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Biological Control of Oomycete Soilborne Diseases Caused by *Phytophthora capsici*, *Phytophthora infestans*, and *Phytophthora nicotianae* in Solanaceous Crops

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ABSTRACT

Oomycete pathogens that belong to the genus *Phytophthora* cause devastating diseases in solanaceous crops such as pepper, potato, and tobacco, resulting in crop production losses worldwide. Although the application of fungicides efficiently controls these diseases, it has been shown to trigger negative side effects such as environmental pollution, phytotoxicity, and fungicide resistance in plant pathogens. Therefore, biological control of *Phytophthora*-induced diseases was proposed as an environmentally sound alternative to conventional chemical control. In this review, progress on biological control of the soilborne oomycete plant pathogens, *Phytophthora capsici*, *Phytophthora infestans*, and *Phytophthora nicotianae*, infecting pepper, potato, and tobacco is described. Bacterial (e.g., *Acinetobacter*, *Bacillus*, *Chryseobacterium*, *Paenibacillus*, *Pseudomonas*, and *Streptomyces*) and fungal (e.g., *Trichoderma* and arbuscular mycorrhizal fungi) agents, and yeasts (e.g., *Aureobasidium*, *Curvibasidium*, and *Metschnikowia*) have been reported as successful biocontrol agents of *Phytophthora* pathogens. These microorganisms antagonize *Phytophthora* spp. via antimicrobial compounds with inhibitory activities against mycelial growth, sporulation, and zoospore germination. They also trigger plant immunity-inducing systemic resistance via several pathways, resulting in enhanced defense responses in their hosts. Along with plant protection, some of the microorganisms promote plant growth, thereby enhancing their beneficial relations with host plants. Although the beneficial effects of the biocontrol microorganisms are acceptable, single applications of antagonistic microorganisms tend to lack consistent efficacy compared with chemical analogues. Therefore, strategies to improve the biocontrol performance of these prominent antagonists are also discussed in this review.

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solanaceous crop

1. Introduction

Solanaceous crops such as tomato, potato, eggplant, tobacco, and pepper are economically important worldwide; however, their production is significantly limited by numerous plant diseases [1–3]. Diseases of solanaceous crops are generally caused by fungal pathogens, with the highest disease rates occurring in oomycete soilborne pathogen-infected crops.

Microorganisms belonging to class Oomycetes are distinct from what is now called the true fungi [4]. Unlike the true fungi, oomycetes are characterized by the presence of two flagella that enhance the swimming ability of zoospores [5]. Moreover, the vegetative stage of oomycetes is diploid, whereas that of fungi is haploid or dikaryotic [6]. Further, cellulose rather than chitin comprises cell walls of oomycetes, making them more closely related to plants than fungi [7]. Control techniques for true fungal pathogens have been extensively researched and numerous approaches, mostly based on cell wall

decomposition or antibiosis, have been developed. However, owing to the difference in oomycete cell wall structure, a different strategy is required to control oomycetes-initiated diseases.

Depending on the inoculum source, plant pathogens can be divided into soilborne (e.g., *Phytophthora* and *Pythium*) and airborne (e.g., *Alternaria* and *Botrytis*) categories. Control of soilborne and airborne diseases is carried out in a similar way; however, several important differences must be noted. For instance, the application of liquid control agent formulations is suitable for airborne diseases as they are applied directly on the plant tissues. However, in soilborne disease management, soil particles complicate the delivery of active ingredients to the plant surface, impeding the protection of host plants from pathogen propagules. Moreover, the rhizosphere environment provides additional nutrients for plant pathogens and other soil microbes in the form of root exudates.

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Therefore, the rhizosphere can represent a more favorable environment for pathogen survival. Additionally, the harsh environmental conditions of the phyllosphere (humidity fluctuations, UV radiation, etc.) are not present in the soil environment, making it harder to control soilborne pathogens.

Historically, plant disease management shifted from more conventional measures represented by cultivation techniques (e.g., water management, crop rotation, and eradication of inoculum sources) to the application of synthetic fungicides. Although cultivation techniques greatly contribute to plant disease management, they are unable to provide sustainable control of disease outbreaks. In contrast, pesticides with a wide spectrum of action against various pathogens provide efficient and sustainable protection of plant health and production yield. Even though fungicide treatment is still the most efficient control strategy, its application has resulted in a number of negative environmental side effects [8–10]. Hence, more environmentally sound alternatives are needed. Soon after the introduction of chemical agents to agriculture, the problem of toxicity to non-target organisms, including host plants themselves, was detected [11]. Furthermore, the application of uncontrolled amounts of fungicides has resulted in their accumulation in the environment (including water reservoirs and soil) and led

to the poisoning of vertebrate animals and even humans [8]. Additionally, accumulation of chemical fungicide residues in plant tissues makes their consumption unsafe. Microorganisms that naturally inhabit soil environments have been proposed as antagonistic agents against plant pathogens that are capable of controlling disease outbreaks. Thus, a biocontrol approach involving the replacement of fungicides with microorganisms that demonstrate outstanding biocontrol performance without toxicity to the environment is seen as promising.

An even more important reason for biocontrol demand is the constantly increasing number of pathogens that are developing resistance to chemical fungicides [12,13]. As most synthetic fungicides act as single site-targeted molecules, genetic mutations in this site can lead to the tolerance of previously susceptible pathogens to those fungicides. In contrast, living organisms inhibit *Phytophthora* pathogens simultaneously through several mechanisms that postpone resistance development. In general, biocontrol agents (BCAs) function *via* different mechanisms (Figure 1): production of metabolites with antifungal properties, induction of plant resistance, and competition for nutrients [14–17]. Antifungal molecules can be diffused into a medium (diffusible antimicrobials) or the air (volatile antimicrobials) [15,16,18–23]. Such molecules can inhibit

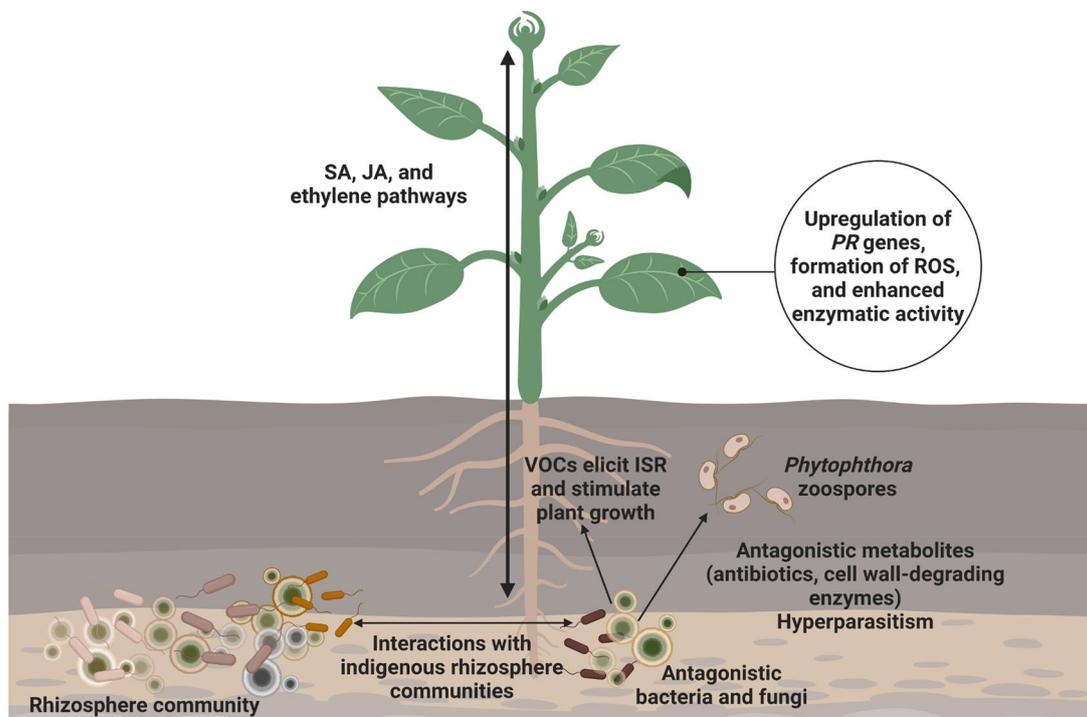


Figure 1. Actions of various microbes antagonistic against *Phytophthora* spp. Antagonistic microbes introduced to the plant rhizospheres can colonize roots, where they interact with plants, *Phytophthora* spp., and indigenous microbes. These antagonists release various metabolites [e.g., antibiotics or volatile organic compounds (VOCs)], directly inhibiting growth of *Phytophthora* spp. populations and/or inducing induced systemic resistance (ISR) in plants. Resistance in plants can be achieved *via* salicylic acid (SA), jasmonic acid (JA), or ethylene pathways. Pathogenesis-related (PR) genes are upregulated as a result of plant resistance induction. The plant defense response includes accumulation of reactive oxygen species (ROS) and/or enhanced chitinase, β -1,3-glucanase, and peroxidase enzymatic activities. The graph was created using BioRender.com.

mycelial growth [14,24–26], sporulation [19,23,27,28], zoospore germination [19,29,30], or a combination of these activities, functioning similarly to antibiotics or fungicides. Other extracellular metabolites include enzymes that degrade pathogenic cell wall or cell membrane components, disrupt cell integrity, and induce cell death [31,32]. Plant resistance elicited by bacterial molecules allows plant tissues to be primed for potential pathogenic attack, resulting in a faster response when the inoculation occurs [33,34]. Moreover, consumption of available nutrients by beneficial microbes results in starvation of pathogens and prevents further disease development [17,35].

Yield increase and growth promotion can be seen as positive side effects of biocontrol treatment [33,36]. Some microbial agents used for protective measures can increase crop production in terms of the number and weight of fruits [36]. Plant-growth promoting metabolites can also contribute to plant disease management. Nitrogen fixation, phosphate solubilization, and siderophore production increase plant nutrient supply [37,38]. Additionally, increased consumption of the minerals from soil lowers their availability to pathogenic organisms. Thus, plant growth promotion by stimulation of mineral consumption is closely associated with nutrient competition with pathogens [39–41]. The production of metabolites similar to phytohormones is another mechanism of plant promotion by BCAs [42]. These molecules are multifunctional, affecting both plant biomass increase and primed resistance to pathogen invasion.

Several *Phytophthora* species, such as *Phytophthora capsici*, *Phytophthora infestans*, and *Phytophthora nicotianae*, are known to infect solanaceous crops, resulting in yield reduction or complete death of plants [40,43,44]. These pathogens heavily infect eggplant, pepper, potato, tobacco, and tomato crops, which are important cash crops worldwide [45]. Pathogens originating from soil, water splash, or irrigation water cause disease at all growth stages owing to their soilborne nature [45]. During their disease life cycle, *Phytophthora* spp. typically infect plants asexually; however, sexual spores such as oospores occur rarely and infect hosts (Figure 2). Depending on the environmental temperature, sporangia can germinate either directly by forming a germ tube or *via* zoospores.

The most common sources of biocontrol agents that control these pathogens are plant tissues and the rhizosphere [38,46–49]. The microorganisms adapted to coexist with plants are more likely to have developed mechanisms of plant protection. Bacteria belonging to the genera *Bacillus* and *Pseudomonas* are common soil inhabitants and have

been extensively reported as plant growth-promoting rhizobacteria (PGPR) and biocontrol agents [23,50–52]. Additionally, numerous other organisms have proven their efficiency in the control of solanaceous crop diseases. Several strains of *Chryseobacterium* have been reported as biocontrol agents for *Phytophthora* blight of pepper [48,53,54]. *Trichoderma*, the most common fungus with antagonistic properties against fungal pathogens, is often applied alone [17] or in combination with other microbial agents [14]. In this review, progress on the development of biocontrol strategies against *P. capsici*, *P. infestans*, and *P. nicotianae* infecting pepper, potato, tobacco, and tomato crops is summarized. In addition, approaches to enhance the biocontrol performance of successful BCAs are discussed.

2. Biological control of *Phytophthora capsici*

2.1. Source of bacterial biocontrol agents

Several bacterial BCAs, mainly *Bacillus* spp. and *Pseudomonas* spp. [19,41,42,55,56], have been reported to control *Phytophthora* blight of pepper caused by *P. capsici* (Table 1) [14–17,19,21–26,30,33,34,38–42,46–49,51,53,56–73]. Rhizosphere soil is one of the most common sources of potential biocontrol agents. This niche is typical of *Bacillus* spp. and *Pseudomonas* spp.; therefore, it comes as no surprise that these groups are the most abundant among rhizospheric microbes [38,46,47]. For instance, Li et al. [74] reported that *Bacillus* spp. and *Pseudomonas* spp. are predominant in the rhizosphere of peppers under various cultivation conditions.

Although the majority of BCAs are isolated from the rhizospheres or root interiors of plants [19,38,46,47,56,60], phyllospheres could serve as alternative BCA sources [48,49]. Yang et al. [48] reported that majority of bacterial strains with antagonism to *P. capsici* could be obtained from phyllospheres. The proteolytic activity of the isolated microbes was more common in leaf interior strains, suggesting enzyme production of the strains is an inhibition mechanism. On the other hand, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production is an additional mechanism of plant growth stimulation [75]. ACC deaminase is an enzyme that reduces levels of ethylene in plants, and thus inhibits stress-induced plant growth stunting. ACC deaminase-producing strains R13 and R33 of *Bacillus subtilis* promoted root and shoot growth of red pepper and provided significant protection against *Phytophthora* blight [76]. Another biocontrol strain K11 of *Bacillus licheniformis* increased

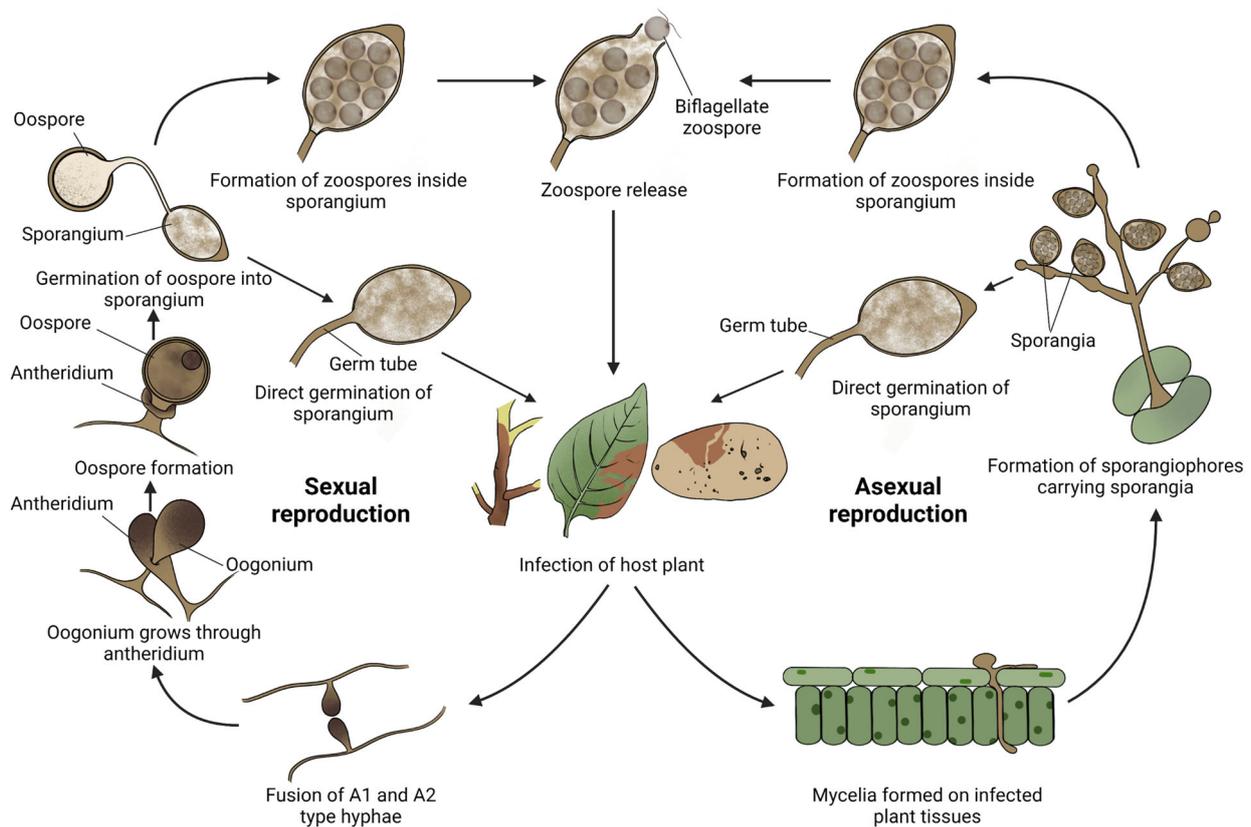


Figure 2. Disease cycle of soilborne diseases of solanaceous crops, such as pepper, potato, and tobacco, caused by *Phytophthora* spp. A host plant is infected by either germinated sporangium or zoospores. Asexual reproduction in infected plants commonly occurs, whereas sexual reproduction rarely takes place. Asexual reproduction is carried out through mycelia formation with sporangiophore production in infected tissues. Sporangiophores produce sporangia that can germinate directly by forming germ tubes (at high temperatures: 20–23 °C) or biflagellate zoospores (at low temperatures: 12–15 °C). Germinated sporangia form appressoria for penetration of plant tissues. Mycelia invade plant tissues intra- and intercellularly and also form haustoria, which allow the pathogen to obtain nutrients from infected plants and enable mycelial growth. In sexual reproduction, which often occurs between A1 and A2 mating types, the hyphae fuse and oogonium grows through the antheridium forming an oospore using the antheridium as a source of nutrients and genetic material. Oospores covered with thick walls can withstand harsh environments and they germinate into sporangium under favorable environmental conditions. The graph was created using BioRender.com.

pepper resistance to drought stress *via* ACC deaminase synthesis [77,78].

2.2. Bacterial biocontrol agents

2.2.1. Acinetobacter

Antibiotics of *Acinetobacter* spp. have also shown antagonistic activity against *Phytophthora* similar to that of *Bacillus* spp. [57]. Iturin A isoforms synthesized by *Acinetobacter baumannii* are effective against *P. capsici* *in vitro* [57]. However, no results on its performance in the plant-BCA-pathogen system are available; thus, further studies are required. In addition, further studies may reveal higher activity in *Acinetobacter*-derived antibiotics as they tend to outperform biocontrol by *Bacillus* spp. For instance, *Acinetobacter* sp. effectively reduced *P. capsici* populations in chili pepper and consequently reduced disease severity to a higher extent than *Bacillus* BCAs used in the same study [38].

2.2.2. Bacillus

Bacillus spp. are dominant among rhizospheric inhabitants with antagonistic activity against *P. capsici*. In some cases, *Bacillus* antagonists are known to exceed 50% of the total antagonistic consortium [74]. Rhizosphere soil is characterized by considerably poor nutrient conditions and high competition among soil microorganisms. Therefore, *Bacillus* spp. naturally inhabiting rhizospheres often provide high *in vitro* antagonism and sustainable *in vivo* performance owing to their high fitness in the niche. Recently, Ngo et al. [47] reported on the high *in vitro* inhibition activity (more than 60% inhibition rate) of the pepper rhizosphere strains *Bacillus siamensis*, *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus methylotrophicus*. These BCAs can also inhibit more than 90% of lesion development caused by *P. capsici* on pepper shoots.

Although *Bacillus* spp. can function *via* several mechanisms, their most efficient trait is the production of antibiotics; these species are well-known

Table 1. List of biocontrol agents (BCAs) and their antagonistic activities against *Phytophthora capsici*.

Type of BCA	Genus	Species and strains	Activity antagonistic to the target pathogen	Reference
Bacteria	<i>Acinetobacter</i>	<i>A. baumannii</i> LCH001 <i>Acinetobacter</i> sp. UQ202	Mycelial growth inhibition Mycelial growth inhibition; hyphal abnormalities formation; emission of VOCs ^a with inhibitory effect on mycelial growth	[57] [38,58]
	<i>Bacillus</i>	<i>B. amyloliquefaciens</i> BS211, EB.DC6, EB.DL1, EB.DM3, FY11, IBFCBF-1, PsL, UQ154, ZY44 <i>B. licheniformis</i> BL06, HS10 <i>B. megaterium</i> IISRBP17 (=BP17) <i>B. methylotrophicus</i> EB.KN13 <i>B. siamensis</i> EB.CP36 <i>B. subtilis</i> Bs 8B-1, HS93, S25 <i>B. thuringiensis</i> IMC8 <i>B. vallismortis</i> Ps, ZZ185 <i>B. velezensis</i> EB.KN12, UQ156 <i>B. cepacia</i> MPC7 <i>Chromobacterium</i> sp. C-61 <i>Chryseobacterium</i> sp. R98 <i>C. phosphatilyticum</i> ISE14 <i>C. wanjijense</i> KJ9C8 <i>F. anhuiense</i> GSE09	Mycelial growth inhibition Mycelial growth inhibition; hyphal abnormalities formation; emission of VOCs ^a with inhibitory effect on mycelial growth; protease enzymatic activity; siderophore production; root colonization; emission of VOCs ^a with inhibitory effect Mycelial growth inhibition; chitinase and protease enzymatic activity Mycelial growth inhibition; chitinase and protease enzymatic activity Mycelial growth inhibition; hyphal abnormality formation; production of antibiotics, HCN ^e , IAA ^d , and siderophores; cellulase, chitinase, glucanase, and protease enzymatic activities; emission of VOCs ^a with inhibitory effect on mycelial growth; root colonization Mycelial growth inhibition Mycelial growth inhibition Mycelial growth inhibition; hyphal abnormality formation; cellulase, chitinase, and protease enzymatic activities; emission of VOCs ^a with inhibitory effect on mycelial growth Mycelial growth inhibition by benzoic acid and phenylacetic acid; hyphal abnormality formation Mycelial growth inhibition; chitinase enzymatic activity Mycelial growth inhibition; cellulase and protease enzymatic activities Root colonization; biofilm formation; IAA ^d production Protease enzymatic activity; HCN ^e production; swarming activity; root colonization Emission of VOCs ^a with inhibitory activity against mycelial growth, sporulation, and zoospore germination; protease enzymatic activity; swimming and swarming activities; root colonization Mycelial growth inhibition; chitinase enzymatic activity; emission of VOCs ^a with inhibitory activity against mycelial growth, sporulation, and zoospore germination Mycelial growth inhibition; ISR ^b induction Mycelial growth inhibition Root colonization; swimming and swarming activities; chemotaxis to root exudates; biofilm formation; tolerance to H ₂ O ₂ Inhibition of mycelial growth, sporangia production, zoospore release and germination; biosurfactant production; siderophore, HCN ^e , and IAA ^d production; protease enzymatic activity; emission of VOCs ^a with inhibitory effect on mycelial growth and sporangia production Mycelial growth inhibition; root colonization (epiphytic and endophytic); emission of VOCs ^a with inhibitory effect on mycelial growth Mycelial growth inhibition; hyphal abnormality formation; inhibition of sporangia formation, zoospore release and zoospore motility; zoospore lysis; biosurfactant production; siderophore, HCN ^e , and IAA ^d production; protease enzymatic activity Mycelial growth inhibition; chitinase enzymatic activity Mycelial growth inhibition; hyphal abnormality formation; chitinase and β -1,3-glucanase enzymatic activities Mycelial growth inhibition; hyphal abnormality formation; zoospore germination inhibition; synthesis of borrelidin inhibiting mycelial growth Mycelial growth inhibition; cellulase and protease enzymatic activities; ISR ^b induction	[38,47,49,51,56,58,59] [19,60] [21,46] [47] [47] [16,42,61] [51,62] [51,62] [38,47,58] [63] [64] [48] [41,65] [53] [22,66,67] [64,67] [34] [14,46] [23] [40,68] [46,69,70] [26,40] [64] [25] [15] [33,39]
	<i>Burkholderia</i>			
	<i>Chromobacterium</i>			
	<i>Chryseobacterium</i>			
	<i>Flavobacterium</i>			
	<i>Lyso bacter</i>	<i>L. enzymogenes</i> C-3, ISE13	Mycelial growth inhibition; chitinase enzymatic activity; emission of VOCs ^a with inhibitory activity against mycelial growth, sporulation, and zoospore germination	[64,67]
	<i>Paenibacillus</i>	<i>P. polymyxa</i> SC09-21	Mycelial growth inhibition	[34]
	<i>Pseudomonas</i>	<i>P. aeruginosa</i> BJ10-86, IISRBP35 <i>P. corrugata</i> CCR04, CCR80 <i>P. fluorescens</i> 3k9, 3ss9, 6ba6, 6L10, 6L14, IISR-6, IISR-8, IISR- 11, IISR-13, IISR-51, ke, mbj <i>P. putida</i> IISRBP25 (=BP25) <i>Pseudomonas</i> sp. B-1, B-10, B-17, 3tg8, 6ba2, 6ba3, 6t14 <i>S. plymuthica</i> C-1 <i>S. griseus</i> H7602 <i>S. plicatus</i> B4-7 <i>S. rochei</i> IT20	Mycelial growth inhibition Root colonization; swimming and swarming activities; chemotaxis to root exudates; biofilm formation; tolerance to H ₂ O ₂ Inhibition of mycelial growth, sporangia production, zoospore release and germination; biosurfactant production; siderophore, HCN ^e , and IAA ^d production; protease enzymatic activity; emission of VOCs ^a with inhibitory effect on mycelial growth and sporangia production Mycelial growth inhibition; root colonization (epiphytic and endophytic); emission of VOCs ^a with inhibitory effect on mycelial growth Mycelial growth inhibition; hyphal abnormality formation; inhibition of sporangia formation, zoospore release and zoospore motility; zoospore lysis; biosurfactant production; siderophore, HCN ^e , and IAA ^d production; protease enzymatic activity Mycelial growth inhibition; chitinase enzymatic activity Mycelial growth inhibition; hyphal abnormality formation; chitinase and β -1,3-glucanase enzymatic activities Mycelial growth inhibition; hyphal abnormality formation; zoospore germination inhibition; synthesis of borrelidin inhibiting mycelial growth Mycelial growth inhibition; cellulase and protease enzymatic activities; ISR ^b induction	[23] [40,68] [46,69,70] [26,40] [64] [25] [15] [33,39]

(continued)

Table 1. Continued.

Type of BCA	Genus	Species and strains	Activity antagonistic to the target pathogen	Reference
Fungi	<i>Trichoderma</i>	<i>Streptomyces</i> sp. IISRBPAct1, IIRPBAc25, IISRBPAct42	Mycelial growth inhibition; cellulase, lipase, and protease enzymatic activities	[71]
		<i>S. vinaceusdrappus</i> S514	Mycelial growth inhibition; cellulase and protease enzymatic activities; ISR ^b induction	[33,39]
		<i>T. asperillum</i> T34	Inhibition of zoospore germination	[17]
		<i>T. brevicompactum</i>	Mycelial growth inhibition	[24]
		KACC40931, KACC41707	Mycelial growth inhibition with gliotoxin	[72]
		<i>T. doratopsis</i> HZA14	Mycelial growth inhibition	[14]
		<i>T. hamatum</i> THSW13	Mycoparasitism: inhibition of hyphal growth, disintegration of hyphae; hyphal abnormality formation	[73]
		<i>T. harzianum</i>	Mycelial growth inhibition; ISR ^b induction	[24]
		<i>T. koningiopsis</i> KACC40553	Mycelial growth inhibition; ISR ^b induction	[24]
		<i>T. petersenii</i>	Mycelial growth inhibition; ISR ^b induction	[24]
		KACC40552, KACC40557	Mycelial growth inhibition	[24]
		<i>T. virens</i> KACC40717, KACC40929	Mycelial growth inhibition	[24]
		Unclassified Ascomycete GPUAT	Mycelial growth inhibition; inhibition of zoosporangia formation and zoospore germination	[30]

^aVolatile organic compounds.

^bInduced systemic resistance.

^cHydrogen cyanide.

^dIndole-3-acetic acid.

producers of antibiotic metabolites with a wide range of actions [18,20,62,79–81]. *Bacillus licheniformis* BL06 was reported to have a variety of inhibitory mechanisms against *P. capsici*, including lysis of hyphae and zoospores and inhibition of sporangia development [19]. Zoospore motility, germination, and germ tube elongation were significantly inhibited and the bacterial treatment caused final lysis of zoospores. These inhibitory properties are attributed to an antifungal protein identified as carboxypeptidase [60].

Moreover, *Bacillus* spp. are known to have dualistic characteristics such as biocontrol and plant growth-promoting activities. For example, *B. amyloliquefaciens* strain IBFCBF-1 was reported to show strong biocontrol performance against *P. capsici* while also significantly promoting pepper growth [56]. Phosphate solubilization and ammonium and indole acetic acid (IAA) production were also found to be possible mechanisms of growth promotion [56].

2.2.3. Chitinolytic bacteria

Consideration of the metabolism traits of BCAs can greatly benefit plant protection efforts. Addition of favorable substrates that are involved in BCA metabolism to the soil environment results in higher BCA growth rates. This is particularly the case for agents with a high level of enzyme production. For example, chitin-degrading microbe populations can be easily increased by introducing external chitin to the environment. Additionally, although chitin is not a component of the mycelial cell walls of *Phytophthora* spp., it is a key component of their zoospores [27]. Thus, chitin-degrading microbes can also contribute to disease management at the sporulation level. Chitinolytic bacteria (*B. licheniformis* LS674 and *B. subtilis* HS93) and *Trichoderma harzianum* were selected by Sid Ahmed et al. [61] based on their strong biocontrol performance in fields. Of these BCAs, only *B. subtilis* HS93 exhibited consistently successful performance in a 2-year greenhouse test, and chitin amendment enhanced its efficiency. Similarly, Kim et al. [64] developed an effective product consisting of three chitinolytic bacterial strains: *Serratia plymuthica* C-1, *Chromobacterium* sp. C-61, and *Lysobacter enzymogenes* C-3. They were batch-cultivated in a 1/5 diluted-chitin medium that proved to enhance the control of *Phytophthora* blight in fields, regardless of crop rotation and solarization in greenhouses as auxiliary control measures. Interestingly, the bacterial combination consisting of chitinolytic strains provided 66% control efficacy in *P. capsici*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Fusarium solani* simultaneously inoculated into soil

[64]. Similarly, chitinolytic bacteria applied in chitin-containing compost were shown to significantly reduce the disease severity of Phytophthora blight of pepper [82].

2.2.4. *Chryseobacterium*

Chryseobacterium as a biocontrol agent of *P. capsici* was first reported by Yang et al. [48]. In particular, *Chryseobacterium* sp. R98 was reported to have the highest biocontrol potential among 17 antagonistic strains, reducing 92.3% of Phytophthora blight severity [48]. Furthermore, strain R98 increased pepper biomass, indicating its PGPR potential. Subsequently, more reports on *Chryseobacterium* biocontrol efficiency against Phytophthora blight of pepper became available [41,53]. *Chryseobacterium wanjuae* KJ9C8 demonstrated high protective performance of pepper plants from Phytophthora blight infection, using colonization and production of proteolytic enzymes as possible biocontrol mechanisms [53]. Similarly, *Chryseobacterium phosphatilyticum* ISE14 significantly reduced Phytophthora blight severity and promoted pepper growth [41,65]. Strain ISE14 was also reported to promote phosphate solubilization significantly, proving its PGPR activity.

2.2.5. *Pseudomonas*

Another common bacteria represented among root endophytes is *Pseudomonas* [38,46]. *Pseudomonas corrugata* CCR04 and CCR80 isolated from the pepper rhizosphere were reported to be successful BCAs [23]. These results are supported by those of other reports on several *Pseudomonas* strains with strong mycelial growth inhibition [26]. These strains had antagonistic properties that targeted not only mycelial growth but also sporangia formation and zoospore release and motility. Similarly, *Pseudomonas aeruginosa* BJ10-86 and *Pseudomonas putida* BP25 strongly inhibited *in vitro* mycelial growth of *P. capsici* [14,70]. However, *Pseudomonas* spp. sometimes lack significant biocontrol efficiency [38]. Their antagonistic potential seems to be a strain-specific trait. Out of 100 strains of *Pseudomonas* spp. analyzed by Özyimal and Benlioglu [40], only 24 strains were able to inhibit mycelial growth in plate tests and only 4 strains demonstrated consistent blight control *in vivo*.

Pseudomonads synthesize secondary metabolites antagonistic to *P. capsici*, including proteolytic enzymes and volatile organic compounds (VOCs) [40,69,70]. Several reports highlight the importance of biosurfactants among other anti-*Phytophthora* metabolites in *Pseudomonas*-mediated biocontrol [29,40]. In particular, all biosurfactant-producing *Pseudomonas* spp. were able to reduce Phytophthora blight severity with no phytotoxicity to the host

plants [40]. *Pseudomonas*-derived surfactants are also known to have zoosporicidal activity, although their involvement in biocontrol is debatable [29]. Interestingly, despite the strong lytic activity of *Pseudomonas putida* strain 267 surfactants against *P. capsici* zoospores, they failed to inhibit not only the mycelial growth of *P. capsici*, but also that of other oomycete pathogens such as *P. infestans*, *Pythium aphanidermatum* and *Pythium ultimum*, suggesting that they target zoospores [29]. Despite evidence of *Pseudomonas*-derived extracellular metabolites being involved in biocontrol, a recent report showed that extracellular metabolites applied in the form of cell-free culture filtrates are less efficient in inhibition of zoospore release and motility and lysis than bacterial cell suspensions [26]. In addition to antimicrobials, *Pseudomonas* BCAs carry a wide range of biocontrol mechanisms, including high swimming and swarming activities [23], strong colonization ability [23,70], siderophore production [40,70], and hydrogen cyanide (HCN) emission [40]. Along with these traits, many *Pseudomonas* spp. are plant growth-promoting rhizobacteria (PGPR), functioning *via* IAA synthesis [40,70], phosphate solubilization [40], and nitrogen fixation [70], which makes them beneficial not only in terms of plant protection but also plant growth stimulation.

2.2.6. *Streptomyces*

Streptomyces BCAs belong to phylum Actinobacteria and are widely distributed in plant rhizospheres. Many studies have shown the high production level of secondary metabolites with antifungal properties produced by *Streptomyces* spp. [15,25,83,84]. For example, *Streptomyces plicatus* B4-7 culture filtrates containing borrelidin as an active antifungal ingredient inhibited mycelial growth and zoospore germination in *P. capsici* [15]. Borrelidin at 5 ppm caused abnormal branching and damage of *P. capsici* mycelia [15]. Correspondingly, crude extracts of *Streptomyces griseus* H7602 were shown to strongly inhibit *P. capsici* mycelial growth [25]. In addition to these antibiotics, a large share of actinobacterial cell metabolites accounted for cell wall-degrading enzymes. This *S. griseus* H7602 can contribute to disease control by producing chitinase, glucanase, lipase, and protease [25]. Thampi and Bhai [71] reported that three *Streptomyces* spp. isolated from pepper rhizospheres exhibited strong antifungal activities against several plant pathogens, including *P. capsici*. Further study on the possible mechanisms of antagonism of these species revealed that they produced lytic enzymes and siderophores, and stimulated nutrient solubilization [71]. Similar results were reported for *Streptomyces rochei* IT20 and *Streptomyces vinaceusdrappus* SS14, with

positive cellulase, chitinase, and protease production, and phosphorus solubilization [39]. Interestingly, the cellulolytic activity of *Streptomyces* strains was reported to be correlated with growth inhibition in *P. capsici* [39]. Therefore, *Streptomyces* spp. are promising BCAs as they tend to have better biocontrol performance than commonly applied fungicides [39]. However, *Streptomyces* antagonism against *P. capsici* varies between species [39]. Nevertheless, strains with proven efficiency can control Phytophthora blight not only in plants but fruits [33]. In addition, *Streptomyces* BCAs act as plant growth-promoting beneficial bacteria [33,39]. Further, owing to their mycelial form, *Streptomyces* spp. are able to exhibit hyperparasitism as a mechanism of biocontrol similar to that of *Trichoderma* species. The mycelium of *S. plicatus* B4-7 was reported to coil around *P. capsici* mycelia and produce its sporangia [15].

It should be noted that alterations to an indigenous microbial community are undesirable because changes in biodiversity might favor pathogenic survival in soil environments. However, soil applications of *Streptomyces* BCAs could increase the biodiversity of rhizosphere bacteria [33]. Increased diversity is associated with the enrichment of bacteria with soil suppressive and biocontrol properties and correlated with plant growth stimulation. The positive influence of *Streptomyces* spp. on rhizosphere communities has triggered interest in more in-depth studies on this subject.

2.2.7. Other bacteria

Other microbes that are less abundantly represented in the rhizosphere also have the potential for biological control. For instance, Aravind et al. [46] reported on the high antagonistic properties of *Micrococcus* sp. and *Curtobacterium* sp. against *P. capsici* *in vitro* and *in vivo*. *Burkholderia cepacia* mainly antagonizes *R. solani* [85,86], but its antagonism against *P. capsici* is poorly studied. *B. cepacia* strain MPC-7 antagonizes *P. capsici* with chitinolytic enzymes and two antimicrobial compounds: benzoic acid and phenylacetic acid [63]. Both anti-oomycete compounds were also proven to be antagonistic to pathogenic bacteria, yeasts, and fungi.

Screening of new biocontrol agents with more pronounced antagonism against plant pathogens sometimes leads to their discovery among unclassified organisms. A report on the antagonism of an unclassified Ascomycete and its filtrate against *P. capsici* is one such example [30]. This Ascomycete had a fungistatic effect on *P. capsici* growth, which was achieved with relatively thermostable unidentified metabolites. Additionally, it was able to

completely inhibit formation of zoospore germination and zoospore germination at certain concentrations.

2.3. Fungal biocontrol agent: *Trichoderma*

Some species of *Trichoderma* are biocontrol fungi efficient against *P. capsici* [17,24]. They often exhibit several direct and indirect mechanisms of biocontrol, resulting in strong antagonism. Indirect mechanisms include competition for nutrients and space, and secondary metabolites with antagonistic modes of action [17,72]. Direct mechanisms refer to mycoparasitism or hyperparasitism of the fungal species. *Trichoderma* mycelia make direct contact with pathogenic hyphae, sometimes coiling around them [73]. Lytic enzymes abundantly generated by *Trichoderma* degrade the cell walls of pathogens, resulting in cell death and release of inner contents. Eventually, the nutrients obtained from pathogenic oomycete cells are successfully consumed by *Trichoderma*. *Trichoderma asperellum* demonstrates antagonistic activities against *P. capsici* via hyperparasitism or competition [17]; *Trichoderma atroviride* antagonizes *P. capsici*, inhibiting mycelial growth [24]; *Trichoderma virens* was reported to hydrolyze *P. capsici* hyphae [72]. Recently, Tomah et al. [72] reported that *T. virens* isolate HZA14 exhibited antibiotic activity since it produced highly active gliotoxin that completely inhibited *P. capsici* growth at 5 $\mu\text{g ml}^{-1}$. Bae et al. [24] first reported detailed effects of *T. atroviride* KACC40557 on host plants. Ethyl acetate extract of isolate KACC40557 altered expression of stress-related genes in pepper and tomato leaves. Moreover, levels of phytohormones in pepper leaves were affected by *Trichoderma* KACC40557 extract. Generally, BCA extracts resulted in priming of protective plant response or prevented initiation of harmful processes, including reactive oxygen species (ROS) accumulation.

Colonization of roots is an important trait in *Trichoderma*-provided biocontrol. It represents an interaction between hyphae and root cells and involves delivery of stimulatory secondary metabolites from the biocontrol agent to the plant host. Different impacts on tomato plant growth were reported based on the colonization development stage of *T. atroviride* [35]. In the absence of direct contact between mycelia and plant roots, primary root growth was stimulated. However, after the establishment of colonization, hypocotyl length was not affected while lateral root formation was initiated. Attraction of beneficial microbes to the root surface is provided by root exudates consisting of carbohydrates, amino acids, lipids, and organic acids. These exudates are host-specific, favoring different PGPRs and/or BCAs, reciprocally increasing

their growth. Exudates also stimulate microbial growth, resulting in faster development of physical contact and its subsequent beneficial effects. Moreover, tomato root exudates contribute to biocontrol of *Phytophthora* spp. by *T. atroviride*, assisting in the competition for space and nutrients in a dose-dependent manner [35].

There is a high level of interest in researches on the biocontrol efficacy of *Trichoderma* spp. owing to their prominent plant growth-promoting activities [87]. Strong correlation between biocontrol efficacy and growth promotion has been observed by Segarra et al. [17]; however, no evidence proving either direct growth stimulation or weight increase as a side effect of plant protection was provided. In another study, the direct impact of *Trichoderma*-provided growth promotion in tomato in the absence of the pathogen was proven [87].

2.4. Microbial volatile organic compounds

In addition to diffusible anti-oomycete compounds, biocontrol agents are capable of VOC synthesis. Owing to their volatile nature, they can inhibit disease development or induce resistance in plants that are spatially separated from the original inoculation location. VOCs demonstrate a range of antagonistic functions, including mycelial growth inhibition [21,22,58,68–70], sporangia formation reduction [22,58,68], and zoospore motility [58] and germination inhibition [22,58]. *B. siamensis* was found to produce two volatile compounds with antifungal activities [88]: 2,6-di-*tert*-butyl-4-methylphenol (BTH) and 2,4-di-*tert*-butylphenol (2,4-DTBP). In this study, antifungal effects were observed against the raspberry postharvest pathogens *Botrytis cinerea* and *Rhizopus stolonifer*. Further, the inhibitory properties of 2,4-DTBP against *P. capsici* have been reported by other studies [22,67]. Anti-*P. capsici* BCAs *Lysobacter enzymogenes* ISE13 and *Flavobacterium anhuiense* GSE09 [66] producing 2,4-di-*tert*-butylphenol exhibit high inhibitory activities against mycelial growth and sporulation, and promote fruit ripening. An *F. anhuiense* GSE09 cell density of as little as 10^6 cells ml^{-1} provides significant inhibition of *P. capsici* growth and sporulation [22]. Khabbaz et al. [16] reported that *P. fluorescens* Pf 9A-14 and *B. subtilis* Bs 8B-1 inhibit *P. capsici* growth *in vitro* via VOCs. Applying the same experimental procedure, Munjal et al. [21] observed the production of volatile compounds by *B. megaterium* BP17.

VOCs belong to several classes of chemical compounds, including aldehydes, alcohols, ketones, and phenolic compounds [58]. Among them, pyrazine derivatives are often reported as components of the

microbial VOC profiles of different BCAs with especially high efficacy against *P. capsici* [21,69,70]. Pyrazines are aromatic compounds that are grouped based on the nitrogen atom located in the *para* position [69]. They are natural components of plant crops and are considered to be safe because they do not affect non-target organisms [89]. The 2-ethyl-3-methyl pyrazine of *B. megaterium* BmBP17 VOCs completely inhibits *P. capsici* mycelial growth at $504 \mu\text{g ml}^{-1}$ concentration [21]. Similarly, VOCs of *P. putida* BP25 containing pyrazine derivatives inhibit 90% of *P. capsici* mycelial growth [70]. A detailed study on the effects of pyrazine derivatives comprising the VOC profile of *P. putida* BP25 was conducted by Agisha et al. [69]. All the tested pyrazine derivatives inhibited a wide range of plant pathogens including *Athelia rolfsii*, *Colletotrichum gloeosporioides*, *Gibberella moniliformis*, *Magnaporthe oryzae*, *P. capsici*, *P. myriotylum*, and *R. solani*, with 2-ethyl-3,6-dimethyl pyrazine having the strongest antagonism. It also reduced *Phytophthora* blight on pepper shoots, along with some other pyrazine derivatives (2,5-dimethyl pyrazine, 2-ethyl-5-methyl pyrazine, 2-methyl pyrazine), exceeding the protection rate conferred by the chemical fungicide metalaxyl. Dimethyl trisulfide, another component of the VOCs produced by *P. putida* BP25 not related to pyrazines, acted as a successful soil biofumigant [69].

In addition to antagonistic activity, bacterial VOCs have been proven to stimulate plant growth [58]. For example, VOCs of *B. amyloliquefaciens* UQ154, *B. velezensis* UQ156, and *Acinetobacter* sp. UQ202 promoted the growth of pepper seedlings and plants in I-plates and under controlled conditions, respectively. Specifically, growth stimulation was observed in terms of increased biomass and primary and lateral root lengths.

2.5. Volatile organic compounds and induced systemic resistance

As briefly mentioned in the previous section, microbial VOCs provide not only direct antagonistic properties against pathogens but also act as elicitors of systemic plant resistance [90]. Upon induction of systemic resistance in plants, defense-related genes are activated and, in turn, enhance the defense response associated with enzymatic activity [34]. Plant genes associated with defense response and stimulated by BCAs can be differentiated by their functions. For instance, *CaBPR1* is a basic pathogenesis-related (*PR*) gene with a high level of identity similar to those of tobacco and tomato. It is activated by *P. capsici* infection, which is correlated with ethylene biosynthesis [91]. Similarly, the *CaPR*-

4 gene is homologous to its analogues of other solanaceous crops and is triggered by jasmonic acid and ethylene [92]. Other *PR* genes encode enzymes capable of pathogen cell wall degradation (β -1,3-glucanase), defense response metabolite production (phenylalanine ammonia lyase), or ROS accumulation (catalase, peroxidase, and superoxide dismutase). ROS H_2O_2 serves a dual role, triggering cell death and stimulating antioxidative defenses at the same time. Furthermore, suppression of H_2O_2 production promotes root colonization by rhizobacteria [33]. Therefore, regulation of H_2O_2 can increase the biocontrol performance and induced systemic resistance (ISR) of bacteria. Levels of the glutathione S-transferase (*GST*) gene responsible for scavenging ROS were upregulated by a combination of two *Streptomyces* biocontrol strains, proving their involvement in ISR [33]. In this case, it was determined that ISR is regulated in an ethylene-dependent manner [33]. Several reports on ISR induction by volatiles of microbial BCAs are available; however, further research to elucidate the involvement of microbial VOCs in plant metabolism is required. Moreover, less is known about the pathways activated in plant tissues for resistance distribution along the plant. Therefore, understanding these mechanisms can help to enhance the overall biocontrol performance of antagonistic microbes and ISR.

2.6. Strategies for enhancing the biocontrol efficacy of biocontrol agents

Screening of potential biocontrol agents in native rhizosphere communities is a promising tool for finding new organisms with higher or wider biocontrol properties. However, identification and characterization of isolated strains is highly laborious to the extent that it is inefficient. Therefore, thanks to pioneering research, the major pool of BCAs was established. Eventually, the importance of environmental factors was realized, resulting in more greenhouse and field tests being conducted. However, the performance of BCAs in outdoor tests was lower than that observed in Petri dishes. Therefore, using a combination of several BCAs to control *P. capsici* is gaining much attention. In particular, microbes functioning through different modes of action that can cause a synergistic effect, resulting in a higher disease suppression rate, are proposed [33,36,49]. For instance, Yang et al. [49] reported that two different strains of *B. amyloliquefaciens*, Zy44 and Fy11, applied simultaneously resulted in lower severity of Phytophthora blight than single strain treatments. Identification of the biocontrol mechanism revealed that strain Zy44 synthesizes lipopeptides with high antifungal activity, whereas strain

Fy11 induces systemic resistance in host plants. Thus, a combination of direct fungal inhibition and priming of plant resistance can increase protection efficiency. Similarly, combining *P. aeruginosa* BJ10-86 with *T. hamatum* THSW13 resulted in synergistic inhibition of Phytophthora blight [14]. A combination of two *Streptomyces* strains, IT20 and SS14, postulated as efficient BCAs on their own, resulted in a synergistic effect [33]. Their synergism was not limited to stronger inhibition of *P. capsici* mycelia alone: they also enhanced pepper growth, flowering, and yield. Combined treatment with strains IT20 and SS14 outperformed single strain applications and the chemical ISR inducer β -aminobutyric acid (BABA) in terms of control of Phytophthora blight. Moreover, combined treatment reduced H_2O_2 production in plants, thereby mediating defense responses [33]. Contradictory to the previous reports, combined application of several *Bacillus* BCAs was reported to have lower disease reduction compared to single strain application [51]. Accordingly, microbial fungicides can negatively impact other beneficial microbes, which should be taken into account and researched further before applying combined BCA treatments.

Aside from using a combination of BCAs, the addition of plant residues or plant-derived composts may enhance biological control. Brassica plants are often used in combination with BCAs and are widely applied in agronomy as cover crops. They are cultivated in the same fields as host crops and disrupt the disease cycle of pathogens owing to their natural resistance [93]. Moreover, brassica crops are known as biofumigants, emitting antifungal compounds and contributing to disease control. Antifungal volatiles are stored inside cells and released during harvest, interacting directly with pathogen propagules [94]. Mixing of bacterial dilutions with rapeseed residue demonstrated the highest rates of disease inhibition and yield increase [36]. Additionally, rapeseed residue and bacterial suspensions were alternately sprayed on fields. Although this type of treatment showed less efficacy than that of a mixture, it still performed better than bacterial treatment alone. Similarly, Wang et al. [59] reported synergistic effects of *B. amyloliquefaciens* and rapeseed meal in disease incidence inhibition. Rapeseed was incompatible with the bacterial agent and suppressed its growth. Based on these results, compatibility of BCAs should be considered when developing an integrated management strategy.

3. Biological control of *Phytophthora infestans*

Biological control of *P. infestans*, which causes potato late blight, has been studied extensively

Table 2. List of biocontrol agents (BCAs) and their antagonistic activities against *Phytophthora infestans*.

Type of BCA	Genus	Species and strains	Activity antagonistic to the target pathogen	References	
Bacteria	<i>Bacillus</i>	<i>B. amyloliquefaciens</i> 17A-B3	Mycelial growth inhibition; cellulase and protease enzymatic activities; production of siderophores and biosurfactants	[55]	
		<i>B. subtilis</i> 30B-B6	Mycelial growth inhibition; cellulase and protease enzymatic activities; production of siderophores and biosurfactants	[55]	
	<i>Pseudomonas</i>	<i>B. velezensis</i> G341	Mycelial growth inhibition with diffusible and volatile antimicrobials	[20]	
		<i>P. brenneri</i> 43R-P1	Mycelial growth inhibition; cellulase and protease enzymatic activities; production of siderophores and biosurfactants	[55]	
		<i>P. chlororaphis</i> R47	Mycelial growth inhibition; inhibition of sporangia germination, zoospore release, and germ tube elongation; root colonization (epiphytic and endophytic); emission of VOCs ^a with inhibitory activity against mycelial growth and sporangial germination	[28,43,95–98]	
		<i>P. fluorescens</i> LBUM636, R76, S35, S49	Mycelial growth inhibition; inhibition of sporangia germination, zoospore release, and germ tube elongation; emission of VOC ^a (1-undecane) with inhibitory activity against mycelial growth and sporangial germination; root colonization (epiphytic and endophytic)	[43,95–99]	
		<i>P. frederikbergensis</i> S04, S19	Mycelial growth inhibition; inhibition of sporangia germination, zoospore release, and germ tube elongation; emission of VOCs ^a with inhibitory effects on mycelial growth and sporangial germination; root colonization (epiphytic and endophytic)	[28,43,95–98]	
		<i>P. jessenii</i> S34	Mycelial growth inhibition; inhibition of sporangia germination, zoospore release, and germ tube elongation; root colonization (epiphytic and endophytic)	[28,43,97,98]	
		<i>P. koreensis</i> 2.74	Production of biosurfactant with inhibitory activity against mycelial growth	[100]	
		<i>P. marginalis</i> R84	Mycelial growth inhibition; inhibition of sporangia germination, zoospore release, and germ tube elongation; root colonization (epiphytic and endophytic); emission of VOCs ^a with inhibitory effects on mycelial growth and sporulation	[28,43,95,97,98]	
		<i>P. protegens</i> 44R-P8	Mycelial growth inhibition; cellulase and protease enzymatic activities; production of siderophores and biosurfactants	[55]	
		<i>P. putida</i> R32	Mycelial growth inhibition; inhibition of sporangia germination, zoospore release, and germ tube elongation, zoospore lysis; emission of VOCs ^a with inhibitory effects on mycelial growth and sporangial germination	[28,43,95,97]	
	Fungi	<i>Chaetomium</i>	<i>C. aureum</i>	Mycelial growth inhibition; production of antibiotics	[101]
			<i>C. cochliodes</i>	Mycelial growth inhibition; production of antibiotics	[101]
<i>C. globosum</i> Cg-6, F0142			Mycelial growth inhibition; production of antibiotics (chaetomin, chaetoviridins); glucanase enzymatic activity	[102, 103]	
<i>Rhizopogon</i> <i>Aureobasidium</i>		<i>R. irregularis</i> MUCL41833	Root colonization	[104]	
		<i>A. pullulans</i> L1, L8	Mycelial growth inhibition; emission of VOCs ^a with inhibitory activity against mycelial growth; induction of ISR ^b	[105]	
		<i>C. pallidicorallinum</i> strain 46	Reduction of leaf lesion size	[106]	
Yeasts	<i>Curvibasidium</i> <i>Metschnikowia</i>	<i>M. pulcherrima</i>	Reduction of leaf lesion size	[106]	

^aVolatile organic compounds.^bInduced systemic resistance.

(Table 2) [20,28,43,55,95–106]. As previously mentioned, biocontrol of *P. capsici* is commonly carried out using *Bacillus* spp., whereas late blight biocontrol is achieved using *Pseudomonas* spp. [28,55,100].

3.1. Bacterial biocontrol agents

3.1.1. Bacillus

Studies have shown that *P. infestans* is efficiently controlled by *Bacillus* spp. For instance, *B. subtilis* and *B. pumilus* could significantly inhibit late blight in a 2-year field experiment [107]. In another study, *B. subtilis*, as the biocontrol-formulated product Serenade, not only provided protective effects but also caused a reduction in disease pressure when applied simultaneously with the pathogen [108]. However, it was revealed that the liquid formulation of the treatment played a crucial role in biocontrol performance, which emphasizes the importance of secondary metabolites.

3.1.2. Pseudomonas

Pseudomonas spp. are great producers of secondary metabolites with strong anti-oomycete activity, including biosurfactants, volatiles, diffusible antibiotics, HCN, and siderophores [28]. In particular, a cyclic lipopeptide (CLP) lokisin of *Pseudomonas koreensis* strain 2.74 provided strong control activity against potato blight at concentrations as low as 0.2 mg ml⁻¹ [100]. The mechanism of the CLP lokisin was determined to be a disruption of zoospore integrity with subsequent lysis. Moreover, no phytotoxicity was observed, even at a 10-fold efficient control concentration, suggesting that the CLP lokisin is environmentally sound.

Extensive studies of *Pseudomonas* spp. that mainly focused on the effects of their VOCs have greatly contributed to the biocontrol of late blight [28,43,96–98]. Initial studies of antagonistic strains of *Pseudomonas* isolated from the phyllosphere and rhizosphere revealed VOC-related inhibition of *P. infestans* and postulated 1-undecene as a prominent, antagonistic volatile compound [98]. This VOC inhibits mycelial growth of *P. infestans* and antagonizes zoosporangia and zoospore formation when it is directly applied to the mycelia and zoosporangia. Zoospores are key elements of the pathogen infection process; thus, their inhibition by biocontrol agents is highly desirable because it completely prevents disease initiation. Although further studies have confirmed that high concentrations of 1-undecene are produced by biocontrol pseudomonads, GC-MS analysis of the pseudomonads has revealed various VOCs with even higher inhibition properties [95]. Interestingly, the screening of VOC-related antagonistic activity revealed that sulfur-containing

metabolites (DMTS and MMTS) tend to inhibit growth and development (mycelial growth, sporangia and zoospore formation, and zoospore motility), whereas simple ketones target zoospore germination. As a result, a combination of several metabolites exploiting different mechanisms can provide more complex and productive control of the disease. As biocontrol agents are seen as an environmentally sound measure of plant protection, side effects on plants and harmful influences on non-target organisms should be eliminated. Although some *Pseudomonas* species, especially *P. aeruginosa*, are known as human pathogens [109], biocontrol-effective strains lack the virulence factors of human pathogenicity [55].

3.2. Fungal biocontrol agents

3.2.1. Arbuscular mycorrhizal fungi

Glomeromycota is a group of arbuscular mycorrhizal fungi (AMF), which are beneficial organisms involved in plant growth promotion. AMFs are natural symbionts of nearly 80% of vascular plants, including pepper and tomato [110]. Therefore, their introduction as biocontrol agents is prominent and studies on their performance are needed. *Chaetomium globosum* (Kunze ex Fr.) is known as a BCA that antagonizes a wide range of plant pathogens, including several *Phytophthora* species [111]. It was registered as the commercial biofungicide Ketomium[®] and is actively applied worldwide. However, studies on its activity against *Phytophthora* spp. that infects solanaceous crops are limited. Several reports suggest that *C. globosum* provides antagonistic activity against *P. infestans* in potato and tomato [102,103]. Biocontrol effects are associated with fungistatic metabolites (chaetomin and chaetoviridins) and glucanolytic activity. The direct biocontrol effect of chaetoviridin A isolated from *C. globosum* culture was demonstrated both *in vitro* and *in vivo* by Park et al. [102]. Other strains of the genus *Chaetomium* act as potential *P. infestans* biocontrol agents; *in vitro* inhibition of *P. infestans* mycelial growth and sporangium germination has been reported in *Chaetomium cochliodes*, *Chaetomium aureum*, *Chaetomium nozdrenkoae*, and *Chaetomium elatum*, and complete inhibition has been demonstrated by *C. aureum* [101]. Analysis of the antifungal metabolites of these *Chaetomium* species revealed previously non-identified metabolites. Therefore, further studies are needed to identify these metabolites.

Because of the living nature of AMFs, their performance under field conditions can be controversial. For instance, *Rhizophagus irregularis* failed to significantly reduce *Phytophthora* blight severity

under favorable conditions, despite its high efficiency under dry and hot cultural conditions [104].

3.2.2. Yeasts

The long history of *P. infestans* research has led to the proposed use of all types of BCAs, even those that are not used against other *Phytophthora* pathogens, including yeast-like organisms. *Aureobasidium pullulans* (De Bary) is a yeast-like fungus known to control several postharvest pathogens; however, little is known about its preharvest performance. Di Francesco et al. [105] were the first to determine its antagonistic potential against *P. infestans* in tomato. *A. pullulans* exhibited both protective and curative properties functioning via plant defense enzyme stimulation and antagonistic metabolite production, respectively. Both diffusible and volatile metabolites provided significant pathogen inhibition. Furthermore, the biocontrol potential of two other yeasts, *Curvibasidium pallidicorallinum* and *Metschnikowia pulcherrima*, against *P. infestans* on potato has been reported by Hadwiger et al. [106].

3.3. Gene-based metabolite analysis against *P. infestans*

As described above, the involvement of antagonistic metabolites in biocontrol cannot be neglected. Antifungal metabolites produced by microbial BCAs are routinely identified by conventional methods such as chromatography. Although accurate and easy to perform, they can be outperformed by polymerase chain reaction (PCR) screening of genes that confer production of antagonistic metabolites. This technique is especially beneficial when high numbers of biocontrol candidates are available. Caulier et al. [55] used PCR screening procedures for various strains of *Bacillus* and *Pseudomonas* spp. with proven antagonistic activity against several potato pathogens, including *P. infestans*. PCR screening provided knowledge on the predominance of bacilysin-related genes among *Bacillus* BCAs, which was partly associated with their strong inhibition activity. Moreover, this method aroused an interest in further investigation of the biocontrol nature of *Pseudomonas brenneri*, which exhibited high biocontrol activity and lacked any of the common antibiotic-related genes. Screening for possible virulence factors harmful to non-target organisms is another important application of the PCR screening method [55]. Caulier et al. [55] reported that all tested *Pseudomonas* strains lacked virulence factor-encoding genes. Furthermore, several *Bacillus* agents were identified as harmful to humans because they carried genes that encode for non-hemolytic enterotoxins (Hemolysin BL and Cerolysin-O). Despite its

obvious benefits, genome screening is dependent on the availability of sequences referring to metabolite-encoding genes. However, owing to the limited availability of such sequences to date, further studies are needed to expand genetic libraries.

Whole genome analyses of BCAs rather than targeting specific genes can provide a more in-depth understanding of what underpins biocontrol performance. Genomes of nine *Pseudomonas* strains belonging to the *P. fluorescens* subgroup were investigated to detect genes previously reported as being involved in antagonism [28]. Inhibition of *P. infestans* mycelial growth was reported to be correlated with HCN production by bacterial strains. Non-HCN producing inhibitors were thought to use the enzymatic activity of chitinases and exoproteases to degrade pathogenic cell barriers. Further, none of the metabolites were associated with sporulation inhibition, including sporangia germination and zoospore release. Genes of CLPs that are involved in zoospore lysis were detected in strains that reduce germ tube formation. Biocontrol mechanisms retrieved from the genome analysis demonstrated the complexity and abundance of these mechanisms. HCN, lytic enzymes, CLPs, siderophores, and bacteriocins were mentioned as biocontrol-involved metabolites functioning in a single organism against different pathogenic stages.

3.4. Importance of in vivo biocontrol tests

Extensive biocontrol studies on late blight resulted in numerous reports describing strong antagonisms of BCAs against *P. infestans* [28,55,105]. However, *in vivo* experiments are limited in their coverage of environmental factors given they are performed in strongly controlled environments or do not even represent natural infection development [43]. Morrison et al. [99] attempted to assess the biocontrol efficiency of the antibiotic phenazine-1-carboxylic acid-producing *P. fluorescens* LBUM636 in tuber and growth chamber tests. Surprisingly, strong consistency between the protection effect *in vitro* and *in vivo* (tuber and growth chamber tests) was observed. In contrast, biocontrol ability was lost when the BCA and pathogen were inoculated with no physical contact between them (two-hole design tuber test). Although these tuber tests were designed to mimic the natural interactions between the pathogen and biocontrol microbe, they still used single-site application of the pathogen and its antagonist, which rarely occurs in fields. Therefore, loss of biocontrol efficacy can be caused by a lack of direct interaction between a BCA and pathogen that commonly occur in nature owing to low colonization activity.

3.4.1. Rhizosphere populations of *P. infestans*

Field tests are important because they consider the influence of environmental conditions on rhizosphere populations. Rhizosphere pathogen populations are always represented by strains with different morphological characteristics, virulence levels, and fungicide resistance. Therefore, these traits should be taken into account when determining biocontrol efficiency. De Vrieze et al. [96] screened *Pseudomonas* BCAs against *P. infestans* isolates in Switzerland. Natural populations of *P. infestans* were shown to vary in sporangial production rate, while their size, virulence, and aggressiveness was correlated with their susceptibility to biocontrol agents. It was shown that *P. infestans* aggressiveness is negatively correlated with sporangial size and positively correlated with sporangial production rate. The most virulent isolates were the most aggressive, producing the highest number of small sporangia that were the most infectious. Furthermore, increased virulence was detected for isolates obtained from areas where chemical fungicides were extensively applied. As to biocontrol susceptibility, it was shown to be negatively correlated with virulence [96].

Consistent with such strong variations in natural *P. infestans* populations, antagonistic strains of *Pseudomonas* failed to provide universal performance against all pathogenic isolates [96]. For instance, *Pseudomonas* sp. R47 successfully inhibited all 10 isolates collected across Switzerland, whereas other strains failed to do so [96]. Interestingly, repeated pathogen exposure to other *Pseudomonas* strains was followed by significant mycelial growth recovery between treatments. However, single exposure to *Pseudomonas* sp. R47 resulted in the complete absence of growth recovery between the treatments. Overall, no loss of susceptibility of *P. infestans* isolates to the antagonistic strain was postulated after the first exposure to any of the BCAs, providing no possibility of rapid biocontrol resistance occurrence. According to the different mechanisms of biocontrol strains, they might be applied at different time points to provide long-term bioprotection and control [28]. Strong colonizers with high sporangia inhibiting potential should be applied preventively as a protective measure. Mycelial growth inhibitors inhibit infection dispersal, whereas zoospore release inhibitors prevent the infection from being passed on to the next generation.

As shown in the aforementioned studies, biocontrol efficiency is heavily dependent on both the biocontrol agent and the pathogenic isolate. Natural pathogen populations vary greatly in morphological and physiological characteristics as well as pathogenicity. Thus, selection of a *P. infestans* isolate to determine the efficiency of any BCA should be unbiased and representative of the natural

occurrence of the pathogen to the greatest extent possible. Preferably, several isolates of the pathogen with varying morphology, physiology, and pathogenicity should be used to prove the efficiency of the BCA.

3.4.2. Rhizosphere populations of biocontrol agents

Despite the high antagonistic performance of many BCAs *in vitro*, certain BCAs, such as *Pseudomonas* spp., fail to provide the same protection levels in *in vivo* tests [97,98]. Possible reasons for low *in vivo* performance include initial low populations of the antagonists in the rhizosphere or low persistence in soil. In a leaf disk test of direct confrontation between *P. infestans* sporangia and *Pseudomonas* strains R47, R76, and S35, BCAs provided significant control at a high cell concentration (2×10^8 cells ml⁻¹), but were ineffective at lower cell densities. Ten-fold dilutions of the respective strains were unable to provide sufficient control. The soil populations of antagonistic strains were estimated to be 10^{2-3} and 10^2 CFU g⁻¹ under greenhouse and field conditions, respectively, explaining poor performance. Therefore, when developing a biocontrol strategy, populations of biocontrol agents must be maintained at a specific level. The gap between high *in vitro* and poor *in vivo* performance is commonly associated with the inability of BCAs to colonize plant roots. Analysis of nine strains of *Pseudomonas* sp. revealed that the majority of these strains were epiphytic colonizers [28]. Gene mining proposed that the type VI secretion system is a component that improves colonization abilities. This gene was detected in the genome of a high-performing colonizer strain that was previously reported to be involved in bacterial competition.

3.5. Combined biocontrol agent treatments to improve biocontrol efficacy

Nature has a complex structure consisting of a branched net of interactions between organisms requiring a balance that is possible only in terms of normal relations between these organisms. Anthropogenic activities, including plant disease control using fungicides, often lead to the disruption of this fragile balance, resulting in negative side effects. Application of agricultural chemicals results in a phytotoxic effect and gradual increase in resistant pathogenic populations [40,113]. Despite the fact that biological control is considered an environmentally sound measure, artificial population increase of a single organism as a result of soil applications can potentially cause a decline in soil suppressiveness. Furthermore, soil populations of beneficial indigenous organisms may decline if they

are incompatible with biocontrol agents. Moreover, despite the employment of various strategies, biological control is still less efficient and sustainable than chemical control [55]. Therefore, combined application of several biocontrol microbes is a promising strategy for enhancing biocontrol efficiency. De Vrieze et al. [43] performed a large-scale *in vivo* experiment focusing on *Pseudomonas* antagonists and their combinations in their search for synergistic interactions. They observed inconsistencies in the interactions between the combined BCA treatments. For instance, the combination of strains S19 and S49 resulted in significant biocontrol enhancement compared to respective single-strain applications. The synergistic effect was likely due to the different mechanisms exploited by these strains (i.e., zoospore inhibition and mycelium inhibition by strains S19 and S49, respectively). By contrast, *Pseudomonas* strain S35 had great biocontrol potential when applied alone, but its efficiency declined when it was combined with other strains. Competence ability varies between different organisms; growth of strain S35 was often inhibited by other biocontrol pseudomonads, which consequently led to a decrease in biocontrol performance. Combining several biocontrol agents might be an alternative strategy for improving biocontrol performance; thus, the competence ability and biocontrol mechanisms of agents should be thoroughly studied.

Some studies highlight the importance of alternative biocontrol mechanisms used by single BCAs for more sustainable biocontrol [28,95,99]. For example, although *P. fluorescens* LBUM636 is unable to synthesize antibiotic phenazine-1-carboxylic acid, its main antagonistic metabolite, it still provides a significant level of biocontrol, implying the involvement of other metabolites [99]. It has been shown that *Pseudomonas* and *Bacillus* strains that produce not only antibiotics but also siderophores and biosurfactants have more effective control properties, highlighting their contribution to biocontrol and ecological fitness [55].

4. Biological control of *Phytophthora nicotianae*

Phytophthora nicotianae (syn. *Phytophthora parasitica*) is an oomycete pathogen that causes black shank in tobacco plants. Recently, biocontrol of black shank was extensively studied and several successful BCAs were proposed, including *Bacillus* spp., *Paenibacillus polymyxa*, *P. aeruginosa*, *Trichoderma* spp., and *Aspergillus flavipes* (Table 3) [24,31,32,37,44,83,114–122]. These BCAs commonly inhibit mycelial growth in *P. nicotianae*, causing abnormalities such as twisted and wrinkled hyphae,

Table 3. List of biocontrol agents (BCAs) and their antagonistic activities against *Phytophthora nicotianae*.

Type of BCA	Genus	Species and strains	Activity antagonistic to the target pathogen	References
Bacteria	<i>Bacillus</i>	<i>B. amyloliquefaciens</i> FZB42	ISR ^a induction	[114]
		<i>B. atrophaeus</i> HAB-5	Mycelial growth inhibition; hyphal abnormality formation; cellulase, chitinase, and protease enzymatic activities; siderophore production	[37]
		<i>B. subtilis</i> Tpb55	Mycelial growth inhibition; hyphal abnormality formation; root colonization; ISR ^a induction	[32,114]
		<i>B. velezensis</i> Ba168, GUMT319	Mycelial growth inhibition; hyphal abnormality formation; root colonization; chemotaxis to root exudates	[116,117]
Fungi	<i>Paenibacillus</i>	<i>P. polymyxa</i> C5	Mycelial growth inhibition; root colonization	[118]
		<i>P. aeruginosa</i> NXHG29	Mycelial growth inhibition; root colonization	[44,119]
		<i>A. flavipes</i> ATCC24487	Mycelial growth inhibition; hyphal abnormality formation; inhibition of zoospore germination and viability	[31]
	<i>Glomus</i> <i>Trichoderma</i>	<i>G. mosseae</i> BEG12	Mycelial growth inhibition; root colonization; ISR ^a induction	[120–122]
		<i>T. asperellum</i> IMI393899	Mycelial growth inhibition; ISR ^a induction	[87]
		<i>T. atroviride</i> TS	Mycelial growth inhibition; ISR ^a induction	[87]
		<i>T. harzianum</i> KACC40871	Mycelial growth inhibition	[24]
		<i>T. petersenii</i> KACC40552, KACC40557	Mycelial growth inhibition; hyphal abnormality formation	[24]
		<i>T. virens</i> KACC40929	Mycelial growth inhibition	[24]

^aInduced systemic resistance.

deformed or swollen hyphal tips, and protoplasm leakage [32,37,44,118].

One of the main limitations of biocontrol using antagonistic microorganisms is the poor or inconsistent performance of these agents under field conditions even though their antagonistic potential *in vitro* is high, as mentioned previously. Therefore, selected agents need to provide significant control not only under experimental conditions but also at a field scale. Accordingly, Han et al. [32] demonstrated a decrease in tobacco black shank under both greenhouse and field conditions using a biocontrol treatment. In the 3-year field trial, the level of disease control provided by *B. subtilis* Tpb55 was equivalent to that provided by the fungicide metalaxyl [32]. Similarly, in another 3-year field trial, *B. velezensis* GUMT319 suppressed tobacco black shank with higher protection levels than those of fungicide and commercial biocontrol product treatments [116]. All antagonistic microorganisms had a protection rate of between 70% and 80% [32,116,118].

Some AMF are also efficient against black shank, mainly *via* induction of systemic resistance in host plants. The involvement of *Glomus mosseae* in biological control of *P. parasitica* was reported recently [121]. *Glomus mosseae*-colonized tomato roots demonstrated a strong protective effect with a significant reduction in infection loci. This disease reduction seems to be associated with AMF-induced plant resistance [119]. Mycorrhizae can cause not only local histological alterations but *PR* gene inductions that induce systemic resistance in plants. A combination of local and systemic resistance provided by a mycorrhizae *G. mosseae* has been postulated by Pozo et al. [121]. The effects of local resistance were provided by new isoforms of cell-wall degrading enzymes (chitinases, chitinases, and β -1,3-glucanases) detected in mycorrhizal plant roots. On the other hand, the effects of systemic resistance were achieved by the increased lytic activity in non-colonized roots of mycorrhizal plants, which were demonstrated in a split root system.

4.1. Root colonization by biocontrol agents

Colonization of host plants by BCAs is crucial to biocontrol performance. BCAs often function through direct antagonism against plant pathogens or induced plant resistance, which provides indirect biocontrol. In both strategies, physical contact between the antagonistic microorganism and the plant is required for plant protection or induction of plant resistance. Loss of biocontrol performance in *in planta* trials is sometimes attributed to the inability of BCAs to colonize plant roots. Several

biocontrol bacteria, including *B. subtilis* Tpb55, *B. velezensis* GUMT319, *P. polymyxa* C5, and *P. aeruginosa* NXHG29, were reported to successfully colonize tobacco roots [32,44,116,118]. Root exudates of host plants serve as a source of nutrients that are generally consumed by BCAs and involved in bacterial metabolism. Therefore, BCA growth and colonizing ability are highly dependent on root exudates. Exudates also establish cross-talk between a plant and the BCA triggering induced resistance. Tobacco root-colonizing bacteria are mainly detected in root tips and elongation zones [32,44,118]. Further, *B. subtilis* Tpb55 cells have been detected in vascular systems [32]. Ren et al. [118] reported that cells of *P. polymyxa* strain C5 were not detected inside tobacco root tissues. By contrast, previous reports [123,124] postulated endophytic colonization of spruce and potato roots by *P. polymyxa*. Therefore, endophytic colonization by *P. polymyxa* might be a strain-specific trait and further studies are required to determine this. With the maturation of roots, colonizing bacteria are capable of migrating to elongation and maturation zones, and sometimes up to stems [44].

Despite the similarity between the colonization patterns of several biocontrol bacteria, their populations fluctuate differently [32,44,118]. Populations of *B. velezensis* Tpb55 increased until 4 days post-inoculation, reaching 10^7 CFU g⁻¹, and then gradually decreased [32]. By contrast, populations of *P. polymyxa* C5 decreased constantly from 10^8 (right after inoculation) to 10^6 CFU g⁻¹ and 10^5 CFU g⁻¹ by 6 days and 18 days post-inoculation, respectively [118]. Another pattern was reported for *P. aeruginosa* NXHG29: its populations decreased from 10^8 to 10^7 CFU g⁻¹ by 3 days post-inoculation, then increased up to 10^8 CFU g⁻¹ at 6 days post-inoculation, followed by another decrease to 10^7 CFU g⁻¹ at 9 days post-inoculation, after which it stabilized at 10^6 CFU g⁻¹ by 12 days post-inoculation and then maintained the same level until the end of experiment (20 days post-inoculation). It is difficult to compare and analyze the results of these studies because the bacterial populations tested were different at different sampling time points. Therefore, a more universal approach is required to eliminate experimental design-related fluctuations.

Root-colonizing bacteria appear to discern between plant hosts. Root exudate composition varies between plants and this composition regulates the microbial community in the rhizosphere [125]. *Bacillus velezensis* GUMT319 was reported to form biofilm structures on the roots of pepper and tobacco plants; however, colonization activity on tobacco plants was significantly higher than that on pepper plants [115]. Lauric acid was identified as

one of the components of pepper root exudates, whereas it was absent among tobacco root exudates. Biofilm formation by *B. velezensis* GUMT319 was negatively affected by lauric acid, which explains its lower colonization activity on pepper roots.

4.2. Chemotactic activity of root-colonizing biocontrol agents

Chemotaxis of microorganisms to root exudates is a key step for successful colonization that provides a competitive advantage for chemotactic bacteria. Composition of root exudates varies not only in different crops but also in the different developmental stages of single plants. Therefore, depending on the composition of root exudates, rhizosphere communities can vary greatly. Root exudates are known to positively affect chemotactic activity [115]. Ma et al. [118] conducted a detailed study of the effect of tobacco root exudates on *P. aeruginosa* chemotaxis and its physiological processes. Nicotine made up 46.7% of total tobacco exudates induced by chemotaxis of *P. aeruginosa* in a dose-dependent manner between 10 and 40 μM . Interestingly, a 40 μM concentration of nicotine served as a threshold as growth and antagonistic activity against *P. nicotianae* and *Ralstonia solanacearum* were 4–7 times higher than those of 10–30 μM nicotine concentrations. The same concentration (40 μM) of nicotine enhanced control of bacterial wilt and black shank in tobacco as well as root populations of *P. aeruginosa*. By contrast, lower concentrations had no effect on either biocontrol performance or bacterial populations. The importance of the 40 μM concentration of nicotine can be explained by its similarity to naturally occurring concentrations of nicotine in rhizosphere environments or by functional activation of a bacteria due to “sensing” of the particular nicotine concentration. Contradictory to previous reports [125–127] stating that root exudates favor sporulation, microconidia germination, and mycelial growth of pathogens, supplementation of nicotine facilitated control of bacterial wilt and black shank caused by *P. aeruginosa*. Although the mechanism of interaction between the bacterial control agent and nicotine is not known, increased populations on tobacco roots under nicotine treatment might favor bacterial competence under rhizosphere conditions. Therefore, a higher colonization rate of *P. aeruginosa* might favor disease management for these plants.

4.3. Biocontrol agents with a wide range of antagonism

One of the most promising and beneficial traits of biocontrol agents, especially compared to those of

agricultural chemicals, is a broad range of antagonism. By exploiting the same mechanism, for example cell-wall degradation with lytic enzymes, one antagonistic microorganism can be efficient against several plant pathogens with the same cell wall composition. Recently, Ding et al. [116] showed that *B. velezensis* inhibits the growth of various fungal fungi belonging to oomycetes and ascomycetes (*P. nicotianae*, *Alternaria alternata*, *Colletotrichum scovillei*, *Colletotrichum capsici*, *Exserohilum turcicum*, *Fusarium carminascens*, *Phomopsis* sp., *Phyllosticta sorghina*, and *Sclerotinia sclerotiorum*). *Bacillus atrophaeus* has an even wider activity range, including *A. alternata*, *Alternaria brassicola*, *C. gloeosporioides* species, *Colletotrichum musae*, *Corynespora cassicola*, *F. oxysporum* f. sp. *cubense*, *Fusarium proliferatum*, *Phyllosticta theaeifolia*, and *Trichothecium roseum* [37]. Among the fungal BCAs, several *Trichoderma* strains tested for antagonistic activity against *Phytophthora* spp. inhibited the mycelial growth of *Phytophthora cactorum*, *P. capsici*, *Phytophthora drechsleri*, *P. infestans*, *Phytophthora melonis*, *P. nicotianae*, and *Phytophthora sojae* [24]. Along with these, *A. flavipes* showed to inhibit various *Phytophthora* spp., such as *Phytophthora arecae*, *P. capsici*, *Phytophthora cinnamomi*, *Phytophthora palmivora*, *P. parasitica*, and *Phytophthora tropicalis*, and had the highest inhibition of *P. parasitica* and the fungi *Alternaria solani*, *C. gloeosporioides*, *F. oxysporum*, and *R. solani* [31]. However, little data are available on biocontrol activity against multiple pathogens in natural conditions and thus further investigations are required.

Most pathogens in fields can infect their host crops independently; however, some of the pathogens have a tendency to infect a crop at the same time, which complicates their management. One of the strategies to control complex infections caused by several pathogens is the selection of BCAs with a broad spectrum of activity. Some reports on the multiple biocontrol performance of BCAs are available, but their number is limited. For instance, *B. atrophaeus* HAB-5 demonstrated the ability to control black shank of tobacco in a square dish system [37]. Additionally, its extract prevented disease initiation of anthracnose in mango fruits. Ma et al. [44] proposed the dually antagonistic bacterium *P. aeruginosa* NXHG29 as a measure to control black shank caused by *P. nicotianae* and bacterial wilt caused by *R. solanacearum*, which often emerge simultaneously. A combination of *P. aeruginosa* NXHG29, which demonstrated antagonism against both pathogens *in vitro*, and organic fertilizer reduced the incidence of both diseases in tobacco plants. However, application of the BCA without fertilizer resulted in worse control performance.

Organic fertilizers serve as a source of organic matter that promotes plant growth by improving soil structure, fertility, and overall quality. Moreover, organic fertilizers act as a supply of nutrients for antagonistic microorganisms and thus promote their growth. Therefore, biocontrol agents can control multiple diseases occurring simultaneously and their performance can be enhanced by organic fertilizers serving as a source of nutrients for both host plants and BCAs.

4.4. Anti-microbial metabolites against *P. nicotianae*

4.4.1. Antimicrobials

Owing to the fact that the majority of reported tobacco black shank biocontrol agents are *Bacillus* spp., their secondary metabolites often have an antagonistic activity that contributes to their performance. For instance, culture filtrates of *B. velezensis* Ba168 inhibited 99% of *P. nicotianae* growth *in vitro* [117]. Several proteins known to be involved in direct antagonism against *P. nicotianae* or induction of systemic resistance were identified in *B. velezensis* Ba168 liquid culture [117].

However, a more conservative approach is still employed to identify antimicrobial metabolites of BCAs and determine their antagonistic effects [31,117]. Recently, genome sequence analysis has been used to identify antagonistic metabolites [116]. Genome sequence-based experiments can obtain a more redundant list of microbial secondary metabolites. They also eliminate the necessity to use several selective methods to detect specific antimicrobials, thereby consuming less time and labor. Using the genome sequence of *B. velezensis* GUMT319, Ding et al. [116] discovered 13 clusters of putative genes involved in biosynthesis of metabolites with potential antimicrobial activity. They most frequently contain antimicrobials such as bacillaene, bacilysin, diffidin, fengycin, macrolactin, surfactin, and terpene, which are commonly conserved for *Bacillus* spp. [116].

Most of these antimicrobials interact with the membrane lipid layer of pathogenic cells. For instance, iturin, fengycin, and surfactin can damage cell membrane integrity or increase permeability; thus, they disrupt membrane transport and lead to cell death [116]. In addition, *B. velezensis* Ba168 extract was reported to increase the cell conductivity of *P. nicotianae* in a dose-dependent manner [116]. Simultaneous increases in the extracellular pH of *P. nicotianae* indicated damage of oomycete membranes, which led to cell content leakage. Finally, visual observations identified cell disintegration as mycelial hyphae were perforated as a result of bacterial treatment. By contrast, some of the microbial metabolites targeted reproductive structures

(zoosporangia and zoospores) rather than pathogenic mycelia. Culture filtrates of *A. flavipes* demonstrated higher inhibitory effects on zoospore germination than mycelial growth or sporangia formation in *P. nicotianae*. Success in *in vitro* antagonism of microbial culture filtrates against *P. nicotianae* led to further determination of their efficiency *in planta*. Some of the extracts proved their strong biocontrol performance in tobacco plants, reducing disease severity of black shank [31,37].

Bacterial extracts containing antimicrobials demonstrate high performance under a wide range of environmental conditions. Extracts of *Bacillus* sp. were resistant to temperature fluctuations [37,117] and pH values between 5 and 10 [37]. They are also resistant to proteolytic enzymes, including amylase, chymotrysin, pepsin, pronase, protease K, and trypsin [37,117]. Although there are few reports on localization of anti-oomycete compounds in biocontrol microorganisms, some suggest that they are localized intracellularly [31].

4.4.2. Phytotoxicity of antimicrobials to host plants

The antagonistic activity of bacteria-derived antibiotics must be efficient to provide adequate disease control. Biocontrol, an environmentally sound alternative to agricultural chemicals, is expected to be harmless to non-target organisms of microbial antibiotics. Therefore, potential BCAs must prove their lack of phytotoxicity to host plants and non-toxicity to other non-target organisms. Extracts of *A. flavipes* proved to be safe for tobacco and tomato seedlings when applied at rates higher than those required for *P. nicotianae* inhibition [31]. Additionally, *A. flavipes* had no interference in tobacco and tomato development and physiology, making it safe to apply for disease protection. By contrast, extracts of the biocontrol *B. atrophaeus* HAB-5 applied in high concentrations were moderately toxic to zebra fish used as a model object in toxicological tests, making its safety debatable [37]. Therefore, determination of toxicity to non-target organisms is a key factor for not only successful control of plant diseases but also for environmental soundness.

4.4.3. Lytic enzymes and siderophores

In addition to antibiotics, *Bacillus* BCAs effective against tobacco black shank commonly produce enzymes that are involved in lysis of pathogenic cell wall components [37,117]. In particular, chitinolytic, proteolytic, and cellulolytic activities of *B. atrophaeus* were demonstrated on selective media by Rajaofera et al. [37]. Additionally, cellulose degradation enzymes were detected in *B. atrophaeus* HAB-

5 extracts, suggesting that fungal hyphae had been dissolved [117]. *Trichoderma* isolates are known to use mycoparasitism as a basic mechanism of biocontrol, attaching to pathogenic hyphae and dissolving them using cell-wall degrading enzymes. *T. atroviride* and *T. asperellum* consistently demonstrated mycoparasitic activities against *P. nicotianae* via increased chitinase production upon pathogen exposure [87].

Siderophores are heavily involved in plant iron metabolism, facilitating the transformation of soil forms into available forms for plant utilization. Further, there are also reports on their antimicrobial properties [129]. Contributions of siderophores produced by *B. atrophaeus* HAB-5 to inhibition of *P. nicotianae* were also reported [37].

4.5. Induced systemic resistance against *P. nicotianae*

Plant pathogens and biocontrol agents are both known to induce resistance in host plants upon recognition of microbe-associated molecular patterns (MAMPs) (e.g., bacterial flagellin and fungal chitin) [129]. Activities of defense-related enzymes, including ROS-associated (peroxidase, catalase, and superoxide dismutase) and lytic (β -1,3-glucanase and chitinase) enzymes, are enhanced by the induction of resistance. In particular, *B. subtilis* Tpb55 can up-regulate peroxidase, catalase, and β -1,3-glucanase-related genes [113]. Systemic acquired resistance induced by pathogenic infection utilizes a salicylic acid (SA) pathway for signal transduction and systemic occurrence of resistance. Furthermore, ISR was reported to function via a jasmonic acid (JA) and ethylene (ET) pathway as well as an SA pathway to confer resistance to the whole plant. *Bacillus amyloliquefaciens* FZB42 was reported to induce resistance in tobacco, reducing black shank symptoms by up to 60% [114]. SA as well as JA/ET pathways were shown to be activated in this ISR induction. Moreover, *B. amyloliquefaciens* FZB42 affected stomatal closure in tobacco leaves and thus prevented pathogenic penetration through natural openings. Bacterial treatment resulted in increased abscisic acid and SA levels and demonstrated their importance for stomatal closure [115].

ISR response can be enhanced by combining bacterial treatments with chemical ISR inducers. For instance, defense-related genes in tobacco were more up-regulated by a combination of *B. subtilis* Tpb55 and riboflavin than a single bacterial application [114]. Moreover, superoxide dismutase (SOD) activity failed to be induced by *B. subtilis* Tpb55 alone; however, the addition of riboflavin activated SOD. Therefore, further studies on measures to

improve ISR induction in plants may be required to enhance bacterial performance.

4.6. Effect of biocontrol agents on microbial community and plant growth

An important aspect of the environmental impact of biocontrol agents is their ability to affect indigenous microbial communities. Ideally, the application of high cell densities of a single organism should not affect community composition; however, it is typically not feasible. For example, *Proteobacteria* (particularly, *Alphaproteobacteria* and *Gammaproteobacteria*) are predominant in soil communities [131,132]; treatment of tobacco with *B. subtilis* Tpb55 as a soil application increased the abundance of *Proteobacteria* and certain bacteria, such as *Bradyrhizobium*, *Rhizobium*, and *Rhodanobacter*, which are known as PGPRs and BCAs [132]. Further, fungicide treatment resulted in higher populations of pesticide-degrading microbes (*Rhodopseudomonas*, *Acidisphaera*, and *Phenylobacterium*) and pollution-related bacteria (*Verrucomicrobia*). This highlights the fact that although BCAs alter microbial communities in the rhizosphere, their impact is rather positive. A recent study focused on a combination of compost and the biocontrol fungi *T. asperellum* and *T. harzianum* [131]. Unlike *B. subtilis* Tpb55 treatment, neither of the *Trichoderma* treatments altered microbial composition. However, in this case, the importance of compost cannot be neglected as all compost-associated communities (either with or without *Trichoderma*) presented a different microbial community than that of control samples. Therefore, the impact of *Trichoderma* spp. on rhizosphere microorganisms cannot be evaluated as it was neutralized by compost amendment. Additionally, the *Trichoderma* species enhanced the relative abundance of some *Bacteroidetes* (*Pedomicrobium*, *Hyphomicrobium*, *Bacillus*, and *Bdellovibrio*) and *Gammaproteobacteria* in *P. nicotianae*-inoculated soil, indicating their contribution to soil suppressiveness against the pathogen.

In addition, *P. nicotianae* infection suppresses plant growth, which leads to a decrease in the heights and weights of plants and eventually leads to crop losses. Therefore, antagonistic microbes that eliminate plant pathogens and reduce disease pressures on host plants can be viewed as indirect promoters of plant growth [118]. Furthermore, many BCAs exhibiting biocontrol potential are microbes originally described as having outstanding plant growth-promoting (PGP) properties. Such properties may include the production of siderophores and plant phytohormones, such as auxins, as well as

phosphate solubilization [37,117]. Siderophores can promote iron uptake by plants, while solubilization of insoluble phosphate enhances plant phosphorus content. Although screening for PGP mechanisms is widely available, genome screening of microbes can be a beneficial alternative to preliminarily determine the PGP potential of selected microbes [117]. Further understanding of the effects of BCAs on non-target organisms, including host plants and soil microbes, will enable the creation of an environmentally sound, beneficial strategy for crop production.

4.7. Measures of biocontrol enhancement

Despite an extensive search for efficient BCAs for tobacco black shank management, no organism capable of outperforming agricultural chemicals has been identified. Therefore, new approaches to supplementation of biocontrol microbes with stimulators is still needed. Among recent studies, supplementation of black shank-managing BCAs with compost, riboflavin, and root exudates were proposed [44,114,131]. Composts serve as a source of nutrients for plants and biocontrol agents. They also contain microbes that contribute to biocontrol and facilitate pathogen suppression [82]. Therefore, their combination with antagonistic microbes is more efficient than BCA treatment on its own [59]. Fortification of compost with *T. asperellum* and *T. harzianum* suppressed the number of *P. nicotianae* in soil [131]. Additionally, composts carrying their own microbiome greatly alter rhizosphere microbial composition. Drastic changes in rhizosphere community composition are unpredictable and can lead to decreased soil suppressiveness over the long-term. Therefore, more nature-derived measures for biocontrol enhancement are desirable. Nicotine, a component of tobacco root exudates was reported to positively affect *P. aeruginosa* growth, root colonizing activity, and *in vitro* antagonism against *P. nicotianae* [44]. It also enhanced biocontrol efficiency *in vivo*, increasing bacterial populations in rhizosphere soil. Zhang et al. [113] proposed stimulation of antagonistic microorganisms with a chemical ISR inducer (riboflavin), which eventually proved to be successful. Riboflavin stimulated the growth of *B. subtilis* Tpb55 in a dose-dependent manner and was shown to be compatible with Tpb55 overall. It enhanced Tpb55 colonizing ability by up to 200% when applied at a rate of 0.2 mg/ml, and improved peroxidase, catalase, and β -1,3-glucanase activities. Therefore, combining BCAs with stimulatory components is a promising strategy for biocontrol enhancement.

5. Conclusions

Phytophthora spp. such as *P. capsici*, *P. infestans*, and *P. nicotianae* remain substantial threats to sustainable production of solanaceous crops, including pepper, potato, and tobacco. It is well known that biological control, an environmentally sound strategy for managing these pathogens, is carried out by bacterial, fungal, and yeast BCAs, among which *Bacillus* spp. and *Pseudomonas* spp. predominate. Other bacterial (e.g., *Acinetobacter*, *Chryseobacterium*, and *Flavobacterium* spp.) and fungal (*Aspergillus flavipes* and *Chaetomium* spp.) BCAs also produce new metabolites that are highly efficient against *Phytophthora* spp. Further research on these novel BCAs will provide new biocontrol strategies with potentially higher efficiency. Microbial BCAs produce secondary metabolites that directly inhibit growth and development of plant pathogens or indirectly antagonize pathogens *via* induction of plant resistance. Direct inhibition of pathogenic propagules is hindered by the requirement for physical contact between metabolites and their targets; therefore, ISR is a more beneficial biocontrol strategy. Several microbial BCAs that bring about ISR against *Phytophthora* spp. have been reported recently. Further studies that focus on the molecular responses of host plants are required to improve biocontrol efficacy. Examples of BCA inhibitory activities against numerous plant pathogens were also discussed. Thus, studies should consider the efficacy of BCAs under *in vivo* conditions, and their effects on plant hosts and non-target organisms.

In this review, the importance of interactions between BCAs and rhizosphere communities is highlighted. Microbial BCAs were shown to affect the indigenous rhizomicrobiome, increasing populations of beneficial microbes. Further studies are needed on the long-term effects of BCAs on soil microbial communities. Moreover, composts used to fertilize soil might alter microbial populations, which may be undesirable. Thus, microbes with both biocontrol and PGP properties could be studied to develop a universal treatment that maximizes crop production and eliminates the necessity for compost application. Owing to the somewhat inconsistent biocontrol efficacy of antagonistic microbes, there is a need to develop approaches that improve their sustainability. Combining several BCAs exhibiting various biocontrol mechanisms or combining microbial BCAs with stimulators, such as composts, chemical ISR inducers, and components of root exudates, may enhance the biological control efficacy of promising microbes. Further research on combined treatments may promote biological control.

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Disclosure statement

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