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Biological control of plant diseases

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A B S T R A C T

Biological control of plant diseases seems to be the best alternative for plant disease control. It is ecofriendly, reliable, cost effective and is an integral component of integrated plant disease management framework for sustainable crop production. A considerable number of biocontrol agents have been established successfully for their bioconrol potential including strains belonging to bacterial genera such as *Agrobacterium, Pseudomonas, Streptomyces* and *Bacillus*, and fungal genera such as *Gliocladium, Trichoderma, Ampelomyces*, *Candida* and *Coniothyrium*. *Trichoderma* and *Pseudomonas fluorescens* are the most important and widely used bioconrol agents being used across the world for the management of several plant diseases. Some bacterial biocontrol agents can inhibit both bacterial and fungal pathogens. Smilarly, fungal-fungal, fungal-bacteria and actinomycete-fungal/actinomycete interactions exist in nature and opens a new area of research in biocontrol of plant diseases. The mechanisms of action of these biocontrol agents against target pathogens are antibiosis, mycoparasitism, competition for space and nutrient, production of certain toxins and secondary metabolites, solublization and sequestration of immobilized plant nutrients, plant growth prowth promoting properties and induced resistance. Multiple interactions between bacteria-fungi-actinomycete, may exist in nature and that is why consortium of microorganisms is needed for tackling several plant pathogens simultaneously instead of only one biocontrol agent-one pathogen,which is the most common and established system of biocontrol of plant diseases.

***Keywords*:** Biocontrol agents, bacteria, fungi, actinomycete, plant diseases

Plant diseases are a major concern to food production worldwide as they can cause serious epidemics leading to catastrophic famine. With the intensification of agricultural production over the past few decades, producers became more and more dependent on agrochemicals as a relatively reliable method of .crop protection. However, indiscriminate use of chemical pesticides causes several deleterious effects, i.e., development of pathogen resistance to the applied chemicals, and their nontarget environmental impacts. Chemical methods, are not economical in the long run because they pollute the atmosphere, damage the environment, leave harmful residues, and can lead to the development of resistant strains among the target organisms. Furthermore, the growing cost of pesticides, particularly in less-affluent regions of the world, and consumer demand for pesticide-free food products has led to search for suitable substitutes for these products. One of the most promising means to achieve this goal is by the use of new tools based on biocontrol agents (BCAs) for disease control alone, or in integration with reduced doses of chemicals resulting in minimal impact of the chemicals on the environment (Harman & Kubicek 1998). A number of BCAs have been registered and are available as commercial products, including strains belonging to bacterial genera such as *Agrobacterium, Pseudomonas, Streptomyces* and *Bacillus*, and fungal genera such as *Gliocladium, Trichoderma, Ampelomyces*, *Candida* and *Coniothyrium*. *Trichoderma* spp. are among the most frequently isolated soil fungi and present in plant root ecosystems (Harman *et al.* 2004). These fungi are opportunistic, avirulent plant symbionts, and function as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from disease. So far, *Trichoderma* spp. are the most studied fungal BCAs and commercially

marketed as biopesticides, biofertilizers and soil amendments (Harman 2000; Harman *et al.* 2004; Lorito *et al.* 2004).

Biocontrol of bacterial diseases by bacteria

Some bacterial strains can be effectively be used as biocontrol of several phyto-pathogenic bacteria. Application of non-pathogenic strains of *Streptomyces* to control scab disease of potato caused by *Streptomyces scabies* is one of example. In this case, biocontrol may operate through antibiosis or competition for space or nutrients in the rhizosphere. In contrast, *Pseudomonas fluorescens* was shown to control the soft rot potato pathogen *Erwinia carotovora* subsp. atroseptica by production of the antibiotic 2,4-diacetylphloroglucinol (DAPG) and competition was not a feature of biocontrol in this system (Cronin *et al.* 1997). Pseudomonas species may also control crown gall disease in many dicotyledonous plants caused by Agrobacterium tumefaciens (Khmel *et al.* 1998). However, the classic, and still commercially successful, bacterial-based biocontrol system is the use of non-pathogenic Agrobacterium strains to control A. tumefaciens. Long-term molecular and ecological studies of this system have identified how the biocontrol works and have also allowed potential problems associated with its use in the field to be overcome. The most widely used non-pathogenic *Agrobacterium* strain K84 produces a highly specific antibiotic agrocin 84, which is encoded by a plasmid. Inundative inoculation of *Agrobacterium* strain K84 to roots by dipping in cell suspensions prior to exposure to the pathogen effectively controls those strains of pathogen susceptible to agrocin 84. Production of other antibiotics such as agrocin 434 or ALS 84 may also play a part (McClure *et al*. 1998), but the ability to survive and compete on roots may also be important.

Biocontrol of fungal diseases by bateria

Although a range of different bacterial genera and species have been studied, the overwhelming number of studies have involved the use of *Pseudomonas* species. Pseudomonads are characteristically fast growing, easy to culture and manipulate genetically in the laboratory, and are able to utilize a range of easily metabolizable organic compounds, making them amenable to experimentation. But, in addition, they are common rhizosphere organisms and must be adapted to live in the rhizosphere to a large extent. Having appropriate rhizosphere competence may be a key feature for reproducible biological control activity in the spermosphere and rhizosphere. A few specific examples of the modes of action involved with bacterial biocontrol of fungal pathogens in the rhizosphere and spermosphere are given below.

Antibiosis

There are numerous reports of the production of antifungal metabolites (excluding metal chelators and enzymes) produced by bacteria *in vitro* that may also have activity *in vivo*. These include ammonia, butyrolactones, 2,4-diacetylphloroglucinol (Ph1), HCN, kanosamine, Oligomycin A, Oomycin A, phenazine-1-carboxylic acid (PCA), pyoluterin (Plt), pyrrolnitrin (Pln), viscosinamide, xanthobaccin, and zwittermycin A as well as several other uncharacterized moieties (Milner *et al.* 1996; Nielson *et al.* 1998; Kim *et al.* 1999; Thrane *et al.* 1999; Nakayama *et al.* 1999). Indeed, isolation and characterization of genes or gene clusters responsible for antibiotic production have now been achieved (Kang *et al*. 1998). Significantly, both Phl and PCA have been isolated from the rhizosphere of wheat following introduction of biocontrol strains of Pseudomonas (Raaijmakers *et al.* 1999), confirming that such antibiotics are produced in vivo. Further, Ph1 production in the

rhizosphere of wheat was strongly related to the density of the bacterial population present and the ability to colonize roots (Raaijmakers *et al.* 1999). Antibiotic production by bacteria, particularly Pseudomonads, seems to be closely regulated by a two-component system involving an environmental sensor and a cytoplasmic response factor (Keel & Défago 1997). These findings may be of considerable significance for bacterial – fungal interactions in general and has major implications for the control of gene expression in complex microbial communities.

Competition for iron

Although competition between bacteria and fungal plant pathogens for space or nutrients as a biocontrol mechanism has been known to exist for many years (Whipps1997a), the largest interest has involved competition for iron. Under iron-limiting conditions, bacteria produce a range of iron chelating compounds or siderophores which have a very high affinity for ferric iron. These bacterial iron chelators are thought to sequester the limited supply of iron available in the rhizosphere making it unavailable to pathogenic fungi, thereby restricting their growth (Loper & Henkels 1999). Recent studies have clearly shown that the iron nutrition of the plant influences the rhizosphere microbial community structure (Yang & Crowley 2000). Pseudomonads also produce two other siderophores, pyochelin and its precursor salicylic acid, and pyochelin is thought to contribute to the protection of tomato plants from *Pythium* by *Pseudomonas aeruginosa* (Buysens *et al*. 1996). However, siderophores are not always implicated in disease control by Pseudomonads (Ongena *et al*. 1999). The dynamics of iron competition in the rhizosphere are often complex. Different environmental factors can also influence the quantity of siderophores produced (Duffy & Défago 1999). There is also the further complication that pyoverdine and salicylate may act as elicitors for inducing systemic resistance against pathogens in some plants (Leeman *et al*. 1996*b*).

Parasitism and production of extracellular enzymes

The ability of bacteria, especially actinomycetes, to parasitize and degrade spores of fungal plant pathogens is well established (El-Tarabily *et al.* 1997). Assuming that nutrients pass from the plant pathogen to bacteria, and that fungal growth is inhibited, the spectrum of parasitism could range from simple attachment of cells to hyphae (Nelson *et al.* 1986) to complete lysis and degradation of hyphae (Mitchell & Hurwitz 1965). Considerable effort has gone into identifying cell wall-degrading enzymes produced by biocontrol strains of bacteria even though relatively little direct evidence for their presence and activity in the rhizosphere has been obtained. Similar techniques involving Tn*5* insertion mutants and subsequent complementation demonstrated that biocontrol of *Pythium ultimum* in the rhizosphere of sugar beet by *Stenotrophomonas maltophila* was due to the production of extracellular protease (Dunne *et al*. 1997).

Induced resistance

Perhaps the interest in biocontrol in the last few years has been concerned with induced resistance defined as 'the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents (inducing agents)' (Kloepper *et al*. 1992). This has resulted through the interactions between microbiologists, plant pathologists and plant scientists armed with an appropriate battery of molecular tools. Such machanism had previously often been overlooked through inadequate techniques or controls as well as the biocontrol agent exhibiting other modes of action at the same time. Most work in this

context has focused on the systemic resistance induced by non-pathogenic rhizosphere - colonizing *Bacillus* and *Pseudomonas* species in systems where the inducing bacteria and the challenging pathogen remained spatially separate for the duration of the experiment, and no direct interaction between the bacteria and pathogen was possible (Sticher *et al*. 1997; van Loon 1997). Bacteria differ in ability to induce resistance, with some being active on some plant species and not others; variation in inducibility also exists within plant species (van Loon 1997). The full range of inducing moieties produced by bacteria is probably not yet known, but lipopolysaccharides (Leeman *et al*. 1995) and siderophores (Métraux *et al*. 1990) are clearly indicated. The definition of induced resistance suggested by Kloepper *et al*. (1992) covered both biotic and abiotic inducers. The major differences are that pathogenesis-related (PR) proteins such as chitinases, β-1,3-glucanases, proteinase inhibitors and one or two other rarer types, are not universally associated with bacterially induced resistance (Hoffland *et al*. 1995) and salicylic acid (a known inducer of SAR) is not always involved in expression of ISR, but this is dependent on bacterial strain and host plant involved (Chen *et al*. 1999). Ethylene responsiveness may also be required at the site of inoculation of the inducing bacteria for ISR to occur (Knoester *et al*. 1999). Changes that have been observed in plant roots exhibiting ISR include: (1) strengthening of epidermal and cortical cell walls and deposition of newly formed barriers beyond infection sites including callose, lignin and phenolics (2) increased levels of enzymes such as chitinase, peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase (3) enhanced phytoalexin production (4) enhanced expression of stress-related genes. However, not all of these biochemical changes are found in all bacterial–plant combinations. Similarly, the ability of bacteria to colonize the internal tissue of the roots has been considered to be an important feature in many of the bacterial–root interactions involving ISR, but is not a constant feature of them all.

Plant growth-promoting rhizobacteria (PGPR)

The concept of PGPR is now well established (Bashan 1998; Shishido & Chanway 1999). The relationship of PGPRs to biocontrol is worthwhile. PGPR increase plant growth indirectly either by the suppression of well-known diseases caused by major pathogens or by reducing the deleterious effects of minor pathogens). PGPR may increase plant growth in other ways, for example, by associative N_2 fixation, solubilizing nutrients such as P, promoting mycorrhizal function, regulating ethylene production in roots, releasing phytohormones, and decreasing heavy metal toxicity. It has been suggested that the two groups should be reclassified into biocontrol plant growth-promoting bacteria (biocontrol PGPB) and PGPB (Bashan & Holguin 1998). To date this proposal does not seem to have been widely accepted, but it does highlight the need to consider the full ecological interactions taking place following application of bacteria to seeds and roots that lead to plant growth promotion. It is also important to remember that deleterious rhizobacteria that inhibit plant growth are also known which can influence such interactions.

Irrespective of mode of action, a key feature of all PGPR is that they all colonize roots to some extent. In some cases this may involve specific attachment through, for example, pili, as with the attachment of *P. fluorescens* to the surface of wheat roots (Vesper 1987). However, such specific attachment does not seem to be an absolute requirement for colonization.

Colonization may involve simply root surface development but, endophytic colonization of the root is also known, and the degree of endophytic colonization depends on bacterial strain and plant type. The endophytic bacteria may be in a particularly advantageous ecological position in that they may be able to grow and compete on the root surface, but also may be capable of developing within the root, relatively protected from the competitive and high-stress environment of the soil. Indeed, many seeds, roots and tubers are normally colonized by endophytic bacteria. The localized signalling between plant and bacteria within the root environment needs further study. Use of mutants and promoter probe techniques are beginning to identify genes in bacteria that are important in colonization and these are often related to nutrient uptake. Such nutrient uptake genes may also play a role in biocontrol by aiding the uptake and metabolism of nutrients that stimulate germination of pathogen propagules.

Biocontrol of bacterial diseases by fungi

In the last few years there have been no clear examples of fungi used to control bacterial plant pathogens in the rhizosphere or spermosphere. The reasons for this are unclear but could perhaps indicate an area that deserves further research in the future.

Biocontrol of fungal diseases by fungi

Interactions between biocontrol fungi and fungal plant pathogens continue to be the focus of a large number of researchers, on a par with work on bacterial–fungal plant pathogen interactions described earlier. However, there is an extra dimension in the quality of the interactions between fungi as biocontrol fungi have much greater potential than bacteria to grow and spread through soil and in the rhizosphere through possession of hyphal growth. Some recent examples of fungal–fungal interaction concerning biocontrol in the rhizosphere and spermosphere are given in Table-2. There are a variety of fungal species and isolates that have been examined as biocontrol agents but *Trichoderma* species clearly dominate, perhaps reflecting the ease of their growth and wide host range (Whipps & Lumsden 2001). There has been an upsurge in interest in non-pathogenic *Pythium* species, particularly *P. oligandrum* where additional modes of action have been determined recently, and a continued interest in well-established saprotrophic antagonists such as non-pathogenic *Fusarium* species, non-pathogenic binucleate *Rhizoctonia* isolates and *Phialophora* species, as well as mutualistic symbionts including mycorrhizal fungi such as Glomus intraradices The most common pathogen targets are *Pythium* species, Fusarium species and *Rhizoctonia solani* reflecting their world-wide importance and distribution perhaps the relative ease of their control under protected cropping systems, although numerous other pathogens have also been examined. Some specific examples of the modes of action found to occur in the rhizosphere and spermosphere during interactions between fungi and fungal plant pathogens are given below.

Competition

There have been relatively few studies on competition for nutrients, space or infection sites between fungi in the rhizosphere and spermosphere. Competition for carbon, nitrogen and iron has been shown to be a mechanism associated with biocontrol or suppression of Fusarium wilt in several cropping systems by non pathogenic *Fusarium* and *Trichoderma* species (Mandeel and Baker 1991; Sivan & Chet 1989) and competition for thiamine as a significant process in the control of *Gaeumannomyces graminis* var. tritici by a

sterile red fungus in the rhizosphere of wheat (Shankar *et al.* 1994). Many studies have shown a relationship between increased colonization of the rhizosphere by a non-pathogen, with disease suppression. Rapid production of spores which are then carried down the root by water continue the root colonization process and this is suggested to be a key feature in the establishment of the biocontrol agent on the root (Douglas & Deacon 1994). Mycorrhizal fungi are also strong candidates for providing biocontrol through competition for space by virtue of their ecologically obligate association with roots. Ectomycorrhizal fungi because of their physical sheathing morphology may well occupy normal pathogen infection sites. Arbuscular mycorrhizas also have potential to occupy space and infection sites on roots, but evidence suggests that biocontrol provided by arbuscular mycorrhizas relates more to induced resistance, improved plant growth and changes in root morphology rather than competition.

Antibiosis

Although production of antibiotics by fungi involved in biocontrol is a well documented phenomenon (Howell 1998; Sivasithamparam & Ghisalberti 1998), there is hardly any recent work clearly demonstrating production of antibiotics by fungi in the rhizosphere and spermosphere. Unlike the situation with biocontrol bacteria, there appear to be no detailed studies in biocontrol fungi of genes coding for antibiotic synthesis. Mutants with raised or decreased production of antibiotics are either natural spontaneous ones or generated by UV or chemical mutagenesis, with inherent problems of pleiotropic gene effects, rather than targeted gene disruption (Howell & Stipanovic 1995). Antibiotic production by fungi exhibiting biocontrol activity has most commonly been reported for isolates of *Trichoderma/ Gliocladium* (Howell 1998) and *Talaromyces flavus* (Kim *et al.* 1990) although in the last few years antibiotics have been at least partially characterized in *Chaetomium globosum* (Di Pietro *et al.* 1992). Gliotoxin production by Trichoderma is also thought to be responsible for cytoplasmic leakage from *R. solani* observed directly on membranes in potting mix (Harris & Lumsden 1997). Production of hydrogen peroxide in the rhizosphere, catalysed by glucose oxidase from *Talaromyces flavus* is thought to be responsible for the biocontrol of *Verticillium* wilt caused by *Verticillium dahliae* on eggplant (Stosz *et al.* 1996). Purified glucose oxidase significantly reduced the growth rate of *V. dahliae* in the presence, but not the absence, of eggplant roots, suggesting that a supply of glucose from the roots was of major importance (Fravel & Roberts 1991). Glucose oxidase also suppressed growth of *V. dahliae in vitro* and killed microsclerotia of *V. dahliae in vitro* and in soil.

Induced resistance

Likewise the bacteria described earlier, the ability of fungi to induce resistance in plants and provide biocontrol has gradually been receiving more attention in the last few years. A considerable number of fungi previously described to provide biocontrol by mechanisms such as competition, antibiosis, mycoparasitism or direct growth promotion are now thought to provide control, at least in part, by this mechanism. These include saprotrophs such as non-pathogenic *Fusarium* isolates, *Trichoderma* species (Yedidia *et al.* 1999), *Pythium oligandrum* (Benhamou *et al.* 1997), non pathogenic binucleate *Rhizoctonia* isolates (Poromarto et al. 1998), and *Penicillium oxalicum* (de Cal *et al.* 1997) as well as mutualistic biotrophs such as mycorrhizal fungi (St Arnaud *et al.* 1997). However, not all these studies used the strict criterion of spatial separation between application of the biocontrol

fungus and the challenging pathogen to define induced resistance. Some simply measured changes in enzymes, PR-proteins or cell wall characteristics found to be induced in plants through SAR without involvement of a pathogen at all (Yedidia *et al.* 1999). Certainly with some mycorrhizal fungi it has been questioned whether the biochemical responses similar to induced resistance found following infection are of sufficient magnitude or quality, or too transient, to provide disease control. Indeed, during some mycorrhizal colonization there is little or no induced resistance response detected (Mohr *et al.* 1998). However, spatial or temporal separation experiments have indicated that increased levels of chitinases, β-1,3 glucanases, β-1,4 glucosidase, PR-1 protein, and peroxidase as well as cell wall appositions and phenolics may be associated with induced resistance due to fungi (Benhamou *et al.* 1997). Nevertheless, more work is needed to identify the biochemical changes taking place in a larger number of fungal–plant combinations as not all these biochemical markers were found to be important in each system examined. *Trichoderma* species produce a 22-kDa xylanase that, when injected in plant tissues, will induce plant defence responses including K^+, H^+ and Ca^{2+} channelling, PR protein synthesis, ethylene biosynthesis, and glycosylation and fatty acylation of phytosterols (Bailey & Lumsden 1998).

Mycoparasitism

There is a voluminous literature on the ability of fungi to parasitize spores, sclerotia, hyphae, and other fungal structures and many of these observations are linked with biocontrol (Madsen & de Neergaard 1999; Mischke 1998; Al-Rawahi & Hancock 1998; Bhagat & Pan 2008, 2010). However, most of the microscopical observations concerning mycoparasitism have come from *in vitro* studies or sterile systems (Benhamou & Chet 1997; Bhagat & Pan 2008, 2011) and examples clearly demonstrating mycoparasitism in the rhizosphere or spermosphere are rare (Lo *et al*. 1998; Bhagat & Pan 2008 2011). The process involved in mycoparasitism may consist of sensing the host, followed by directed growth, contact, recognition, attachment, penetration, and exit. Although not all these features occur in every fungal–fungal interaction, the key factor is nutrient transfer from host to mycoparasite. Directed growth of hyphae of *Trichoderma* on hyphae of *Rhizoctonia solani* prior to penetration has often been observed (Chet *et al*. 1981) and the presence of host sclerotia have been shown to stimulate germination of conidia of *Coniothyrium minitans* (Whipps *et al*. 1991) and *Sporidesmium sclerotivorum* (Mischke & Adams 1996). This process may involve hydrophobic interactions or interactions between complementary molecules present on the surface of both the host and the mycoparasite such as between lectins and carbohydrates. With *Trichoderma*, there is ample evidence of lectin production by both parasite and host *Cortiium* (*Sclerotium*) *rolfsii* and involvement of lectins in the differentiation of mycoparasitism-related structures. Penetration or cell wall degradation are frequently observed during mycoparasitism, great emphasis has been placed on characterizing and cloning the extracellular enzymes such as β-1,3 glucanases, chitinases, cellulases, and proteases produced by fungal biocontrol strains. Several fungi have been examined in this context including *Talaromyces flavus,* but most such work has essentially focused on *Trichoderma* species. Degradation products of the cell wall were considered to act as inducers of these enzymes in this host–pathogen system. Interestingly, when examining *T. harzianum* P1 interaction with *Botrytis cinerea,* ech42 gene

transcription was found to be triggered by physiological stress reflecting carbon source depletion rather than the presence of chitin as the inducer. The final evidence for a role for cell wall-degrading enzymes in biocontrol involves the expression of fungal genes in transgenic plants. One feature often overlooked with mycoparasitism is that it may not always be confined to control of plant pathogens. A mycoparasite may also have the potential to attack beneficial fungi such as those forming mycorrhiza. For example, *T. harzianum* T-203 was shown to attack mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* in an axenic system (Rousseau et al. 1996). However, in a soil-based system, G. intraradices was unaffected by the presence of *T. harzianum*. Indeed *T. harzianum* appeared to be suppressed through nutrient competition. These results may reflect the different isolates of fungi and plants used, and the experimental systems applied, but they clearly demonstrate the complexity of the interactions that can occur in the rhizosphere.

Plant growth promotion and rhizosphere competence

The terminology associated with biocontrol in the rhizosphere and with soil–plant–microbe interactions has gradually become more complex through the use of a range of descriptive rather than mechanistic terms such as plant growth promotion and rhizosphere competence. Much like the situation with PGPR, many saprotrophic fungi, particularly certain isolates of *Trichoderma* species, can provide plant growth promotion in the absence of any major pathogens (Whipps 1997*a*; Inbar *et al*. 1994; Bhagat & Pan 2008 2010). In many cases these studies are restricted to simple observations of improved plant growth with no indication of the possible mechanisms involved, although there are exceptions. For example, *Trichoderma harzianum* was shown to solubilize phosphate and micronutrients that could be made available to provide plant growth (Altomare *et al*. 1999). This situation is compounded by the fact that many proven fungal biocontrol agents including some *Trichoderma* species, binucleate *Rhizoctonia* isolates and *Pythium oligandrum* can provide improved plant growth in the absence of pathogens. Further, colonization of the surface of the seeds or roots or the behaviour as endophytes has frequently been seen to be a desirable trait for biocontrol activity. Although there is a clear relationship between rhizosphere colonization and biocontrol activity with most isolates of biocontrol fungi such as *Trichoderma* species, non pathogenic Fusaria, *P. oligandrum, Verticillium biguttatum*, and *Talaromyces flavus*, this is not always the case. It is important to appreciate that just because a microorganism can grow in the rhizosphere or spermosphere, it may not automatically provide biocontrol or plant growth promotion. Similarly, the converse is also true. A proven biocontrol agent of a soil-borne plant pathogen may not always be capable of colonizing the rhizosphere or providing plant growth promotion.

The situation is much clearer with mycorrhizal fungi where, through ecologically mutualistic symbiosis with the plant, the major feature involves improving plant nutritional status, perhaps water balance and thus plant growth. Biocontrol of plant pathogens is generally viewed as a secondary role. It has been suggested that ability to produce cellulases and thus utilize substrates available in the rhizosphere may also be an important feature. However, UV mutants of *Trichoderma harzianum* lacking cellulase production were found to have enhanced rhizosphere competence whereas two cellulase overproducers were found not to colonize the rhizosphere of bean plants.

Multiple microbial interactions

The majority of interactions considered so far concern a single pathogen and a single biocontrol agent in the rhizosphere. However, one way of improving biocontrol in the rhizosphere may be to add mixtures or combinations of biocontrol agents, particularly if they exhibit different or complementary modes of action or abilities to colonize root microsites. Such multiple interactions are the normal situation in the rhizosphere. Numerous permutations have been considered, including combinations of different bacteria, fungi and both bacteria and fungi. For example, a seed application of a combination of three PGPR, *Bacillus pumilus* , *Bacillus subtilis* and *Curtobacterium flaccumfaciens* provided greater control of several pathogens on cucumber than when any were inoculated singly (Raupach & Kloepper 1998), combinations of *Paenibacillus* sp. and a *Streptomyces* sp. suppressed *Fusarium* wilt of cucumber better than when either was used alone (Singh et al. 1999) and a combination of *Pseudomonas fluorescens* and *Stenotrophomonas maltophila* improved protection of sugar beet against Pythium mediated damping-off in comparison with either applied individually (Dunne *et al.* 1998). Combinations of fungi and bacteria have also been shown to provide enhanced biocontrol. For instance, *Trichoderma koningii* combined with either Pseudomonas chlororaphis or *P. fluorescens* provided greater suppression of take-all of wheat than *T. koningii* alone (Duffy *et al*. 1996), *Trichoderma* (*Gliocladium*) *virens* GL-3 combined with *Burkholderia cepacia* provided stands of pepper greater protection than either antagonist used alone in the presence of a mixture of up to four soil-borne pathogens (Mao et al. 1998) and non-pathogenic *Fusarium oxysporum* combined with *Pseudomonas putida* provided better suppression of *Fusarium* wilt of flax caused by *F. oxysporum* f. sp. *lini* than either alone (Duijff et al. 1999). Enhanced plant growth promotion has also been recorded in the absence of pathogens by applications of combinations of bacterial or fungal plant growth promoting microorganisms. However, it is important when considering the use of mixtures or combinations of strains that no member of the mixture is inhibitory to another or interferes excessively with the existing, normal and non-pathogenic microbiota associated with the roots. Several biocontrol agents including isolates of *Pseudomonas*, *Gliocladium* and *Trichoderma* species have been shown to have little or no adverse effect on establishment and function of arbuscular mycorrhizas although there are reports of adverse effects of some isolates of *Trichoderma* and *Streptomyces griseoviridis* on arbuscular mycorrhiza formation (McAllister *et al*. 1994). Clearly, the complex interactions that can take place in the rhizosphere between biocontrol agents and the indigenous microbiota needs to be considered during development of commercial microbial products.

Actinomycetes- Biocontrol of plant diseases

Besides the enormous numbers of agroactive metabolites produced by actinomycetes, they also play an important role in agriculture as biocontrol agents. Antagonism against an extensive variety of plant pathogens has been reported. A microorganism that colonizes roots is ideal for use as a biocontrol agent against soil-borne diseases. Actinomycetes, especially *Streptomyces,* are qualitatively and quantitatively important in the rhizosphere where they actively colonize plant root systems. To better understand how these bacteria may act as biocontrol agents, we must understand how they colonize the rhizosphere environment, and how they utilize the different mechanisms of biocontrol once they are established there.

Conclusion and future directions

Successful biocontrol of plant disease requires an intricate array of interactions. Understanding these interactions at the molecular and ecological level will make possible the rational development of biocontrol for agriculture. Application of genetic analysis to microorganisms involved in biocontrol has led to substantial progress in understanding the microbial metabolite and regulatory genes involved in biocontrol. Ecological analyses have begun to describe the responses of microbial communities to introduction of biocontrol agents. The integrated use of genetic, molecular, and ecological approaches will form the basis for significant future advances in biocontrol research. In particular, additional effort in these areas will be essential for developing a more complete understanding of biocontrol and for making practical use of biocontrol strategies for agriculture. First, understanding mechanisms of pathogen resistance to the action of biocontrol agents is critical to sustain disease suppression with long-term use. Strategies to minimize resistance and prevent its spread should be designed. The second area that is ripe for study is genetic diversity within species of both biocontrol agents and host plants. Exploitation of genetic variation among members of a microbial species that suppresses disease may provide a solution to the variability across space and time that has been observed with many biocontrol agents. The genetics of the host should be exploited for supportiveness of biocontrol, and hospitality to biocontrol agents should be enhanced through directed breeding or genetic modification of the host plant. The third, and most challenging, area of research needed to explain the biological context for biocontrol is microbial community ecology. A better understanding of the microbial interactions that enhance or detract from biocontrol will determine the long-term success of biocontrol. In particular, attention should be paid to nonculturable members of the rootassociated and soil communities because these microorganisms may be numerically dominant and have not been studied. Molecular methods developed for the study of microorganisms in their environments are key tools for the study of the influences of the microbial community on biocontrol.

Finally, perhaps the greatest interest in the future lies with the application of modern molecular techniques and their integration with conventional experimental procedures to understand and utilize soil–plant–microbe interactions. The significance of these techniques has already been described with the monitoring of biocontrol agents and their impact on microbial populations, in the construction of *Agrobacterium radiobacter*, and in understanding the modes of action of biocontrol agents, particularly with induced resistance in plants. Further, much effort has gone into developing transgenic bacteria and fungi expressing genes that provide enhanced biocontrol activity, and to transgenic plants expressing genes that provide disease resistance, while also allowing a greater understanding of the mechanisms operating in the rhizosphere. With environmental concerns and the regulatory barriers, it remains to be seen whether transgenic micro-organisms and plants for disease control become universally accepted both as research tools and as commercial products.

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