2**. Moreover, detailed mechanism of interaction against *P. infestans* was developed only with β-Rubromycin, Iturin A and phenazine-1-carboxylic acid. β-Rubromycin belongs to the quinone antibiotics that have the ability to inhibit retroviral reverse transcriptase but also act as inhibitors of DNA polymerases [101]. [94] evaluated the activity of β-Rubromycin produced by *Streptomyces* isolated from soil against *P. infestans*, showing that this compound was capable of inhibiting the infection caused by sporangia and zoospores in tomato plants. The mechanism of action seems to be related to the up regulation of the RIO kinase-like gene that are involved in morphological development, altering processes as important in *P. infestans* as cyst germination and hyphal elongation. [95] evaluated the biocontrol capacity of *Bacillus subtilis* WL-2 against *P. infestans*, establishing that Iturin A was the metabolite involved in the inhibition capacity against this phytopathogen, causing cell membrane disruption and an irregular internal cell structure. Iturin A is a lipopeptide that exerts its antimicrobial action through the alteration of the cell membrane via the production of pores that generate osmotic perturbation [102]. In addition to its activity in the membrane it was observed that iturin A was capable of generating mitochondrial damage in *P. infestans*, causing oxidative stress and alterations in the respiratory chain which alter ATP synthesis. [54] reported the effect of phenazine-1-carboxylic acid (PCA) produced by a strain of *Pseudomonas fluorescens* on the transcriptome of *P. infestans*, establishing that this

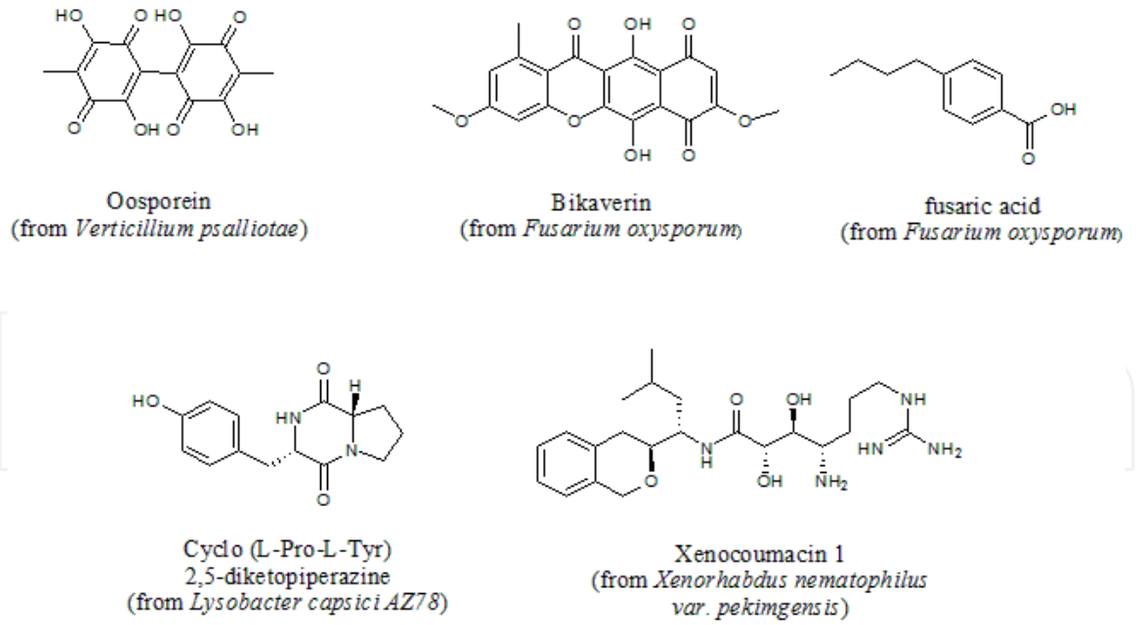

Figure 1.
*Anti-*Phytophthora infestans *compounds produced by fungi microorganisms.*

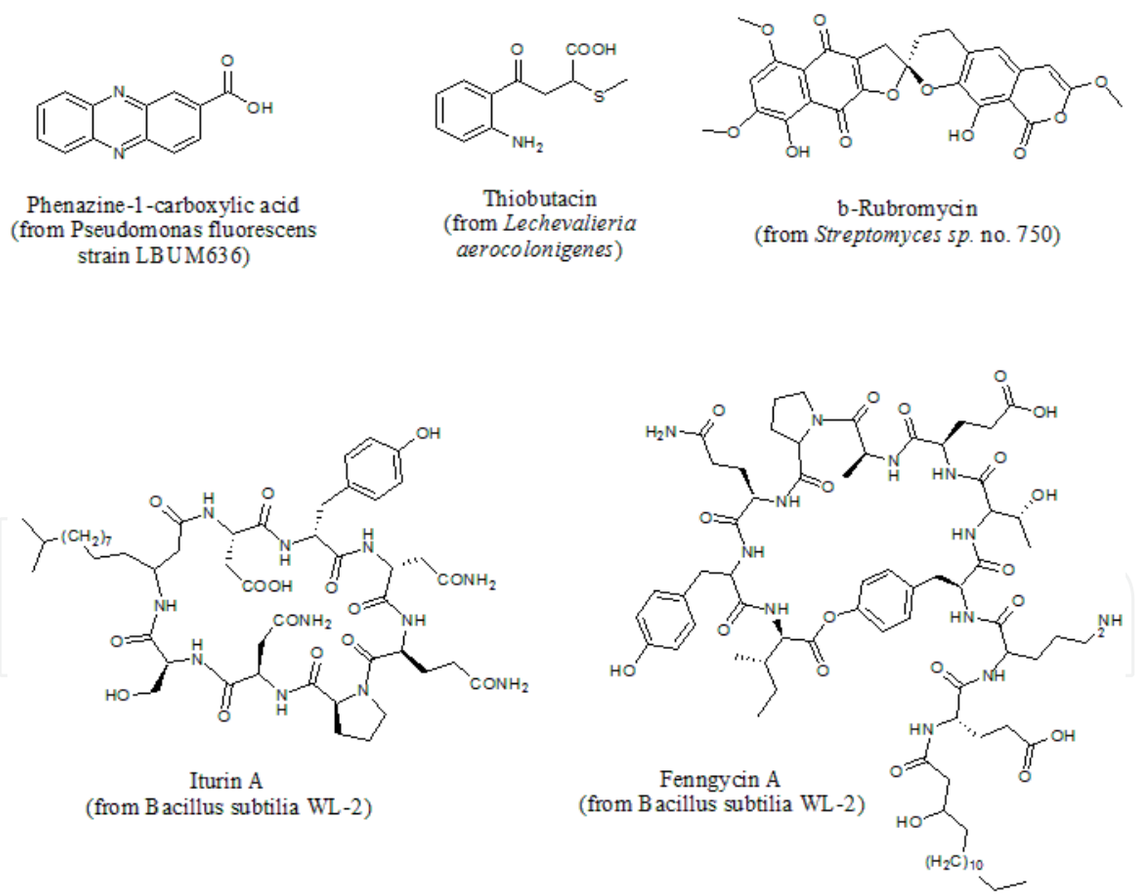

Figure 2.
*Anti-*Phytophthora infestans *compounds produced by bacteria microorganisms.*

compound alters the expression of genes involved in functions like phosphorylation mechanisms, transmembrane transport and oxydo-reduction activities.

Another method of disease control, so-called Mycroparasitism, is able to antagonize plant pathogens and promote plant growth by treatment with other microorganisms. Mycoparasitism is a direct mechanism in which microogranism colonizes the

pathogen through detection, parasitization and growth actions [103, 104]. This protection strategy has been recognized as an important mechanism of biological control. Mycoparasitics such as the oomycete *Pythium oligandrum* [105], *Pythium periplocum* [106] and different species from *Trichoderma* including *T. asperellum*, *T. atroviride*, *T. virens*, and *T. harzianum* are successfully used against *P. infetans*. These mycoparasitic grow faster than their pathogenic plant counterparts, which means that they can occupy rhizosphere space and nutrition, thus promoting both plant growth [107] resistance in host plants [108, 109]. The mechanism of Trichoderma spp., for example, appear to be very complex involving the detection of plant pathogen through chemotropism; lysis of the pathogen's cell wall (the key to mycoparasitism) [110]; pathogen's hyphal penetration by appresorial formation; production of cell wall-degrading enzymes (CWDEs) and peptaibols, mediated by heterotrimeric G-proteins and mitogen-activated protein (MAP) kinases [111]; and parasitizing pathogen's cell wall contents [112]. Degradation of pathogen's cell wall during mycoparasitism is mediated by a set of hydrolytic enzymes including β-(1,6)-glucanases, chitinases, and proteases. Several members from each of these classes have been shown to be involved in mycoparasitism and/or to be induced under mycoparasitism-related growth conditions [113]. Although these microorganisms demonstrate their potential as mycoparasitic biological control agents, fewer mechanistic studies have been investigate the molecular or genetic determinants of their mycoparasite lifestyle.

However, rather than directly expanding into infected plant, microorganism might compete with the pathogen producing secondary metabolites able to partially or totally inhibit the pathogenic fungi. This classical mechanism occurs when special and nutritious resources are limited. Consequently, the antagonistic microorganisms feed on the available resources for growth, causing therefore a reduction in the growth of the pathogens. A published example of metabolite-pathogen protection is that produced by *Phoma eupatorii* 8082. This endophyte has a remarkable potential to produce the anthocyanin product [114]. The latter could be produced as a result of a stress response positively regulated by jasmonic acid [115–118]. Hence, it is possible that tissue colonization with *Pho. Eupatorii* induce jasmonic acid dependent defense responses, which may play a role in the inhibition of the *P. infestans* infection. Indeed, [119] reported that jasmonic acid induced reduction of infection in the leaves of tomato and potato plants and [120] testified the mandatory existence of jasmonic acid to activate the defensive responses elicited by a peptide secreted by *P. infestans*. Some microorganisms including *Trichoderma* spp., produce inorganic compounds able to alter soil pH and therefore able to modify micronutrients (phosphate, iron and Manganese) [121]. In these extreme conditions *Trichoderma* spp., were able to produce various kinds of Siderophore products [122], including: caprogens, ferrichromes and fusarinines [123], thanks to the change in non-ribosomal peptide synthetase products and diverse non-ribosomal peptide synthetase-encoding genes [124]. Siderophores play a dual role, an antagonistic agent by inhibiting or even suppressing the growth of pathogens by divesting source of iron, as well as an agonist agent that helps to solubilize iron that was not available to the plant. These abilities explain the competition mechanism on the nutrient resources.

Alternative mechanism of disease control against attack of pathogens is based on the induction of systemic and local resistances [125]. Such resistances result from complex interactions between plants and antagonist elicitors, provoking physiological and biochemical alteration of cells. Indeed, two major kinds of systemic resistances have been studied; systemic acquired resistance (SAR) [126] and induced systemic resistance (ISR) [127]. Both systemic resistances are based on distinct phytohormonal signals. Various compounds have been proposed as potential signals for systemic resistances activation. The non-protein amino acid, β-Aminobutyric acid

(BABA), is known to induce resistance against various pathogens on a wide range of plants. Indeed, DL- β-Aminobutyric acid-induced resistance of potato against late blight pathogen *P. infestans* trough the signaling compound salicylic acid [128]. BABA also provided significant control against *P. infestans* on tomato [129]. The systemic defense is induced in a salicylic acid dependent manner; furthermore various inorganic chemicals including indole acetic acid, di-potassium hydrogen orthophosphate, hydrogen peroxide, calcium chloride, ferric chloride and metalaxyl were able to induce resistance against the disease caused by *P. infestans*. Treatment with those agents promotes the synthesis of defense enzymes like peroxidase, polyphenol oxidase (POX) and phenylalanine ammonia lyase (PAL) [130]. In addition, Curdlan b-1,3-Glucooligosaccharides has shown to enhance plant resistance against the pathogen *P. infestans* in foliar tissues of potato (*Solanum tuberosum* L. cv. McCain G1) by accumulation of H2O2 and salicylic acid and the activities of phenylalanine amino-lyase, b-1,3-glucanase and chitinase [131].

6. Conclusion

The application of beneficial bacteria and fungi as biocontrol agents is an interesting building block of sustainable and environmentally sound management strategies of *Phytophtora infestans*. A holistic approach should be considered to reach satisfactory levels of *P. infestans* control by a beneficial microbes. Based on the number of currently known isolates with biocontrol activity against *P. infestans*, the predominant genera are *Pseudomonas*, *Bacillus*, *Streptomyces*, *Trichoderma* and *Penicillium*. The ability to affect survival structures, sharing the same ecological niche as *Phytophtora*, inducing resistance responses in the plant and promoting plant growth are desirable characteristics of a competent BCA against *P. infestans*. However, among several criteria the potential bottlenecks such as large-scale production, formulation, preservation conditions, shelf life, application methods, and combination potentiality of one or more microbes should be tackled early in the selection process.

Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

BABA	Acide bêta-aminobutyrique.
BCA	Biological control agent.
CWDE	Cell wall degrading enzymes.
HCN	Hydrogen cyanide.
ISR	Induced systemic resistance.
IAA	Indole acetic acid.
MAP	Mitogen-activated protein.
PAL	Phenylalanine ammonia lyase.
PCA	Phenazine-1-carboxylic acid.
PGPM	Plant growth promoting microbes.
POX	Peroxidase.
PR-protein	Pathogenesis related protein.
SAR	Systemic acquired resistance.

TLC	Thin layer chromatography.
VFA	Virdiofungin A.
VOC	Volatile organic compounds.

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