Chapter 12
Soil Suppressive Microorganisms and Their Impact on Fungal Wilt Pathogens

M.K. Mahatma and L. Mahatma

12.1 Introduction

Saprophytic microorganisms are indispensible members of food chain and play vital role in recycling of carbon and nutrients by decomposition in the ecosystem. Availability of the nutrients creates favorable conditions for the growth of plants. Starvation of saprophytes poses natural selection pressure and adaption to it leads to the evolution of parasitism. Some of these microorganisms evolved complex mechanisms in response to the host defense and adapted to utilize nutrients from the living organisms, and gradually facultative parasite, facultative saprophyte, obligate parasite, and hyperparasitism developed making the soil ecosystem highly complex. In soil, many microorganisms occur in close proximity, and they interact in a unique way. The sum total of all of the individual interactions establishes the equilibrium population. Odum (1959) proposed seven relationships between the different living organisms in the equilibrium as follows: (a) *neutralism*, in which two organisms behave entirely independently; (b) *symbiosis*, the two symbionts relying upon one another and both benefiting by the relationship; (c) *protocooperation*, an association of mutual benefit to the two species but without the cooperation being obligatory for the existence or for their performance of some reaction; (d) *commensalism*, in which only one species derives benefit while the other is unaffected; (e) *competition*, a condition in which there is a

M.K. Mahatma (✉)
ICAR-Directorate of Groundnut Research, Ivnagar Road, P. Box # 5, Junagadh 362001, GJ, India
e-mail: maheshmahatma@gmail.com

L. Mahatma
Department of Plant pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari 396 450, GJ, India
e-mail: mahatmalalit@yahoo.co.in

M.K. Meghvansi, A. Varma (eds.), *Organic Amendments and Soil Suppressiveness in Plant Disease Management*, Soil Biology 46,
DOI 10.1007/978-3-319-23075-7_12

suppression of one organism as the two species struggle for the limiting quantities of nutrients, O_2, space, or other common requirements; (f) *amensalism*, in which one species is suppressed while the second is not affected, often the result of toxic production; and (g) *parasitism* and *predation*, the direct attack of one organism upon another. Existence of these relationships and their predominance characterize the soil. Conveniently, the soil has been classified in to two different categories, viz., conducive and suppressive soil. If in the soil, plant pathogenic microorganisms develop well and provide congenial conditions for the severe diseases, it is known as conducive soil. To be conducive, there should be the appropriate population density of the particular pathogen in the soil. Whereas, soils in which the pathogen does not establish, or establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil is known as suppressive soil (Baker and Cook 1974). Suppressive soil provides hostile environmental conditions for the pathogen to build up inoculum potential and penetration. Numerous biotic and/or abiotic factors cumulatively make the soil suppressive. Many antagonistic, pathogenic, as well as unapparent microorganisms remain in equilibrium proportion in the soil which predominately determines its characteristics. As long as the equilibrium remains ideal or shifted towards the antagonistic microorganisms by selectively favoring its activities, the soil suppresses the disease and support good crop. However, if the equilibrium shifts towards the pathogenic microorganisms and increases its potentiality, it becomes conducive soil. Range of the suppressiveness has been observed, and there may be intermediate or ideal suppressive soil. Suppressive soils have been described for many soil-borne pathogens, viz., *Gaeumannomyces graminis* var. *tritici* (take-all of wheat, which causes blackening of the plant base, stunting, and, in severe cases, white inflorescence with shrivelled grains and no yield); *Fusarium oxysporum* (wilt diseases of tomato, radish, banana, and others); *Phytophthora cinnamon* (root rot of eucalyptus); *Pythium* spp. and *Rhizoctonia solani* (damping-off of seedlings of several crops, including sugar beet and radish); *Thielaviopsis basicola* (black root rot of tobacco, bean, cherry trees, and others); *Streptomyces scabies* (bacterial potato scab; i.e., lesions on potato tubers); *Ralstonia solanacearum* (bacterial wilt of tomato, tobacco, and others); *Meloidogyne incognita* (root swelling and root-knot galls on several crops, mostly in tropical and subtropical countries). In the present chapter, different aspects of microbial soil suppressiveness and their impact on wilt disease have been discussed in detail.

12.2 Historical Landmark

Soil suppressiveness and microorganisms in the suppression of disease were first time realized by Sanford (1926) while working on potato scab disease caused by *S. scabies*. He observed that the incidence of potato scab caused by *S. scabies* is reducing in the green manuring crop in Canada. Attention of Millard in England

was drawn to this observation and soon reported that the reduced disease incidence was due to the presence of inhibitory effect of nonpathogenic bacteria (Millard and Taylor 1927). Henery (1932) from the University of Alberta, Canada, reported that with the increasing temperature, the infection curve for *G.graminis* on wheat seedling was downwards in unsterilized but upward in sterilized soil. Decrease in the infection with the raise in the temperature in the unsterilized soil was also due to enhanced effect of soil mycroflora. The result was confirmed by Garrett (1934) of Waite Institute, Adelaide. This has guided to focus on different soil inhabiting microorganisms for the management of devastating diseases. Weindling (1932) showed that *Trichoderma* sp., a common saprophytic fungus, was able to parasitize the mycelia of other fungi. The first report of fusarium wilt suppressive soil was made by Stover (1962). Suppression of fusarium wilt of radish by growth-promoting effects of fluorescent pseudomonads was first published by Kloepper and Schroth (1978) and later by Geels et al. (1985). Substantial work on the biological control and suppressive soil has been done by the different scientists throughout the word. Weller et al. (2002) thoroughly reviewed the microbial populations responsible for specific soil suppressiveness to plant pathogens.

12.3 Fungal Wilt Disease

Vascular wilt characterized by the presence of pathogen in the vessels of angiosperm is one of the most destructive diseases. Four genera of fungi, viz., *Fusarium*, *Verticillium*, *Ceratocystis*, and *Ophiostoma* cause the vascular wilt. Among the different genera, *Fusarium* and *Verticillium* are most important and cause disease to the wide range of plants. *Verticillium* sp., a cold loving fungus that thrives best in heavy soils, does not require injury for infection whereas *Fusarium* sp., found in the tropical and subtropical region, grows best in sandy soil and causes more damage when root-knot, reniform, or sting nematodes injure the roots. *Fusarium* sp. prefers acidic condition and can be transmitted internally in seed, while *Verticillium* prefers alkaline conditions and is not transmitted internally in the seeds. High nitrogen fertilizer, excessive soil moisture, thin stands, and deep cultivation during the growing season favor wilt disease. Both fungi survive long periods in soil in the absence of a cultivated host. Fusarium wilt was first recognized in the nineteenth century by Atkinson (1892) and was later described for other soils around the globe. *Verticillium* (*Verticillium albo-atrum* and *V. dahliae*) causes vascular wilts of vegetables, flowers, field crops, perennial ornamentals, and fruit and forest trees in the temperate region. All vascular wilts have certain disease symptoms in common and are almost similar to the physiological drought, however, are irreversible. In cross sections of infected stems and twigs, discolored brown areas appear as a complete or interrupted ring consisting of discolored vascular tissues. Vessels may be clogged with mycelium, spores, or polysaccharides produced by the fungus. Clogging is increased further by gels and gums formed by the breakdown products of plant cells by the enzymatic action of the fungi. In some hosts, balloon-

like tyloses are produced by parenchyma cells adjoining some xylem vessels (Agrios 2005).

12.4 Classification of Suppressive Soils

Two different categories, viz., general and specific suppressiveness, are most commonly used by many scientists to classify soil suppressiveness. The widespread but limited ability of soils to suppress the growth or activity of soil-borne pathogens has been referred to as "general suppression." Nonspecific antagonism or biological buffering terminologies (Weller et al. 2002) have also been used to simplify the nomenclature; however, they are less accepted. Accordingly, specific suppressiveness is due to antagonistic effect of individual or selected groups of microorganisms during some stage in the life cycle of a pathogen. Though the general and specific suppressiveness is most widely used terminology, it seems ambiguous and represents the notation of general suppressiveness encompassing wide range of pathogens. Similarly, specific suppressiveness gives the notation of specific suppression of the disease. However, general suppressiveness is also effective against the given class of pathogen only. This is clearly illustrated for the soil suppressive to the fusarium wilt which is not even suppressive to the disease caused by *F. solani, F. roseum*, and other soil-borne pathogens (Alabouvette 1986; Deacon and Berry 1993; Steinberg et al. 2007). Suppressiveness is so specific and sometimes is cultivar specific. Hopkins et al. (1987) from Florida in a long-term monoculture of watermelon cultivar observed that most of the cultivars wilted severely after 4–5 years regardless of previously described levels of resistance to *Fusarium oxysporium* f. sp. *niveum*. Only the resistance in Smokylee and Crimson Sweet was stable in the monoculture, and only Crimson Sweet continued to have acceptable level of yields throughout the monoculture. Crimson Sweet only moderately resistant to fusarium wilt in greenhouse tests had a unique resistance that was effective throughout the 7 years monoculture. Instead, if the horizontal suppressiveness and vertical suppressiveness are used to notify the general and specific suppressiveness, respectively, it would be more comprehensive. The classification can be metaphorically comprehended as horizontal resistant (horizontal suppressive) and vertical resistant (vertical suppressive). However, in the present chapter old terminologies, viz., general and specific suppressiveness, are used. Various characteristics of suppressive soils are given in Table 12.1 and described suitably in the different subheading in the chapter.

Table 12.1 Characteristics of nonspecific and specific suppressive soils

Sr. No.	Characteristics	General suppressive	Specific suppressive	References
1	Synonym	Nonspecific suppressive soil Nonspecific antagonist or biological buffering, horizontal suppressive soil	Specific antagonist or biological buffering, vertical suppressive soil	Baker and Cook (1974), Alabouvette (1986), Weller et al. (2002)
2	Number of microorganisms associated	Many	One or few	Alabouvette (1986), Weller et al. (2002)
3	Effect of soil organic matter	Enhanced on addition	Not affected much	Hopkins et al. (1987), Weller et al. (2002)
4	Transferability	Less	More	Menzies (1959), Cook and Rovira (1976), Weller et al. (2002)
5	Inducibility	No	Yes	Cook and Rovira (1976), Alabouvette (1986), Weller et al. (2002)
6	Effect of edaphic, climatic, and agronomic conditions	More	Less	Cook and Rovira (1976)
7	Duration	Retain from the longer period	Retain from the shorter period	Hopkins et al. (1987)
8	Effectiveness in the absence of plants	Not affected	Affected	Hopkins et al. (1987), Weller et al. (2002)
9	Reversibility	Difficult to convert in conducive soil	Easy to convert in conducive soil	Cook and Rovira (1976), Larkin et al. (1993)

12.5 Wilt Suppressive Soils

Among the different wilts, fusarium wilt suppressive soil has only been observed and studied extensively. Suppressive soil has been reported from the four places, viz., in the Salinas Valley, California, United States; the Chateaurenard region, near Cavaillon, France; the Canary Islands and the Broye Valley, Switzerland. Among these, the Chateaurenard soils in France and the Salinas Valley soil in California are known for their natural suppressiveness to fusarium wilt diseases (Louvet et al. 1976; Kloepper et al. 1980; Scher and Baker 1980). Monoculture-induced suppressiveness to fusarium wilt of watermelon was studied at Central Florida Research and Education Centre, Leesburg, Florida (Larkin et al. 1993). Alabouvette

(1986) extensively worked on the fusarium wilt suppressive soils from the Châteaurenard region and reviewed the results. They coined the concept of soil receptivity to soil-borne pathogens while working on fusarium wilt in melon which reflects the capacity of a soil to allow a pathogen to establish, develop, persist, and express its pathogenicity on host plants (Alabouvette et al. 1982). Study reveled that the absence of disease could not always be accounted for the absence of the pathogen (*F. oxysporum* f. sp. *melonis*). This was demonstrated by introducing into various soils increasing amounts of a given pathogen; at similar inoculum densities, severity of disease on a population of susceptible host plants varies significantly according to soils indicating the various degrees of soil receptivity to Fusarium wilt (Alabouvette et al. 1982). It is thus possible to identify disease suppressiveness.

12.6 Microorganisms in Soil Suppressiveness and Its Mechanisms

Suppressiveness of soil is mainly related to its biological properties; however, physical, chemical, and meteorological factors affect the biological factors and thereby indirectly affect the suppressiveness of the soil. Both general and specific suppression are eliminated by autoclaving and gamma radiation which support the biological basis of disease suppression. General suppression is reduced but not eliminated by soil fumigation, and 70 ± C moist heat (Cook and Rovira 1976). The specific suppressiveness was eliminated by pasteurization (Shipton et al. 1973; Scher and Baker 1982; Alabouvette 1986; Raaijmakers and Weller 1998; Westphal and Becker 2000). Numerous kinds of antagonistic microorganisms have been found to increase in suppressive soils; most commonly, however, pathogen and disease suppression has been shown to be caused by fungi, such as *Trichoderma* sp., *Penicillium* sp., and *Sporidesmium* sp., or by bacteria of the genera *Pseudomonas* sp., *Bacillus* sp., and *Streptomyces* sp. However, populations of nonpathogenic *F. oxysporum* and fluorescent *Pseudomonas* spp. have been repeatedly shown to be involved in suppression of fusarium wilts in naturally occurring disease suppressive soils. Other antagonistic microorganisms have been proposed having lesser roles in the suppression of fusarium wilts (Alabouvette 1990; Larkin et al. 1996). Suppressiveness to *F. oxysporum* f. sp. *melonis* (Scher and Baker 1980) and *F. oxysporum* f. sp. *niveum* (Hopkins et al. 1987; Larkin et al. 1993) was induced following continuous cropping of melon and watermelon, respectively. Interestingly, the induction of suppressiveness in these cases was associated with continuous cropping of partially resistant cultivars, whereas induction of suppressiveness against other soil-borne pathogens normally involves monoculture of susceptible cultivars (Whipps 1997). Evidence of a similar induction of suppression in the early 1900s was reviewed by Kommedahl et al. (1970), in which long-term monoculture of cultivar resistant to flax wilt resulted in a marked decline in disease following

several years of increases at Ventura Count, California, USA. Whereas, cropping to susceptible cultivars resulted in complete wilt (100 %) every year. Schneider (1982) also observed islands of healthy celery plants in fields uniformly devastated by wilt. In both of these cases, the organisms responsible for suppressiveness were nonpathogenic *F. oxysporum*. Transfer of suppressiveness to a raw conducive, fumigated, or sterilized soil by addition of 0.1–10 % or less (w/w) of the suppressive soil further consolidated the role of microorganisms in suppressiveness. Mechanisms in suppression of fusarium wilt by microorganisms may involve competition for substrate and root surface, antagonism, PGPR activities, and cytological modification of host plant holistically.

12.6.1 Competition for Nutrients and Root Surface

After the germination of the pathogen, it has to travel to some distance before it comes in the contact of the host surface, and host parasite relationship is established. Till the distance is travelled, the pathogen need to remain dependent on some other source of nutrients. Presence of other microorganisms may exert competition for the nutrients and site of infection which is a general phenomenon regulating the population dynamics of microorganisms sharing the same ecological niche and having the same physiological requirements (Alabouvette et al. 2009). Carbon, nitrogen, and phosphorous are the important nutrients required for the growth of fungi. Among the different nutrients, competition for the carbon is most significant and is responsible for the inhibition of germination and subsequent growth of the fusarium wilt pathogen in Châteaurenard region (Bouches-du-Rhône, France) in melon and cotton field (Alabouvette et al. 1977; Sivan and Chet 1989; De Boer et al. 2003). Larkin et al. (1996) isolated 400 different microorganisms including actinomycetes, bacteria, and fungi from watermelon root growing in the suppressive and non-suppressive soil to fusarium wilt of watermelon and concluded that nonpathogenic *F. oxysporium* was the primary antagonist responsible for the disease suppressiveness. Other than *F. oxysporum, Trichoderma* spp., Arbuscular mycorrhizal fungi (AMF), fluorescent *Pseudomonas spp*., *Bacillus* spp., *Alcaligenes* sp., etc. have been reported to control *fusarium* diseases in different crops (Park et al. 1988; Duijff et al. 1991; Lemanceau and Alabouvette 1991; Chen et al. 1995; Tanwar et al. 2013). *Trichoderma* spp. and *Pseudomanas spp*. colonize near to the root surface and exert multiple effects on the pathogen by competing for the nutrient & infection site and parasitizing the pathogen either directly or indirectly by secreting many growth limiting metabolites (Perelló et al. 2003). In artificially developed suppressive soil, application of a combination of biocontrol agents is likely to more closely mimic the natural situation and may, therefore, represent a more viable control strategy of wilt diseases in many crops. Lemanceau et al. (1992, 1993) described increased suppression of fusarium wilt of carnation by combining *P. putida* WCS358 with nonpathogenic *F. oxysporum* Fo47. The enhanced disease suppression by this

combination is due to siderophore-mediated competition for iron by *P. putida* WCS358, which makes the pathogenic *F. oxysporum* strain more sensitive to competition for glucose by the nonpathogenic strain *F. oxysporum* Fo47. Furthermore, Leeman et al. (1996) showed that combining strains of nonpathogenic *Verticillium lecanii*, *Acremonium rutilum*, or *F. oxysporum* with the fluorescent *Pseudomonas* spp. strains WCS358, WCS374, or WCS417 resulted in significantly better suppression of fusarium wilt of radish compared to the single organisms. This mechanism was proved using a GUS-marked strain of pathogenic *F. oxysporum* f. sp. *lini* and a *pvd-in a Z*-marked derivative *of P. putida* WCS358. The study confirmed that suppression by the nonpathogenic *Fusarium* sp., strain is related to reductions in both population density and metabolic activity of the pathogen on the root surface, and that competition for iron contributes to the suppression by *Pseudomonas* sp., and enhances the biological activity of the nonpathogenic *F. oxysporum* strain. The significant role for pyoverdine production by *P. putida* WCS358 in this interaction was ascertained as the siderophore deficient mutant did not enhance disease control achieved by use of the nonpathogenic *F. oxysporum* alone (Duijff et al. 1999).

Competition for the infection court by quantifying root colonization by a nonpathogenic and a pathogenic strain of *F. oxysporum* was observed by Eparvier and Alabouvette (1994). Glucuronidase activity of the GUS-transformed pathogen was reduced in the presence of the protective strain and concluded that these strains were competing for root colonization. It is evident that stable suppressiveness such as suppressiveness of the soil from the Salinas Valley or Chateaurenard is based on the collective effects of several microorganisms and mechanisms (Schippers 1992).

12.6.2 Antagonism

Antagonism which involves the destruction or inhibition of the growth of the pathogen by other microorganisms is a well-known phenomenon in the ecosystem. The parasitic activity of strains of *Trichoderma spp*. towards various pathogens has been studied and reviewed thoroughly (Harman et al. 2004; Motlagh and Samimi 2013; El-Rahman and Mohamed 2014; Lelavthi et al. 2014). Chitin and β-1,3-glucan are the main structural components of fungal cells walls, except those from members of the class oomycetes, which contain β-1,3-glucan and cellulose. Antagonism by the *Trichoderma spp*. involves specific recognition between the antagonist and its target pathogen and triggers cell wall-degrading enzymes, viz., β-(1,3)- glucanases, chitinases, lipases, and proteases. These enzymes penetrate the hyphae of the pathogen resulting into death of the target organism (De la Cruz et al. 1992; Sivan and Chet 1989). In addition, they produce some lytic enzymes during the parasitic interaction between *Trichoderma* spp. and some pathogenic fungi (Haran et al. 1996). Other cell wall-degrading enzymes, including hydrolyzing minor polymers (proteins, β-1,6-glucans, α-1,3-glucans, etc.), may be involved in the effective and complete degradation of mycelial or

conidial walls of phytopathogenic fungi by *Trichoderma* spp. Mycoparasitism describes the type of biotrophic interactions in which organisms benefit at the expense of the fungi (Druzhinina et al. 2011). Partial degradation of the host cell wall is normally observed in later stages of the parasitic process. Initially, the mycoparasite grows directly towards its host and often coils around it or attaches to it by forming hook-like structures and apressoria. Following these interactions, *Trichoderma* spp. sometimes penetrate the host mycelium, apparently by partially degrading its cell walls (Elad et al. 1984). Heterotrimeric G-proteins and mitogen-activated protein (MAP) kinases affected biocontrol-relevant processes such as the production of hydrolytic enzymes and antifungal metabolites and the formation of infection structures. MAPK signaling was also found to be involved in induction of plant systemic resistance in *T. virens* and in the hyperosmotic stress response in *T. harzianum*. *Trichoderma* mycoparasitism combines processes such as nutrient competition (Chet 1987), the secretion of antifungal metabolites (Lorito et al. 1996), and formation of morphological changes such as coiling around the host and development of appressorium-like structures (Lu et al. 2004).

Antibiosis is also a very common phenomenon of antagonism of many biocontrol agents (BCAs) such as fluorescent *Pseudomonas* spp., *Bacillus* spp., *Streptomyces* spp., and *Trichoderma* spp. Various secondary metabolites have been reported from these microorganism with their role in the suppression of several plant pathogens (Weller and Thomashow 1993; Alabouvette et al. 2009). Production of antibiotics including phenazine-1-carboxylic acid, 2,4 diacetylphloroglucinol (2,4- DAPG), pyoluteorin, and pyrrolnitrin play an important role in the biological control of soil-borne pathogens by certain strains of fluorescent *Pseudomonas* spp. that produce these antibiotics (Keel et al. 1992; Kraus and Loper 1995). There are some evidences of the activity of phenazines and anthranilate in the antagonism of *Pseudomonas aeruginosa* toward *F. oxysporum* (Anjaiah et al. 1998).

12.6.3 PGPR Activities

Microbial activities are 10–1000 times higher in the vicinity of plant roots than in unplanted soil (Lugtenberg and Bloemberg 2004). Plant Growth Promoting Rhizobacteria (PGPR), viz., *Azotobacter* spp., *Azospirillum* spp., *Acetobacter* spp., *Rhizobium* spp., *Bacillus* spp., AMF, *Trichoderma* spp., *Pseudomonas* spp., etc., competitively colonize near plant roots and stimulate plant growth and/or inhibit the pathogenic activities. Signal molecules secreted by the root surface of the susceptible plant activate the germination of prologues pathogenic fungi; however, presence of PGPR and its colonization prevents subsequent growth of pathogenic microorganisms near to the root surface. Supply of nutrients either by fixing or solubilizing, production of phytohormones (such as auxin and cytokinin), and volatile growth stimulants such as ethylene and 2,3-butanediol help plants in growing better and controlling diseases (Haas and Défago 2005; Ayed et al. 2006;

Daami-Remadi et al. 2006; Chowdappa et al. 2013). Efficiency and level of disease suppression depend upon efficacy, population dynamics, and location of these microorganisms. Biodegradation activities of PGPR, through the action of ACC deaminase activity that hydrolyzes ACC into ammonia and α-ketobutyrate, prevent the synthesis of plant growth inhibiting levels of ethylene in the roots (Viterbo et al. 2010). ACC deaminase has previously been reported for *Pseudomonas spp*., and its activity has been associated with an increase in root elongation due to the reduced inhibition caused by ethylene (Avis et al. 2008). *Trichoderma* strains colonize the plant roots and influencing the synthesis of chloroplast enzymes that increases rate of photosynthesis (Abo-Ghalia and El-Khallal 2005) or establishing chemical communication and systemically altering the expression of numerous plant genes that alter plant physiology and photosynthetic efficiency (Harman et al. 2004; Hermosa et al. 2012). Further, *Trichoderma* sp. has the ability to increase the solubility of nutrients with low solubility like phosphates and other micronutrients like zinc, copper, iron, and manganese (Altomare et al. 1999), and the soluble form of phosphorus was easily absorbed by the extensive plant roots. Thus, through an increased nutrient uptake, bioagents compensate for the losses caused by pathogen attack. Biocontrol potential of AMF could be explained in terms of its ability to change root architecture, improved nutrient uptake, competition with the pathogen for infection site, activation of plant defense enzymes (chitinase, chitosanase, β-1,3-glucanase, and superoxide dismutase), phenolic and phytoalexin production (Avis et al. 2008).

12.6.4 Induced Systemic Resistance

Induced systemic resistance (ISR) is the process whereby the detrimental effect of a pathogen on plant is induced by prior treatment with an elicitor, either an organism or chemical. It has been proposed that, in suppressive soils, plant roots are associated with microbial communities that have an overall beneficial effect on plant health. ISR allows plants to withstand pathogen attack to the leaves or roots, without offering total protection (Harman et al. 2004). Many effective PGPMs elicit ISR, irrespective of antibiotic production (Zehnder et al. 2001; Ongena et al. 2004). Systemic induced resistance (SAR) by *P. fluorescens* WCS417r was established in carnation, radish, Arabidopsis tomato (Van Peer et al. 1991; Leeman et al. 1995; Pieterse et al. 1996; Duijff et al. 1998). Indeed, a mutant of this bacterial strain reduced endophytic root colonization and a lower ability to induce systemic resistance (Duijff et al. 1997). The effects of three different strains of *Pseudomonas* spp. mediating ISR in *Arabidopsis thaliana* have been investigated through transcriptome analysis of plants with roots that were colonized by one of these strains (*P. fluorescens*WCS417r, *P. thivervalensis*, or *P. fluorescens* CHA0). Studies with *A. thaliana* mutants indicate that the jasmonate/ethylene-inducible defense pathway is important for ISR, whereas the salicylate-inducible pathway mediating SAR seems to be less important. Total six classes of antibiotic compounds, viz.,

phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides (all of which are diffusible), and hydrogen cyanide (HCN; which is volatile) were reported from *P. fluorescens* which suppress root disease. The modes of action of these secondary metabolites are partly understood. These antibiotics exert inhibition of electron transport chain and fungal respiratory chains and cause membrane damage (Reviewed by Haas and Défago 2005). In bean, ISR elicited by a *P. putida* strain was associated with elevated levels of hexenal, which is a volatile antifungal compound, and with enhanced expression of enzymes that are involved in hexenal synthesis (Ongena et al. 2004). The ability of nonpathogenic *F. oxysporum* to induce resistance has been shown in carnation, cucumber, chickpea, and tomato (Kroon et al. 1991; Mandeel and Baker 1991; Hervás et al. 1995; Fuchs et al. 1997). However, the efficacy of the induced resistance varies according to the fungal biocontrol strain (Olivain et al. 1995). The spatial separation between the biocontrol strains used to induce resistance and the challenging pathogen in the split root system led to the conclusion that the reduction of the disease incidence by the inducing microorganisms was plant mediated (Hoffland et al. 1996). Further, inoculation with nonpathogenic *F. oxysporum strain* Fo47 increased chitinase, β-1,3-glucanase, and β-1,4-glucosidase activity in plants, confirming the ability of Fo47 to induce resistance in tomato Fuchs et al. (1997). This study suggests that Fo47 may act as an inducer of resistance through a classic SAR-like mechanism and induces PR proteins. *T. harzianum* strain T-39 also found to induce resistance and made leaves of bean plants resistant to diseases that are caused by the fungal pathogens *Botrytis cinerea* and *Colletotrichum lindemuthianum*, even though T-39 was present only on the roots and not on the foliage Bigirimana et al. (1997).

12.6.5 Cytological Modification

Induction of cytological modification in response to the presence and activities of nonpathogenic, antagonistic, plant growth promoting microorganisms tends to make the root surface incompatible for the penetration and subsequent establishment. Treating tomato plants with *Trichoderma* species has resulted in the formation of hemispherical cell wall appositions and the occlusion of some intercellular spaces by an amorphous material (Hibar 2007). Similarly, Benhamou and Thériault (1992) showed that treating tomato plants with *Pythium oligandrum* before inoculation with *F. oxysporum f.* sp. *radicis-lycopersici* has entailed cytological changes, mainly characterized by the elaboration of structural barriers, cell wall thickenings, and plugging of most intercellular spaces. Bao and Lazarovits (2001) observed reduced wilt disease incidence due to cell wall thickening in tomato plants after treatment of nonpathogenic strain of *F. oxysporum* (70T01).

12.7 Techniques to Study the Soil Suppressiveness

Haas and Défago (2005) discussed that the complexity of the disease suppression and observed four phenomena: First, certain suppressive soils when pasteurized (e.g., by wet heat at 70 °C for 30 min) lose their suppressiveness, and other harsher antimicrobial treatments (e.g., gamma radiation or autoclaving) have the same effect (Shipton et al. 1973; Scher and Baker 1980). Second, suppressiveness can be transferable: an inoculum of 0.1–10 % of a specific suppressive soil introduced into a conducive soil can establish disease suppression (Menzies 1959; Cook and Rovira 1976; Weller et al. 2002). Third, when the pH of a fusarium wilt suppressive soil is lowered from 8 to 6 by the addition of H_2SO_4, the soil looses suppressiveness (carnation to the wilt disease) because of the change in the soil environment. Fourth, several years of monoculture can induce disease suppression in some soils. The best-studied example is suppressiveness to *F. oxysporum* f. sp. *melonis* (Scher and Baker 1980) and *F. oxysporum* f. sp. *niveum* (Hopkins et al. 1987; Larkin et al. 1993) which was induced following continuous cropping of melon and watermelon, respectively. All these decisively establish that microorganisms are invariably associated with the soil suppressiveness; however, the soil environmental conditions also play role in making the soil suppressive either directly or indirectly by making the environment conducive for the antagonistic microorganism. To study the soil suppressiveness, these four phenomena should be systematically studied by using various techniques. Even with the advent of the advanced soil monitoring techniques, the nature of the soil microbiota, its dynamics, activities, and interactions are still largely enigmatic. One or few microorganisms may primarily be responsible for the suppressiveness, but interactions with other members of the rhizosphere community can significantly modulate its degree. Moreover, the phenomenon of disease suppression might be related to specific functions or activities of soil microorganisms rather than the simple presence or abundance of particular populations in the soil. Traditional approaches to study microbial communities in soils were based on culture-dependent techniques. These approaches were useful for isolation purposes, but were very limited in their scope to understand microbial communities and diversity. Recent developments in new types of media and methods have led to considerable advances in this composition and diversity of soil microbial communities; however, still less than 1 % of the microorganisms present in soil may be readily isolatable whereas remaining 99 % microorganisms viable but nonculturable (VBNC) stage (Torsvik et al. 1996; Kuske et al. 1997; Oliver 2005). It is generally admitted that disease suppressiveness is related to a global increase in soil microbial biomass. A large biomass would create a competitive environment deleterious for the pathogens (Janvier et al. 2007). To overcome the dependence on the culture dependence techniques and expand our understanding, culture-independent techniques to "first identify and then recover" important antagonists are extensively useful. These are holistic, high throughput, accurate, and comprehensive techniques; however, they have not been used to study the soil suppressiveness. For better understanding, it is recommended to use

combination of culture-dependent and culture-independent techniques (Liesack et al. 1997).

12.7.1 Culture-Dependent Techniques

Culture-dependent techniques involves soil sampling, isolation of bacteria, and determination of colony forming units; screening of isolates for in vitro antagonistic activity towards pathogen; screening of antagonists for production of siderophores and cell-wall degrading enzymes; and identification of the isolated microorganism based on various biochemical and molecular techniques. The identification techniques include biochemical as well as nucleic acid based identifications which are quite accurate and reproducible. A thorough understanding of the mechanisms of action is needed to maximize consistency. In the *F. oxysporum* wilt suppressive soil, many studies dealing with nonpathogenic *F. oxysporum* have proven that not all the nonpathogenic strains are effective in controlling wilts. Since there is currently no known genetic marker to identify these strains, the only available and reliable method to screen for efficient strains is a bioassay in which the potential biocontrol agents are confronted with the pathogen in the presence of the host plant and disease incidence or severity is monitored. In general, the closer the screening method is to the production system, the greater the chances are for success.

12.7.2 Culture-Independent Techniques

Culture-independent techniques allow the study of a much greater part of the soil microflora. These techniques may be biochemical or molecular depending upon the test performed (Table 12.2). Biochemical techniques involve different assays, viz., Ability of microbial communities to degrade different carbon substrates (BIOLOG); Phospholipid fatty acid (PLFA); Fatty acid methyl ester (FAME); Enzyme activities and Metabolites (volatile and nonvolatile) profiling. Molecular techniques involves ITS/IGS or NTS sequencing ITS/IGS sequencing; Terminal-restriction fragment length polymorphism (T-RFLP); Denaturing gradient gel electrophoresis (DGGE and PCR-DGGE); RAPD and Gene-specific primers; 16S rRNA microarray probes; etc.

12.7.2.1 Biochemical Techniques

The community level physiological patterns established using the BIOLOG systems have been used to detect differences in the ability of microbial communities to degrade different carbon substrates (Garland and Mills 1991). Pérez-Piqueres

Table 12.2 Various techniques to identify microorganism in suppressive soil

Sr. No.	Techniques	Instruments	Suppressive soil to pathogen/disease	Microorganism Identified/specific method	References
1.0	Biochemical				
1.1	Carbon utilization	Biolog	South-East Asian isolates Take-all disease and *Rhizoctonia solani*	*Trichoderma* spp. *Pseudomonas corrugata*	Kubicek et al. (2003) Barnett et al. (1999)
1.2	FAME and PLFA)	GC	Take-all disease, *Rhizoctonia solani* *Verticillium dahliae* Kleb *Fusarium* crown and root rot of asparagus	*Pseudomonas corrugata* *Pseudomonas putida* Bacteria, Fungi, and Mycorrhiza	Barnett et al. (1999) Berg et al. (2002) Hamel et al. (2005)
1.3	Enzyme activity	Spectrophotometer/ substrate degradation in medium	Soil suppressiveness to seedling blight of barley *(Fusarium culmorum)* Suppressive soil to *F. oxysporum* on melon plants	Higher activities of β-glucosidase and cellobiohydrolase Higher phosphatase and β-glucosidase	Rasmussen et al. (2002) Ros et al. (2005)
1.4	Metabolites DAPG and other	HPLC	*Fusarium oxysporum* f. sp. *Lycopersici* and *F. oxysporum* f. sp. *albedinis*	*Pseudomonas chlororaphis* (phenazine carboxylic acid, 2-hydroxy phenazine carboxylic acid, and 2-hydroxy phenazine)	Mezaache-Aichour et al. (2012)
1.5	Volatile organic antifungal molecules	GC-MS	*Sclerotinia sclerotiorum*	*Pseudomonas* spp.	Fernando et al. (2005)
2.0	Molecular				
2.1	ITS/IGS or NTS sequencing	PCR	*Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *albedinis* Biocontrol isolates from different countries Fusarium Wilt of cucumber and other crops	*Pseudomonas chlororaphis* (16S rDNA and sequencing) *Trichoderma* spp. (ITS1 region of rDNA) Nonpathogenic *Fusarium oxysporum* (FIGS11/FIGS12 primers of IGS region	Mezaache-Aichour et al. (2012) Hermosa et al. (2004)

				of rDNA produced 500-bp DNA fragment)	Wang et al. (2013)
2.2	T-RFLP DGGE	PCR	Effect of Biocontrol Agent *Pseudomonas fluorescens* 2P24	Soil Fungal Community in Cucumber Rhizosphere using T-RFLP and DGGE	Gao et al. (2012)
2.3	3.RAPD M13 and D7 primers	PCR	Take-all disease suppressive soil *Fusarium oxysporum* f. sp. *radicis-lycopersici*	DAPG-producing *Pseudomonas spp*.	Raaijmakers and Weller (2001)
2.4	Gene-specific primers	PCR	Bio control isolates *Verticillium dahliae* Kleb (Isolated from potato, oilseed rape, strawberry, and from bulk soil)	*Trichoderma* spp. (tef1 gene) *P. putida* (phlD and chiA genes)	Hermosa et al. (2004) Berg et al. (2002)
2.5	5.16S rRNA probes	Microarray	(*Tobacco basicola*) tobacco black root rot suppressive soil	Fluorescent Pseudomonas (with Pseudomonas probe:Pseu1, PseuD, PseubC2BC3-2, and PseubC2-10)	Kyselkova et al. (2009)

et al. (2006) compared the BIOLOG profiles of different soil mixes suppressive to *R. solani* from the non-amended highly conducive control soil. Similarly, Benizri et al. (2005) compared the BIOLOG profiles of the bacteria inhabiting two healthy and one sick soil, mimicking peach tree replant disease. Analysis separated the soil bacteria isolated from healthy soils from those isolated from sick soils. Kubicek et al. (2003) identified *Trichoderma* spp. and *Pseudomonas corrugita* from the suppressive soil to Take-all disease from South East Asian Isolates. Barnett et al. (1999) characterized a collection of 14 spontaneous phenotype variants, derived from in vitro and in vivo cultures (wheat roots) of *P. corrugata* 2140, using fatty acid methyl ester profiles (GC-FAME), carbon substrate utilization (BIOLOG), and in vitro inhibition against seven soil microorganisms. All three phenotype profiles indicated marked differences between some variants and the parent isolate. Some variant types were classified taxonomically by GC-FAME as different species to their wild-type parent, and up to a Euclidian distance of 11 from their parent. Taxonomic identification by the BIOLOG assay was more consistent than others. Phospholipid-derived fatty acids (PLFA) are chemotaxonomic markers of bacteria and other organisms. Phospholipids are primary lipids found in cell membranes that are saponified, releasing fatty acids contained in their diglyceride tail. Phospholipids are extracted from the whole soil and analyzed. Once the phospholipids of an unknown sample are saponified, the composition of the resulting PLFA can be compared to the PLFA)of known organisms to determine the identity of the sample organism. Many fatty acids have been isolated and are representative of specific microbial groups, making PLFA analysis a useful tool to describe microbial diversity and structure (Bossio et al. 1998; Ibekwe and Kennedy 1998). Various fatty acid biomarkers have been reported for microorganism identification, viz., PLFA C18:2ω6 was taken as indicator of fungal biomass (Frostegard and Baath 1996); C16:1ω5, as indicator of extra radical mycorrhizal hyphae and spores (Olsson 1999); while 16:0 and 16:1 (equivalent proportions) along with 18:1ω7c/ω9t/ω12t fatty acids as biomarkers for *Pseudomonas* spp. (Piotrowska-Seget and Mrozik 2003).

The types and proportions of fatty acids present in cytoplasm membrane and outer membrane (gram negative microorganisms) lipids of cells are major phenotypic traits. FAME is a type of fatty acid ester that is derived by transesterification of fats with methanol. Since every microorganism has its specific FAME fingerprint, it can be used as a tool for microbial source tracking (MST). FAME microbial markers would be a useful indicator of soil health and that the soil odd number fatty acid proportion changed due to organic amendment, which also reduced the disease incidence (Cai et al. 2003). From Fusarium wilt suppressive soil of Chateaurenard, France, total 37 species of bacteria with 71 antagonists were identified using FAME and/or 16S rRNA gene sequencing. A high proportion of the antagonists isolated from this soil produced siderophores (94 % of 71) and chitinase activity (46 %). Interestingly, suppressive soil of Chateaurenard, France, displayed higher diversity of antagonistic bacteria (Adesina et al. 2007). Soil enzymes and metabolites play vital roles for the maintenance of soil ecology and soil health. Enzymatic activities in the soil are mainly of microbial origin; therefore, microorganisms are acting as

the indicators of soil health and can be used as measures of microbial activity and characteristics of the soil. The potential enzymes playing major roles in maintaining soil health are—amylase, arylsulphatase, β-glucosidase, cellulase, chitinase, dehydrogenase, phosphatase, protease, and urease. These enzymes and other metabolites can be studied by the spectrophotometric techniques. Higher activities of β-glucosidase, cellobiohydrolase, phosphatase, and β-glucosidase was observed by in the soil suppressive to seedling blight of barley *(F. culmorum)* and to *F. oxysporum* on melon plants (Rasmussen et al. 2002; Ros et al. 2005). Phenazine carboxylic acid, 2-hydroxy phenazine carboxylic acid, and 2-hydroxy phenazine have been observed by HPLC in the *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *albedinis* suppressiveness (Mezaache-Aichour et al. 2012).

12.7.2.2 Molecular Techniques

All the molecular techniques are based on the nucleic acid of the microbial communities which involves amplification of the DNA and sometimes its sequencing to validate the result with higher precision. Depending upon the specificity of the DNA fragment and primers used for the amplification of the DNA, various techniques have been named. Random Amplified Polymorphic DNA (RAPD) employ short primers (8–12 nucleotides) to amplify large template of genomic DNA without its prior knowledge, expecting that fragments will amplify. This makes the method popular for comparing the DNA of biological systems that have not been resolved. Other PCR uses gene-specific primers sets from the different part of the DNA. Gene-specific primers (phlD and phz) for the biosynthesis genes 2,4-diacetylphloroglucinol (2,4- DAPG) and phenazine-1-carboxylic acid (PCA) in pseudomonads in soils have been used to characterize wilt suppressive soil (Raaijmakers et al. 1997). Internal transcribed spacer (ITS) is a piece of nonfunctional RNA situated between 5′ external transcribed sequence (5′ ETS), 18S rRNA, ITS-1, 5.8S rRNA, ITS-2, 28S rRNA, and finally the 3′ ETS. During rRNA maturation, ETS and ITS pieces are spliced. Genes encoding ribosomal RNA and spacers occur in tandem repeats that are thousands of copies long, each separated by regions of non-transcribed DNA termed intergenic spacer (IGS) or non-transcribed spacer (NTS). Sequence of the ITS region is highly conserved because of low evolutionary pressure and widely used in taxonomy. Isolation of these from the soil samples is easy as they are in high copy number. Several taxon-specific primers have been described that allow selective amplification of fungal sequences. By using oligonucleotide primers targeted to conserved regions in the 16S and 23S genes, RISA (Ribosomal intergenic spacer analysis) fragments can be generated from most of the dominant bacteria in the soil sample. Amplification results in complex banding pattern that provides a community-specific profile where each DNA band corresponds to a bacterial population on the original assemblage. Majority of the rRNA operon serves a structural function; portions of the 16S-23S intergenic region can encode tRNAs depending on the bacterial species. *P. chlororaphis, Trichoderma* spp., nonpathogenic *F.oxysporum*, and

many more biocontrol agents have been identified by RISA (Mezaache-Aichour et al. 2012; Hermosa et al. 2004; Wang et al. 2013). Terminal Restriction Fragment Length Polymorphism (T-RFLP) is a molecular tool for the profiling of microbial communities based on the position of a restriction site closest to a labeled end of an amplified gene. The method is based on digesting a mixture of PCR-amplified variants of a single gene using one or more restriction enzymes and detecting the size of each of the individual resulting in terminal fragments using a DNA sequence. Muyzer et al. (1993) described a technique based on the separation of all the same length PCR-amplified fragments coding for 16S rRNA, by denaturing gradient gel electrophoresis (DGGE). DGGE analysis of different microbial communities demonstrated the presence of up to 10 distinguishable bands in the separation pattern, which were most likely derived from as many different species constituting these populations, and thereby generated a DGGE profile of the populations. These techniques allow the analysis of both culturable and nonculturable microorganisms and provide a rapid method for observing changes in community structure in response to different environmental factors. Besides total bacterial and fungal communities, the structure of specific subgroups can also be assessed (Garbeva et al. 2006). In a soil having received pig slurry or compost and showing an increased suppressiveness to *R. solanacearum* biovar 2 on potato, PCR-DGGE revealed differences in the bacterial community structure (Schonfeld et al. 2003; Gorissen et al. 2004). These amendments resulted in the appearance of several novel bands and different relative intensities of bands common to the treated and non-treated soils. In the case of compost amendment, several discriminate DGGE bands and PCR products were cloned and/or sequenced in order to identify the corresponding microorganisms; but their involvement in disease suppressiveness remains to be tested. Nevertheless, even if the microorganisms are not directly responsible, these DNA markers might serve as indicators of these treatments and thus as indicator of the *R. solanacearum*-suppressive status of soil. Comparing bacterial DGGE patterns of soils receiving different treatments, Kowalchuk et al. (2003) found that except for a sterilized and then amended soil, all DGGE patterns from the treated and control soils were highly similar. The same samples were also examined by fungal PCR-DGGE. The profiles obtained were much simpler than those obtained for bacteria. Once again the sterilized and amended soil was very different from the others. Yang et al. (2001) compared DGGE fingerprinting of rhizospheric bacterial communities associated with healthy or *Phytophthora cinnamomi* infected avocado roots. An assay clearly revealed that bacterial communities from healthy roots, both of control trees or trees treated with biocontrol bacteria, were highly similar, but different from the communities on infected roots. Gao et al. (2012) studied soil fungal community in cucumber rhizosphere using T-RFLP and)DGGE and observed *Pseudomonas fluorescens* 2P24 as biocontrol agent. Pérez-Piqueres et al. (2006) used the T-RFLP method to characterize microbial communities. Correspondence analyses clearly separated both fungal and bacterial community structures of the most suppressive amended soil from the other treatments. All these results demonstrate that the microbial community structure and diversity are often sensitive to the phytopathological

status of soils, but until now, no microbial component was identified as potential indicator of disease suppression from such studies. Indeed, after the whole community fingerprinting, it is necessary to select the discriminating markers and to identify the microorganisms "hidden" behind.

DNA microarray technique is accurate and helps in handling large number of samples. Kyselkova et al. (2009) assessed 64 16S rRNA microarray probes whose signals correlated with tobacco black-root-rot (*Tobacco basicola*) suppressiveness in greenhouse analyzed to discriminate suppressive from conducive soils under field conditions. Rhizobacterial communities of tobacco and wheat sampled in 2 years from four farmers' fields of contrasted suppressiveness status were compared. The 64 previously identified indicator probes correctly classified 72 % of 29 field samples, with 9 probes for *Azospirillum*, *Gluconacetobacter*, *Sphingomonadaceae*, *Planctomycetes*, Mycoplasma, *Lactobacillus crispatus*, and *Thermodes ulforhabdus* providing the best prediction. The whole probe set (1033 probes) revealed strong effects of plant, field location and year on rhizobacterial community composition, and a smaller (7 % variance) but significant effect of soil suppressiveness status. Study signifies the use of subset of 16S rRNA probes targeting diverse rhizobacteria as indicator of suppressiveness under field conditions.

12.8 Conclusion

In soil, many microorganisms occur in close proximity and interact in a unique way. Soils in which the pathogen does not establish, or establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil, are known as suppressive soils. Two different categories, viz., general or horizontal (widespread but limited ability of soils to suppress the growth or activity of soil-borne pathogens) and specific or vertical (due to antagonistic effect of individual or selected groups of microorganisms during some stage in the life cycle of a pathogen) suppressiveness is most commonly observed. Wilt suppressive soils have been reported from the four places, viz., in the Salinas Valley, California, United States; the Chateaurenard region, near Cavaillon, France; the Canary Islands and the Broye Valley, Switzerland. Among these, the Chateaurenard soil in France and the Salinas Valley soil in California are known for their natural suppressiveness to Fusarium wilt diseases. Numerous kinds of antagonistic microorganisms have been found to increase in suppressive soils; most commonly, however, pathogen and disease suppression has been shown to be caused by fungi, such as *Trichoderma* sp., *Penicillium* sp., and *Sporidesmium* sp., or by bacteria of the genera *Pseudomonas* sp., *Bacillus* sp., and *Streptomyces* sp. Populations of nonpathogenic *F. oxysporum* and fluorescent *Pseudomonas* spp. have been repeatedly shown to be involved in suppression of fusarium wilts in naturally occurring disease suppressive soils. Mechanisms in suppression of fusarium wilt by microorganisms are may be involving; competition for substrate and root surface; antagonism; PGPR activities; and

cytological modification of host plant holistically. Less than 1 % of the microorganisms present in soil may be readily isolatable whereas remaining 99 % microorganism viable but nonculturable (VBNC) stage. To overcome the dependence on the culture dependence techniques and expand our understanding, culture-independent techniques to "first identify and then recover" important antagonists are extensively useful. For better understanding, it is recommended to use combination of culture-dependent and culture-independent techniques. Culture-independent techniques allow the study of a much greater part of the soil microflora. These techniques may be biochemical or molecular depending upon the test performed.

References

Abo-Ghalia H, El-Khallal SM (2005) Alleviation of heavy metal stress by arbuscular mycorrhizal fungi and jasmonic acid in maize plants. Egypt J Bot 45:55–77

Adesina MF, Lembke A, Costa R, Speksnijder A, Smalla K (2007) Screening of bacterial isolates from various European soils for *in vitro* antagonistic activity towards *Rhizoctonia solani* and *Fusarium oxysporum*: site dependent composition and diversity revealed. Soil Biol Biochem 39:2818–2828

Agrios GN (2005) Plant pathology, 5th edn. Elsevier, New York

Alabouvette C (1986) Fusarium wilt suppressive soils from the Chateaurenard region: review of a 10-year study. Agronomie 6:273–284

Alabouvette C (1990) Biological control of Fusarium wilt pathogens in suppressive soils. In: Hornby D (ed) Biological control of soilborne plant pathogens. CAB International, Wallingford, pp 27–43

Alabouvette C, Rouxel F, Louvet J (1977) Recherches sur la résistance des sols aux maladies. III. Effets du rayonnement a sur lamicroflore d'un sol et sa resistance a la fusariose vasculaire du melon. Ann Phytopathol 9:467–471

Alabouvette C, Couteaudier Y, Louvet J (1982) Comparaison dela réceptivité de différents sols et substrats de culture aux fusarioses vasculaires. Agronomie 2:1–6

Alabouvette C, Olivain C, Migheli Q, Steinberg C (2009) Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing *Fusarium oxysporum*. New Phytol 184:529–544

Altomare C, Norvll WA, Bjrkman T, Harman GE (1999) Solubilization of phosphates and micronutrients by plant growth promoting and biocontrol *Trichoderma harzianum* strain 1295–22. Appl Environ Microbiol 65:2926–2933

Anjaiah V, Koedam N, Nowak-Thompson B, Loper E, Hofte M, Tambong LT, Comelis P (1998) Involvement of phenazines and anthranilate in the antagonism of *Pseudomonas aeruginosa* PNAI and Tn5 derivatives toward *Fusarium* spp. and *Pythium* spp. Mol Plant Microbe Interact 9:847–854

Atkinson GF (1892) Some diseases of cotton. Al Agric Exp Stn Bull 41:65

Avis TR, Gravel V, Antoun H, Tweddell RJ (2008) Multifaceted beneficial effects of rhizospheric microorganisms on plant health and productivity. Soil Biol Biochem 40:1733–1740

Ayed F, Daami-Remadi M, Jabnoun-Khiareddine H, El-Mahjoub M (2006) Potato vascular Fusarium wilt in Tunisia: incidence and biocontrol by *Trichoderma* spp. Plant Pathol J 5:92–98

Baker KF, Cook RJ (1974) Biological control of plant pathogens. Freeman, San Francisco, p 433

Bao JR, Lazarovits G (2001) Differential colonization of tomato roots by non-pathogenic and pathogenic *Fusarium oxysporum* may influence Fusarium wilt control. Phytopathology 91:449–456

Barnett SJ, Alami Y, Singleton I, Ryder MH (1999) Diversification of *Pseudomonas corrugata* 2140 produces new phenotypes altered in GC-FAME, BIOLOG, and *in vitro* inhibition profiles and taxonomic identification. Can J Micobiol 45:287–298

Benhamou N, Thériault G (1992) Treatment with chitosan enhances resistance of tomato plants to the crown and root rot pathogen *Fusarium oxysporum* f. sp. *radicis-lycopercisi*. Physiol Mol Plant Pathol 41:33–52

Benizri E, Piutti S, Verger S, Pages L, Vercambre G, Poessel JL, Michelot P (2005) Replant diseases: bacterial community structure and diversity in peach rhizosphere as determined by metabolic and genetic fingerprinting. Soil Biol Biochem 37:1738–1746

Berg G, Roskot N, Steidle A, Eber L, Zock A, Smalla K (2002) Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different Verticillium host plants. Appl Environ Microbiol 68:3328–3338

Bigirimana J, De Meyer G, Poppe J, Elad Y, Höfte M (1997) Induction of systemic resistance on bean (*Phaseolus vulgaris*) by *Trichoderma harzianum*. Med Fac Landbouww Univ Gent 62:1001–1007

Bossio DA, Scow KM, Gunapala N, Graham KJ (1998) Determinants of soil microbial communities: effects of agricultural management, season and soil type on phospholipid fatty acid profiles. Microb Ecol 36:1–12

Cai Y, Zhao S, LiaoZ He C, Zhuang X (2003) Effect of fertilization on tomato bacterial wilt biocontrolling and soil health restoration using FAME analysis. Agric Sci China 2:779–785

Chen C, Bauske EM, Musson G, Rodríguez-Kábana R, Kloepper JW (1995) Biological control of Fusarium wilt on cotton by use of endophytic bacteria. Biol Control 5:83–91

Chet I (1987) Innovative approaches to plant disease control, Trichoderma - application, mode of action, and potential as a biocontrol agent of soilborne pathogenic fungi. Wiley, New York, pp 137–160

Chowdappa P, Kumar SPM, Lakshmi MJ, Upreti KK (2013) Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. Biol Control 65:109–117

Cook RJ, Rovira AD (1976) The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. Soil Biol Biochem 8:269–273

Daami-Remadi M, Hibar K, Jabnoun- Khiareddine H, Ayed F, El Mahjoub M (2006) Effect of two *Trichoderma* species on severity of potato tuber dry rot caused by Tunisian *Fusarium* complex. Int J Agric Res 1:432–441

De Boer W, Verheggen P, Klein Gunnewiek PJA, Kowalchuk GA, Van Veen JA (2003) Microbial community composition affects soil fungistasis. Appl Environ Microbiol 69:835–844

De la Cruz J, Hidalgo-Gallego A, Lora JM, Benítez T, Pintor-Toro JA, Llobell A (1992) Isolation and characterization of three chitinases from *Trichoderma harzianum*. Eur J Biochem 206:856–867

Deacon JW, Berry LA (1993) Biocontrol of soil-borne plant-pathogens–concepts and their application. Pestic Sci 37:417–426

Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV, Kubicek CP (2011) *Trichoderma*: the genomics of opportunistic success. Nat Rev Microbiol 9:749–759

Duijff BJ, Bakker PAHM, Schippers B (1991) Suppression of fusarium wilt of carnation by Pseudomonas in soil: mode of action. In: Keel C, Knoller B, Defago G (eds) Plant growth-promoting rhizobacteria, progress and prospect, vol 14. International Organization for Biological and Integrated Control of Noxious Animals and Plants/West Palaearctic Regional Section, Interlaken, pp 152–157

Duijff BJ, Gianinazzi-Pearson V, Lemanceau P (1997) Involvement of the outermembrane lipopolysaccharides in the endophytic root colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS417r. New Phytol 135:325–334

Duijff BJ, Pouhair D, Olivain C, Alabouvette C, Lemanceau P (1998) Implication of systemic induced resistance in the suppression of fusarium wilt of tomato by *Pseudomonas fluorescens* WCS417r and by nonpathogenic *Fusarium oxysporum* Fo47. Eur J Plant Pathol 104:903–910

Duijff BJ, Recorbet G, Bakker PAHM, Loper JE, Lemanceau P (1999) Microbial antagonism at the root level is involve in the suppression of fusarium wilt by the combination of nonpathogenic *Fusarium oxysporum* Fo47 and *Pseudomonas putida* WCS358. Phytopathology 89:1073–1979

Elad Y, Barak R, Chet I (1984) Parasitism of sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum*. Soil Biol Biochem 16:381–386

El-Rahman SSA, Mohamed HI (2014) Application of benzothiadiazole and Trichoderma harzianum to control faba bean chocolate spot disease and their effect on some physiological and biochemical traits. Acta Physiol Plant 36:343–354

Eparvier A, Alabouvette C (1994) Use of ELISA and GUS-transformed strains to study competition between pathogenic and non-pathogenic *Fusarium oxysporum* for root colonization. Biocontrol Sci Technol 4:35–47

Fernando WGD, Ramarathnam R, Krishnamoorthy AS, Savchuk SC (2005) Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil Biol Biochem 37:955–964

Frostegard A, Baath E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol Fertil Soils 22:59–65

Fuchs JG, Moenne-Loccoz Y, Défago G (1997) Non-pathogenic *Fusarium oxysporum* strain Fo47 induces resistance to Fusarium wilt in tomato. Plant Dis 81:492–496

Gao G, Yin D, Chen S, Xia F, Yang J, Qing L, Wang W (2012) Effect of biocontrol agent *Pseudomonas fluorescens* 2p24 on soil fungal community in cucumber rhizosphere using T-RFLP and DGGE. PLoS ONE 7(2), e31806

Garbeva P, Postma J, Van Veen JA, Van Elsas JD (2006) Effect of above-ground plant species on soil microbial community structure and its impact on suppressionn of *Rhizoctonia solani* AG3. Environ Microbiol 8:233–246

Garland JL, Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Appl Environ Microbiol 57:2351–2359

Garrett SD (1934) Factors affecting the pathogenicity of cereal root-rot fungi. Biol Rev 9:351–361

Geels FP, Schmidt EDL, Schippers B (1985) The use of 8- hydroxyquinoline for the isolation and prequalification of plant growth-stimulating rhizosphere pseudomonads. Biol Fertil Soils 1:167–173

Gorissen A, Van Overbeek LS, Van Elsas JD (2004) Pig slurry reduces the survival of Ralstonia solanacearum biovar 2 in soil. Can J Microbiol 50:587–593

Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319

Hamel C, Vujanovic V, Jeannotte R, Nakano-Hylander A, St- Arnaud M (2005) Negative feedback on a perennial crop: Fusarium crown and root rot of asparagus is related to changes in soil microbial community structure. Plant Soil 268:75–87

Haran S, Schickler H, Chet I (1996) Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. Microbiology 142:2321–2331

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* spicies.-Opportunistic avirulent plant symbionts. Nat Rev Microbiol 2:43–56

Henery MD (1932) A study of scab resistance in the potato. J Agric Res 54:305–317

Hermosa MR, Keck E, Chamorro I, Rubio B, Sanz L, Vizaino JA, Grondona I, Monte E (2004) Genetic diversity shown in *Trichoderma* biocontrol isolates. Mycol Res 108:879–906

Hermosa R, Viterbo A, Chet I, Monte E (2012) Plant-beneficial effects of *Trichoderma* and of its genes. Microbiology 158:17–25

Hervás A, Trapero-Casas JL, Jiménez-Díaz RM (1995) Induced resistance against Fusarium wilt of chickpea by nonpathogenic races of *Fusarium oxysporum* f. sp. *ciceris* and nonpathogenic isolates of *F. oxysporum*. Plant Dis 79:1110–1116

Hibar K (2007) Induction of resistance in tomato plants against *Fusarium oxysporum* f. sp. *radicis-lycopersici* by *Trichoderma* spp. Tunis J Plant Prot 2:47–58

Hoffland E, Hakulinen J, Van Pelt JA (1996) Comparison of systemic resistance induced by avirulent and non-pathogenic *Pseudomonas* species. Phytopathology 86:757–762

Hopkins DL, Larkin RP, Elmstrom GW (1987) Cultivar-specific induction of soil suppressiveness to fusarium wilt of water melon. Phytopathology 77:607–611

Ibekwe AM, Kennedy AC (1998) Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. FEMS Microbiol Ecol 26:51–163

Janvier C, Villeneuve F, Alabouvette C, Edel-Hermann V, Mateille T, Steinberg C (2007) Soil health through soil disease suppression: which strategy from descriptors to indicators. Soil Biol Biochem 39:1–23

Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, Burger P, Wirthner P, Haas D, Défago G (1992) Suppression of root diseases by *Pseudomonas fluorescens* CHAO: importance of the secondary metabolite 2,4-diacetylphloroglucinol. Mol Plant Microbe Interact 5:4–13

Kloepper JW, Schroth MN (1978) Plant growth-promoting rhizobacteria on radishes. In: Proceedings of the 4th international conference on plant pathogenic bacteria, vol 2. Station de Pathologie Végétale et de Phytobactériologie, INRA, Angers, France, pp 879–882

Kloepper JW, Leong J, Teintze M, Schroth M (1980) *Pseudomonas* siderophores: a mechanism explaining diseasesuppressive soils. Curr Microbiol 4:317–320

Kommedahl T, Christensen JJ, Frederickson RA (1970) A half century of research in Minnesota on flax wilt caused by *Fusarium oxysporum*. Minn Agric Exp Stn Tech Bull 273:35

Kowalchuk GA, Van Os GJ, Aartrijk J, Veen JA (2003) Microbial community responses to disease management soil treatments used in flower bulb cultivation. Biol Fertil Soils 37:55–63

Kraus J, Loper JE (1995) Characterization of a genomic region required for production of the antibiotic pyoluteorin by the biological control agent *Pseudomonas fluorescens* Pf-5. Appl Environ Microbiol 61:849–854

Kroon BAM, Scheffer RJ, Elgersma DM (1991) Induced resistance in tomato plants against Fusarium wilt invoked by *Fusarium oxysporum* f. sp. *dianthi*. Neth J Plant Pathol 97:401–408

Kubicek CP, Bissett J, Druzhinina I, Kullnig-Gradinger C, Szakacs G (2003) Genetic and metabolic diversity of *Trichoderma*: a case study on South-East Asian isolates. Fungal Gen Biol 38:310–319

Kuske CR, Barns SM, Busch JD (1997) Diverse uncultivated bacterial groups from soils of the arid southwestern United States that are present in many geographic regions. Appl Environ Microbiol 63:3614–3621

Kyselkova M, Kopecky J, Frapolli M, Defago G, Sagova-Mareckova M, Grundmann GL, Moenne-Loccoz Y (2009) Comparison of rhizobacterial community composition in soil suppressive or conducive to tobacco black root rot disease. ISME J 3:1127–1138

Larkin RP, Hopkins DL, Martin FN (1993) Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to fusarium-wilt of watermelon. Phytopathology 83:1097–1105

Larkin RP, Hopkins DL, Martin FN (1996) Suppression of Fusarium wilt of watermelon by nonpathogenic *Fusarium oxysporum* and other microorganisms recovered from a disease-suppressive soil. Phytopathology 86:812–819

Leeman M, Van Pelt JA, Den Ouden FM, Heinsbroek M, Bakker PAHM, Schippers B (1995) Induction of systemic resistance against fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. Phytopathology 85:1021–1027

Leeman M, den Ouden FM, van Pelt JA, Cornelissen C, Matamala-Garros A, Bakker PAHM, Schippers B (1996) Suppression of fusarium wilt of radish by co-inoculation of fluorescent *Pseudomonas* spp. and root-colonizing fungi. Eur J Plant Pathol 102:21–31

Lelavthi MS, Vani L, Pscal R (2014) Antimcrobial activity of *Trichodema harzinum* against bacteria and fungi. Int J Curr Micobiol Appl Sci 3:96–103

Lemanceau P, Alabouvette C (1991) Biological control of fusarium diseases by fluorescent Pseudomonas and nonpathogenic *Fusarium*. Crop Prot 10:279–286

Lemanceau P, Bakker PAHM, Dekogel WJ, Alabouvette C, Schippers B (1992) Effect of pseudobactin 358 production by *Pseudomonas putida* WCS358 on suppression of fusarium wilt of carnations by nonpathogenic *Fusarium oxysporum* Fo47. Appl Environ Microbiol 58:2978–2982

Lemanceau P, Bakker PAHM, Dekogel WJ, Alabouvette C, Schippers B (1993) Antagonistic effect of nonpathogenic *Fusarium oxysporum* Fo47 and pseudobactin 358 upon pathogenic *Fusarium oxysporum* f. sp. Diant. Appl Environ Microbiol 59:74–82

Liesack W, Janssen PH, Rainey FA, Ward-Rainey NL, Stackenbrandt E (1997) Microbial diversity in soil: the need for a combined approach using molecular and cultivation techniques. In: van Elsas JD, Trevors JT, Wellington EMH (eds) Modern soil microbiology. Marcel Dekker, New York, pp 375–439

Lorito M, Woo SL, D'Ambrosio M (1996) Synergistic interaction between cell wall degrading enzymes and membrane affecting compounds. Mol Plant Microbe Interact 9:206–213

Louvet J, Rouxel F, Alabouvette C (1976) Recherches sur la resistance des sols aux maladies. I. Mise en evidence de la nature microbiologique de la resistance d'un sol au développement de la fusariose vasculaire du melon. Ann Phytopathol 8:425–436

Lu Z, Tombolini R, Woo S et al (2004) *In vivo* study of *Trichoderma*-pathogen-plant interactions, using constitutive and inducible green fluorescent protein reporter systems. Appl Environ Microbiol 70:3073–3081

Lugtenberg BJJ, Bloemberg GV (2004) Life in the rhizosphere. In: Ramos JL (ed) Pseudomonas, vol 1. Kluwer Academic/Plenum, New York, pp 403–430

Mandeel Q, Baker R (1991) Mechanism involved in biological control of fusarium wilt of cucumber with strain of non-pathogenic *Fusarium oxysporum*. Phytopathology 81:462–469

Menzies JD (1959) Occurrence and transfer of a biological factor in soil that suppresses potato scab. Phytopathology 49:648–652

Mezaache-Aichour S, Guechi A, Nicklin J, Drider D, Prevost HRN, Strange RN (2012) Isolation, identification and antimicrobial activity of pseudomonads isolated from the rhizosphere of potatoes growing in Algeria. J Plant Pathol 94:89–98

Millard WA, Taylor CB (1927) Antagonism of microorganisms as the controlling factor in the inhibition of scab by green manuring. Ann Appl Biol 14:202–215

Motlagh MRS, Samimi Z (2013) Evaluation of Trichoderma spp., as biological agents in some of plant pathogens. Ann Biol Res 4:173–179

Muyzer G, De Waal EC, Uitterlinden AC (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes for 16S rRNA. Appl Environ Microbiol 55:695–700

Odum EP (1959) Fundamentals of ecology. W.B. Saunders Co, Philadelphia

Olivain C, Steinberg C, Alabouvette C (1995) Evidence of induced resistance in tomato inoculated by nonpathogenic strains of *Fusarium oxysporum*. In: Manta M (ed) Environmental biotic factors in integrated plant disease control 3rd EFPP conference, Poznan, pp 427–430

Oliver JD (2005) The viable but nonculturable state in bacteria. J Microbiol 43:93–100

Olsson PA (1999) Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiol Ecol 29:303–310

Ongena M, Duby F, Rossignol F, Fauconnier ML, Dommes J, Thonart P (2004) Stimulation of the lipoxygenase pathway is associated with systemic resistance induce in bean by a nonpathogenic *Pseudomonas* strain. Mol Plant Microbe Interact 17:1009–1018

Park CS, Paulitz TC, Baker R (1988) Biocontrol of fusarium wilt of cucumber resulting from interactions between *Pseudomonas putida* and non-pathogenic isolates of *Fusarium oxysporum*. Phytopathology 78:190–194

Perelló A, Mónaco C, Sisterna M, Dal Bello G (2003) Biocontrol efficacy of Trichoderma isolates for tan spot of wheat in Argentina. Crop Prot 22:1099–1106

Pérez-Piqueres A, Edel-Hermann V, Alabouvette C, Steinberg C (2006) Response of soil microbial communities to compost amendments. Soil Biol Biochem 38:460–470
Pieterse CMJ, Van Wees SCM, Hoffland E, Van Pelt JA, Van Loon LC (1996) Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. Plant Cell 8:1225–1237
Piotrowska-Seget Z, Mrozik A (2003) Signature lipid biomarker (SLB) analysis in determining changes in community structure of soil microorganisms. Pol J Environ Stud 12:669–675
Raaijmakers JM, Weller DM (1998) Natural plant protection by 2,4-diacetylphloroglucinol producing *Pseudomonas*spp. in take-all decline soils. Mol Plant Microbe Interact 11:144–152
Raaijmakers JM, Weller DM (2001) Exploiting genotypic diversity of 2,4-Diacetylphloroglucinol-producing Pseudomonas spp.: characterization of superior root-colonizing P. fluorescens strain Q8r1-96. Appl Environ Microbiol 67:2545–2554
Raaijmakers JM, Weller DM, Thomashow LS (1997) Frequency of antibiotic producing *Pseudomonas* spp. in natural environments. Appl Environ Microbiol 63:881–887
Rasmussen PH, Knudsen IMB, Elmholt S, Jensen DF (2002) Relationship between soil cellulolytic activity and suppression of seedling blight of barley in arable soils. Appl Soil Ecol 19:91–96
Ros M, Hernandez MT, Garcia C, Bernal A, Pascual JA (2005) Biopesticide effect of green compost against Fusarium wilt on melon plants. J Appl Microbiol 98:845–854
Sanford GB (1926) Some factors affecting the pathogenicity of *Actinomyces scabies*. Phytopathology 16:525–547
Scher FM, Baker R (1980) Mechanism of biological control in a Fusarium- suppressive soil. Phytopathology 70:412–417
Scher FM, Baker R (1982) Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to fusarium wilt pathogens. Phytopathology 72:1567–1573
Schippers B (1992) Prospects for management ofnatural suppressiveness to control soilborne pathogens. In: Tjarnos EC, Papavizas GC, Cook RJ (eds) Biological control of plant diseases. Plenum, New York, pp 21–34
Schneider RW (1982) Suppressive soils and plant disease. American Phytopathology Society, St. Paul, MN, p 88
Schonfeld J, Gelsomino A, Overbeek LSV, Gorissen A, Smalla K, Elsas JDV (2003) Effects of compost addition and simulated solarisation on the fate of *Ralstonia solanacearum* biovar 2 and indigenous bacteria in soil. FEMS Microbiol Ecol 43:63–74
Shipton PJ, Cook RJ, Sitton JW (1973) Occurrence and transfer of a biological factor in soil that suppresses takeall of wheat in eastern Washington. Phytopathology 63:511–517
Sivan A, Chet I (1989) The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. Phytopathology 79:198–203
Steinberg C, Edel-Hermann V, Alabouvette C, Lemanceau P (2007) Soil suppressiveness to plant diseases. In: van Elsas JD, Jansson J, Trevors JT (eds) Modern soil microbiology, 2nd edn. CRC, Boca Raton, pp 455–501
Stover RH (1962) Fusarial wilt (Panama disease) of bananas and other Musa species. Commonwealth Mycological Institute, p 117
Tanwar A, Aggarwal A, Panwar V (2013) Arbuscular mycorrhizal fungi and *Trichoderma viride* mediated *Fusarium* wilt control in tomato. Biocontrol Sci Technol 23:485–498
Torsvik V, Sorheim R, Goksoyr J (1996) Total bacterial diversity in soil and sediment communities – a review. J Ind Microbiol 17:170–178
Van Peer R, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81:728–734
Viterbo A, Landau U, Kim S, Chernin L, Chet I (2010) Characterization of AAC deaminase from the biocontrol and plant growth-promoting agent Trichoderma asperellum T203. FEMS Microbiol Lett 305:42–48

Wang C, Lin Y, Lin Y, Chung W (2013) Modified primers for the identification of nonpathogenic *Fusarium oxysporum* isolates that have biological control potential against fusarium wilt of cucumber in Taiwan. PLoS ONE 8, e65093

Weindling R (1932) *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology 22:837–845

Weller DM, Thomashow LS (1993) Microbial metabolites with biological activity. In: Lumsden RD, Vaughn JL (eds) Pest management: biologically based technologies. American Chemical Society, Washington, DC, pp 173–180

Weller DM, Raaijmakers JM, Gardener BBM, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348

Westphal A, Becker JO (2000) Transfer of biological soil suppressiveness against *Heterodera schachtii*. Phytopathology 90:401–406

Whipps JM (1997) Developments in the biological control of soil-borne plant pathogens. Adv Bot Res 26:1–134

Yang C, Crowley DE, Menge JA (2001) 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and *Phytophthora* infected avocado roots. FEMS Microbiol Ecol 35:129–136

Zehnder GW, Murphy JF, Sikora EJ, Kloepper JW (2001) Application of rhizobacteria for induced resistance. Eur J Plant Pathol 107:39–50