Chapter 7
Biological Control of Peronosporomycete Phytopathogen by Bacterial Antagonist

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7.1 Introduction

The peronosporomycetes are phylogenetic relatives of brown algae and diatoms under the kingdom of Straminipila (Dick 2001). They are devastating pathogens of plants, animals, fishes, crustaceans, and microorganisms (Margulis and Schwartz 2000). Several species of this group of microorganisms such as *Phytophthora infestans* and *Plasmopara viticola* are listed among the top ten economically most important plant pathogens, resulting in multibillion-dollar crop losses worldwide (Agrios 1997; Haverkort et al. 2008). *Phytophthora* spp. have long been recognized worldwide as destroyer of plants. About 80 identified species of *Phytophthora* are causing some of the world's most economically important and devastating diseases in over 2,000 plant species (Abad et al. 2008). Among them, *Ph. infestans*, the cause of potato and tomato late blight disease was responsible for the Great Irish Potato Famine in the mid-nineteenth century. This disease is still widespread throughout potato-growing regions of the world and is virtually impossible to grow potato without some form of late blight disease control. Several other species such as *Ph. cinnamomi*, *Ph. capsici*, *Ph. megasperma*, *Ph. parasitica*, *Ph. erythroseptica*, *Ph. fragariae*, and *Ph. palmivora* are also extremely destructive on their hosts causing primarily root and lower stem rots, but also some cankers, twig blights, and fruit rots (Yang et al. 1994; Carruthers et al. 1995; Valois et al. 1996; Ko et al. 2009; Schisler et al. 2009; Timmusk et al. 2009). One of the dangerous aspects of *Phytophthora* pathogens is emergence of new and more virulent species. Record of new species of *Phytophthora* has become customary from new hosts as well as from new countries or even continents.

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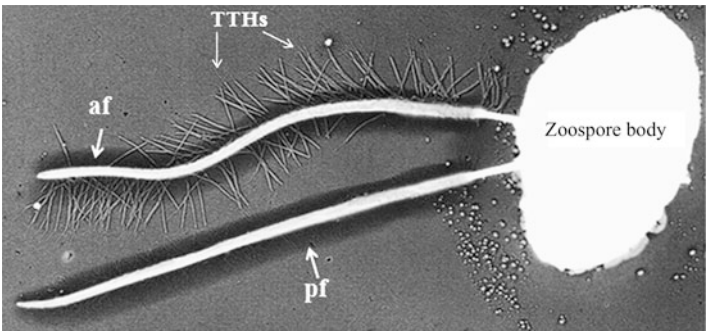

Fig. 7.1 Transmission electron micrograph (TEM) of a glutaraldehyde-fixed *Aphanomyces cochlioides* zoospore with flagella. *af* Anterior flagellum ornamented with two rows of tubular hairs, *pf* Posterior flagellum covered with very fine hairs, *TTHs* Tripartite tubular hairs on an anterior flagellum

The soilborne phytopathogenic peronosporomycetes such as *Pythium* spp. and *Aphanomyces* spp. cause devastating damping-off as well as root rot of seedlings in many crop and forest plants (Whipps and Lumsden 1991). They attack seeds after planting and rot them before they germinate. Moreover, root and crown rot caused by *Pythium* spp. and *Aphanomyces* spp. has become an increasing problem in different crops (Deora et al. 2006; Timmusk et al. 2009) and resulting inadequate plant stand in both nursery and field. Another historically infamous peronosporomycete is *Plasmopara viticola*, a causal agent of downy mildew disease of grapevine, which almost inundated the European wine industry in late nineteenth century. This obligate biotrophic pathogen was introduced into Europe from North America in 1976 (Gobbin et al. 2006). Downy mildews caused by various species of biotrophic peronosporomycete genera such as *Plasmopara*, *Basidiophora*, *Hyaloperonospora*, *Sclerospora*, etc. are serious pathogens of many crops and responsible for substantial amount of annual yield losses (Shetty et al. 1995). Because of the obligate parasitic mode, they are difficult to study at molecular level. Hence, our knowledge on their biology is very limited.

The peronosporomycetes infect their host plants through asexually generated characteristic biflagellate motile zoospores (Fig. 7.1). The zoospores have powerful sensory transduction system to locate and aggregate potential infection sites of the host and then rapidly undergo necessary morphological alterations for invading host tissues (Islam et al. 2001, 2002). The infection cycles of the peronosporomycetes are extremely rapid, which result epidemic for large area of crops within a few days under favorable environment. Despite the economical and environmental importance of the peronosporomycete diseases, they are difficult to control due to their unique cellular features, such as cell wall composition and/or lack of sterol metabolism (Nes 1987). Rapid development of resistance against agrochemical is also complicated in traditional agrochemical-based plant protection approaches against the peronosporomycetes. Moreover, the deleterious effects and consequences of the use of synthetic chemicals to the environment and nontargeted organisms are also discouraging their

use against the peronosporomycetes. Therefore, development of effective biologically rational management strategies against the peronosporomycete phytopathogens are badly needed.

Biological control involves the use of organisms, their products, genes, or gene products to control undesirable organisms (pests) and favor desirable organisms, such as crops, trees, beneficial organisms, and insects (National Academy of Sciences 1987). The organism that suppresses the disease or pathogen is referred to as the biological control agent (BCA). In last three decades, we have witnessed a dramatic development in research on biological control of plant diseases including those caused by the peronosporomycetes. Plant pathologists have been fascinated by the perception that disease suppressing soil or antagonistic plant-associated microorganisms could be used as environment-friendly biocontrol agents (Haas and Defago 2005). The concept of biological control is becoming popular not only because of increasing public concern about the use of hazardous chemical pesticides, but also uncertainty or inefficiency of current disease control strategies against the peronosporomycetes (Cook 1993; Islam et al. 2005a, b). Biological control strategies attempt to enhance the activities of BCA either by introducing high populations of a specific BCA or by enhancing the conditions that enable a BCA in their natural habitat to suppress the diseases (Nelson 2004). BCAs are easy to deliver, increase biomass production, and yield and improve soil and plant health (Burr et al. 1978; Kloepper et al. 1980b; Stockwell and Stack 2007). Isolation and characterization of new potential BCAs and understanding their ecology, behavior, and mode of action are considered as major foci in current biocontrol research (Islam et al. 2005b, 2011). The rapid development of convenient techniques in molecular biology has revolutionized this field by facilitating the identification of the underlying molecular mechanism of pathogen suppression (Islam et al. 2005b, 2011; Islam 2008) and by providing means for construction of "superior" BCAs through genetic engineering (Fenton et al. 1992; Bainton et al. 2004).

A large body of literature indicates that biological control agents such as bacterial antagonists can significantly suppress the disease caused by peronosporomycete phytopathogens and increase the yield of crops. Bacterial antagonists commonly studied and deployed for the control of peronosporomycete diseases include *Pseudomonas*, *Bacillus*, *Burkholderia*, *Lysobacter*, *Actinobacter*, *Enterobacter*, *Paenibacillus*, and *Streptomyces* (Anjaiah et al. 1998; Handelsman et al. 1990; Liu et al. 2007a; Islam et al. 2004, 2005b, 2011). Suppression of pathogens or diseases by the biocontrol agents is accomplished by several ways, such as production of antibiotics or lytic enzymes (Osburn et al. 1995; Palumbo et al. 2005; Perneel et al. 2008; Islam et al. 2011), competition for specific nutrient (e.g., iron or carbon) (van Dijk and Nelson 1998; Heungens and Parke 2000; Lee et al. 2008), induction of systemic resistance in the host plants (Yan et al. 2002; Zhang et al. 2010), and parasitizing pathogen's hyphae (Tu 1978) and/or reproductive structures (Khan et al. 1997).

Although several good reviews on biocontrol of plant diseases have been published (McSpadden Gardener and Fravel 2002; Compant et al. 2005; Haas and Defago 2005; Weller 2007), however, there is no review so far been published

specifically on biocontrol of peronosporomycete phytopathogens by bacterial antagonists. Recently, potentials for biological control of plant diseases by *Lysobacter* spp. (Islam 2011) and *Bacillus* spp. (Borriss 2011) have been reviewed. In this chapter, we attempt to review current knowledge on biocontrol of peronosporomycete phytopathogen by the bacterial antagonists. This review covers activities of BCAs against pathogens in both *in vitro* and *in vivo* conditions. The mode of action and application of BCAs in the practical field are also discussed.

7.2 Bioassay Methods to Screen Antagonistic Bacteria, Detect and Identify Antiperonosporomycetal Compounds

Selection of a potential isolate is the first and foremost important step in biological control by bacterial antagonist. Isolation of bacteria in a suitable culture medium from soil, plant, and water generates huge number of taxonomically diverse population. It is always challenging for researchers to find a convenient method to screen these vast population of isolated bacteria and identify the potentially active strain based on *in vitro* antagonism against a target pathogen. Over the years, some convenient *in vitro* bioassay methods have been developed in different laboratories. Some widely used bioassay methods are briefly discussed in this section.

Among the various bioassay methods, dual culture assay is a relatively simple and rapid screening method, which involves the cocultivation of two organisms on a soft agar medium in a Petri dish (Fig. 7.2). The area of the inhibition zone is taken as a measure of antagonistic potential of the isolates. However, microscopic observation on growth inhibited hyphal tips reveals that antagonistic bacteria exert diverse morphological alterations on the approaching hyphae and thus inhibit normal polar growth of the peronosporomycetes which can be seen in naked eye (Deora et al. 2006). The diversity of morphological alterations in the affected hyphae appears to be associated with the mode of action of antagonism by the bacteria (Fig. 7.2).

Other bioassay methods for assessment of activities of BCAs include (1) double layer agar method (Kraus and Loper 1992); (2) homogeneous solution method for motility and viability assay of zoospores (Islam et al. 2005b, 2011); (3) detached leaf assay (Islam et al. 2011); (4) inverted lid method (Ongena et al. 1999); (5) hyphal column assay (Yang et al. 1994); (6) bioassay to detect antiperonosporomycetal substances such as thin layer chromatography (TLC) (Islam et al. 2005b, 2011), high performance liquid chromatography (HPLC) (Nakayama et al. 1999), gas chromatography (GC) (Ko et al. 2009); (7) test with negative mutants (Becker and Cook 1988); (8) molecular detection of antibiotic biosynthesis genes (Chung et al. 2008); (9) use of antibiotic gene transcription in situ (Meyer et al. 2010); (10) lytic enzyme assay (Ko et al. 2009); (11) antiperonosporomycetal protein assay (Woo et al. 2002); (12) detection of siderophores (Schwyn and Neilands 1987); and (13) advanced microscopic study to understand mode of action (Islam 2008, 2010).

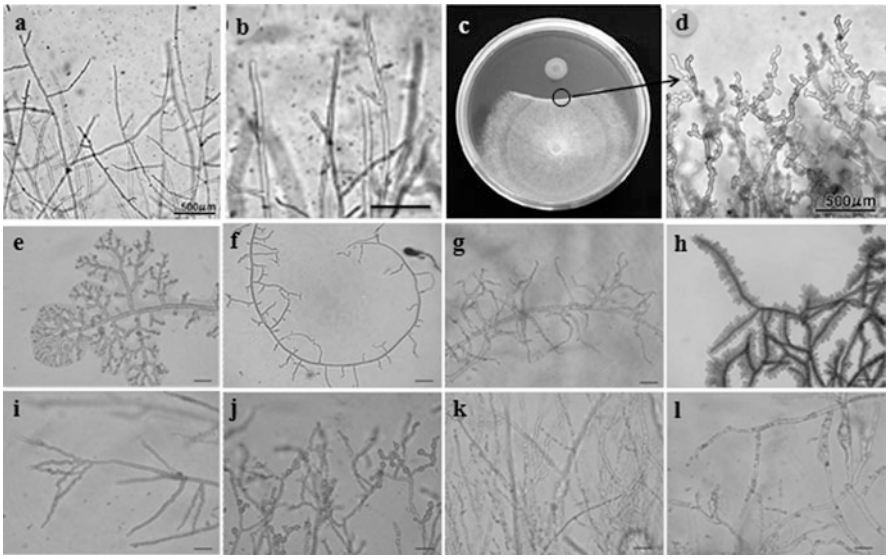

Fig. 7.2 Morphological alterations due to *in vitro* interactions between antagonistic bacteria and peronosporomycete phytopathogens in a dual culture on agar plate (adapted from Islam et al. 2005b and Deora et al. 2006). (**a**), (**c**)–(**h**) *Aphanomyces cochlioides* AC-5, and (**b**), (**i**)–(**l**) *Pythium aphanidermatum* PA-5. (**a**) Normal hyphal growth of AC-5; (**b**) normal hyphal growth of PA-5; (**c**) inhibition of AC-5 mycelial growth in the presence of *Lysobacter* sp. SB-K88 (Islam et al. 2005b); (**d**) curly growth of AC-5 hyphae approaching an SB-K88; (**e**) *Pseudomonas jessenii* strain EC-S101: excessive hyphal branching or hyperbranching; (**f**) *Stenotrophomonas maltophilia* EC-S105: curling; (**g**) *Delftia* sp. EC-107: longer and pointed tip with irregular growth; (**h**) *Bacillus subtilis* EC-S108: colonization of bacteria on hyphae; (**i**) *P. jessenii* EC-S101: apical branching; (**j**) *Pseudomonas* sp. EC-S102: swelling; (**k**) *Pseudomonas* sp. EC-S102: extensive vacuolation; (**l**) *Bacillus subtilis* EC-S108: decrease in normal branching, swollen hyphae and necrosis. Scale bars, (**e**)–(**l**) 50 μm

7.3 *In Vivo* Disease Suppression by Various Biocontrol Bacteria

Bacteria from diverse origins and taxonomic genera such as *Bacillus*, *Pseudomonas*, *Streptomyces*, *Burkholderia*, *Lysobacter*, and *Enterobacter* have been shown high potentials to suppress various plant diseases caused by the peronosporomycete phytopathogens (Table 7.1). Literature of some notable groups or genera of biocontrol bacteria are reviewed in this section.

7.3.1 Pseudomonas spp.

Pseudomonas spp. are most researched BCAs against peronosporomycete phytopathogens. Members of this bacterial genus are ubiquitous in soils and rhizosphere of plants. They have many traits that make them one of the best

Table 7.1 List of biocontrol bacteria, source of isolation, target pathogens, and mode of application on suppression of plant diseases caused by the peronosporomycete phytopathogens

Species/strain	Source	Mode of application	Target pathogen	Disease	Reference
Acinetobacter sp. LCH001	*Cinnamomum camphora* stems	–	*Ph. capsici*	–	Liu et al. (2007a)
Actinoplanes spp.	–	Sporangia on clay granules or as a root dip	*Py. ultimum*	Damping-off and root rot of Geraniums and poinsettias	Filonow and Dole (1999)
		Sporangia on clay granules	*Py. ultimum*	Damping-off of table beet and bush bean	Khan et al. (1997)
Ac. campanulatus, *Micromonospora chalcea*, *Streptomyces spirali*	Cucumber roots	Pruned root-dip method	*Py. aphanidermatum*	Damping-off and crown root rot of cucumber	El-Tarabily et al. (2010)
Bacillus cereus UW85	Roots of alfalfa	Seeds coated with UW85 grown in TSB, CB, or Min 3C and dried. Methyl cellulose is used as adhesive	*Ph. megasperma* f. sp. *medicaginis*	Damping-off of alfalfa	Handelsman et al. (1990), Silo-Suh et al. (1994)
		Spore-based formulation	*Ph. sojae*	Damping-off and root rot of soybean	Osburn et al. (1995)
Ba. megaterium	Rhizosphere of pepper plants	Seedlings drench with bacterial inoculum	*Ph. capsici*	Blight or crown blight disease on pepper plants	Akgül and Mirik (2008)
Ba. mycoides MW27	Pea rhizosphere soil	Prill, granule, and seed coat formulations	*A. euteiches*	Root rot of pea	Wakelin et al. (2002)
Ba. pumilus SE34 *Ps. fluorescens* 89B61	–	Bacterial suspensions to soilless growth media	*Ph. infestans*	Late blight of tomato	Yan et al. (2002)
Ba. pumilus INR7	–	Seed treatment with INR7	*Pl. halstedii*	Downy mildew of sunflower	Nandeeshkumar et al. (2008)
Ba. subtilis BBG100 (transformant)	*Ba. subtilis* ATCC 6633	Seed treatment with BBG100	*Py. aphanidermatum*	Damping-off of tomato	Leclère et al. (2005)

Ba. subtilis HS93	Roots of pepper plants	Seeds soaked in saccharose suspension containing bacteria	*Ph. capsici*	*Phytophthora* root rot of sweet pepper	Sid Ahmed et al. (2003a)
		Seed treatment and root drenching with bacteria or its chitin formulation	*Ph. capsici*	*Phytophthora* root rot of pepper plant	Sid Ahmed et al. (2003b)
Ba. subtilis strain M4	Soil	Seeds treated with vegetative cells of bacteria	*Py. aphanidermatum*	Damping-off of tomato	Ongena et al. (2005)
Ba. subtillis	Rhizosphere soil of healthy red pepper	Seeds soaked in bacterial suspension	*Ph. capsici*	*Phytophthora* blight of red pepper	Lee et al. (2008)
Ba. subtilis cot1	Contaminated *Continus*-tissue culture	Dipping microplants and seedlings into bacterial suspension	*Py. ultimum Phytophthora* spp.	Damping-off of *Astilbe*, *Photinia*, *Hemerocallis*, and *Brassica*	Berger et al. (1996)
Ba. subtilis ME488	Soil	Seed treated with bacteria in methyl cellulose	*Ph. capsici*	*Phytophthora* blight of red pepper	Chung et al. (2008)
Ba. subtilis KS1	Grape berry skin	–	*Pl. viticola*	Downy mildew of grapevine	Furuya et al. (2011)
Ba. subtilis (commercial formulation serenade)	Commercial formulation	Spray bacterial suspension	*Ph. infestans*	Potato late blight	Stephan et al. (2005)
Ba. safensis, *Lysinibacillus boronitolerans*, *Ba. subtilis* subsp. *subtilis*, *Ba. pumilus*, *Ba. macauensis*	Roots of soybean seedling	Soil drench for all strains. Seed treatment for strain 1PC-11 (one or more strain separately or jointly)	*Ph. capsici*	*Phytopthora* blight of squash plant	Zhang et al. (2010)
Bu. cepacia AMMD, *Ps. fluorescens* PRA25	Rhizosphere of pea	Seed mixed with bacteria and then air-dried	*Pythium* spp.	Preemergence damping-off	Parke et al. (1991), Bowers and Parke (1993), King and Parke (1993)

(continued)

Table 7.1 (continued)

Species/strain	Source	Mode of application	Target pathogen	Disease	Reference
Bu. cepacia AMMDR1	Pea rhizosphere	Seed treatment or root dip inoculation	*Py. aphanidermatum*	Preemergence damping off of pea	Heungens and Parke (2000)
			Py. aphanidermatum *A. euteiches*	Postinfection stage of preemergence damping-off and root rot of pea	Heungens and Parke (2001)
Bu. cepacia BC1	Soybean root	Preinfiltrated seed coated with bacterial antagonist	*Py. ultimum* *Py. arrhenomanes* *Fusarium graminearum*	Damping-off of corn	Mao et al. (1998)
E. clocae	Cucumber seeds and cotton hypocotyls	Seed soaked in bacterial suspension with methocel adhesive	*Pythium* spp.	Seed rot and preemergence damping-off of cotton	Nelson (1988)
E. clocae, *Acinetobacter calcoaceticus*, *Pseudomonas* spp., *Pantoea agglomerans*	Seeds of cotton, perennial ryegrass, zucchini, tomato, onion, and cotton hypocotyls	Sand containing cylinders drenched with bacterial suspension	*Py. ultimum*	Seed rot and damping-off of cotton	van Dijk and Nelson (1998)
E. clocae EcCT-501R3	Cotton hypocotyls	Seed placed on soil and drenched with bacteria	*Py. ultimum*	Damping-off of cotton	van Dijk and Nelson (2000)
		Seed treatment	*Py. ultimum*	Seed infection of cucumber	Windstam and Nelson (2008a,b)
E. clocae	–	Bacterial suspension poured on seed placed in soil	*Py. ultimum*	Damping-off of carrot, cotton, cucumber, lettuce, radish, tomato, and wheat	Kageyama and Nelson (2003)

Lysobacter enzymogenes 3.1T8	Root tips of cucumber grown on used rock wool	Addition of unwashed bacterial suspension grown on R2A medium or its filtrate to seed and/or nutrient solution	*Py. aphanidermatum*	Root and crown rot of cucumber	Folman et al. (2003, 2004)
		Washed bacterial cells plus chitosan on top of each rock wool block	*Py. aphanidermatum*	Root and crown root rot of cucumber	Postma et al. (2009)
L. enzymogenes strain C3	Turf grass leaf surface	Bacterial seed coating using methyl cellulose as adhesive	*Py. ultimum* var. *ultimum*	Damping-off of sugar beet	Palumbo et al. (2005)
L. (*Stenotrophomonas*) sp. strain SB-K88	Fibrous roots of sugar beet	Seeds are mixed with bacterial pellet and air dried	*Pythium* spp.	Damping-off of sugar beet	Nakayama et al. (1999)
			Ap. cochlioides	Damping-off of sugar beet and spinach	Islam et al. (2004, 2005b), Islam (2010)
L. antibioticus HS124	Rhizosphere soil	Seedling pot is amended with bacterial culture	*Ph. capsici*	*Phytophthora* blight of pepper plant	Ko et al. (2009)
Paenibacillus sp., *St. sioyaensis*	Soil and peat moss	Unknown	*Py. aphanidermatum*	Unknown	Hong and Meng (2003)
Paenibacillus sp. strain B2	Mycorrhizosphere of *Sorghum bicolor*	Strain B2 alone or with *Glomus mosseae*	*Ph. parasitica*	Root rot of tomato plants	Budi et al. (1999)
Pae. polymyxa	Wheat (B2), and peanut (B5 and B6) rhizosphere	Roots of 2 week seedlings soaked in cultures of *P. polymyxa*	*Py. aphanidermatum*	Damping-off of *Arabidopsis*	Timmusk et al. (2009)
Ps. fluorescens Pf5	Cotton rhizosphere	Seeds treatment with bacteria	*Py. ultimum*	Damping-off of cotton seedlings	Howell and Stipanovic (1980)
		Seed treatment with bacteria suspended in methyl cellulose	*Py. ultimum*	Damping-off of cucumber	Kraus and Loper (1992)

(continued)

Table 7.1 (continued)

Species/strain	Source	Mode of application	Target pathogen	Disease	Reference
Ps. fluorescens Pf1	Crop rhizosphere	Seed treatment with bacteria suspended in methyl cellulose or with talc formulation	*Py. aphanidermatum*	Damping-off of tomato and hot pepper	Ramamoorthy et al. (2002)
Pseudomonas sp. DSS73	Rhizoplane of sugar beet seedlings	Bacterized seed	*Py. ultimum*	*Pythium*-root disease	Koch et al. (2002), Andersen et al. (2003)
Ps. fluorescens DR54	Rhizosphere of sugar beet	Bacterial suspension applied on seed surface	*Py. ultimum*	Damping-off of sugar beet	Nielsen et al. (1998, 1999)
Ps. aeruginosa PNA1	Rhizosphere of chickpea	Seeds are soaked in bacterial suspension.	*Py. splendens*	Damping-off of bean	Anjaiah et al. (1998)
		Bacteria applied to soil	*Py. splendens* *Py. myriotylum*	Damping-off of bean root rot of cocoyam	Tambong and Höfte (2001), Perneel et al. (2008)
		–	*Ph. capsici*	*Phytophthora* blight of pepper	Kim et al. (2000)
Ps. fluorescens strain F113 and M114 (pCU2031)	Sugarbeet rhizosphere	Seed dipping into bacterial suspension	*Py. ultimum*	Damping-off sugar beet	Fenton et al. (1992)
Ps. fluorescens	Sugar beet rhizosphere	Seeds soaking in bacterial suspension	*Py. ultimum*	Damping-off of sugar beet	Delany et al. (2001)
Ps. fluorescens	Rhizosphere of sugar beet	Seed soaking in bacterial suspension	*Py. ultimum*	Damping-off of pea	Bainton et al. (2004)
Pseudomonas spp. strain B324 and A708	Roots of wheat	Seed treated with bacteria suspended in methyl cellulose	*Pythium* spp.	Preemergence seedling blight of wheat	Becker and Cook (1988)
Ps. aeruginosa 7NSK2	Rhizosphere of barley	Seeds soaked in bacterial suspension	*Py. splendens*	Damping-off of tomato	Buysens et al. (1996)
Ps. fluorescens AB254	Rhizosphere soil	Seed coated with bacteria (methyl cellulose) and dried	*Py. ultimum*	Damping-off of sweet corn (*Zea mays*)	Callan et al. (1990)

Ps. chlororaphis Tx-1	–	Addition to hydroponic nutrient solution	*Py. aphanidermatum* *Py. dissotocum*	Root rot peppers	Chatterton et al. (2004) Khan et al. (2003)
		–	*Ph. megasperma*	Root rot asparagus	Carruthers et al. (1995)
Ps. fluorescens, *Ba. cereus* HY06, *Ps. chlororaphis*, *Ps. corrugata*, *Bu. gladioli*, *Comamonas acidovorans*	–	Bacterial application to hydroponic nutrient solution	*Py. phanidermatum*, *Py. dissotocum*	*Pythium* root rot of *Chrysanthemum*	Liu et al. (2007b)
Ps. fluorescens CHA0 *Ps. fluorescens* strain CHAO and CHAO/pME3090	Tobacco rhizosphere	Unknown	*Py. ultimum*	Damping-off of cucumber, cress, and wheat	Maurhofer et al. (1994, 1995)
Ps. fluorescens CHA0r	Tobacco rhizosphere	Soaking pots of 1-week-old seedlings with bacterial suspension	*Hyaloperonospora parasitica*	Arabidopsis downy mildew	Iavicoli et al. (2003)
Ps. fluorescens CHA0, *Ps. fluorescens* KD	Rhizosphere of tobacco (CHAO) and wheat (KD)	Soil inoculation	*Py. ultimum*	*Pythium* root rot of wheat	Meyer et al. (2010)
Ps. fluorescens strains B5 and X *Ps. corrugate* strain R117 *Bacillus subtilis* strains B2 and B6	–	Seed treatment (sugar beet) or drenching (cucumber) or seed coating	*Py. ultimum*	Damping-off of sugar beet and cucumber	Georgakopoulos et al. (2002)
Ps. corrugata, *Ps. fluorescens*, *Ps. marginalis*, *Ps. putida*, *Ps. syringae*, *Ps. viridiflava*	Peat, barks, and composts	bacterial application to rock wool	*Py. ultimum* *Py. aphanidermatum*	Damping-off of tomato	Gravel et al. (2005)

(continued)

Table 7.1 (continued)

Species/strain	Source	Mode of application	Target pathogen	Disease	Reference
Ps. fluorescens 2-79R, *Bu. cepacia*1-23, *Pseudomonas* sp. 1-30	Wheat roots	Seed soaking in bacterial suspension with methyl cellulose as adhesive	*Pythium* spp.	*Pythium* root rot of winter wheat	Milus and Rothrock (1997)
Pseudomonas sp. (fluorescent pseudomonads)	Rhizosphere and the rhizoplane of cotton	Bacterial suspension pipetted over seed	*Py. ultimum*	Root disease in cotton	Hagedorn et al. (1989)
Ps. fluorescens strain Hv37aR2	Barley roots	Seeds soaked in bacterial suspension and air dried	*Py. ultimum*	*Pythium* disease of cotton	Howie and Suslow (1991)
Ps. fluorescens strain (PfT-8)	Tomato rhizosphere	Bacterial suspension in lignite-based formulation	*Pythium* spp.	Damping-off of tomato	Jayaraj and Radhakrishnan (2008)
Ps. fluorescens 3551	Cotton rhizosphere	Seeds dipped in bacterial suspension	*Py. ultimum*	Preemergence damping-off of cotton	Loper (1988)
Ps. fluorescens (EBL 20-PF)	Chili leaf	Seed or soil treatment with talc-based formulation	*Py. aphanidermatum*	Damping-off of chili	Muthukumar et al. (2011)
Pseudomonas strains BTP1, BTP7	Barley and tomato roots	Addition of bacterial suspension to hydroponic nutrient	*Py. aphanidermatum*	*Pythium* root rot of cucumber	Ongena et al. (1999)
Ps. fluorescens-putida ML5	ML5 from pericarp of sugar beet seed.	Dry seed coating with bacteria in methyl cellulose and talc	*Py. ultimum*	Seed rot and damping-off of sugar beet	Osburn et al. (1989)
Ps. putida R20	R20 from rhizosphere of lima bean				
Ps. putida N1R	–	Seed soaked in bacterial suspension with pelgel as sticker	*Py. ultimum*	Damping-off of pea, cucumber, and soybean	Paulitz and Loper (1991)
Ps. fluorescens B5, *Ps. corrugata* 2140	–	Pelleted seeds were soaked in bacterial suspension and air-dried	*Py. ultimum*	Damping-off of sugar beet	Schmidt et al. (2004a, b, c)

Ps. fluorescens strain SS101	Rhizosphere of wheat	Bulb or soil treatment	*Py. intermedium*	Root rot hyacinth bulb	de Souza et al. (2003b)
		Leaves were immersed into bacterial suspension	*Ph. infestans*	Tomato late blight	Tran et al. (2007)
		Bacteria applied to soil as an atomized mist	*Pythium* spp.	*Pythium* disease of apple seedling	Mazzola et al. (2007)
Ps. fluorescens, *Serratia* spp., *Bacillus* spp.	Soil from pea field	Bacterial suspensions were applied to soil	*Py. ultimum*	Damping-off of field pea	Wang et al. (2003)
Pseudomonas spp., *Ba. megaterium*, *Arthrobacter histidinolovorans*, *Cytophaga johnsonae*	Roots of sugar beet	Washed and unwashed bacteria applied to pelleted seed and then partially dried	*Py. ultimum*, *A. cochlioides*	Sugar beet damping-off complex	Williams and Asher (1996)
Ps. fluorescens, *Pseudomonas* sp. *Enterobacter* sp., *E. cloacae*	–	Mixed microbial suspension was used to inoculate shallow puncture wounds on tubers.	*Ph. erythroseptica*	Pink rot of potato tubers	Schisler et al. (2009)
Pseudomonas sp. strain EC-S101, *Stenotrophomonas maltophilia* EC-S105	Rhizoplanes of spinach and sugar beet	Sterilized seeds coated with bacteria partially	*Ap. cochlioides*	Damping-off of sugar beet and spinach	Deora et al. (2005)
Pseudomonas jessenii strain EC-S101	Rhizoplane of spinach	Seed coating	*Ap. cochlioides*	Damping-off of sugar beet	Deora et al. (2006)
Ps. fluorescens ECO-001	Rhizoplane of a weed *Plantago asiatica*	–	*Ap. cochlioides AC-5*	Damping-off of sugar beet	Islam and Fukushi (2010)
Ps. fluorescens UOM SAR14	–	Seed treatment	*Sclerospora graminicola*	Downy mildew of pearl millet	Raj et al. (2003)
Ps. fluorescens WCS417r	–	Soil inoculation	*H. parasitica*	Downy mildew in *Arabidopsis thaliana*	Van der Ent et al. (2008)

(continued)

Table 7.1 (continued)

Species/strain	Source	Mode of application	Target pathogen	Disease	Reference
Ps. fluorescens	Rhizosphere of pearl millet	Seed treatment, and/or foliar application	*Sc. graminicola*	Downy mildew of pearl millet	Umesha et al. (1998)
Ps. fluorescens 0990, *Ps. putida* 06909	Citrus rhizosphere soil	Unknown	*Ph. parasitic*	Citrus root rot	Yang et al. (1994)
Rhizobium japonicum	–	Inocula of *Rhizobium* applied to potted soil	*Ph. megasperma*, *Ph. megasperma*, *Py. ultimum*	Root rot of soybean	Tu (1978)
Serratia plymuthica HRO-C48	Rhizosphere of oilseed rape	Seeds are soaked in bacterial suspension	*Py. apahnidermatum*	Damping-off of cucumber	Pang et al. (2009)
Se. marcescens 90-166, *Ps. Fluorescens* 89B-61, *Ba. pumilus* SE34, *Ba. pumilus* T4, and *Ba. pasteurii* C-9	–	Seed treatment or root drenches	*Peronopora. tabacina*	Blue mold of tobacco	Zhang et al. (2001)
Se. plymuthica C-1, *Chromobacterium* sp. C-61, *L. enzymogenes* C-3	Soils	Bacteria in chitin media applied as soil drench	*Ph. capsici*	*Phytophthora* blight	Kim et al. (2008)
Stenotrophomonas maltophilia strain W81	Rhizosphere of sugar beet	Seed dipping into bacterial suspension	*Py. ultimum*	Sugar beet	Dunne et al. (1997, 2000)
Streptomyces spp.	Soils	Potting mix inoculated with the spores	*Py. ultimum*	Root disease of lettuce	Crawford et al. (1993)
Streptomyces spp.	Raspberry roots, potato rhizosphere, and soil	Bacterial agar blocks set around the developing roots of each plantlet	*Ph. fragariae* var. *rubi*	Root rot of raspberry	Valois et al. (1996)
Streptomyces spp.	Soil	Spore suspension applied over each seed after sowing	*Ph. medicaginis* and *Ph. sojae*	Root rot of alfalfa and soybean	Xiao et al. (2002)

Streptomyces sp. AMG-P1	–	Culture extract sprayed on seedlings	*Phytophthora* and *Pythium* spp.	Late blight of red pepper and tomato	Lee et al. (2005)
Streptomyces spp. strain BSA25 and WRA1 and *Mesorhizobium ciceri* WSM1666 or Kaiuroo 3	Roots of lentil, chickpea, pea, faba bean, and wheat	Dry seed coating in *Streptomyces* with Arabic gum and *Rhizobium* applied over-head soil	*Ph. medicaginis*	Root rot of chick pea	Misk and Franco (2011)
Streptomyces sp. ANK313 *Streptomyces* sp. ANK302	Soil	Tested on motility of zoospore	*Pl. viticola*	Downy mildew of grapevine	Abdalla et al. (2011) Zinada et al. (2011)
Streptomyces sp. strain B5136	Sea water	Leaf disk assay	*Pl. viticola*	Downy mildew of grapevine	Islam et al. (2011)
Streptomyces sp. AP77	Seawater of a *Porphyra yezoensis* cultivation facility	–	*Py. porphyrae*	Red rot of red alga (*Porphyra* spp.)	Woo et al. (2002)
St. halstedii AJ-7	Soil	Red-pepper seeds were soaked in the culture broth	*Ph. capsici*	*Phytophthora* blight of red pepper	Joo (2005)

BCAs against various phytopathogens (Weller 2007). It is now over 40 years since *Pseudomonas* spp. were first recognized as a potential BCA. Within this period, intensive research has given rise to many well-characterized *Pseudomonas* BCAs (Table 7.1). Among them, the fluorescent *Pseudomonas* spp. received paramount importance due to their efficacy in biocontrol activity. A well-characterized fluorescent pseudomonad is *Ps. fluorescens* Pf5, suppresses several *Pythium* diseases, including damping-off disease in cotton (Howell and Stipanovic 1980) and cucumber caused by *Py. ultimum* (Kraus and Loper 1992). Another strain of *Ps. fluorescens*, Pf1 controls damping-off diseases in tomato and hot pepper caused by *Py. aphanidermatum* (Ramamoorthy et al. 2002). A strain of fluorescent *Pseudomonas*, DSS73 isolated from the rhizoplane of sugar beet seedlings, displayed suppression of root-pathogenic *Py. ultimum* by producing biosurfactant antibiotics (Sørensen et al. 2001; Nielsen et al. 2002; Andersen et al. 2003). *Ps. fluorescens* strain DR54 isolated from sugar beet rhizosphere showed high biocontrol activity against *Pythium* damping-off in sugar beet (Nielsen et al. 1998). Similarly, *Ps. fluorescens* strain F113 isolated from sugar beet in Ireland displayed damping-off disease suppression in sugar beet and pea caused by *Py. ultimum* (Fenton et al. 1992; Delany et al. 2001; Bainton et al. 2004).

An important strain CHA0 of *Ps. fluorescens* was isolated from roots of tobacco grown near Payern, Switzerland, in a soil naturally suppressive to black root rot of tobacco caused by *Thielaviopsis basicola* (Stutz et al. 1986). This strain has shown one of the broadest range of potential biocontrol and growth-promoting mechanisms of any PGPR described so far. CHA0 suppresses *Pythium* damping-off of cucumber and wheat and infection of *Arabidopsis* by *Hyaloperonospora parasitica* (Maurhofer et al. 1995; Iavicoli et al. 2003; Meyer et al. 2010). It is also equally effective against nonperonosporomycete fungi, viruses, and nematodes (Keel et al. 1992; Maurhofer et al. 1994; Siddiqui and Shaukat 2003). Application of *Ps. fluorescens* SS101 to soil or bulbs effectively controls root rot of flower bulb crops caused by *Py. intermedium* in both laboratory and small-scale field experiments (de Souza et al. 2003b). Strain SS101 when applied to soil controls *Pythium*-root rot of apple seedlings (Mazzola et al. 2007) and when applied to leaves controls late blight of tomato caused by *Ph. infestans* (Tran et al. 2007).

Ps. aeruginosa PNA1, isolated from the rhizosphere of chickpea, has widely been shown biocontrol efficacy against a number of phytopathogenic fungi and peronosporomycetes (Anjaiah et al. 1998). This strain demonstrated *in vivo* biocontrol activity against various peronosporomycetes including *Py. splendens* on bean (Anjaiah et al. 1998), *Py. myriotylum* on cocoyam (Tambong and Höfte 2001; Perneel et al. 2008), and *Ph. capsici* on pepper (Kim et al. 2000). Another strain of *Ps. aeruginosa*, 7NSK2 isolated from the rhizosphere of barley, promotes the growth of several crops and suppresses *Py. splendens*-induced damping-off in tomato (Buysens et al. 1996). Isolates of *Ps. chlororaphis* (previously *Ps. aureofaciens*) strongly suppressed *Py. aphanidermatum* in roots of pepper, cucumber, and chrysanthemum (Chatterton et al. 2004; Khan et al. 2003; Liu et al. 2007b), and *Ph. megasperma* in roots of asparagus (Carruthers et al. 1995) and moderately suppressed *Py. dissotocum* in roots of hydroponic

chrysanthemums (Liu et al. 2007b). *Ps. putida* strain N1R provides biocontrol of *Ps. ultimum* on soybean, pea, and cucumber (Paulitz and Loper 1991). Similarly, *Ps. jessenii* strain EC-S101 and *Stenotrophomonas maltophilia* EC-S105 efficiently suppressed *in vivo* damping-off disease caused by *Ap. cochloides* (Deora et al. 2005). Some pseudomonads have been found to protect plants from various pathogens by inducing systemic resistance. For example, *Ps. fluorescens* strains UOM and SAR14Ps control *Sclerospora graminicola* on pearl millet (Raj et al. 2003) and *Ps. fluorescens* WCS417r controls *Hy. Parasitica* on *Arabidopsis* (van der Ent et al. 2008).

7.3.2 *Bacillus* spp.

The spore-forming bacteria, *Bacillus* spp. are considered one of the most effective biocontrol candidates for plant diseases caused by peronosporomycetes. A large body of literature is available concerning biological control of peronosporomycete phytopathogens by various strains of *Bacillus* spp., some important research findings are reviewed (Table 7.1). Bacterial strain antagonistic to a certain peronosporomycete on plate assay may not necessarily be effective in suppressing disease in in vivo or vice versa. For example, *Ba. cereus* UW85 is not inhibitory to *Ph. megasperma* f. sp. *medicaginis* on plates, but its application to the alfalfa seeds suppresses the damping-off disease caused by the same pathogen in the field conditions (Handelsman et al. 1990). In contrast, the same strain was inhibitory to *Ph. sojae* on plate assay, but increased yields only on the susceptible cultivar where *Phytophthora* root rot was a factor. At another site where *Phytophthora* root rot was not a factor, UW 85 increased plant stands significantly over untreated seeds regardless of *Phytophthora* root rot resistance (Osburn et al. 1995). Both additive and nonadditive responses were also observed when several strains were applied together for disease control. For example, a nonadditive response was demonstrated by Everts and Armentrout (2001) for powdery mildew of pumpkin. Several *Bacillus* spp. were also found effective in suppressing *Phytophthora* blight in pepper and squash caused by *Ph. capsici*. Preinoculation of pepper plants with three strains of *Ba. megaterium* alone or in combination, significantly reduced disease severity of *Phytophthora* blight or crown blight caused by *Ph. capsici* in field experiments (Akgül and Mirik 2008). Several strains of *Ba. subtilis* isolated from the rhizoplane and rhizosphere pepper have been found useful for suppression of *Phytophthora* blight of pepper when applied as seed coating (Sid Ahmed et al. 2003a, b; Lee et al. 2008; Chung et al. 2008). The mixture of *Ba. safensis* T4 + *Lysinibacillus boronitolerans* SE56 significantly improved control efficacy compared to the individual strain (Zhang et al. 2010).

Several lines of evidence suggest that inoculation of *Bacillus* spp. may induce systemic resistance in the host plant. Several strains of *Ba. safensis*, *Lysinibacillus boronitolerans*, *Ba. pumilus*, and *Ba. macauensis* have also been shown to induce protection squash against *Phytophthora* blight (Zhang et al. 2010). Similarly,

Ba. pumilus INR7 + T4 + SE56 and INR7 + *Ba. subtilis* IN937a + T4 + SE56 tended to induce higher levels of disease reduction compared to individual strains. *Ba. pumilus* strains INR7 and SE34 were also successful to elicit systemic protection against downy mildew caused by *Plasmopara halstedii* in sunflower (Nandeeshkumar et al. 2008) and late blight on tomato caused by *Ph. infestans* (Yan et al. 2002), respectively. Downy mildew, caused by *Pl. viticola*, was also reduced on grape berry skins and leaves by treatment with *Ba. subtilis* KS1 (Furuya et al. 2011).

A common soil bacterium, *Paenibacillus* spp., displayed biocontrol activity against several diseases caused by peronosporomycetes. *Paenibacillus* sp. strain B2 isolated from the mycorrhizosphere of *Sorghum bicolor* displayed antagonistic activities against some soilborne pathogens when applied with Glomus mosseae (Budi et al. 1999). Application of strain B2 alone or with *G. mosseae* to tomato plants significantly reduced root necrosis caused by *Ph. parasitica*. Besides, this bacterium also enhanced colonization of mycorrhizae in the rhizosphere of tomato. A strain of *Pae. illinoisensis* KJA-424 isolated from soil in the west coast of Korea reduced root mortality of pepper plants caused by *Ph. capsici* when it (in 0.2 % colloidal chitin) was applied into the pot soil. Timmusk et al. (2009) reported that pretreatment of *Arabidopsis* root with some strains of *Pae. polymyxa* showed significant protection against subsequent infection by *Py. aphanidermatum*. The survival rates of *Py. aphanidermatum* infected plants were higher when the seedlings were pretreated with B2 and B5 strains than that of B6 treatment.

7.3.3 *Burkholderia* spp.

Burkholderia spp. formerly erroneously identified as *Pseudomonas* spp. showed biocontrol activities against several peronosporomycetal diseases. For example, pea seeds treated with *Burkholderia* (*Pseudomonas*) *cepacia* strains AMMD and AMMDR1 (rifampicin resistant mutant) resulted in increased seedling stand and reduced preemergence damping-off caused by *Py. ultimum* and *Py. sylvaticum*. Seed treatment with *Bu. cepacia* (strain AMMD) and *Ps. fluorescens* (PRA25) alone or in combination with captan effectively suppressed damping-off disease of pea (Parke et al. 1991). Application of *Bu. cepacia* increased seedling emergence (40 %) and yield (48 %) compared with captan alone. Strains AMMD or PRA25 significantly suppressed early stage of diseases, while strain AMMD also suppressed the final incidence of disease at harvest over 2 successive years (Bowers and Parke 1993). *Bu. cepacia* AMMDR1 significantly reduced *Py. aphanidermatum* postinfection colonization and damping-off of pea seeds, even when the bacteria were applied 12 h after zoospore inoculation (Heungens and Parke 2001). The level of biocontrol of *Pythium* damping-off by AMMD depended on the host genotype, while the seed treatment with bacteria did not reduce the symptoms of *Aphanomyces* root rot (King and Parke 1993). *Bu. cepacia* AMMDR1 significantly reduced colonization

of taproots by *Ap. euteiches* mycelium, when roots were dip inoculated in a concentrated cell suspension (Heungens and Parke 2001). Both root-dip inoculation of bacterial suspension at lower concentrations and seed inoculation resulted in lower numbers of bacteria near the root tip early on in the infection process.

7.3.4 *Enterobacter cloacae*

Enterobacter cloacae is a common seed-associated bacterium (Hadar et al. 1983), which is an effective biological control agent that suppresses seed infections, protecting a number of plant species from *Py. ultimum*-induced damping-off (Table 7.1) (Nelson 1988; van Dijk and Nelson 1998, 2000). Seeds previously inoculated with *E. clocae* strains NRRL B-14095 and NRRL B-14096 suppressed preemergence *Pythium* damping-off of cucumber compared to control. The performance of biocontrol by *E. clocae* was varied in different plant species. *E. cloacae* was equally effective in controlling *Pythium* damping-off when placed on the seeds of various crops such as carrot, cotton, rye, lettuce, radish, tomato, and wheat (Nelson 1988; Kageyama and Nelson 2003). However, it was ineffective in biocontrol of diseases in corn (Kageyama and Nelson 2003); pea (Hadar et al. 1983; Kageyama and Nelson 2003); and soybean, snap bean, and lima bean.

7.3.5 *Lysobacter* spp.

The *Lysobacter* spp. are ubiquitous inhabitants in the diverse environment that have some unique features including gliding motility, high genomic G + C ratio (65–72 %), and brush-like polar fimbriae (Islam et al. 2005a, b). This genus have gained broad interest for several reasons such as (1) rich source for production of a variety of novel antibiotics, such as lysobactins or katanosins (Bonner et al. 1988), cephabacins (Lee et al. 2008), tripropeptins (Hashizume et al. 2001, 2004), and macrocylic lactams such as xanthobaccins (Hashidoko et al. 1999; Nakayama et al. 1999; Yu et al. 2007); (2) production of a wide variety of extracellular cell wall degrading enzymes such as β-lytic proteases (Sid Ahmed et al. 2003b), endopeptidase (Muranova et al. 2004), keratinases, β-1,3 glucanases (Palumbo et al. 2003), cellulase (Ogura et al. 2006), and lysoamidase (Riazanova et al. 2005); (3) ability to suppress plant diseases and colonize plant surfaces (Martin 2002; Islam et al. 2004, 2005b; Islam 2008, 2010); and (4) exhibition of wolf-pack-like micropredatory behavior (Martin 2002; Islam 2010). Some of these unique features of *Lysobacter* spp. are advantageous for using them as BCAs against phytopathogens (Zhang et al. 2001; Folman et al. 2004; Islam et al. 2005a; Kobayashi et al. 2005; Ji et al. 2008). Among 21 identified species, biocontrol activity of *L. enzymogenes* strains C3 and 3.1T8 and *Lysobacter* sp. SB-K8 has extensively been investigated. The biocontrol potentials of *Lysobacter* spp. including their mode of action have recently been reviewed (Islam 2011).

7.3.6 Actinomycetes

Numerous surveys of soil bacteria have identified considerable number of strains of actinomycetes as potential BCAs (Khan et al. 1997; Filonow and Dole 1999; Crawford et al. 1993; Misk and Franco 2011). One of the advantages of actinomycetes is their ability to produce spore. These spores are long lived and resistant to heat and desiccation, and maintain a stable population over the time. Actinomycetes such as *Actinoplanes* spp. have shown great promise for reducing *Pythium* root rot in horticultural plants in the greenhouse. Several strains of *Actinoplanes* spp. (W57, W257, or 25844) were applied on clay granules at 5 % or 0.5 % w/w to *Py. ultimum* oospores-infested soil-less potting mix 5 days prior to replanting geranium or poinsettia seedlings. Application of *Actinoplanes* spp. generally reduced root rot severity and increased plant stand compared to nontreated plants after 6 week grown in a greenhouse (Filonow and Dole 1999). When strain W257 was applied as granules or as a root dip, it was as effective as the fungicide metalaxyl in reducing the root rot. When strains 25844, W57, and W257 were applied as granules at 5 % (w/w) to field plots infested with oospores of *Py. ultimum*, only strain 25844 consistently increased emergence and reduced root rot of table beets compared to controls (Khan et al. 1997). In the same study, strain 25844 at 1 % (w/w) also increased the emergence of bush beans at 28 days after planting in *Py. ultimum*-infested plots, but lower rates were found ineffective. Similarly, El-Tarabily et al. (2010) showed that the endophytic actinomycetes such as *Ac. campanulatus*, *Micromonospora chalcea*, and *Streptomyces spiralis*, when applied individually or in combination significantly promoted plant growth and reduced damping-off and crown and root rot of cucumber caused by *Py. aphanidermatum* under green house conditions. These isolates when applied individually or in combination to cucumber seedlings, also promoted growth and yield and reduced seedling damping-off and root and crown rot of mature cucumber plant in the field (El-Tarabily et al. 2010).

The use of streptomycete actinomycetes as biological control agents against the peronosporomycete disease has received considerable attention (Crawford et al. 1993; Valois et al. 1996; Xiao et al. 2002; Joo 2005; Lee et al. 2005; Misk and Franco 2011; Abdalla et al. 2011; Islam et al. 2011) (Table 7.2). Eleven strains of actinomycetes belong to the genus *Streptomyces* significantly reduced the root rot index caused by *Ph. fragariae* var. *rubi* when inoculated on raspberry plantlets (Valois et al. 1996). In contrast, antiperonosporomycetal compounds originating from actinomycetes appear to be selectively active against *Phytophthora* and *Pythium*. Spraying tomato seedlings with culture broth of *Streptomyces* sp. AMG-P1 resulted highly inhibitory effect against late blight disease caused by *Ph. infestans* at 500 μg freeze-dried weight per milliliter, whereas its antibiotic paromomycin showed potent in vivo activity against red pepper and tomato late blight diseases with 80 and 99 % control value, respectively, at 100 μg/ml (Lee et al. 2005).

The use of *Streptomyces* spp. in biocontrol of plant diseases depends on their potential inhibitory effects on the pathogen. Eight isolates having potential pathogen-inhibitory capabilities were subsequently tested for their ability to control *Phytophthora* root rots on alfalfa and soybean in sterilized vermiculite and naturally infested field soil. The *Streptomyces* isolates significantly reduced root rot severity in alfalfa and soybean caused by *Ph. medicaginis* and *Ph. sojae*, respectively (Xiao et al. 2002). Similarly, in a greenhouse experiment, *Strepmyces* sp. BSA25 and WRA1 with the highest antagonistic capabilities against broad spectrum of pathogens were tested for their ability to control *Phytophthora* root rot of chickpea caused by *Ph. medicaginis* and found that both isolates promote vegetative growth of chick pea and successfully suppressed *Phytophthora* root rot when coinoculated with either *Mesorhizobium ciceri* WSM1666 or Kaiuroo 3 (Misk and Franco 2011).

7.3.7 Disease Suppression by Other BCAs

There are a few other bacterial genera which have been proved to be effective BCAs against peronosporomycete pathogens, but have been used in a limited study. Tu (1978) reported that *Phytophthora* root rot of soybean was lessened in the green house when rhizobia were applied to the potted soil immediately after planting. *Serratia plymuthica* HRO-C48 isolated from the rhizosphere of oilseed rape was effective in suppressing damping-off of cucumber caused by *Py. aphanidermatum* (Pang et al. 2009). *Stenotrophomonas maltophilia* W81, isolated from a sugar beet rhizosphere is capable of conferring protection against *Py. ultimum*-mediated damping-off (Dunne et al. 1997).

7.4 Mechanism of the Biological Control

Biological control of peronosporomycete phytopathogens is a multifaceted process in which several mechanisms are involved. This section reviews current knowledge on widely recognized mechanisms of disease suppression by the biocontrol bacteria.

7.4.1 Direct Antagonism or Antibiosis

A condition in which one or several metabolites that are excreted by an organism have a harmful effect on other organisms is known as antibiosis (Haas and Defago 2005). The microbial metabolites that can suppress growth and reproduction or kill other microorganisms at low concentration are known as antibiotics. A large number of structurally diverse chemical compounds (antibiotics) have been identified as principles of biocontrol of peronosporomycetal diseases by antagonistic bacteria

Table 7.2 Some antiperonosporomycetal compounds (antibiotics) produced by antagonistic bacteria

Antibiotic	Producing bacteria	Peronosporomycete	Bioactivity	References
2,4-diacetylphloroglucinol (DAPG)	*Ps. fluorescens* strain F113, M114, Pa 21, CHAO, and CHAO/pME3090	*Py. ultimum*	Mycelial growth inhibition	Shanahan et al. (1992), Fenton et al. (1992), Delany et al. (2001), Bainton et al. (2004), Maurhofer et al. (1995)
	Ps. fluorescens CHA0r	*Peronospora parasitica*	Induction of ISR in host plant	Iavicoli et al. (2003)
	Ps. fluorescens ECO-001	*Ap. cochlioides* AC-5	Growth inhibition, excessive branching, and disruption of F-actin in AC-5 hyphae	Islam and Fukushi (2010)
		Ap. cochlioides AC-5 and *Pl. vitivola*	Inhibition of zoosporogenesis and the motility of zoospores	Islam and von Tiedemann (2011)
Phenazine	*Ps. aeruginosa PNA1*	*Py. splendens*	Mycelial growth inhibition	Anjaiah et al. (1998)
		Py. splendens, *Py. myriotylum*	Mycelial growth inhibition. Induced aggregation of cell content and vacuolization within the hyphae	Perneel et al. (2008)
	Ps. fluorescens 2-79	*Py. aristosporum*	Mycelial growth inhibition	Gurusiddaiah et al. (1986)
	Ps. chlororaphis Tx-1 (*Ps. aureofaciens PA 147–2*)	*Ph. megasperma*	Mycelial growth inhibition	Carruthers et al. (1995)
Pyoluteorin (4,5-dichloro-1H-pyrrol-2yl-2.6-dihydroxyphenyl ketone)	*Pseudomonas fluorescens* Pf5	*Py. ultimum*	Mycelial growth inhibition	Howell and Stipanovic (1980), Kraus and Loper (1992), Maurhofer et al. (1994, 1995)
	Ps. fluorescens CHA0			
	Ps. fluorescens strain CHAO and CHAO/pME3090			
Oomycin A	*Ps. fluorescens* strain Hv37aR2	*Py. ultimum*	Decrease production of secondary inoculum	Howie and Suslow (1991)
Anthranilate	*Ps. aeruginosa* PNA1 and mutant FM13	*Py. splendens*	Mycelial growth inhibition.	Anjaiah et al. (1998)

HCN	*Pseudomonas* spp. strain group A1-A4 *Pseudomonas* sp. DSS73	*Py. ultimum*	Mycelial growth inhibition	Nielsen et al. (2002) Andersen et al. (2003)
	Ba. subtillis (R13, R33)	*Ph. capsici*	Mycelial growth inhibition	Lee et al. (2008)
	Streptomyces spp.	*Ph. medicaginis*	Mycelial growth inhibition	Misk and Franco (2011)
Massetolide A	*Ps. fluorescens* strain SS101	*Py. intermedium*	Had surface activity and caused lysis of zoospores	de Souza et al. (2003b)
		Ph. infestans	Lysis of zoospores and induce systemic resistance	Tran et al. (2007)
Viscosinamide	*Ps. fluorescens* DR54	*Py. ultimum*	Mycelial growth inhibition	Nielsen et al. (1998)
			Aiding in surface colonization of plant roots, surfaces of soil, and reduces growth and aerial mycelium development	Nielsen et al. (1999)
	Pseudomonas spp. strain group V1	*Py. ultimum*	Surface tension reduction and mycelial growth inhibition	Nielsen et al. (2002)
Amphisin	*Pseudomonas* spp. strain group A1 *Pseudomonas* sp. DSS73	*Py. ultimum*	Surface tension reduction and mycelial growth inhibition	Nielsen et al. (2002) Andersen et al. (2003)
Lokisin, hodersin, and tensin	*Pseudomonas* spp. strain group A2, A3, and A4, respectively	*Py. ultimum*	Surface tension reduction and mycelial growth inhibition	Nielsen et al. (2002)
Rhamnolipid A	*Ps. aeruginosa* PNA1 (B5)	*Ph. capsici*, *Py. splendens*, *Py. myriotylum*	Inhibition of hyphal growth and germination of zoospores and lysis of zoospores	Kim et al. (2000), Perneel et al. (2008)
Mycosubtilin	*Ba. subtillis* BBG100 (Transformant)	*Py. aphanidermatum*	Mycelial growth inhibition	Leclère et al. (2005)

(continued)

Table 7.2 (continued)

Antibiotic	Producing bacteria	Peronosporomycete	Bioactivity	References
Iturin A	*Ba. subtilis* KS1 *Acinetobacter* sp. LCH001 *Ba. subtilis* ME488	*Pl. viticola* *Ph. capsici* *Ph. capsici*	Mycelial growth inhibition	Furuya et al. (2011) Liu et al. (2007a) Chung et al. (2008)
Zwittermicin A	*Bacillus cereus* UW85	*Ph. megasperma* f. sp. *medicaginis*, *Ph. sojae*	Inhibition of germ tube length and mycelial growth	Handelsman et al. (1990), Silo-Suh et al. (1994), Osburn et al. (1995)
Antibiotic B	*Ba. cereus* UW85	*Ph. megasperma* f. sp. *medicaginis*	Inhibition of germ tube elongation and swollen and deformation of germ tube tips	Handelsman et al. (1990), Silo-Suh et al. (1994)
Bacilysin	*Ba. subtilis* ME488	*Ph. capsici*	Mycelial growth inhibition	Chung et al. (2008)
4-hydroxyphenylacetic acid	*L. antibioticus HS124* *Burkholderia* sp. MP-1	*Ph. capsici*	Deformation, lysis, and bending of hyphae, inhibition of mycelial growth	Ko et al. (2009) Mao et al. (2006)
Xanthobaccin A	*Lysobacter* (*Stenotrophomonas*) sp. strain SB-K88	*Pythium* spp., *Ap. cochlioides*	Inhibition of mycelial growth, motility of zoospores, and lysis of zoospores	Nakayama et al. (1999), Islam et al. (2005b), Islam (2008, 2010)
Pyrrolnitrin	*Serratia plymuthica* HRO-C48	*Py. apahnidermatum*	Mycelial growth inhibition	Pang et al. (2009)
Paromomycin	*Streptomyces* sp. AMG-P1	*Phytophthora* and *Pythium* spp.	Mycelial growth inhibition	Lee et al. (2005)
Khatmiamycin	*Streptomyces* sp. ANK313	*Pl. viticola*	Motility inhibition and lysis of zoospores	Abdalla et al. (2011)
Staurosporine	*Streptomyces* sp. strain B5136	*Pl. viticola*	Inhibition of motility of zoospores by inhibiting protein kinase C activity	Islam et al. (2011)
Isocoumarins	*Streptomyces* sp. ANK302	*Pl. viticola*	Motility inhibition and lysis of zoospores	Zinada et al. (2011)
(+)-4,5-Didehydroacaterin and 3[(1R)-hydroxyhexyl]-5-methylene-2(5H)-furanone	*Ps. jessenii* EC-S101	*Aphanomyces cochlioides*	Induction of hyperbranching and inhibition of hyphal growth	Deora et al. (2010), Hatano et al. (2007)

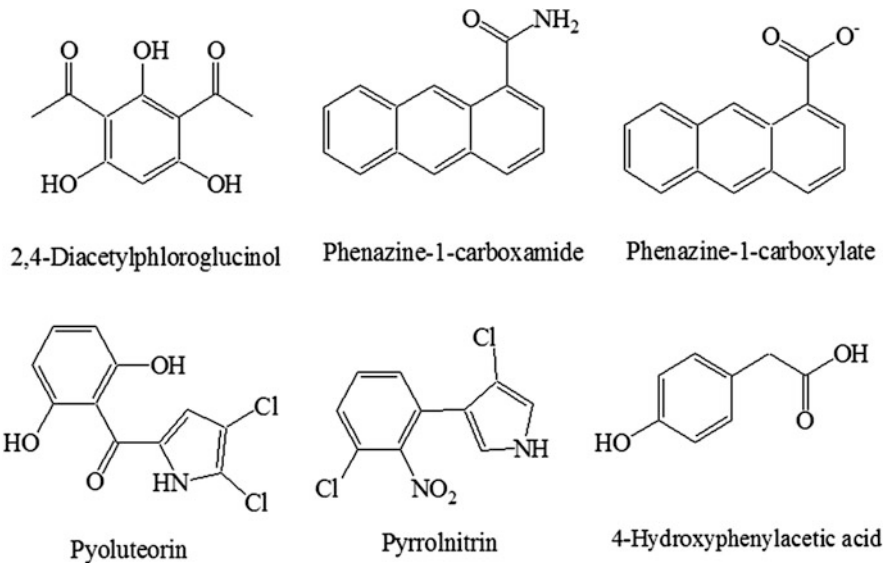

Fig. 7.3 Bioactive compounds from different biocontrol strains of *Pseudomonas* spp. against Peronosporomycete phytopathogens

(Figs. 7.3, 7.4, 7.5, and 7.6). The action mechanisms of antibiotics are very diverse and sometime very specific. Indeed, antibiosis is one of the most-studied mechanisms of biological control by bacterial antagonists. Antibiotics such as DAPG, phenazines, pyoluteorin, hydrogen cyanide, oomycin A, anthranilate, and cyclic lipopeptides have been reported to involve in suppression of peronosporomycete phytopathogens by *Pseudomonas* spp. (Table 7.2). The polyketide phenolic antibiotic, DAPG is produced by different strains of *Ps. fluorescens* to suppress the growth of peronosporomycetes (Fig. 7.3) (Shanahan et al. 1992; Fenton et al. 1992; Keel et al. 1992; Maurhofer et al. 1995; Delany et al. 2001; Iavicoli et al. 2003; Bainton et al. 2004; Islam and Fukushi 2010; Islam and von Tiedemann 2011).

DAPG has shown a wide range of inhibitory activities such as antiviral, antibacterial, antifungal, antihelminthic, and phytotoxic properties (Bainton et al. 2004). Production of DAPG is considered as one of the major determinants of disease suppression by *Ps. fluorescens*. It inhibits a diverse group of peronosporomycetes such as *Py. ultimum* (Fenton et al. 1992; Shanahan et al. 1992; Delany et al. 2001), *Ap. cochloides* (Islam and Fukushi 2010), *Peronospora parasitica* (Iavicoli et al. 2003), *Pl. viticola* (Islam and von Tiedemann 2011). DAPG-induced disease suppression is associated with alteration or disruption of a variety of cellular peronosporomycetes. For example, mycelial growth inhibition of *Py. ultimum* and *Ap. cochlioides* through excessive branching and curling (Shanahan et al. 1992; Islam and Fukushi 2010); disruption of the organization of cytoskeletal filamentous actin in *Ap. cochlioides* hyphae (Islam and Fukushi 2010); disorganization in hyphal tips of *Py. ultimum* var. *sporangiiferum*, including alterations (proliferation, retraction, and disruption) of the plasma membrane, vacuolization, and cell content disintegration

(de Souza et al. 2003a); inhibition of zoosporogenesis and the motility of zoospores of *Ap. cochlioides* and *Pl. vitivola* (Islam and von Tiedemann 2011); and induction of systemic resistance in the host plants (Iavicoli et al. 2003). DAPG has recently been reported to inhibit the mitochondrial function in yeast (Gleeson et al. 2010). As cleavage of nuclei and dramatic differentiation of sporangia during zoosporogenesis require supply of energy from the mitochondria, impairment of the mitochondrial function in the *Pl. viticola* sporangia and zoospores by DAPG might be associated with suppression of zoospore release and motility inhibition of zoospores, respectively (Islam and von Tiedemann 2011). To understand structure–activity relationships, Islam and von Tiedemann (2011) tested several phloroglucinol derivatives, namely, phloroglucinol (PG), monoacetylphloroglucinol (MAPG), 2,4,6-triacetylphloroglucinol (TAPG), and 2,4-dipropylphloroglucinol (DPPG) structurally related to the DAPG on zoosporogenesis and motility behavior of two peronosporomycetes, *Pl. viticola* and *Ap. cochlioides* (Fig. 7.3). According to their bioassay results, the activities of the tested compounds ranked DPPG > TAPG > DAPG > MAPG > PG for both zoosporogenesis and motility inhibition of the zoospores. It appeared that (1) the degree of substitution of hydrogen atoms in the benzene ring of phloroglucinol by acyl groups (acetyl or propyl) increased bioactivity; and (2) substitution with a larger aliphatic group (propyl) showed higher activity than the shorter aliphatic group (acetyl).

Phenazines are low molecular weight nitrogen-containing heterocyclic antimicrobial compound consisting of brightly colored pigment produced by the bacterial genera *Pseudomonas* (Gurusiddaiah et al. 1986; Carruthers et al. 1995; Anjaiah et al. 1998; Perneel et al. 2008). Phenazine compounds have antibiotic activity against a wide range of bacterial and fungal pathogens including several peronosporomycetes that cause important root diseases of plants (Carruthers et al. 1995; Anjaiah et al. 1998). Two phenazine antibiotics, phenazine-1-carboxylic acid (PCA) and phenazine-1-carboxamide (oxychlororaphine), and an anthranilate produced by *P. aeruginosa* PNA1 showed a dominant role in suppressing *Pythium* damping-off diseases in chick pea, bean, and lettuce (Fig. 7.3) (Anjaiah et al. 1998; Perneel et al. 2008). PCA produced by *Ps. fluorescens* 2-79 (Gurusiddaiah et al. 1986) and *Ps. chlororaphis* Tx-1 (Carruthers et al. 1995) showed excellent activity against *Py. aristosporum* and *Ph. megasperma*, respectively. The mode of action of phenazines in antiperonosporomycete interactions includes mycelial growth inhibition of *Pythium* (Anjaiah et al. 1998; Perneel et al. 2008; Gurusiddaiah et al. 1986), and *Phytophthora* (Carruthers et al. 1995) and aggregation of cell content and vacuolization of *Pythium* hyphae (Perneel et al. 2008). Another antibiotic, pyoluteorin (4,5-dichloro-1H-pyrrol-2yl-2.6-dihydroxyphenyl ketone) is a chlorinated polyketide antibiotic secreted by the rhizosphere bacterium *Ps. fluorescens* Pf-5 (Howell and Stipanovic 1980; Kraus and Loper 1992) and *Ps. fluorescens* CHA0 (Maurhofer et al. 1994, 1995). This antibiotic inhibits growth of mycelia of a seed- and root-rotting peronosporomycete, *Py. ultimum* (Howell and Stipanovic 1980; Kraus and Loper 1992). *Ps. fluorescens* strain Hv37aR2 produces oomycin A, which is linked to in vivo biocontrol of *Py. ultimum* infection on cotton (Howie and Suslow 1991).

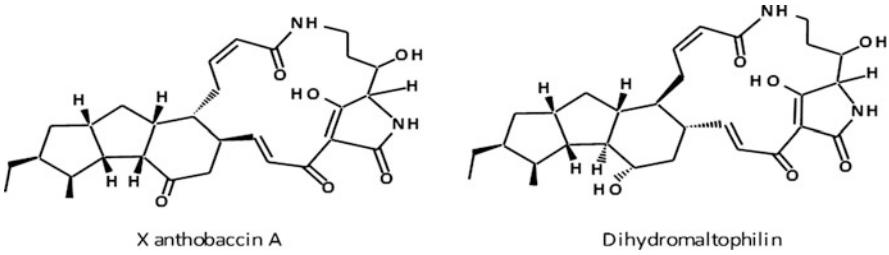

Fig. 7.4 Structures of two tetramic acid-containing macrocyclic lactam antibiotics, xanthobaccin A and dihydromaltophilin produced by *Lysobacter* sp. SB-K88 and *L. enzymogenes* C3, respectively

Zwittermicin A is a linear aminopolyol, which is involved in suppression of *Phytophthora* diseases on alfalfa and soybean by *Ba. cereus* UW85 (Handelsman et al. 1990; Silo-Suh et al. 1994; Osburn et al. 1995). It inhibits elongation of germ tube of zoospores and mycelial growth of *Phytophthora* spp. (Osburn et al. 1995). Four compounds, namely, phenylacetic acid (PA), hydrocinnamic acid (HCA), 4-hydroxyphenylacetic acid (HAA), and 4-hydroxyphenylacetate methyl ester (HPME) were isolated from the culture broth of *Burkholderia* sp. strains MP-1, PA, HCA, and HPME, which moderately inhibited *Ph. capsici*. However, HAA isolated from the culture supernatant of *Lysobacter antibioticus* HS124 induces abnormal hyphae of *Ph. capcisi* (Ko et al. 2009).

The biocontrol bacterium *Lysobacter* sp. SB-K88 suppresses damping-off disease in sugar beet and spinach caused by *Ap. cochlioides* and *Pythium* sp. through production of at least three lytic antibiotics, xanthobaccin A, B, and C (Nakayama et al. 1999; Islam et al. 2005b). Direct application of purified xanthobaccin A to seeds suppressed damping-off disease in sugar beet in soil naturally infested with *Pythium* spp. (Nakayama et al. 1999). The predominant antibiotic, xanthobaccin A produced by SB-K88 inhibits mycelial growth, impairs motility, and causes lysis of zoospores of *Aphanomyces cochlioides* (Nakayama et al. 1999; Islam 2008, 2010) (Figs. 7.4 and 7.5).

The mode of action of this macrocyclic lactam antibiotic includes disruption of ultrastructure and organization of filamentous actin in the cells of *Ap. cochlioides* (Islam 2008). The plane structure of xanthobaccin A is the same as that of a known antibiotic maltophilin, which was isolated from a rhizobacterium of rape *Stenotrophomonas maltophilia* R3089 (Jacobi et al. 1996). Both xanthobaccin A and maltophilin belong to a group of tetramic acid containing macrocyclic lactam antibiotics. An analogue of xanthobaccin A, dihydromaltophilin was identified as a heat stable and potent antiperonosporomycetal compound in the culture fluid of *L. enzymogenes* strain C3 (Yu et al. 2007). This compound exhibits a wide range of antimicrobial activities and shows a novel mode of action by disrupting the biosynthesis of a distinct group of sphingolipids (Giesler and Yuen 1998).

Suppression of *Py. aphanidermatum* damping-off in cucumber by *Serratia plymuthica* HRO-C48 is found to be responsible for its ability to produce antibiotic

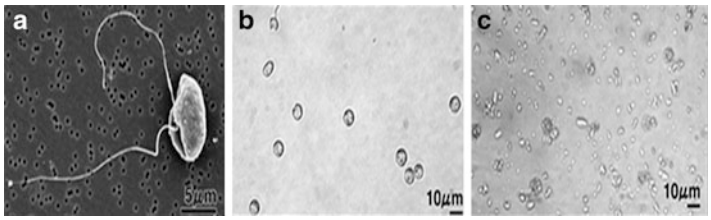

Fig. 7.5 Light and scanning electron micrographs showing *Aphanomyces cochlioides* zoospore-lytic activity of xanthobaccin A isolated from the biocontrol bacterium *Lysobacter* sp. SB-K88 (adapted from Islam et al. 2005b). (**a**) Micrograph of a biflagellate *A. cochlioides* zoospore (untreated control); (**b**) no lysis of zoospore in control. A small portion of (10–15 %) of motile zoospores in the control dish were stopped and changed into round cystospores and then settled to the bottom of the dish; (**c**) complete lysis of all halted zoospores by xanthobaccin A at 1.0 μg/ml

pyrrolnitrin (Pang et al. 2009). The pyrrolnitrin belongs to phenylpyrrole group and inhibits growth, synthesis of protein, RNA, DNA, and uptake of metabolites of pathogens (Pang et al. 2009).The aminoglycoside antibiotic, paromomycin is detected in *Streptomyces* sp. AMG-P1 as a candidate for the control of tomato and potato diseases caused by *Pythium* spp. and *Ph. infestans* (Lee et al. 2005). A new antiperonosporomycetal compound, khatmiamycin was recently isolated from the culture broth of a terrestrial *Streptomyces* sp. ANK313, which exhibits potent motility inhibitory (100 %) and lytic (83 ± 7 %) activities against zoospores of the grapevine downy mildew pathogen, *Pl. viticola* (Fig. 7.6) (Abdalla et al. 2011).

Similarly, four isocoumarins have been isolated from the terrestrial *Streptomyces* sp. ANK302, namely 6,8-dimethoxy-3-methylisocoumarin, 6,8-dihydroxy-3-methylisocoumarin, 6,8-dihydroxy-7-methoxy-3-methylisocoumarin, and 6,7,8-trimethoxy-3-methylisocoumarin displayed varying levels of motility inhibitory effects against *Pl. viticola* zoospores (Zinada et al. 2011). Although mechanism is not known, several other antibiotics such as nonactin, oligomycin F, and antimycin A isolated from marine *Streptomyces* spp. also displayed potent motility inhibitory and lytic activities against zoospores of the grapevine downy mildew pathogen, *Pl. viticola* (Fig. 7.7) (Islam et al. unpublished).

Recently, staurosporine was identified as the active principle in the ethyl acetate extracts of a marine *Streptomyces* sp. strain B5136 that rapidly impaired the motility of zoospores of the grapevine downy mildew pathogen *Pl. viticola* (Fig. 7.6) (Islam et al. 2011). The indolocarbazole antibiotic, staurosporine is a known broad-spectrum inhibitor of protein kinases, including protein kinase C (PKC). To understand the role of specific protein kinase in the maintenance of flagellar motility of zoospores, Islam et al. (2011) tested 22 known kinase inhibitors. Interestingly, the PKC inhibitor chelerythrine was the most potent to arrest the motility of zoospores at concentrations starting from 5 nM. Inhibitors that targeted kinase pathways other than PKC pathways did not practically show any activity in impairing zoospore motility. Both staurosporine and chelerythrine also inhibited the release of zoospores from the *Pl. viticola* sporangia in a dose-dependent manner. In addition, staurosporine completely suppressed downy mildew disease in grapevine leaves at

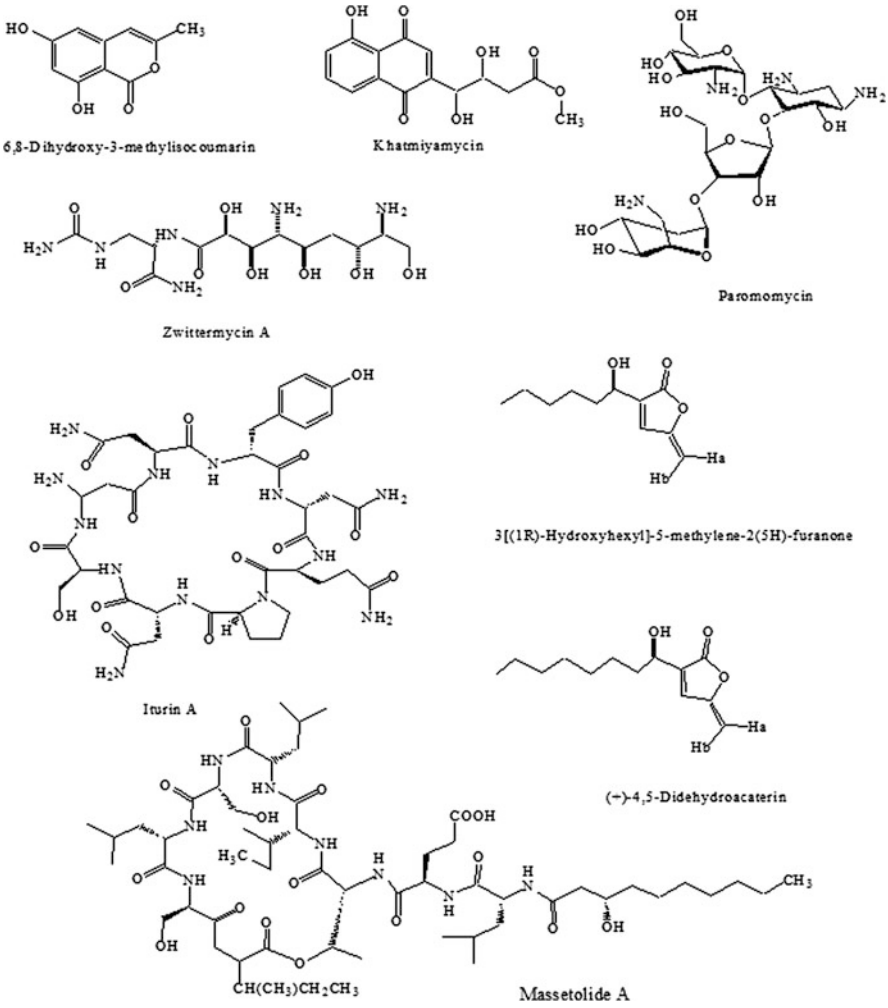

Fig. 7.6 Bioactive compounds from various biocontrol bacteria against peronosporomycete phytopathogens

2 μM, suggesting the potential of small-molecule PKC inhibitors for the control of peronosporomycete phytopathogens (Fig. 7.8). This study for the first time discovered that PKC is as a key signaling mediator associated with zoosporogenesis and the maintenance of flagellar motility in peronosporomycete zoospores (Islam et al. 2011). Interestingly, this finding parallels earlier work on the role of PKC in flagellar motility of mammalian sperm and spermatozoa of aquatic vertebrates (Rotem et al. 1990; White et al. 2007). Because motility is critical for the life cycles and pathogenicity of pathogens, elucidation of the details of signal transduction pathways might help us to design strategies for biorational management of the notorious peronosporomycete phytopathogens.

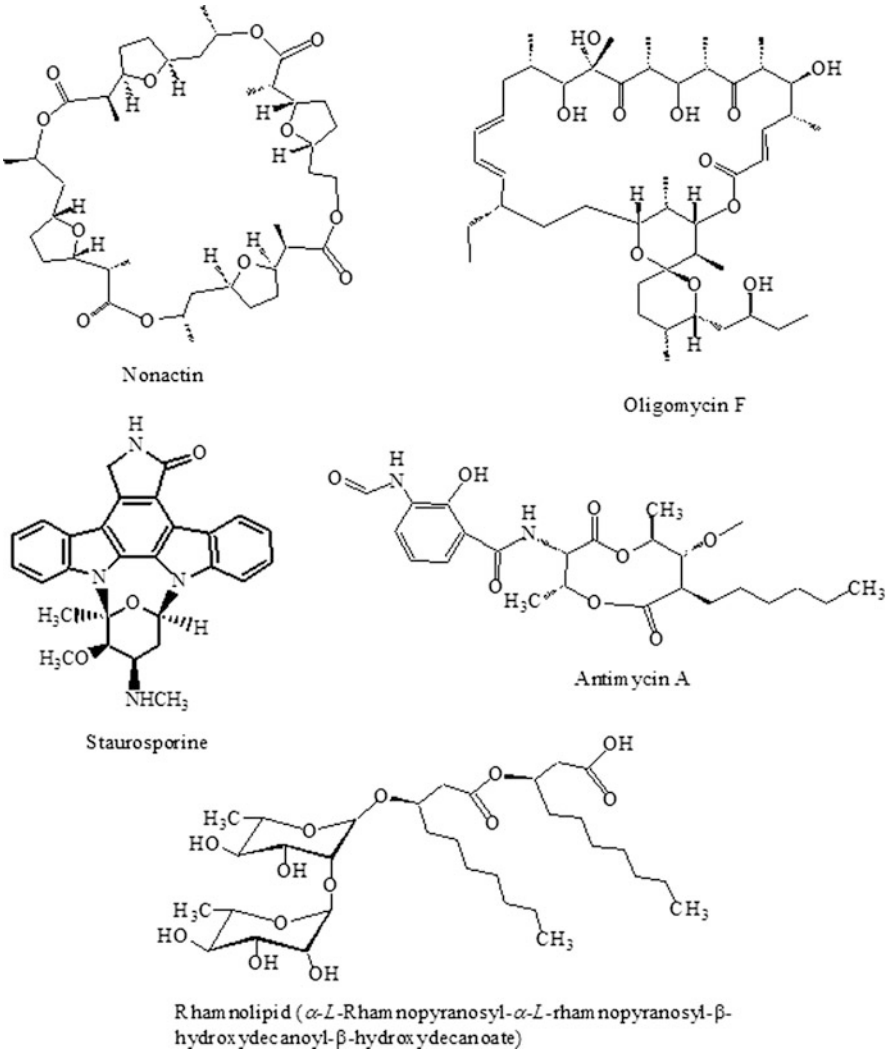

Fig. 7.7 Some antibiotics that affect motility, viability, and developmental transitions of zoospores of phytopathogenic peronosporomycetes

7.4.2 Biosurfactants as Antiperonosporomycetal Agent

Several strains of *Ps. fluorescens* were reported to produce antibiotics with surface-active properties. These antibiotics are designated as biosurfactants, which belong to a family of closely related cyclic lipopeptides (CLP). CLP has been shown destructive effects on zoospores of *Phytophthora* and *Pythium* spp. (Stanghellini and Miller 1997; Nielsen et al. 1999; de Souza et al. 2003b; de Bruijn et al. 2007;

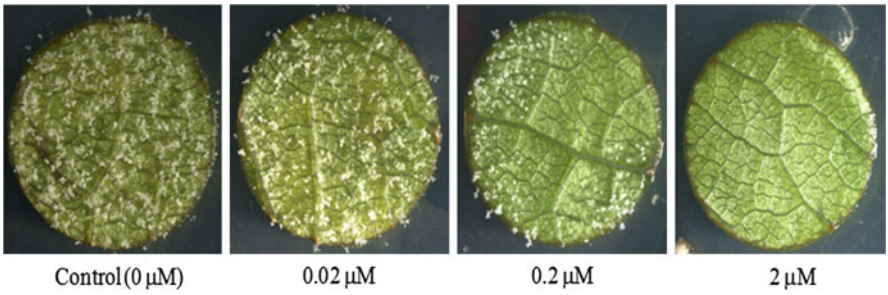

Fig. 7.8 Suppression of sporangial growth of a downy mildew pathogen, *Plasmopara viticola* on grapevine leaf disks by varying doses of staurosporine isolated from *Streptomyces* sp. B 5136 (adapted from Islam et al. 2011). Application of staurosporine at 2 mM concentration completely suppresses downy mildew disease on grapevine leaf. Staurosporine was suspended in aqueous DMSO (1 %) and an appropriate dose was sprayed on leaf disks placed on 1.5 % water agar 12 h before (pre-) or after (post) inoculation with *P. viticola* sporangia (5×10^3/ml). Inoculated leaf disks were incubated at 25 °C in 95 % relative humidity for 6 days

Perneel et al. 2008). Massetolide A, a cyclic lipopeptide with a nine-amino-acid peptide ring linked to 3-hydroxydecanoic acid was isolated from the culture broth of *Ps. fluorescens* strain SS101 (de Souza et al. 2003b). It is a metabolite with versatile functions causing lysis of zoospores, providing significant control of *Ph. infestans*, both locally and systemically via induced resistance and contribute to the colonization of tomato plants by *Ps. fluorescens* SS101 (Tran et al. 2007). Further study by van de Mortel et al. (2009) demonstrated that massetolide A induced the formation of transmembrane pores with an estimated size of between 1.2 and 1.8 nm. Zoospores were found to be most sensitive to massetolide A followed by mycelium and cysts. Massetolide A significantly reduced sporangium formation and caused increased branching and swelling of hyphae. Interestingly, a loss-of-function transformant of *Ph. infestans* lacking the G-protein subunit was more sensitive to massetolide A, whereas a gain-of-function transformant required a higher massetolide A concentration to interfere with zoospore aggregation which suggests that the cellular responses of *Ph. infestans* to this cyclic lipopeptide are, in part, dependent on G-protein signaling. Genome-wide expression profiling by microarray analysis may help to unravel the mode of action of massetolide on *Ph. infestans*.

Another metabolite, viscosinamide is an important component of activity of *Ps. fluorescens* DR54 against *Py. ultimum* (Nielsen et al. 1998, 1999). It aids in surface colonization of plant roots and soil surfaces and as antibiotic, reduces growth and aerial mycelium development of *Py. ultimum* and *Rhizoctonia solani* (Nielsen et al. 1999). Some strains of *Pseudomonas* sp. produce amphisin, lokisin, hodersin, and tensin (Nielsen et al. 2002). The ability of *Ps. fluorescens* DSS73 to efficiently control root-pathogenic *Py. ultimum* is shown to arise from secreting amphisin (Andersen et al. 2003). The biosurfactants, rhamnolipids produced by *Ps. aeruginosa* PNA1 suppressed plant-pathogenic peronosporomycetes through lysis of zoospore and inhibition of mycelia growth (Kim et al. 2000; Perneel et al. 2008). Although an increasing number of CLPs with surfactant properties have

been described in *Pseudomonas* spp., a few are also produced by *Bacillus* spp. *Ba. subtilis* ATCC 6633 produces surfactant mycosubtilin, a member of the iturin family (Leenders et al. 1999). Leclère et al. (2005) showed that a derivative of the *Ba. subtilis* strain BBG100 that overproduces mycosubtilin showed increased activity against *Pythium* on tomato seedlings. Iturin A, another antimicrobial lipopeptide structurally very similar to mycosubtilin has been reported to be produced by several *Bacillus* strains. This compound showed strong inhibitory activity against various peronosporomycetes (Chung et al. 2008; Furuya et al. 2011). Iturin A is also produced by a strain of *Acinetobacter* (Liu et al. 2007a). The cell free filtrate of *Acinetobacter* sp. LCH001 was strongly inhibitory against several phytopathogens including *Ph. capsici*, *F. graminearum*, and *R. solani*, and the bioactive compounds identified were as isomers of iturin A, namely, iturin A2, iturin A3, and iturin A6.

7.4.3 Lytic Enzyme as a Means of Biocontrol

Production of extracellular lytic enzymes has been implicated in plant protection by many biocontrol bacteria (Table 7.3). Exposure of phytopathogens to lytic enzymes can result in the degradation of the polymeric compounds such as chitin, proteins, cellulose, hemicellulose, glucans, and DNA of cell walls of the pathogen and use these as a carbon and energy source (Leah et al. 1991). Proteases, chitinases, glucanases, endopeptidase, lipases, lysoamidase, phospholipases, keratinases, lactamases, and phosphatases produced by bacterial antagonists can degrade structural matrix of cell wall of many phytopathogens including the peronosporomycetes (Lim et al. 1991; Fridlender et al. 1993; Valois et al. 1996; Dunne et al. 1997; Nielsen et al. 2002; Andersen et al. 2003; Hong and Meng 2003; Koch et al. 2002; Palumbo et al. 2005).

It has been reported that some fluorescent pseudomonad produced protease, siderophore, and HCN acted as antimicrobial agents against *Pythium* sp. and *Ph. nicotianae*. Suppression of late blight disease in pepper through secretion of lytic enzymes such as protease, chitinase, β-1,3-glucanase, and lipase in concert with the release of antibiotic compound 4-hydroxyphenylacetic acid by *L. antibioticus* strain HS124 has been demonstrated (Ko et al. 2009). However, chitinases are less essential in causing lysis of cell walls of the peronosporomycetes as they do not contain significant amount of chitin. For example, *Stenotrophomonas maltophilia* strain W81 produces extracellular enzymes chitinase and protease; however, commercially purified chitinase or cell-free supernatants from cultures of the protease-negative mutant W81M1 or the chitinase- and protease-negative mutant W81A1 had no effect on integrity of the essentially chitin-free *Pythium* mycelium and did not prevent subsequent growth of this peronosporomycete (Dunne et al. 1997). The proteolytic enzyme was identified as serine protease and

Table 7.3 Some important secreted lytic enzymes and antimicrobial protein produced by biocontrol bacteria against the peronosporomycete phytopathogens

Enzyme	Producing bacteria	Peronosporomycete	Mode of action	Reference
Protease	*Stenotrophomonas maltophilia* strain W81, *Pseudomonas* sp. DSS73	*Py. ultimum*	Irreversible loss of mycelial growth ability Degradation of proteinaceous cell-wall components and leakage of cell constituents	Dunne et al. (1997, 2000), Nielsen et al. (2002), Koch et al. (2002)
β-1,3-glucanases	*L. enzymogenes* strain C3, *Burkholderia* (*Pseudomonas*) *cepacia*	*Py. ultimum*	Lyse hyphae and/or inhibit hyphal growth	Palumbo et al. (2005) Fridlender et al. (1993)
	Paenibacillus sp., *St. sioyaensis*	*Py. aphanidermatum*	Inhibit mycelial growth and causes degradation or lysis of cell wall of hyphae	Hong and Meng (2003)
β-1,3-, β-1,4- and β-1, 6-glucanases	*Streptomyces* spp. (Strain EF-72, EF-22, EF-34, EF-14, EF-97, EF-76, EF-27, EF-43, DVD3, EF-25, and DVD4)	*Ph. fragariae* var. *rubi*	Hydrolyze glucans from *Phytophthora* cell walls and cause lysis of *Phytophthora* cells	Valois et al. (1996)
SAP (A novel antimicrobial protein)	*Streptomyces* sp. AP77	*Py. porphyrae*	Mycelial growth inhibition	Woo et al. (2002)

the bacterial mutants capable of over producing serine protease showed improved biocontrol activity against *Pythium* spp. on sugar beet (Dunne et al. 2000). Serine protease is antiperonosporomycetal against *Py. ultimum*, causing irreversible loss of mycelial growth ability, degradation of proteinaceous cell-wall components, and leakage of cell constituents (Dunne et al. 1997, 2000). Similarly, the ability of strain DSS73 to inhibit the growth of the root pathogenic peronosporomycete, *Py. ultimum* is believed to originate from the production and excretion of proteases and other biocontrol traits by the bacterium (Nielsen et al. 2002; Koch et al. 2002).

Bacterial enzymes with glucanolytic activity have a very significant role in the suppression of peronosporomycete diseases because β-glucans constitute 80–90 % of the wall dry weight of the peronosporomycete phytopathogens. *L. enzymogenes* strain C3 produces multiple extracellular β-1,3-glucanases encoded by the *gluA*, *gluB*, and *gluC* genes and are thought to contribute to the biological control activity against *Bipolaris* leaf spot of tall fescue and *Pythium* damping-off of sugar beet (Palumbo et al. 2005). *Bu. cepacia* synthesizes β-1,3-glucanase that destroys the integrity of *R. solani*, *S. rolfsii*, and Py. *ultimum* cell walls (Fridlender et al. 1993). Valois et al. (1996) reported that the antagonistic actinomycetes that suppressed the mycelial growth of *Ph. fragariae* var. *rubi* were shown to produce glucanases cleaving β-1,3, β-1,4, and β-1,6. These enzymes could hydrolyze glucans from cell wall structure of growing mycelia of *Pythium* and *Phytophthora*, and cause lysis of hyphal cells (Valois et al. 1996; Hong and Meng 2003; Palumbo et al. 2005). *Streptomyces* sp. AP77 produces an extracellular protein to suppress *Pythium*. Surprisingly, this anti-*Pythium* protein, designated as SAP, had neither *Pythium* cell wall-degrading activity nor any other polysaccharolytic activity, implying that it has a unique inhibitory function different from those of the polysaccharolytic enzymes used in the higher terrestrial plants (Woo et al. 2002).

7.4.4 Competition Between Plant-Associated Bacteria and Peronosporomycete Phytopathogens

Rhizosphere is a battle field or playground for diverse microorganisms including plant pathogens and beneficial bacteria. Competition between plant-associated bacteria and phytopathogens has long been thought to be an important means of suppressing plant diseases. In rhizosphere and spermosphere habitats, common critical resources are shared by both the pathogen and introduced microbial biocontrol strains. They compete with each other for nutrient and/or space. Root and seed exudates are primary source of nutrient for the rhizosphere microorganisms. Rhizosphere competence implies that bacterial antagonists are well adapted to their utilization (Lugtenberg et al. 1999). Germination and hyphal growth of pathogen propagules are stimulated by root and seed exudates (Stanghellini and Burr 1973; Nelson 1990). However, degradation of exudate stimulants by introduced microbial strain limits germination of pathogen propagules and hyphal growth of the pathogen. As a result, chances of the disease development are reduced.

For example, biocontrol of *Py. ultimum* by pseudomonads could be mediated through competition for seed volatiles. Hyphal growth from soilborne sporangia of *Py. ultimum* is stimulated by volatile compounds such as ethanol and acetaldehyde from the germinating seeds of pea and soybean.

However, this stimulation is reduced when seeds are treated with *Ps. putida* NIR. NIR uses these volatiles as carbon source and reduce their concentration in the spermosphere through metabolism. Heungens and Parke (2000) reported that *B. cepacia* AMMDR1 controls *Py. aphanidermatum* largely through antibiosis, but competition for zoospore-attracting compounds can also contribute to the effect. The bacterium AMMDR1 metabolizes the zoospore attractant. Zoospores of *Pythium* spp. are also attracted to water soluble sugars, amino acids, and fatty acids exuded by seeds or seedling (van Dijk and Nelson 1998). van Dijk and Nelson (1998) have shown that *E. cloacae* strain EcCt-501 can utilize seed exudates from a number plant species as a sole carbon source and reduces stimulatory activity of exudate to *Py. ultimum* sporangia by metabolizing the active stimulatory molecules including linoleic acid present in the exudates. Therefore, this trait is important for biological control of *Pythium* seed rot by this bacterial antagonist.

Fatty acids from seeds and roots are required to elicit germination responses of *P. ultimum* (van Dijk and Nelson 2000). Two mutants of *E. cloacae* EcCT-501R3, Ec31 (*fadB*) and EcL1 (*fadL*), reduced in β-oxidation and fatty acid uptake, respectively, fail to metabolize linoleic acid, to inactivate the germination-stimulating activity of cotton seed exudate and linoleic acid, and to suppress *Pythium* seed rot (van Dijk and Nelson 2000). This suggests that *E. cloacae* prevents *Py. ultimum* seed infections by preventing the germination of *Py. ultimum* sporangia through efficient metabolism of fatty acid components of seed exudate. However, the success of *E. cloacae* as a biological control organism is directly related to its ability to rapidly interfere with the early responses of *Pythium* propagules to germinating seeds. *E. cloacae* fails to suppress *Pythium* damping-off, if bacterial cells are added after full sporangial activation, but suppresses *Py. ultimum* seed infections if sporangial activation and germination happens within the first 30–90 min after sowing (Windstam and Nelson 2008a). It has been reported that *E. cloacae* can reduce the stimulatory activity of cucumber but not corn seed exudates (Kageyama and Nelson 2003). This is due to exudate inactivation by *E. cloacae* occurs in the cucumber spermosphere but not in the corn spermosphere (Windstam and Nelson 2008a). Other components of the seed exudates such as elevated level of sugars in the corn spermophere prevent degradation of long-chain unsaturated fatty acids by *E. cloacae*, leading to its failure to suppress *Py. ultimum* sporangial activation, germination, and subsequent disease development (Windstam and Nelson 2008b).

7.4.5 Production of Siderophores by Biocontrol Bacteria

Siderophores are small, high-affinity iron chelating compounds secreted by grasses and microorganisms such as bacteria and fungi. Iron is an important mineral

element essential for growth of all living organisms including microorganisms. The scarcity of bioavailable iron in soil habitats and on plant surfaces leads to a furious competition (Loper and Henkels 1997). Under iron-limiting conditions, some antagonistic bacteria produce low molecular weight compounds called siderophores to competitively acquire ferric ion (Whipps 2001). These metabolites chelate the ferric ion and serve as vehicles for the transport of Fe(III) into bacterial cells. There are various types of bacterial siderophores, which differ in their abilities to sequester iron. However, in general, they deprive pathogenic peronosporomycetes of this essential element since their siderophores have lower affinity (Loper and Henkels 1999; O'Sullivan and O'Gara 1992). Some plant growth promoting microorganisms increase plant growth by supplying the plant with sequestered iron where microbial siderophores are used by plants as a source of iron. In a number of cases, the growth promotion and biocontrol effect of rhizosphere-inhabiting bacteria has been attributed to siderophore-mediated iron acquisition (Kloepper et al. 1980a; Loper 1988). Siderophore production was detected in the isolates of *Ba. subtilis* that increase shoot and root length of red pepper plants and suppress *Phytophthora* blight of the plants (Lee et al. 2008).

Becker and Cook (1988) reported that the plant growth-promoting activity of some strains of fluorescent pseudomonads on wheat results from ability of the strains to suppress *Pythium* by production of siderophores. Siderophore, pyoverdin production has been proved to be important in the biological control of *Pythium*-induced damping-off of cotton by *Ps. fluorescens* 3551(Loper 1988) and in the inhibition of *Ph. parasitica* by *P. fluorescens* and *P. putida in vitro* (Yang et al. 1994). *Ps. aeruginosa* 7NSK2 improves the growth of several crops (Höfte et al. 1991) by producing three siderophores, salicylic acid (Buysens et al. 1996), pyochelin (Höfte et al. 1993), and the fluorescent pyoverdin (Höfte et al. 1993) under iron limiting condition. Production of either pyochelin or pyoverdin by 7NSK2 is necessary to achieve high levels of protection against *Py. splendens*-induced postemergence damping-off in tomato (Buysens et al. 1996). The action of pyoverdin and pyochelin seems to be interchangeable because a mutant producing only pyochelin or pyoverdin is equally antagonistic and with both mutants, wild-type levels of protection are obtained. Mycelial growth (Loper 1988) but not sporangial germination (Paulitz and Loper 1991) of *Pythium* spp. is inhibited by iron starvation. *Streptomyces* species are known for the production of hydroxamate type siderophores, which inhibit phytopathogen growth by competing for iron in rhizosphere soils (Khamna et al. 2009). Suppression of *Ph. medicaginis* as well as enhancement of plant growth by actinobacterial strains is attributed not only to their antibiotic production, but also to the ability to produce siderophores (Misk and Franco 2011).

7.4.6 Induced Systemic Resistance

Some biocontrol bacteria elicit a phenomenon that is known as induced systemic resistance (ISR) in the host plant. ISR of plant against pathogen is a widespread phenomenon that has been intensively investigated with respect to the underlying

signaling pathways as well as to its potential use in plant protection (Heil and Bostock 2002). Elicited by a local infection or colonization of nonpathogenic bacteria, plants respond with a salicylic-dependent signaling cascade that leads to the systemic expression of a broad spectrum and long-lasting disease resistance that is efficient against peronosporomycetes, fungi, bacteria, and viruses. Changes in cell wall composition, de novo production of pathogenesis-related proteins such as chitinases and glucanases, and synthesis of phytoalexins are associated with resistance (Kloepper and Tuzun 1996; Van Loon et al. 1998).

Bacterial agents mediated ISR against peronosporomycete pathogens such as *Ph. infestans*, *Ph. capsici*, *Py. aphanidermatum*, *Pl. halstedii*, *Sclerospora graminicola*, *H. parasitica*, and *Peronospora tabacina* have been demonstrated in many plant species including tomato, sunflower, squash, chili, pearl millet, apple seedling, tobacco, and Arabidopsis (Umesha et al. 1998; Ongena et al. 1999, 2005; Yan et al. 2002; Zhang et al. 2001, 2010; Van der Ent et al. 2008; Mazzola et al. 2007; Muthukumar et al. 2011). Zhang et al. (2010) reported that some strains of *Bacillus* spp. are effective in inducing ISR against *Ph. capsici* on squash, and improved disease control can be achieved by multiplexing them.

Two strains of plant growth-promoting rhizobacteria, *Ba. pumilus* SE34 and *Ps. fluorescens* 89B61, elicited systemic protection against late blight on tomato (Yan et al. 2002). Induced resistance by SE34 and 89B61 is associated with reduction in disease severity and germination of sporangia and zoospore on the leaf surface. Although physical separation between tested bacteria and target pathogens are necessary for ISR to be elicited, strain SE34 is detected in the leaves, suggesting the involvement of additional mechanism beside ISR during SE34-mediated protection (Yan et al. 2002). Localized stimulation of one part of a plant can result in the systemic expression of resistance in other parts; therefore, it has been hypothesized that a signal is generated and mobilized from the initial infection site (Dean and Kuc 1986).

Salicylic acid (SA), jasmonic acid (JA), and ethylene apparently are involved in signaling pathways. Both *Ba. pumilus* SE34 and *Ps. fluorescens* 89B61 elicit ISR in a JA-dependent manner. From a molecular point of view, the onset of rhizobacteria-mediated ISR has not generally been associated with major changes in gene expression. The nonpathogenic rhizobacterial strain *Ps. fluorescens* WCS417r has been shown to trigger ISR against *H. parasitica* in Arabidopsis (Ton et al. 2002). However, root inoculation with *Ps. fluorescens* WCS417r does not lead to an accumulation in the roots or in the leaves of the SA-responsive genes *PR*-1, *PR*-2, and *PR*-5, of the ET-inducible gene *Hel*, of the ET- and JA- responsive genes *ChiB* and *Pdf1.2*, or of the JA-inducible genes *Atvsp*, *Lox1*, *Lox2*, *Pal1*, and *Pin2*. A change could only be observed in the potentiation of the expression of JA-dependent *Atvsp* after pathogen challenge of ISR-expressing plants (van Wees et al. 1999). Higher level of antimicrobial phenolics were accumulated in cucumber plants treated with *Ps. fluorescens* BTP1 and M3 and challenged with *Py. aphanidermatum* (Ongena et al. 1999). Similarly, potentiated activities of phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), and phenolics were observed in *Ps. fluorescens* Pf1 pretreated tomato and hot pepper

plants challenged with *Py. aphanidermatum* (Ramamoorthy et al. 2002). Combined application of talc-based formulation of *Ps. fluorescens* EBL 20-PF and *Trichoderma viridae* and challenge inoculated with *Py. aphanidermatum* recorded maximum induction of PO, PPO, PAL, β-1,3-glucanase, and the accumulation of phenolics in chili plants (Muthukumar et al. 2011).

Further studies using *H. parasitica* have shown that WCS417r-mediated ISR is elicited through JA-, ET-, and *NPR1*-dependent defenses (Ton et al. 2002). T-DNA knockout mutants *myb72*-1 and *myb72*-2 are incapable of mounting ISR against the pathogen *H. parasitica*, indicating that *MYB72* is essential to establish ISR against this pathogen (van der Ent et al. 2008). *MYB72* is an ethylene-inducible R2R3-*MYB*-like transcription factor gene and is specifically activated in the roots upon colonization by WCS417r (Verhagen et al. 2004). Root inoculation of *Arabidopsis thaliana* ecotype Columbia with *Ps. fluorescens* CHA0r also partially protect leaves from the *H. parasitica* where production of DAPG is thought to be the determinant of *Ps. fluorescens* CHA0r for this ISR (Iavicoli et al. 2003). Although DAPG is known for its antibiotic property, in this experiment DAPG produced by bacteria leads to physiological changes that subsequently induce ISR. Another study on pea plants indicates that DAPG can act also as a plant hormone-like substance, inducing physiological and morphological changes that enhance nodulation by *Rhizobium*. ISR induced by CHA0r depends on *NPR1* and JA signaling pathways, indicating that ISR induced by strains WCS417r and CHA0r against *H. parasitica* differ with respect to the sensitivity to ET.

Bacteria-mediated ISR against peronosporomycete phytopathogen is also associated with changes in plant defense-related enzymes (Ongena et al. 2005; Muthukumar et al. 2011). Disease protection by *Ba. subtilis* M4 against *Py. aphanidermatum* in tomato is associated with significant changes in gene transcription in the host plant (Ongena et al. 2005). These enzymes are known to have antifungal properties and thought to play an important role in plant defense by restricting the growth and development of pathogens (Boller 1992). Although several hundred articles on ISR have been published, however, many questions are still unanswered and require further investigation. Strong efforts are required to identify the compounds causing resistance, and future studies should quantify these compounds in combination with the biologically detectable resistance to characterize the induced stage (Heil and Bostock 2002).

7.4.7 Hyperparasitism

Parasitism is a symbiosis in which two phylogenetically unrelated organisms coexist over a prolonged period of time. An avirulent pathogen could be hyperparasite on more virulent pathogens. The activities of various hyperparasites that parasitize virulent plant pathogens can lead to biocontrol. Several bacterial antagonists have been reported to be hyperparasites on several peronosporomycetes. *Actinoplanes* spp. are filamentous bacteria that produce minute sporangia, which when hydrated

release motile spores capable of parasitizing *Pythium* spp. or related microorganisms. Khan et al. (1993) reported that oospores of *Py. aphanidermatum*, *Py. arrhenomanes*, *Py. irregulare*, *Py. myriotylum*, and *Py. ultimum* were parasitized by *Ac. azureus*, *Ac. brasiliensis*, *Ac. caeruleus*, *Ac. ferrugineus*, *Ac. ianthinogenes*, *Ac. italicus*, *Ac. minutisporangius*, *Ac. rectilineatus*, *Ac. teichomyceticus*, *Ac. utahensis*, *Ac. violaceous*, *Ac. yunnahenis*, plus 15 strains of unspeciated *Actinoplanes*. Parasitized oospores had disorganized cytoplasms and hyphae of *Actinoplanes* sp. emerging from them. Filonow and Dole (1999) showed that strains of *Actinoplanes* spp. that are hyperparasites of oospores of *Pythium* spp. reduce root rot severity and increase plant stand of poinsettia and geranium.

Ac. missouriensis and *Pseudomonas* spp. infect oospores of *Ph. megasperma* var. *sojae*, *Ph. cactorum*, *Pythium* sp., and *Ap. euteiches* in natural soil, reducing populations of oospores in soil. Tu (1978) reported that *Rh. japonicum* reduced *Phytophthora* root rot of soybean by parasitizing hyphae of the peronosporomycete. The bacteria colonize growing hyphal tips and prevent contact between *Ph. megasperma* and host root tissue, and thereby reduce the chance of *Ph. megasperma* infection. *Rhizobium* reduces fungal sporulation and causes extensive surface and internal colonization of mycelia of *Ph.megasperma* and *Py. ultirnum* (Tu 1978).

7.4.8 Plant and Hyphal Colonization

Antagonistic bacteria are thought to protect against root pathogens more effectively if they have a strong ability to colonize the root system (Weller 1988). Islam et al. (2005a, b) investigated plant colonization behavior of a *Lysobacter* sp. SB-K88 by the aid of scanning electron microscopy (SEM). SB-K88 colonizes both the root and leaf surfaces in a characteristic perpendicular fashion. In colonized regions, a semitransparent film apparently enveloping the root and microcolonies was observed on the sugar beet root surface (Islam et al. 2005a). That *Lysobacter* strain also efficiently colonized the roots of several plants, including spinach, tomato, *A. thaliana*, and *Amaranthus gangeticus*. Interestingly, the SB-K88 also colonized *Ap. cochlioides* hyphal surface in the same perpendicular manner when grown together on liquid medium. Detailed transmission electron microscopic analysis revealed that the SB-K88 has long (~6 μm) brush-like, fragile fimbriae at one pole of the dividing bacterial cells. As fimbriae are known to function in bacteria to adhere to the substrates, Islam et al. assumed that brush-like fimbriae help SB-K88 to attach perpendicularly on plant and hyphal cell walls as well as for gliding motility. Presence of fimbriae appears to be characteristic structural features of bacteria having gliding motility (Spormann 1999).

In cotton, control of *Pythium* seed rot and preemergence damping-off by *E. cloacae* and *E. herbicola* strains were correlated with suppression of seed colonization by *Pythium* spp. (Nelson 1988). Seed pericarps are favorable ecological niches for species of *Pseudomonas*. *Ps. fluorescens-putida* ML5 and *Ps. putida* R20 readily

colonize pericarp of sugar beet seed. Occupation of this competitive site in spermosphere accounts for the effectiveness of these strains in reducing the incidence of seed rot and damping-off caused by *Py. ultimum* (Osburn et al. 1989). Fukui et al. (1994a, b) also demonstrated the importance of high initial pericarp colonization by *Ps. fluorescens* for antagonism against *Py. ultimum*. The dynamics of bacterial colonization of plant tissues vary among bacteria as influenced by various environmental factors (Loper et al. 1985). For *Ps. fluorescens* B5, the total population size per plant and downward colonization of the root (below 40 mm depth) increased significantly with increasing its inoculum density applied to the seeds, while for *Ps. corrugata* 2140, no significant influence of initial inoculum density on root colonization was observed (Schmidt et al. 2004a). Soil matrix potential and temperature had pronounced influence on seed or root colonization and biological control of *Pythium* spp. by pseudomonads (Mathre et al. 1994; Schmidt et al. 2004b). Population density of *Ps. fluorescens B5* per seedling as well as downward colonization and biocontrol performance by B5 were significantly reduced at high temperatures (25–35 °C), while *Ps. aureofaciens* AB254 (Mathre et al. 1994) showed good biological control against *Py. ultimum* at temperatures above 22 °C. This strain may therefore complement *Ps. fluorescens* B5 in a combined inoculum, although compatibility would have to be confirmed.

In addition to the colonization of plant roots, the colonization of hyphae could be an important mechanism for maintaining a close association between antagonistic bacteria and peronosporomycetal pathogens. Biological control of *Pythium* seed rot and preemergence damping-off of cotton by *E. cloacae* and *Erwinia herbicola* have been attributed to the ability of bacteria to attach to the hyphae and to inhibit the growth of *Py. ultimum* (Nelson 1988). *Ps. putida* 06909 grew extensively on hyphae of *Ph. parasitica* that cause citrus root rot and inhibited, but did not kill, the pathogen *in vitro* (Yang et al. 1994). Hyphal colonization-deficient *Tn5* mutants of *P. fluorescens* and *P. putida* were nonflagellated and were defective in colonization and inhibition of colonies of *Ph. parasitica in vitro*, confirming the linkage between the loss of flagella and the loss of the ability to colonize and inhibit peronosporomycetal mycelia. The loss of flagella in *Tn5* mutants of *P. fluorescens* has also been associated with an inability to colonize potato roots (de Weger et al. 1987). In another study, nonflagellated *Tn5* mutants of *P. fluorescens* were defective in adhesion to sand, suggesting that the flagella, or other outer membrane proteins associated with flagella, were involved in adhesion to surfaces (Deflaun et al. 1990). Islam et al. (2005b) demonstrated that a biocontrol strain *Lysobacter* sp. SB-K88 equally colonize both plant roots and hypahe of damping-off pathogen *Aphanomyces cochlioides* in perpendicular fashion. The SB-K88 does not show penetration of hyphal cells, however, disrupts ultrastructure and cytoskeleton of the *Ap. cochlioides* cells by secreting macrocyclic lactam antibiotics.

7.5 Conclusion and Perspectives

The peronosporomycetes have remarkable biological features including β-1,3-glucan polymers and cellulose made cell wall, energy storage carbohydrate mycolaminarin, diploidy at the vegetative stage, motile zoospores, and sexual cycle, which make them ubiquitous inhabitants in the diverse environment. Application of bacterial antagonists for the management of peronosporomycetal diseases of various plants is strongly connected to our understanding of bacterial diversity, host specificity, mechanism of action, active ingredient identification, formulation, and application. Information on potential biocontrol agents, their *in vitro* antagonistic activity, detection and isolation techniques of antipathogenic compounds, mode of action, in vivo disease suppressive activity, and practical formulation will facilitate to more efficient application methods of inoculant strains and strategy to reduce disease caused by peronosporomycete phytopathogens. A wide diversity of bacterial strains have been reported with antagonistic activity and some have shown high promise in vivo biocontrol effect on economically important peronosporomycete plant diseases through different mechanisms including antibiosis, high plant colonization, induced systematic resistance, and lytic enzyme production. This wealth of information has provided a firm foundation for broader incorporation of these bacterial antagonists into sustainable strategies for the management of peronosporomycete phytopathogens.

Although knowledge on ecological impact of biocontrol agents as alternative means of chemical-based plant disease management has been enhanced in the past decades, however, the greatest challenge facing BCAs is to release them for practical use. BCAs are needed to be transformed from niche market products into mainstream crop protection agents. More technical developments are desired to recognize the high-throughput production process of quality biocontrol products and to identify factors that affect efficacy and shelf life of these living biopesticides. Aside from the fundamental research for biocontrol agent identification, application, and efficacy improvement, continued effort should be taken to establish close linkage between researchers and entrepreneurs to facilitate technology promotion and acceptance by end users. Recent advances on whole genome sequences of several peronosporomycetes and biocontrol bacteria will provide the future basis for a comprehensive understanding of BCA–plant–pathogen interactions and development of improved strains with customized properties that will potentially function as effective biocontrol agents in low input sustainable agriculture.

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