Chapter 9
Microbial Suppressiveness of *Pythium* Damping-Off Diseases

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9.1 Introduction

Soilborne plant pathogens causing wilts, root and crown rots, and damping-off are major yield-limiting factors in the production of fiber, food, and ornamental crops. Most soilborne pathogens are difficult to control by conventional strategies such as the use of synthetic fungicides. The lack of reliable chemical controls, the occurrence of fungicide resistance in pathogens, and the breakdown or circumvention of host resistance by pathogen populations are among the key factors underlying potentials to develop other control measures. The search for alternative strategies has also been stimulated by public concerns about the adverse effects of soil fumigants such as methyl bromide on the environment and human health. Cook and Long (1995) postulated that many plant species have developed a defense strategy against soilborne pathogens that involves the selective stimulation and support of populations of antagonistic rhizosphere microorganism. Over the past century, evidence has accumulated that such plant-associated microorganisms

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account for many examples in which susceptible plants remain almost free of infection despite ample exposure to the virulent inoculum of soilborne pathogens. Natural disease-suppressive soils probably are the best examples in which the indigenous microflora effectively protect plants against soilborne pathogens. Suppressive soils initially become apparent because the incidence or severity of disease is lower than expected for the prevailing environment or as compared to that in surrounding soil (Cook and Baker 1983). Suppressive soils have been described for many soilborne pathogens including *Phytophthora cinnamomi*, *Phytophthora infestans*, *Pythium splendens*, *Pythium ultimum*, *Rhizoctonia solani*, and *Ralstonia solanacearum* (Mazzola 2007). Among the fungal diseases, damping-off is a serious disease complex worldwide of a wide range of seedlings in nurseries, glasshouses, gardens, crops, and forests and can damage both germinating seeds and young seedlings. Two types of damping-off diseases were known, preemergence damping-off and postemergence damping-off (Yang 2001). Damping-off is incited by any of a handful of fungal diseases, including several root rots (*Pythium*, *Phytophthora*) and molds (*Sclerotinia* or white mold, *Botrytis* or gray mold) (Agrios 1997). *Pythium* species cause more than 60 % mortality of seedlings both in nursery and in main field (Manoranjitham et al. 2000). Management of *Pythium* damping-off is very difficult due to its wide host range, soilborne nature, and prolonged survival of propagules in the soil. Traditionally, this disease is remediated by the application of synthetic fungicides. But the excessive use of fungicides resulted in the accumulation of residual toxicity and environmental pollution and altered the biological balance in the soil by attacking the beneficial microorganisms besides development of resistance in *Pythium* spp. against fungicides. Therefore, it is necessary to develop an effective, cheap, and environmentally safe nonchemical method for the control of damping-off disease. So, microbial control has been developed successfully as an alternative strategy and became a good promise in the field of microbial control in the past two decades (Muthukumar et al. 2011; Singh and Sachan 2013). Accordingly, biocontrol of *Pythium* damping-off disease with biological control agents (BCAs) including filamentous fungi, bacteria, actinomycetes, and yeasts has been intensively studied involving *Enterobacter cloacae*, *Gliocladium virens*, *Trichoderma harzianum*, *Rhizoctonia* spp., *Pseudomonas* spp., and *Cladorrhinum foecundissimum*, which are considered as ecologically sustainable and safe crop protection solutions (Khare and Upadhyay 2009; Muthukumar et al. 2011). The biological control products are regulated by governmental regulations for registration and use. Suppression of damping-off by biocontrol agents is the consequence of the interactions between soilborne pathogen, plant, and microbial community. The occurrence and development of soilborne diseases depend on several factors affecting either the pathogen or the plant. The complexity of the interactions between a pathogen and its plant host, influenced by biotic and abiotic factors of the environment, makes the control of the diseases often very difficult (Weller et al. 2002). Mycoparasitism, antibiosis by enzymes and secondary metabolites, competition, and induction of plant defense system are typical mechanisms of biocontrol agents (Singh and Sachan 2013). Soil interferes in many ways in the relationships between microorganisms, pathogens, and host plant. It can even modify the interactions among microorganisms themselves. In disease-suppressive soils, disease incidence or severity commonly remains low in spite of the presence of the pathogen, a susceptible host plant, and favorable climatic conditions. Soil suppressiveness to diseases depends on the pathogen itself, its inoculums density and its intrinsic aggressiveness, and also on different soil factors including both biotic and abiotic components. Soil abiotic components such as texture, organic matter content, pH, and temperature and moisture greatly affect the behavior of the pathogens and determine disease incidence or severity. Soil biotic factors that affect on the occurrence and development of soilborne diseases include: autecology of pathogens, interactions between microorganisms and pathogens, and interactions between plants and pathogens (Messiha et al. 2007; Steinberg et al. 2007). Soil physicochemical and biological factors interact to provide rapidly changing ecological niches and microbial components (Cook and Baker 1983). Soil organic matters also have a profound influence on microorganisms in soil, particularly those, including some pathogen, saprophytic and obligate plant parasites. This chapter presents recent advances and findings regarding the role of beneficial microbes in the *Pythium* damping-off disease suppression and the biological aspects highlighting the mechanisms of action of biocontrol process.

9.2 Damping-Off Diseases

Damping-off diseases are worldwide economically significant on numerous agricultural, ornamental, and horticultural crops and can be caused by soilborne plant pathogenic fungi under various environmental conditions (Salman and Abuamsha 2012). The name damping-off usually refers to the disintegration of stem and root tissues at and below the soil line. The plant tissues become water-soaked and mushy, and the seedling wilts and falls over (Fig. 9.1).

Damping-off diseases, however, can have several phases. The fungi that cause these diseases can attack the seed or the seedling below the soil line before it emerges, causing a preemergence damping-off where seeds become soft and mushy, turn dark brown and germinating seedlings shrivel, and may darken. Preemergence damping-off disease is difficult to be diagnosed because the seeds are not visible; consequently, the losses are often attributed to “poor seed” (Baker 1957). If the germinant has not emerged after a considerable period, the seed should be excavated and examined; if the seed contents are decayed, then damping-off fungi may be involved. On the other hand, postemergence damping-off causes death of seedlings after emergence or transplanting at the soil line where stem tissue near the soil line is weakened and decayed, usually causing plants to topple and die.

When only roots are decayed, plants may continue growing but remain stunted, wilt, and eventually die. As seedlings get older, they become more resistant to damping-off pathogens. Most pathogens that cause damping-off diseases are responsible for diseases as the plant grows to maturity. Root rot, crown rot, stem

Fig. 9.1 Damping-off caused by *Pythium* (Courtesy: "Martin Chilvers, Michigan State University." Reproduced with permission)

lesions, basal rot, crater rot, bottom rot, and stem girdling diseases may all be associated with damping-off pathogens attacking mature plants. Generally, damping-off is caused by over 30 species of fungi such as *Pythium*, *Rhizoctonia*, *Fusarium*, *Alternaria*, *Sclerotinia*, *Phytophthora*, *Thielaviopsis*, and *Botrytis* (Flint 1998; Yang 2001). The most common culprits that are associated with damping-off are *Pythium* species (water molds) and *Rhizoctonia solani* (true fungi). *Pythium* is a cosmopolitan and biologically diverse genus. Most species reside in soil inhabitants, although some are aquatic inhabitants. Most *Pythium* spp. are saprobes or facultative or opportunistic plant pathogens causing a wide variety of diseases, including damping-off (Larkin et al. 1995; Sumner et al. 1990). Damping-off diseases caused by *Pythium* species usually begin as root rot. This group of fungi survives as oospores in the soil that germinate to attack root tips and root hairs, causing a progressive deterioration of the root. The seedling may wilt or rot in the ground. *Pythium* species are often responsible for preemergence damping-off (Agrios 1997). The environmental conditions that favor damping-off vary according to the pathogen. *Pythium* spp. tend to be most active during the spring months when soil temperatures are still cool and soil moisture is plentiful (Flint 1998; Yang 2001). Landis et al. (1990) have been reported that although damping-off disease is usually caused by fungi or oomycetes, stresses such as high surface soil temperatures and chemicals can also cause damping-off symptoms.

9.3 Microbial Control of *Pythium* Damping-Off

Strategies to control soilborne diseases are limited because of their extremely broad host range, their ecological behavior, and the high survival rate of resistant forms such as oospores and sclerotia under different environmental conditions, and cultivars with complete resistance are not available (Li et al. 1995). Many pathologists have investigated that biological control agents offer an environmentally friendly alternative to protect plants from soilborne pathogens (Whipps 2001; Weller et al. 2002). Damping-off suppression can operate directly on fungal plant pathogens in the bulk soil, in the rhizosphere, and in some cases in plants. In the bulk soil, antagonistic soil microbes may act directly on resting spores or on active mycelium during a saprotrophic phase of plant pathogens, thus suppressing the plant pathogen directly. This suppression can be either specific or general. Specific disease suppression is caused by one or a few specific microorganisms. General disease suppression is caused by multiple microorganisms, acts against multiple pathogens, and is quickly restored. General disease suppression is directly related to microbial metabolic activity and mediated by availability of nutrients and energy available for growth of the pathogen through the soil. General disease suppression acts mainly in the bulk soil and is therefore largely congruent with pathogen suppression; it is especially effective against pathogens that have a saprotrophic phase. Also, in the rhizosphere, antagonists may suppress pathogens by interfering directly with germination, growth, and infection processes or indirectly through inducing host resistance (Termorshuizen and Jeger 2008). Effective biological control of damping-off requires careful matching of antagonists to pathosystems to achieve any of the three types of biological control: preventative control, eradicative control, or reductive control. Accordingly, biological control agents are more target specific and hence have fewer negative effects on nontarget organisms or even beneficial organisms in the rhizosphere (Cunniffe and Gilligan 2011).

9.3.1 Microbial Diversity and Disease Suppression

BCAs are beneficial organisms acting as naturally occurring enemies against pathogen such as bacteria and fungi. In last three decades, several antagonists were used to provide direct effects on *Pythium* spp., causal agent of damping-off, reducing their growth and preventing establishment in the rhizosphere (Howell 2003; Faltin et al. 2004). However, most of them showed inconsistent in vitro results, and only very few antagonists were analyzed under open field conditions (Grosch et al. 2005). The ability to control disease is more likely related to the production of specific metabolites or other substances than to the ability to produce fungal reproductive propagules (Lewis and Papavizas 1984). A wide range of aerobic microorganisms are involved in this aspect, and their introduction into soil improves fertility and structure and a range of population effects that may lead to suppression of plant pathogens and eventually of disease. However, it is difficult to determine exact suppression mechanisms as compost represents a “microbial community structure rather than a single species” (Boulter et al. 2002).

9.3.1.1 Bacterial Biocontrol Agents

In the past, Broadbent et al. (1971) found difficulty in controlling *Pythium* damping-off. Few actinomycetes were antagonistic to *P. ultimum* than to the other plant pathogenic fungi (Broadbent et al. 1971), as for antagonistic bacteria effectively acted as a biological control agent against *Pythium* damping-off: *Enterobacter cloacae*, *Bacillus* spp., *P. cepacia*, *P. corrugata*, *P. fluorescens*, *P. marginalis*, *P. putida*, *P. syringae*, *P. viridiflava*, and *Erwinia herbicola* (Gravel et al. 2005). *Stenotrophomonas maltophilia* and *Lysobacter enzymogenes* have been exploited to control *P. ultimum* in sugar beet (Palumbo et al. 2005). *Actinoplanes philippinensis* and *Micromonospora chalcea* were also investigated to control damping-off in cucumber (El-Tarabily 2006). Li et al. (2007) concluded that all paenibacilli prevented preemergence damping-off caused by *P. aphanidermatum*. *Serratia entomophila* strain M6 is a suitable candidate for exploitation as biocontrol agent of *P. aphanidermatum* (Chairat and Pasura 2013). Recently, it was observed that *E. faecalis* is a bactericidal agent producing diffusible metabolites which inhibited *P. ultimum* growth in vitro as shown in Fig. 9.2 (Kilany et al. 2015).

Streptomyces rubrolavendulae (Yen) S4 has been described as a biocontrol agent for controlling *Pythium* damping-off disease of the horticultural plant Joseph’s coat caused by *P. aphanidermatum* (Loliam et al. 2013).

9.3.1.2 Fungal Biocontrol Agents

Fungi have a broad-spectrum antagonistic activity against *Pythium* damping-off. Biocontrol of preemergence damping-off induced by *Pythium* species is achieved by coating radish and pea seeds with *T. harzianum* or *T. koningii* (El-Katatny et al. 2001). Besides, control of *Pythium* spp. in tobacco, sugar beet, and cauliflower by *T. harzianum* through soil application was recorded (Das et al. 2002). The successful application of *Trichoderma* species for the management of damping-off caused by *Pythium* species in chili and tomato has been reported (Jayaraj et al. 2006; Muthukumar et al. 2011). Two biological control agents, *Pythium nunn* and *T. harzianum* isolate T-95, were combined to reduce *Pythium* damping-off of cucumber in greenhouse (Paulitz et al. 1990). *Gliocladium virens* most consistently and effectively controlled damping-off of zinnia, cotton, and cabbage seedlings caused by *P. ultimum* (Lumsden and Locke 1989). Pre- and postemergence damping-off of wheat caused by *P. diclinum* was successfully controlled by *Gliocladium roseum* or *T. harzianum* (Abdelzaher 2004). Eight isolates of binucleate *Rhizoctonia* spp. from South Australian plant nurseries and potting mix

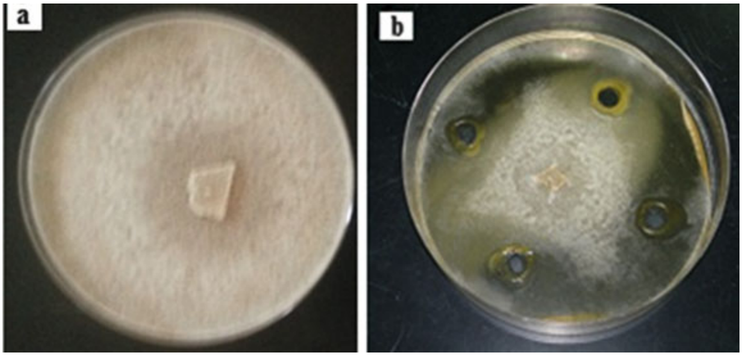

Fig. 9.2 Antifungal activity of *E. faecalis* against *P. ultimum*, where (**a**) refers to the control and (**b**) refers to the sample (Kilany et al. 2015)

suppliers were screened for ability to control damping-off disease caused by *P. ultimum* var. *sporangiiferum* (Harris et al. 1993). *C. foecundissimum* has a considerable potential as a biocontrol agent for damping-off of eggplant and pepper caused by *P. ultimum* (PuZ3). Antagonistic activities of *Aspergillus* species, *Penicillium* species, and *Trichoderma* species against *P. debaryanum* were studied by in vitro dual culture experiment (Hasan et al. 2013).

9.4 Mechanism of Microbial Control of *Pythium* Damping-Off

The antagonists encounter the pathogen either by direct antagonism (physical contact and/or a high degree of selectivity for the pathogen by the mechanism (s) expressed by the BCA(s)) or indirect antagonism (activities that involve stimulating of plant host defense (Pal and Gardener 2006)). *Pythium* damping-off suppression is the consequence of the interactions between the plant, pathogens, and BCAs (parasitism, predation, mutualism, protocooperation, commensalisms, neutralism, and competition), depending on the environmental conditions (Chisholm et al. 2006). Several strategies have been used to study the complex tripartite interaction in order to improve advantageous interactions, enhance the practical application of these beneficial microorganisms, and unravel the mechanisms of biological control (Vinalea et al. 2008; Rey and Schornack 2013). Most described mechanisms of pathogen suppression include the modulation by relative occurrence of other organisms in addition to the pathogen as shown in Fig. 9.3. The most effective BCAs studied appear to antagonize pathogens using multiple mechanisms (Iavicoli et al. 2003).

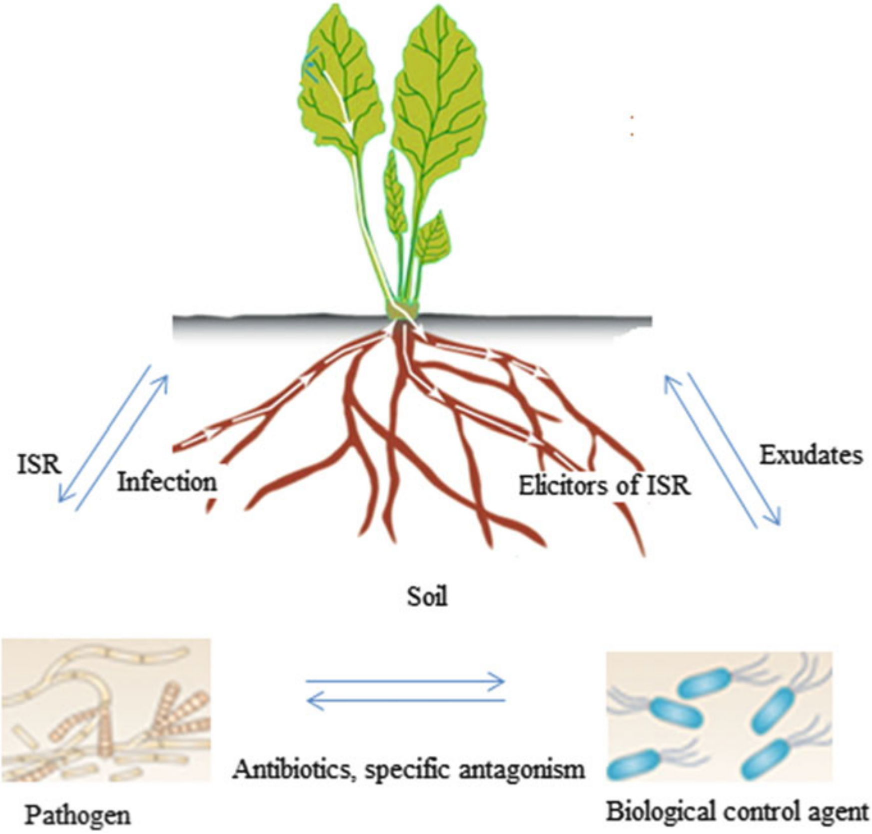

Fig. 9.3 Mechanisms of specific biocontrol agents for controlling plant pathogens

9.4.1 Mycoparasitism

Various microorganisms were recorded as parasites to soilborne pathogenic fungi in many systems (Elad 1995), depending on the production of antibiotics and fungal cell wall-degrading enzymes. It has been reported that antibiotics and hydrolytic enzymes are not only produced together but act synergistically in mycoparasitic antagonism (Schirmböck et al. 1994).

9.4.1.1 Lytic Enzymes

Harman et al. (1980) had suggested that mycoparasitism was the principle mechanism of *Pythium* damping-off when seeds were coated with *Trichoderma hamatum*. Their suggestion was based on evidence that in the presence of *Pythium* spp., *T. hamatum* becomes able to produce hydrolytic enzymes β-1,3-glucanase and cellulase, on observation of hyphal parasitism in vitro on *Pythium* spp. (Elad et al. 1982). *Stenotrophomonas maltophilia* and *Lysobacter enzymogenes* have the potential to antagonize *P. ultimum* infecting sugar beet by production of proteases and glucanases, respectively (Palumbo et al. 2005). The mycoparasitism of *S. rubrolavendulae* S4 against *P. aphanidermatum* was indicated by the degradation of *P. aphanidermatum* mycelium by means of cellulase production which was demonstrated by electron micrographs (Loliam et al. 2013). Moreover, *P. putida* strain N1R provides biocontrol of *P. ultimum* by enzymatic degradation of volatile seed exudates, which would otherwise stimulate the pathogen to cause preemergence damping-off (Paulitz 1991).

9.4.1.2 Antibiotics

Many microbes produce and secrete one or more compounds with antibiotic activity (Shahraki et al. 2009). It has been shown that some antibiotics produced by microorganisms are particularly effective against plant pathogens and the diseases they cause (Islam et al. 2005). Concomitantly, pyoluteorin, a new antibiotic, gliovirin, and gliotoxin had been isolated from *P. fluorescens* Pf-5, *T. virens* (GV-P), and *T. virens* (GL-21), respectively, that are effective antibiotics against damping-off incited by *P. ultimum* (Wilhite et al. 1994). Mutants of *T. harzianum* with altered antibiotic production were found inhibitory to *P. ultimum* (Graeme-Cook and Faull 1991). Further, the mechanism adopted to interpret *Pythium* damping-off biocontrol by *P. fluorescens* was attributed to the production of both antibiotics 2,4-diacetylphloroglucinol and viscosinamide (Thrane et al. 2000). Recently, *T. viride* was found highly inhibitory to *P. indicum* and *P. aphanidermatum*, the causal organisms of damping-off of tomato, by the effect of volatile and nonvolatile metabolites that inhibit the mycelial growth of *P. aphanidermatum* as well as increased the plant growth (Neelamegam 2004; Khare et al. 2010; Muthukumar et al. 2011). Moreover, Leclere et al. (2005) found that *B. subtilis* BBG100 exert profound effect to control damping-off caused by *P. aphanidermatum* by mycosubtilin. The efficacy of *Calothrix elenkenii* against damping-off disease, caused by *P. aphanidermatum* in three vegetable crops, tomato, chili, and brinjal, is due to antifungal compound production (Manjunath et al. 2010). *Chaetomium globosum* control the damping-off in sugar beet caused by *P. ultimum* by production of cheatomin (Lo 1998).

9.4.2 Suppression by Other By-Products

The suppression of *Pythium* damping-off involves production of microbial metabolites such as ethanol, ammonia, siderophore, etc. Toxic metabolites produced by *Trichoderma* spp. on seed coats are the principal mechanism of biological control of *Pythium* damping-off. *E. cloacae* is a potential antagonist against *Pythium* spp.

owing to production of ethanol which is an effective stimulant of sporangium germination, and reductions in ethanol production may reduce or delay sporangium germination of *Pythium* spp. thereby delaying seed colonization (Nelson 1987). Besides, Howell et al. (1988) reported that ammonia produced by *E. cloacae* was involved in the suppression of *P. ultimum*-induced damping-off of cotton. Similarly, *P. aeruginosa* and *P. fluorescens* produced siderophores to control *P. ultimum* damping-off in tomato and potato (Goud and Muralikrishnan 2009). Moreover, glycolipids that are produced by *Pseudomonas* spp. can damage the zoospores that are released from the sporangia of *Pythium* spp. (Stanghellini and Miller 1997). *P. putida* produced volatile metabolites to control *P. ultimum* damping-off in pea and soybean (Lo 1998).

9.4.3 Attachment to Pathogen Surfaces

Biocontrol activity may be requiring the attachment of BCAs to the surface of the host cells. Attachment mechanisms play a vital role in cell-cell interactions between fungi and other microorganisms (Douglas 1987).Cook and Long (1995) successfully used the attachment phenomenon to select potential BCAs among phyllosphere bacteria and yeasts. A common observed feature of the *E. cloacae-Pythium* system in vitro was the ability of *E. cloacae* to attach to the hyphae, agglutinate cell wall fragments of *P. ultimum* inhibiting mycelial growth (Nelson et al. 1986). It was suggested that the agglutination of cell wall fragments of *P. ultimum* occurred in the absence of some sugars or in the presence of others. In the absence of sugars, cells of *E. cloacae* attached to the hyphae. This is consistent with studies of phytoplanktonic bacteria where carbon starvation apparently promotes the attachment of bacteria to surfaces (Marshall 1980). On the other hand, in the presence of glucose or sucrose (sugars that block the agglutination of cell wall fragments by blocking available receptor sites), bacteria did not attach to the hyphae (Nelson et al. 1986). Furthermore, *P. fluorescens* provided superior seed protection from *Pythium* damping-off in naturally infested soils by adhering to hyphae of *P. ultimum* leading to fungal growth inhibition (Callan et al. 1990).

9.4.4 Competition

Generally, nutrient and space competition have been believed to play an important role in disease suppression. Biocontrol by nutrient competition can occur when the biocontrol agent decreases the availability of a particular substance, thereby limiting the growth of the pathogen. Soilborne pathogens, such as species of *Pythium*, infecting through mycelial contact, are more susceptible to competition by other soil- and plant-associated microbes than by those germinating directly on plant surfaces which they invade through appressoria and infection pegs. Rhizosphere or phyllosphere BCAs are generally protecting the plant by rapid colonization, thus consuming completely the limited available substrates so that none is left for pathogens to grow. Apparently, it was suggested that competition for nutrients between germinating oospores of *P. aphanidermatum* and bacteria significantly correlated with suppression of damping-off in the greenhouse (Fldd and Chet 1987). It appears more likely that competition is the primary mechanism by which *P. oligandrum* protects seed from infection by *P. ultimum* resulting in protection of sugar beet seeds from damping-off (Martin and Hancock 1987). Furthermore, Green et al. (2001) explained the biological control using *T. harzianum* by competition with *P. ultimum* for substrates from the seed coat and wounded or infected root tissue. Moreover, effective catabolism of nutrients in the spermosphere has been identified as a mechanism contributing to the suppression of *P. ultimum* by *E. cloacae* (van Dijk and Nelson 2000; Kageyama and Nelson 2003).

9.4.5 Role of Host and Disease Suppression

Apparently, host plants possess a little predictive value for the disease that is actually developing. This can be due to host-induced factors, such as induced systemic resistance (ISR), systemically acquired resistance (SAR), and specific disease suppression. The importance of host species in substrate-induced disease suppression has rarely been investigated. Van Rijin (2007) studied the effect of compost on disease suppression of the same isolate of *P. ultimum* using five different host seedlings (pea, cucumber, tomato, carrot, and sugar beet) and six composts mixed with peat. There was a significant interaction between pathosystem and compost type. Since in this experiment the host was the sole source of variation, host-mediated effects must explain this interaction. The genetic and functional diversity of the rhizosphere community is a key factor of specific disease suppression (Weller et al. 2002). This diversity varies according to plant species through the quantity and quality of root exudation and rhizodeposition (Bergsma-Vlami et al. 2005). Therefore, plants evolve strong defense mechanisms to effectively ward off pathogens while supporting development toward useful interactions (Jones and Dangl 2006; Bonneau et al. 2013). Microbe-associated chemical stimuli can induce plant host defenses through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens. Induction of host defenses can be local and/or systemic, depending on the type, source, and amount of stimuli. Induced systemic resistance (ISR) is mediated by jasmonic acid (JA) and/or ethylene, which are attributed to a variety of microorganisms and can result in control of multiple pathogens (Paulitz and Matta 1999). One of the most important biological agents is *S. plymuthica*, currently used in greenhouses which may provide economical prolonged protection against damping-off by sensitizing susceptible cucumber plants to elaborate a wide range of defense mechanisms (Benhamou et al. 2000). Ramamoorthy et al. (2002) recorded that in addition to direct antagonism of

P. fluorescens and plant growth promotion, induction of defense-related enzymes involved in the phenylpropanoid pathway collectively contributed to enhance resistance against invasion of *P. aphanidermatum* in tomato and hot pepper. Moreover, Howell et al. (2000) and Howell (2003) demonstrated that application of *T. virens* to cotton seedling induced the resistance in the host plant by synthesis of much higher concentrations of the terpenoids desoxyhemigossypol (dHG), hemigossypol (HG), and gossypol (G) in developing roots than those found in untreated controls. Some biocontrol strains of *Pseudomonas* sp. and *Trichoderma* sp. are known to strongly induce plant host defenses against *Pythium* damping-off (Harman et al. 2004; Haas and Défago 2005). The mechanism of *T. harzianum* Rifai for controlling maize seedling disease caused by *P. ultimum* Trow was investigated by proteome technique, and the result suggested that *T. harzianum* strain T22 was not only able to promote seedling growth but also induce the plant resistance by protein accumulation (Chen et al. 2005). *B. subtilis* strain BSCBE4 and *P. chlororaphis* strain PA23 obviously reduced the incidence of damping-off of hot pepper incited by triggering the plant-mediated defense mechanism in response to infection by *P. aphanidermatum* (Nakkeeran et al. 2006).

9.4.6 Metabolism of Germination Stimulants

Preemergence damping-off incited by *P. ultimum* in cotton was controlled using *Trichoderma virens*; this was attributable to metabolism of pathogen germination stimulants by the biocontrol agent released by the seed (Chen et al. 1988). In addition, the mechanisms involved in the biocontrol of preemergence damping-off of cotton seedlings incited by *P. ultimum* were studied by Howell (2002; 2003) who found that control by *T. virens* (G6, G6-5) or protoplast fusants of *T. virens* and *T. longibrachiatum* (Tvl-30, Tvl-35) was due to metabolism of germination stimulants released by the cotton seed. These compounds normally induced pathogen propagules to germinate. It is apparent that *T. virens* completely inhibited mycelial growth and sporangium production of *P. aphanidermatum*, the causal agent of Chinese-kale damping-off (Intana and Chamswarng 2007). It is apparent that *P. fluorescens* had the capacity to inhibit the germination of *Pythium* oospores, its growth, and the infection process (Cook and Long 1995; Ellis et al. 1999). One of the more effective bacterial species studied for its *Pythium* suppressiveness is *E. cloacae*. Molecular evidence showed that strain E6 of *E. cloacae* has the potential to inactivate the fatty acid that stimulates *Pythium* sp. germination, consequently protecting seeds from damping-off disease (van Dijk and Nelson 1997). Researchers provided strong evidence to support a mechanism for the suppression of *Pythium* damping-off by *E. cloacae* through which *E. cloacae* metabolize seed exudate fatty acid stimulants of *P. ultimum* sporangium germination resulting in reduction in sporangium germination and subsequent seed infection (van Dijk and Nelson 2000). *E. cloacae* protect the corn and cucumber seeds from *P. ultimum* infections by reducing sporangial activation and germination

(Windstam and Nelson 2008). *E. cloacae* are also effective in inactivation of the stimulatory activity of the seed exudates, thereby reducing *P. ultimum* sporangium germination on carrot, cotton, cucumber, lettuce, radish, tomato, and wheat (Kageyama and Nelson 2003). Suppressive efficiency of bacterial consortia to *P. ultimum* damping-off was attributed to degradation of seed exudate linolenic acid that stimulates the germination of *P. ultimum* sporangia (McKellar and Nelson 2003).

9.4.7 Soil Dynamics

Soil physicochemical and biological factors interact to provide hastily changing ecological niches and microbial components. Biological control of soilborne pathogens could be possible through manipulation of soil condition (Cook and Baker 1983). Soil organic substances support the largest numbers and types of microorganisms interacting with each other leading to modification or alteration in soil conditions that greatly influence the microbial community and their activity in soil ecosystem (Boulter et al. 2002). The extent of soilborne pathogen suppression will vary substantially depending on the quantity and quality of organic matter present in soil (Hoitink and Boehm 1999). It appears more likely that the primary mechanism by which *P. oligandrum* protects sugar beet seed from *P. ultimum* damping-off infection is alteration of the quality and quantity of sugar beet seed exudates in the spermosphere (Martin and Hancock 1987). Another aspect of the microbial populations studied was their composition and diversity in relation to disease suppression. Broad-spectrum biological control of diseases caused by *Pythium* requires the supplementation of organic nutrients in soil for survival of biocontrol agents where the decomposition level of organic matter significantly affects the composition of bacterial taxa as well as the populations and activities of biocontrol agents (Hoitink and Boehm 1999). Concomitantly, soil microbial community and carbon and nitrogen availability could be exploited as predictors to the relative growth of *P. ultimum* and *P. aphanidermatum* and the incidence of cotton seedling damping-off (Kowalchuk et al. 2003). The influence of microbial community structures in the different rock wool treatments toward *Pythium* disease suppression was investigated (Postma et al. 2005). Furthermore, the findings obtained by Manici et al. (2004) indicate that the green manures suppress *Pythium* sp. and also induced an increase in total soil microbial activity.

9.5 Commercially Available Biocontrol Agents

Currently, biocontrol of damping-off with bacterial and fungal antagonists is being investigated very intensively. The problems associated with the commercial acceptance of biological control agents of *Pythium* damping-off are discussed, and several methods of improving selection, activity, and use are described. Commercially available biocontrol rhizobacteria include *B. subtilis* strains GB03 (Kodiak; Gustafson), MBI 600 (Subtilex; Becker Underwood), and QST 713 (Serenade; AgraQuest), *B. pumilus* strain GB34 (Yield Shield; Gustafson), *B. licheniformis* strain SB3086 (EcoGuard; Novozymes), a mixture of *B. subtilis* strain GB122 and *B. amyloliquefaciens* strain GB99 (BioYield; Gustafson), several *Bacillus* spp. (yield-increasing bacteria in China), *S. griseoviridis* K61 (Mycostop; AgBio Development), and a few strains of *P. fluorescens*, *P. putida*, and *P. chlororaphis* (Cedomon; BioAgri). These biocontrol bacteria can be applied as dry products (granules or powders), cell suspensions (with or without microencapsulation), or seed coatings (Schisler et al. 2004). Several commercial products of *Trichoderma* like Biocure, Antagon, Bioderma, Trichofit, Dermapack, and Trichosan in India and Binab-7, Azadderma, F-Stop, Trichodex, and Trichodermin abroad have appeared in the market which indicate that bioagents are becoming popular (Kanjanamaneesathian et al. 2003; Khare and Upadhyay 2009). In the field, reproducible cost-effective biological control is rare. Nevertheless, *G. virens*, *P. oligandrum*, *T. harzianum*, and *C. minitans* have been exploited commercially for the control of damping-off disease incited by *Pythium*. Fungal antagonists have been introduced into soil or applied to seeds, and biocontrol of damping-off is sometimes equivalent to standard fungicide applications (Whipps 1997; Fravel et al. 1998). Although the number of biocontrol products is increasing tremendously, these products still represent a low proportion of fungicides: a total share of 3.5 % of the total crop protection markets (Fravel 2005).

9.6 Methods of Application of BCAs

Pythium spp. are effectively controlled by seed treatment because the fungus is active early in the season during seed germination (Heydari and Misaghi 2003). Eventually, application of biological control strategies requires more knowledge-intensive management to be effective. So, there are several methods of application of antagonisms: (1) overall application, (2) application to the infection site, (3) one place application, and (4) occasional application (Heydari et al. 2004).

9.7 Conclusion

Generally, damping-off disease is caused by different species of *Pythium* and it represents a major economic problem. Traditionally, chemical pesticides have been used to control most soilborne fungal diseases, but they are restricted by many hazards they cause. An alternative strategy for damping-off disease management was established by a tremendous number of biocontrol agents including bacteria, actinomycetes, and fungi. Such BCAs became successfully popular for control of

Pythium damping-off diseases and are considered as an important economic tool for protecting the crops. BCAs have different suppressive potentials on *Pythium* damping-off diseases in the same particular ecological niche. The study of the population dynamics and tripartite interaction between the plant, pathogen, and antagonist is crucial to understand the mechanistic pathway of biocontrol agents and, consequently, to develop an appropriate biocontrol strategy. Predominantly, the different mechanisms of antagonism occur across a spectrum of directionality related to the amount of interspecies contact and specificity of the interactions. The most effective type of antagonism is direct antagonism resulting from physical contact and/or a high degree of selectivity for the pathogen by the mechanism expressed by the BCA (e.g., hyperparasitism). Conversely, indirect antagonisms result from activities that do not involve sensing or targeting a pathogen by the BCA through two mechanisms, competition and stimulation of plant host defense. The latter mechanism is more prevalent within the indirect antagonism. Mixed-path antagonism has been observed though some mechanisms involved the production of antibiotics, lytic enzymes, and other by-products as well as suppression of germination. Additionally, some microorganisms exhibited one mechanism, while others may work through several mechanisms. The latter microorganisms are likely to be more robust under extreme conditions. In spite of a plethora of examples in the literature of microbes with biocontrol activity against *Pythium* damping-off diseases, very few have given considerable levels of reproducible control across a number of seasons and sites. Two microbial groups, *Pseudomonas* spp. and *Trichoderma* spp., have given the greatest success. Therefore, BCA applications have been used successfully in combination with each other's. In the future, it is expected that better-performing BCAs will be developed. However, there is still great potential for the discovery of microbes with increased biocontrol abilities and to produce novel bioactive products.

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