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Biocontrol of Soilborne Plant Pathogens

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INTRODUCTION

Biocontrol involves harnessing disease-suppressive microorganisms to improve plant health. Disease suppression by biocontrol agents is the sustained manifestation of interactions among the plant, the pathogen, the biocontrol agent, the microbial community on and around the plant, and the physical environment. Even in model laboratory systems, the study of biocontrol involves interactions among a minimum of three organisms. Therefore, despite its potential in agricultural applications, biocontrol is one of the most poorly understood areas of plant–microbe interactions.

The complexity of these systems has influenced the acceptance of biocontrol as a means of controlling plant diseases in two ways. First, practical results with biocontrol have been variable. Thus, despite some stunning successes with biocontrol agents in agriculture, there remains a general skepticism born of past failures (Cook and Baker, 1983; Weller, 1988). Second, progress in understanding an entire system has been slow. Recently, however, substantial progress has been made in a number of biocontrol systems through the application of genetic and mathematical approaches that accommodate the complexity.

Biocontrol of soilborne diseases is particularly complex because these diseases occur in the dynamic environment at the interface of root and soil known as the rhizosphere, which is defined as the region surrounding a root that is affected by it. The rhizosphere is typified by rapid change, intense microbial activity, and high populations of bacteria compared with nonrhizosphere soil. Plants release metabolically active cells from their roots and deposit as much as 20% of the carbon allocated to roots in the rhizosphere, suggesting a highly evolved relationship between the plant and rhizosphere microorganisms. The rhizosphere is subject to dramatic changes on a short temporal scale—rain events and daytime drought can result in fluctuations in salt concentration, pH, osmotic potential, water potential, and soil particle structure. Over longer temporal scales, the rhizosphere can change due to root growth, interactions with other soil biota, and weathering processes. It is the dynamic nature of the rhizosphere that makes it an interesting setting for the interactions that lead to disease and biocontrol of disease (Rovira, 1965, 1969, 1991; Hawes, 1991; Waisel et al., 1991).

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The complexity of the root–soil interface must be accommodated in the study of biocontrol, which must involve whole organisms and ultimately entire communities, if we are to understand the essential interactions in soil in the field. The challenge in elucidating mechanisms of biocontrol is in reducing the complexity to address tractable scientific questions. One of the most effective approaches toward the identification of critical variables in a complex system has been genetics. The study of mutants can be conducted in simplified laboratory systems or in the field, thus making accessible the examination of particular genetic changes and the associated biochemical characteristics in the real world.

This review presents recent advances in our understanding of the biocontrol of root diseases. We emphasize research aimed at enhancing our understanding of the biology of the interactions that result in disease suppression. It is this understanding that will make possible the practical use of microorganisms in the management of plant disease in agroecosystems. Numerous recent reviews present comprehensively the variety of microbial biocontrol agents (Chet, 1987; Weller, 1988; Whipps and Lumsden, 1991; O'Sullivan and O'Gara, 1992; Cook, 1993; Goldman et al., 1994; Cook et al., 1995; Lumsden et al., 1995). In this discussion of current and future directions in biocontrol, our goal is to present key themes in the discipline, drawing on the bacteria *Pseudomonas* and *Bacillus* and the fungi *Trichoderma* and *Gliocladium* as examples representing a range of life strategies and mechanisms of disease suppression. We address the principles of interactions of the biocontrol agent with the pathogen, the host plant, and the microbial community, illustrating each principle with some well-studied examples of successful biocontrol agents.

INTERACTIONS WITH THE PATHOGEN

Antibiosis

Biocontrol is often attributed to antibiosis. In many biocontrol systems that have been studied, one or more antibiotics have been shown to play a role in disease suppression. The fact that antibiosis is a common mechanism of biocontrol may be due to a bias in choice of organisms for study. Alternatively,

it may be due to the attractiveness of the antibiosis hypothesis, or antibiosis may be simply a highly effective mechanism for suppressing pathogens in the rhizosphere. Genetic analyses have been particularly informative in determining the role of antibiotics in biocontrol, in part because mutants can be screened easily in vitro for changes in antibiotic accumulation, providing the means to conduct thorough genetic analyses. Many antibiotics have been implicated in biocontrol only by correlative data; the following section focuses on those that have been implicated by mutant analyses and biochemical studies using purified antibiotics.

Role of Antibiotics

A number of highly effective disease-suppressive agents are found among the fluorescent pseudomonads, making this group of bacteria the most widely studied group of antibiotic producers in the rhizosphere. The first antibiotics clearly implicated in biocontrol by fluorescent pseudomonads were the phenazine derivatives (Figure 1) that contribute to disease suppression by *Pseudomonas fluorescens* strain 2-79 and *P. aureofaciens* strain 30-84, which control take-all of wheat (Weller and Cook, 1983; Brisbane and Rovira, 1988). Evidence for the role of phenazines includes an analysis of transposon insertion mutants that lack the ability to produce phenazine-1-carboxylate and are reduced in disease suppressiveness (Thomashow and Weller, 1988; Pierson and Thomashow, 1992). Furthermore, the antibiotic is produced on roots grown in soil (Mazzola et al., 1992). *P. fluorescens* strain CHA0 produces hydrogen cyanide, 2,4-diacetylphloroglucinol, and pyoluteorin, which directly interfere with growth of various pathogens and contribute to disease suppression (Figure 1; Voisard et al., 1989; Keel et al., 1990, 1992; Maurhofer et al., 1994b). Mutants deficient in production of the antimicrobial substances are reduced in their ability to suppress certain diseases. Furthermore, a quantitative relationship between antibiotic production and disease suppressiveness is suggested by the enhancement of production of 2,4-diacetylphloroglucinol and pyoluteorin accomplished by adding extra copies of a 22-kb fragment of DNA that improves suppression of *Pythium* on cucumber (Maurhofer et al., 1992).

The genes for the biosynthesis of many of the metabolites involved in disease suppression by fluorescent pseudomonads have been isolated, and their regulation has been studied (Pierson and Thomashow, 1992; Pierson et al., 1995; Bangera and Thomashow, 1996). An emerging theme in the fluorescent pseudomonads is that global regulatory elements coordinate production of secondary metabolites. For example, biosynthesis of phenazine derivatives in *P. aureofaciens* is regulated by a quorum sensor, PhzR, that perceives cell population density through the concentration of an autoinducer (Pierson et al., 1994). Interestingly, mutants that lack the ability to produce the autoinducer can use similar molecules produced by other rhizosphere inhabitants, suggesting that the presence of significant populations of other bacteria could influence phenazine production by *P. aureofaciens* in the rhizosphere (Pierson and Pierson, 1996; Wood and Pierson, 1996). Additionally, the environmental sensors ApdA and GacA influence the production of numerous secondary metabolites involved in biocontrol by pseudomonads (Laville et al., 1992; Gaffney et al., 1994; Corbell and Loper, 1995).

Sigma factors also regulate antibiotic production in fluorescent pseudomonads. Sigma factors are subunits of RNA polymerase that direct transcription in bacteria. Each sigma factor has promoter specificity and regulates a distinct set of genes, thereby playing a key role in gene regulation in bacteria. In *Escherichia coli*, gene expression in stationary phase involves sigma^{s} (Kolter et al., 1993). A homolog of sigma^{s} is significant in biocontrol by *P. fluorescens* through its role in expression of stationary phase genes required for the production of certain secondary metabolites and survival on plant material (Sarniguet et al., 1995). The ratio of the housekeeping sigma factor sigma^{70} and sigma^{s} appears critical in the regulation of various metabolites involved in disease suppression (Schnider et al., 1995). Further work delineating the chemical, physical, and biological factors that regulate bacterial gene expression in the rhizosphere will contribute to the understanding of behavior of biocontrol agents and will suggest strategies for improving their performance.

Although bacilli have received less attention as potential biocontrol agents than have the pseudomonads, evidence indicating that they may promote effective disease suppression is accumulating. The bacilli are particularly attractive for practical use because they produce stable endospores, which can survive the heat and desiccation conditions that may be faced by biocontrol agents (Turner and Backman, 1991; Lumsden et al., 1995; Osburn et al., 1995). One well-studied example is *Bacillus cereus* strain UW85, which suppresses diseases caused by the oomycetes, a group of protists that cause severe plant diseases. Analysis of mutants of *B. cereus* shows a significant quantitative relationship between disease suppressiveness and the production of two antibiotics, zwittermicin A and kanosamine (Silo-Suh et al., 1994; Milner et al., 1996c). Zwittermicin A is an aminopolyol representing a new class of antibiotic, and kanosamine is an aminoglycoside (Figure 1). The purified antibiotics suppress disease and inhibit development of oomycetes by stunting and deforming germ tubes of germinating cysts. Thorough analysis of antibiotic biosynthesis and regulation in the bacilli will depend on the development of genetic techniques, such as high frequency transformation, transposon mutagenesis, and reporter gene fusions, similar to those available for the pseudomonads.

Trichoderma and *Gliocladium* are closely related fungal biocontrol agents. Each produces antimicrobial compounds and suppresses disease by diverse mechanisms, including the production of the structurally complex antibiotics gliovirin and gliotoxin (Figure 1; Howell et al., 1993). Mutants of *Gliocladium virens* that do not produce gliotoxin are reduced in their ability to control Pythium damping-off (Wilhite et al., 1994). Mutants with increased or decreased antibiotic production show a corresponding effect on biocontrol (Howell and Stipanovic, 1983).

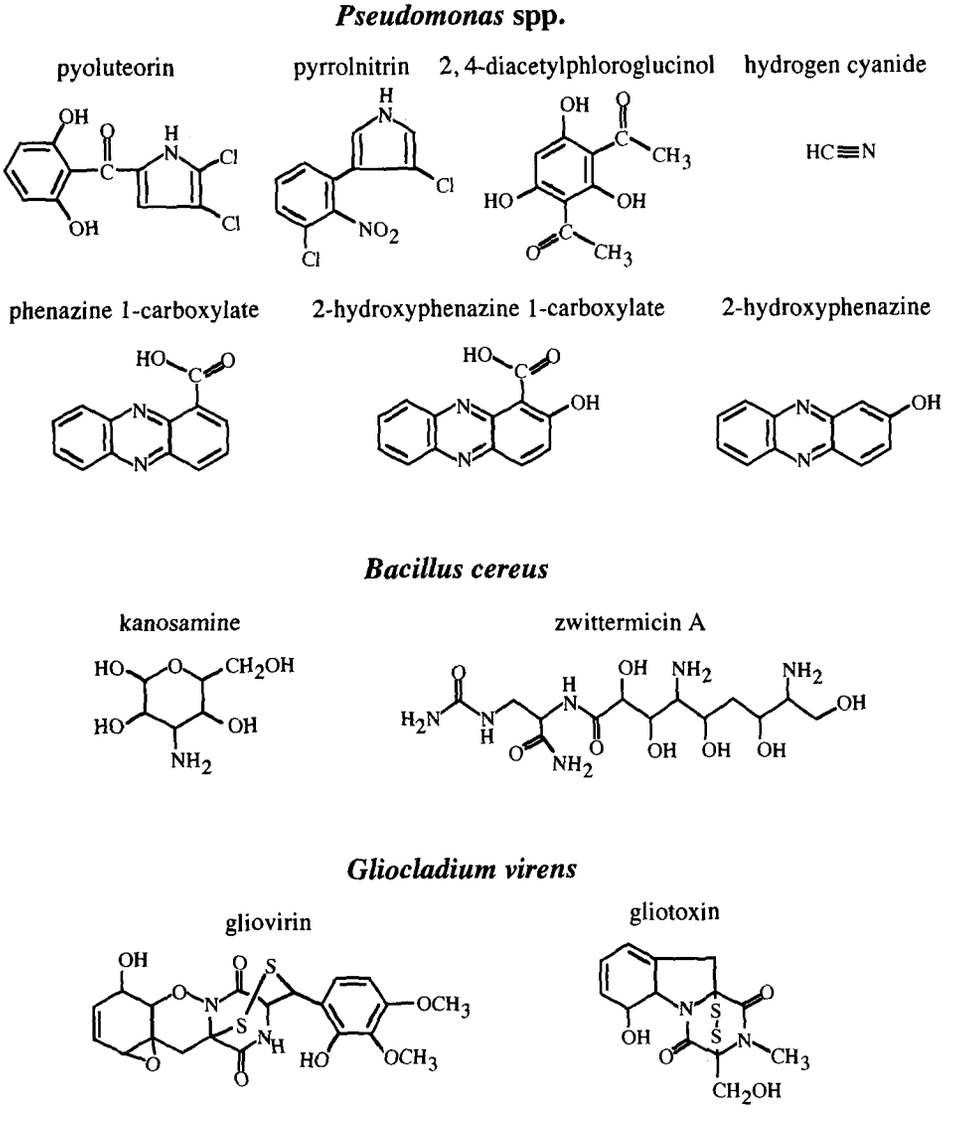

Figure 1. Chemical Structures of Antibiotics Produced by Bacterial and Fungal Biocontrol Agents.

Pseudomonas fluorescens strain Pf-5 produces pyoluteorin, pyrrolnitrin, 2,4-diacetylphloroglucinol, and hydrogen cyanide; and strain CHA0 produces pyoluteorin, 2,4-diacetylphloroglucinol, and hydrogen cyanide; strain BL915 produces pyrrolnitrin and hydrogen cyanide; and strain Q2-87 produces 2,4-diacetylphloroglucinol. *P. fluorescens* strain 2-79 and *P. aureofaciens* strain 30-84 produce the phenazine derivatives. *Bacillus cereus* strain UW85 produces zwittermicin A and kanosamine. *Gliocladium virens* strains G-3 and G-9 produce gliovirin, and strain G-20 produces gliotoxin (Lumsden et al., 1992).

Antibiotic Resistance

One of the goals of the use of biocontrol in agriculture is to avoid the pitfalls associated with synthetic pesticides, including the development of resistance in pest populations. An attractive feature of biocontrol strategies is that populations of pathogens resistant to antibiotics produced by biocontrol agents are likely to develop slowly. There are two reasons why this may be so. First, most biocontrol agents produce more than one antibiotic, and resistance to multiple antibiotics should occur only at a very low frequency. Second, total exposure of the pathogen population to the antibiotics is low because, in general, the populations of biocontrol agents are localized on the root; therefore, selection pressures are minimized. Nevertheless, if sufficient selection pressure is applied to pathogens, the appearance of strains that are not controlled by biocontrol agents is inevitable. The use of antimicrobial agents in human medicine and agriculture has shown that

selection pressures drive the evolution of resistance faster than we expect or hope for. For example, the development of multiple-drug resistance derived from spontaneous mutations was not predicted to occur, but now it is recognized as a common mechanism of antibiotic resistance in bacteria (Hachler et al., 1991; Cohen et al., 1993).

Therefore, research is needed to understand the molecular bases of pathogen resistance to antibiotics produced by biocontrol agents. Resistance should be studied before it occurs in the field so that fundamental knowledge can be applied to anticipate and prevent the breakdown of biocontrol. Examples of such approaches include inhibiting resistance proteins, combining antibiotics that select for different resistance genes, and avoiding use of biocontrol agents against pathogen populations in which a high frequency of resistance is predicted. Similarly, understanding resistance of insects to *B. thuringiensis* has led to innovative strategies to reduce the impact of resistance on insect biocontrol (Tabashnik, 1994).

Resistance to antibiotics usually arises in a sensitive population by spontaneous mutation or by horizontal gene transfer (Davies, 1994; Nikaido, 1994; Spratt, 1994). Mutations conferring resistance may affect antibiotic uptake or target sensitivity. Resistance genes that can be transferred encode antibiotic-modifying enzymes, resistant target molecules, or efflux pumps; such genes, when carried by antibiotic-producing bacteria to prevent them from committing suicide when they produce the antibiotic, are known as self-resistance genes (Cundliffe, 1989; Davies, 1994). Self-resistance genes could be transferred to target pathogens in the soil; therefore, it is essential to understand mutations conferring resistance in target pathogens as well as self-resistance genes in producing organisms.

The importance of self-resistance is illustrated in the crown gall biocontrol system. *Agrobacterium radiobacter* effectively controls crown gall, which is caused by *A. tumefaciens*, largely through the action of the antibiotic agrocin 84. Efficacy was threatened when agrocin 84–resistant strains of the pathogen were isolated from galls on *A. radiobacter*–treated plants (Panagopoulos et al., 1979). These strains were likely the result of transfer of plasmid pAgK84, which carries both agrocin 84 production and resistance genes, from the biocontrol strain to the pathogen (Vicedo et al., 1993; Stockwell et al., 1996). A deletion in the plasmid-encoded functions for conjugal transfer generated a nonmobilizable derivative of pAgK84, which should provide more stable disease suppression (Jones et al., 1988). This example demonstrates both the possibility of antibiotic resistance in target pathogens and the importance of understanding the genetic basis for antibiotic resistance in the design of more robust strategies for biocontrol.

Despite the clear warning indicated by the transfer of agrocin-84 resistance, few other studies have focused on resistance to antibiotics involved in biocontrol. A gene encoding zwittermicin A resistance, *zmaR*, was cloned from the zwittermicin A–producing biocontrol agent *B. cereus* UW85 (Milner et al., 1996a). The *zmaR* locus has been found in diverse *B. cereus* strains, including some that do not produce zwittermicin A, suggesting the possibility of horizontal transfer of zwittermicin A resistance within this species (Raffel et al., 1996). Although *zmaR* can confer zwittermicin A resistance on *E. coli*, it is not known whether *zmaR* has been transferred to or can confer zwittermicin A resistance on microorganisms in the soil or rhizosphere.

Little information is available concerning spontaneous mutations that confer antibiotic resistance on pathogens that are the targets of biocontrol strategies. However, predictions can be made about the types of resistance mechanisms that might be deployed. For example, the wheat pathogen *Septoria tritici* acclimates to 1-hydroxyphenazine by inducing genes for catalase, superoxide dismutase, and melanin production (Levy et al., 1992); therefore, mutants of the pathogen that constitutively produce high levels of these protectants might not be suppressed by phenazine-producing biocontrol organisms. The widespread occurrence of cyanide-resistant respiratory pathways in microorganisms suggests that prolonged application of hydrogen cyanide–producing biocontrol agents may select for pathogens containing cyanide-resistant oxidases (see Osbourn, 1996, in this issue, for a discussion of pathogen resistance to cyanide-producing compounds in plants).

Recent evidence shows variation among strains of *Gaeumannomyces graminis* for sensitivity to antibiotics produced by fluorescent pseudomonad biocontrol agents, and disease induction by these resistant strains is not suppressed effectively by the biocontrol agent (Mazzola et al., 1995). It is not clear whether these resistant strains arose from a sensitive population by spontaneous mutation or gene transfer or whether they are simply immune to the antibiotics because they lack an appropriate uptake system or sensitive target in the cell. Resistance due to mutations, gene transfer, or immunity will present a challenge for the use of biocontrol in the field.

Iron Competition

Biocontrol can involve suppression of the pathogen by depriving it of nutrients. The best understood example of this mechanism is iron competition. Iron is abundant in Earth's crust, but most of it is found in the highly insoluble form of ferric hydroxide; thus, iron is only available to organisms at concentrations at or below 10^{-18} M in soil solutions at neutral pH. This presents a challenge for bacteria, which require iron at micromolar concentrations for growth. Bacteria have evolved high-affinity iron uptake systems to shuttle iron into the cell (Neilands, 1981; Neilands and Nakamura, 1991). The typical system involves a siderophore, which is an iron-binding ligand, and an uptake protein, which transports the siderophore into the cell. The fluorescent pseudomonads produce a class of siderophores known as the pseudobactins, which are structurally complex iron-binding molecules. Analyses of mutants lacking the ability to produce siderophores suggest that they contribute to suppression of certain fungal and oomycete diseases (Duijff et al., 1994a; Buysens et al., 1996).

An interesting aspect of siderophore biology is that diverse organisms can use the same type of siderophore. Microorganisms may use each other's siderophores if they contain the

appropriate uptake protein (Koster et al., 1993; Raaijmakers et al., 1995a), and plants can even acquire iron from certain pseudobactins (Duijff et al., 1994b). Further work is needed to characterize the ability of soilborne organisms to utilize siderophores produced by biocontrol agents. Rapid breakdown of biocontrol would be expected if the target pathogens could circumvent disease suppression predicated on iron deprivation by acquiring the ability to utilize the siderophores from their neighbors in the soil.

Parasitism

In addition to antibiosis and iron deprivation, certain biocontrol agents also reduce plant disease by parasitizing pathogens. For example, *Trichoderma* spp parasitize fungal plant pathogens. The parasite extends hyphal branches toward the target host, coils around and attaches to it with appressorium-like bodies, and punctures its mycelium (Chet et al., 1981; Goldman et al., 1994). These events require specific interactions between the parasite and fungal host, including the detection of chemical gradients and mycelial surface features. The specificity is illustrated by the observation that *Trichoderma* coils around *Pythium ultimum* hyphae but not plastic threads of a similar diameter (Dennis and Webster, 1971). Digestion of host cell walls is accomplished by a battery of excreted enzymes, including proteases, chitinases, and glucanases. These enzymes often have antifungal activity individually and are synergistic in mixtures or with antibiotics (Di Pietro et al., 1993; Lorito et al., 1993a, 1994). Mycoparasitism has been suggested as a mechanism of biocontrol by *Trichoderma* spp and *G. virens*, but its contribution to disease suppression remains uncertain, and clarification will be achieved with mutants lacking the cell wall–degrading enzymes. These mutants will be challenging to generate because *Trichoderma harzianum* produces at least three distinct chitinases (de la Cruz et al., 1992) as well as proteolytic and glucanolytic enzymes. Recent advances in molecular analysis, including the cloning of a *T. harzianum* gene encoding endochitinase, and methods for transformation of *Trichoderma* and *Gliocladium* make the generation of mutants with multiple gene disruptions feasible (Lorito et al., 1993b; Hayes et al., 1994).

Genetic Diversity among Biocontrol Agents

The complexity of the interactions involved in biocontrol and the wide range of environmental conditions found globally in agriculture make it unlikely that any one strain will suppress even a single disease in all settings. The genetic diversity of microorganisms with disease-suppressive potential remains a powerful yet largely untapped resource for biocontrol of plant disease (Kerr and Htay, 1974). There is a need to seek new biocontrol strains, particularly strains adapted to the site where they will be used (Cook, 1993; Stabb et al., 1994), but if random bacterial isolates from each site are screened for disease suppressiveness as has been done for years, the effort will continue to be labor intensive and will not make use of existing knowledge of biocontrol mechanisms.

Recently, there has been some success in identifying diverse biocontrol strains that suppress plant pathogens through common mechanisms (Stabb et al., 1994). This approach has been employed successfully in insect biocontrol programs, most notably in the development of *B. thuringiensis*–based strategies for the control of insects. Initial application of *B. thuringiensis* produced limited and variable insect control. Biocontrol by *B. thuringiensis* is due to accumulation of a large protein toxin, which forms a crystal in the bacterial cell. Identification of the crystal toxin as the basis for insect control led to searches for genetically diverse *B. thuringiensis* strains with related but unique crystal toxins. *B. thuringiensis*–based biocontrol strategies are now employed, and their development has generated a tremendous knowledge base of fundamental genetics and protein chemistry (Deacon, 1983; Hoefte and Whitely, 1989; Feitelson et al., 1992; Carlton, 1993). The limitation of this approach is that widespread use of organisms that share a mechanism of biocontrol will increase the selection for resistance; therefore, this approach must be coupled with an understanding of the mechanism and frequency of resistance and of strategies to avoid it.

Recently, a comparable approach has been applied to biocontrol of plant disease, and there have been some notable successes in finding diverse strains that suppress disease based on a common mechanism. Genes for antibiotic production or antibiotic self-resistance are conserved among antibiotic producers and therefore form the basis of molecular probes for detecting new antibiotic-producing strains. Zwittermicin A–producing *B. cereus* strains and 2,4-diacetylphloroglucinol–producing *P. fluorescens* strains have been identified from geographically and chemically diverse soils (Stabb et al., 1994; Keel et al., 1996; Raffel et al., 1996). Strains that produce the same antibiotic can be phenotypically diverse and may express traits useful under particular conditions (Figure 2). Some of these strains may provide effective control in certain soils in certain geographic regions or on particular crops (Figure 2E). In addition, the genetic diversity of these strains may be tapped by combining them in mixed inoculants. Certain mixtures of fluorescent pseudomonads, or fluorescent pseudomonads and fungi, suppressed disease more effectively than did single-strain inoculants (Park et al., 1988; Pierson and Weller, 1994; Duffy and Weller, 1995; Duffy et al., 1996).

INTERACTIONS WITH THE PLANT

Colonization

It seems logical that a biocontrol agent should grow and persist, or "colonize," the surface of the plant it protects, and colonization is widely believed to be essential for biocontrol (Weller, 1983; de Weger et al., 1987; Parke, 1991). However, colonization, or even the initial population size of the biocontrol

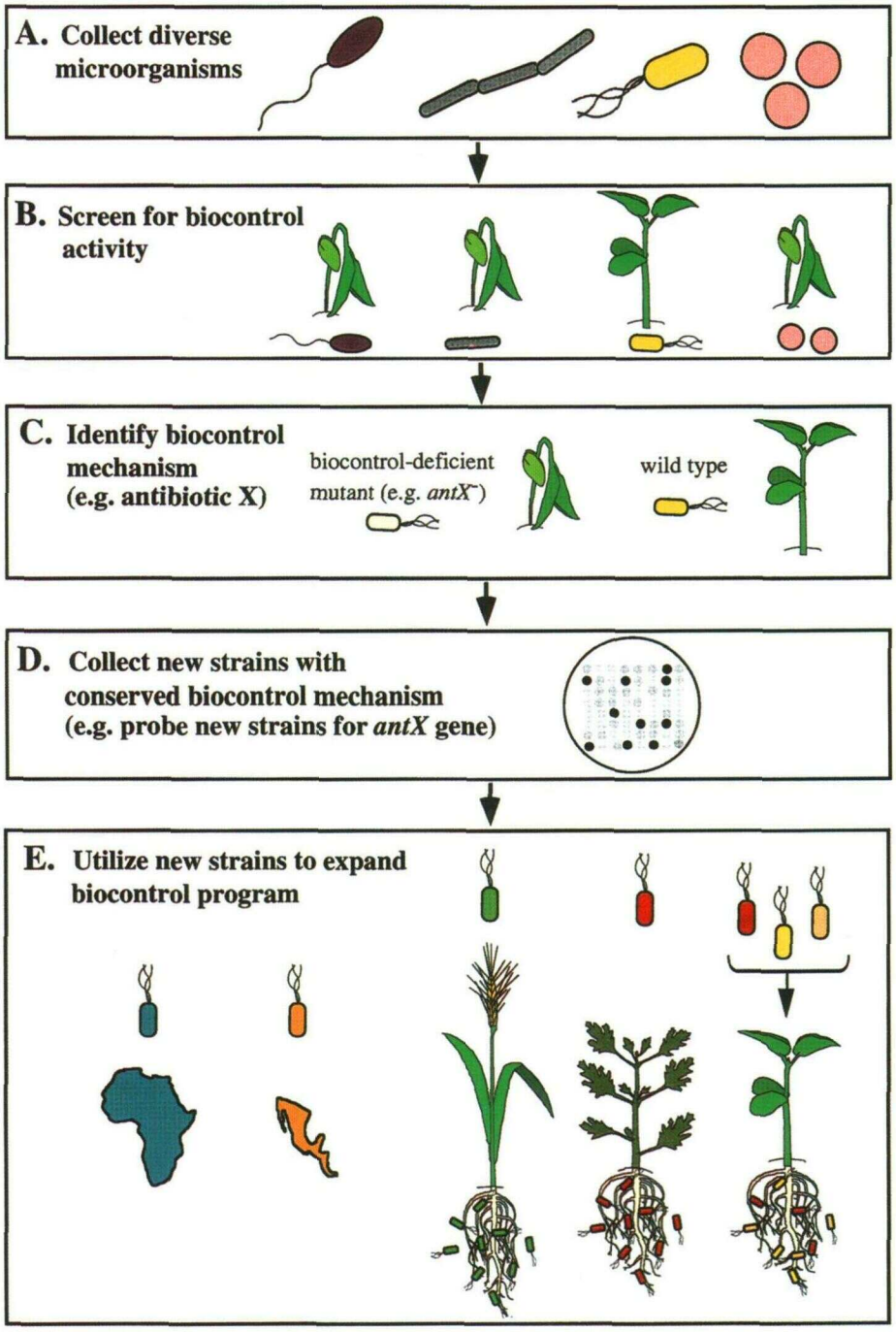

Figure 2. Proposed Model for a Biocontrol Research and Development Program.

(A) and **(B)** Initially, many diverse microorganisms are collected **(A)** and screened for biocontrol activity **(B)**. Many isolates may be screened to identify a disease-suppressive isolate (depicted in **[B]** as a yellow rod). It is unlikely that this strain will be effective under diverse conditions. **(C)** and **(D)** An approach to identifying new strains that, collectively, will be effective under diverse conditions is to elucidate mechanisms of biocontrol **(C)** and to identify new biocontrol agents that share the same mechanism **(D)**. Genetic analyses can elucidate biocontrol mechanisms, such as the contribution of antibiotic X to biocontrol **(C)**. A knowledge of biocontrol mechanisms, and the genes responsible, can lead to the development of nucleic acid probes designed to identify new strains with the same mechanism of biocontrol activity, in this example depicted as a probe for gene *antX* **(D)**.

(E) Although similar in biocontrol mechanism, strains containing *antX* may be genetically diverse in important ways, making some new strains useful on different crops, in different geographic regions, or as part of genetically diverse mixtures. This approach readily leads to identification of large collections of disease-suppressive strains, avoiding the need to repeat the years of research depicted in **(A)** to **(C)**.

agent, has been shown to be significantly correlated with disease suppression in only a few instances (Paulitz and Baker, 1987; Parke, 1990; Bull et al., 1991). For example, in the suppression of damping-off of peas by *P. cepacia* (recently renamed *Burkholderia cepacia*), there is a significant relationship between population size of the biocontrol agent and the degree of disease suppression (Parke, 1990). Also, suppression of take-all of wheat is correlated with colonization of roots by *P. fluorescens* strain 2-79 (Bull et al., 1991). However, even in interactions that require colonization for disease suppression, the biocontrol agent may not be required at high population density. It is intriguing that certain highly effective biocontrol agents, such as *P. cepacia* and *B. cereus*, achieve only modest populations on roots of field-grown plants and appear to replace, not augment, the population of indigenous members of their species (Halverson et al., 1993; King and Parke, 1996). These results suggest that roots have a carrying capacity, or a limit on the size of a population they can support, for certain species of bacteria.

Understanding the interplay between the sizes of the populations of the biocontrol agent and the pathogen has progressed significantly due largely to a theoretical modeling approach introduced by Johnson (1994) and augmented by empirical verification by others (Raaijmakers, 1995b; Montesinos and Bonaterra, 1996). The application of mathematical models provides a depth of analysis that has not been achieved in past work on biocontrol. From this work, it is evident that careful attention must be paid to the dose–response relationship with both microbial partners, because biological outcomes are grossly different when observations are made at very high or very low disease pressure. Elaboration of these mathematical models will introduce a time variable, describing the relationship between disease severity or incidence and populations of the biocontrol agent and pathogen during plant development.

The bacterial characteristics that contribute to active or passive spread on roots are not well understood (Parke, 1991). However, under field conditions, percolating water probably plays an essential role in the passive distribution of bacteria on roots (Parke et al., 1986; Liddell and Parke, 1989). Nevertheless, motility is important for biocontrol in some plant–bacterial pairs under some but not all conditions (de Weger et al., 1987; Bowers and Parke, 1993), and osmotolerance is correlated with colonization ability (Loper et al., 1985). Cell surface characteristics influence attachment to roots, which may be necessary for colonization (Vesper, 1987; Anderson et al., 1988). Certain mutations that affect accumulation of secondary metabolites also influence colonization of plant material in field soil (Mazzola et al., 1992; Pfender et al., 1993; Natsch et al., 1994; Carroll et al., 1995). A promising approach that will likely broaden the array of traits considered to be important for colonization is to screen mutants directly for increased or decreased ability to colonize roots (Lam et al., 1991). Mutants of *Pseudomonas* strains of both phenotypes have been identified, and analysis of these mutants indicates that prototrophy for amino acids and vitamin B1, rapid growth rate, utilization of organic acids, and lipopolysaccharide properties contribute to colonization ability (Lugtenberg et al., 1996).

The study of root colonization by fungal biocontrol agents is more complex. Defining a fungal unit to quantify and the difficulty of conducting genetic analyses are challenges in studying the ecology of fungi. One study focused on mutants of *Trichoderma* that are resistant to the fungicide benomyl. These mutants are dramatically increased in root colonization and biocontrol ability, even in the absence of benomyl (Ahmad and Baker, 1987, 1988a). Benomyl resistance correlates with several phenotypes, including increased cellulase production and altered morphology, making it difficult to determine the basis for increased colonization (Ahmad and Baker, 1988b; Peterbauer et al., 1992). It is possible that increased cellulase production enhances root colonization by enabling *Trichoderma* to utilize plant cell debris and that increased colonization enhances biocontrol.

Induced Resistance

Some biocontrol agents induce a sustained change in the plant, increasing its tolerance to infection by a pathogen, a phenomenon known as induced resistance. In some cases, it is clear that induced resistance by biocontrol agents involves the same suite of genes and gene products involved in the well-documented plant response known as systemic acquired resistance (SAR), but this is not always the case. SAR is typically a response to a localized infection or an attenuated pathogen, which is manifested in subsequent resistance to a broad range of other pathogens (Ross, 1961; Uknes et al., 1992; Ryals et al., 1996, in this issue). The best understood examples of induced resistance occur in the biocontrol of aboveground diseases; these are discussed below.

The idea that biocontrol agents might induce resistance in the host was first suggested on the basis of experiments showing that bacterial treatments protected potato tubers from subsequent infection by *P. solanacearum* (Kempe and Sequeira, 1983). More recently, it has been shown that the biocontrol agent *P. fluorescens* strain CHA0 (Maurhofer et al., 1994a) induces SAR-associated proteins, confers systemic resistance to a viral pathogen, and induces accumulation of salicylic acid, which plays a role in signal transduction in SAR (Gaffney et al., 1993; Ryals et al., 1996, in this issue). Mutants of CHA0 that do not produce the siderophore pyoverdin do not induce SAR, suggesting a novel role for bacterial metabolites in disease suppression (Maurhofer et al., 1994a). Another fluorescent pseudomonad, *P. putida*, induces expression of the gene encoding PR1a, which is associated with the classical SAR response (Zdor and Anderson, 1992). Other strains of *P. fluorescens* do not induce expression of the gene products associated with the classic SAR response but appear instead to induce a functionally analogous response (Hoffland et al., 1995).

Another line of evidence for induced resistance, which may or may not involve SAR, is that some biocontrol agents

suppress disease when they are applied far from the site of infection by the pathogen, and they cannot be found at the infection site (Wei et al., 1991; Zhou and Paulitz, 1994; Liu et al., 1995). Furthermore, in suppression of Fusarium wilt by *P. fluorescens*, preparations of lipopolysaccharides from the bacterial cell surface induce resistance as effectively as the living bacteria, demonstrating that biocontrol is not necessarily due to transport of the bacteria or an antibiotic through the plant (Leeman et al., 1995a, 1995b). Whether or not biocontrol agents suppress disease by inducing resistance, it is essential that SAR and biocontrol strategies be compatible, because future agricultural practices are likely to require the integration of multiple pest control strategies (Chen et al., 1996).

Genetic Variation in the Host

Although much of the research focusing on plant genes affecting interactions with beneficial microorganisms deals with relationships with nitrogen-fixing symbionts (Baldani and Dobereiner, 1980; Baldani et al., 1986; Bliss, 1991; see also Long, 1996, and Pawlowski and Bisseling, 1996, in this issue), there is ample evidence that plants vary in their ability to support and respond to other beneficial microflora. The ability to support certain biocontrol organisms varies among plant species and among cultivars within species. Some plants appear to attract and support communities of microorganisms that are antagonistic to certain pathogens (Neal et al., 1973; Azad et al., 1985). Legume species vary in the magnitude of response to the plant growth-promoting bacterium *B. polymyxa* (Chanway et al., 1988a), and *Bacillus* strains isolated from wheat roots enhance growth of wheat in a cultivar-specific manner (Chanway et al., 1988b). Plant species vary in their ability to induce genes for pyoluteorin biosynthesis in *P. fluorescens* (Kraus and Loper, 1995), presumably due to variation in composition of root exudate among the species. Strains of *P. fluorescens* that overproduce pyoluteorin and 2,4-diacetylphloroglucinol provide superior disease suppression compared with the parent strain in some host–pathogen combinations and not others, and the effects correlate with host, and not pathogen, sensitivity to antibiotics (Maurhofer et al., 1995). Numerous studies have shown that different cultivars vary in survival or disease incidence in the presence of a pathogen and a biocontrol agent (Howie and Echandi, 1983; Vakili and Bailey, 1989; Leeman et al., 1995c; Liu et al., 1995; King and Parke, 1996). The two challenges in assessing plant variation for this trait are to separate effects on the pathogen from those on the host and to partition host resistance and supportiveness of biocontrol (K.P. Smith and R.M. Goodman, unpublished data).

The practical extension of the discovery that plants vary in the ability to support biocontrol is to enhance this characteristic through breeding (Milner et al., 1996b; O'Connell et al., 1996). This has been referred to as breeding for "hospitality" of the host plant (R.M. Goodman, unpublished data) and is likely to have a substantial impact on efficacy of biocontrol of plant disease. Plants that are hospitable to a biocontrol agent might produce root exudates that support growth or induce expression of genes in the microorganism involved in disease suppression, attract the biocontrol agent to the infection site, or respond to the biocontrol agent by mounting a resistance response (Nelson et al., 1986; Liu et al., 1995; O'Connell et al., 1996). Breeding can also be employed to produce isogenic lines that can provide the basis for identifying traits in plants that influence their relationships with microorganisms. Breeding for hospitality to biocontrol agents will be facilitated by demonstrating heritability of the trait and by mapping genes associated with hospitality. This has been initiated in tomatoes, in which inbred lines derived from a wide cross have been assessed for their ability to support biocontrol by *B. cereus*. Substantial variation for the trait is observed among these lines, thus providing the basis for mapping genes that contribute to hospitality to *B. cereus* (Figure 3).

INTERACTIONS WITH THE MICROBIAL COMMUNITY

The interaction of the biocontrol agent with the microbial community may provide clues to explain why many organisms suppress disease effectively in the laboratory but fail to do so in the field. Biocontrol organisms may be affected by microbial communities, and they may influence the communities they enter. In some cases, they may enhance components of the community that work in concert to suppress disease. Recent evidence suggests that deliberate manipulation of microbial communities may be a highly robust and effective form of biocontrol (English and Mitchell, 1988; Boehm and Hoitink, 1992).

The ability of *P. fluorescens* to suppress Fusarium wilt in radish seems partly due to its effects on the fungal community, in particular on the nonpathogenic strains of *Fusarium oxysporum* (Schippers, 1993). Certain fluorescent pseudomonads have also been shown to displace resident fungi and bacteria, in some cases reducing populations of deleterious microorganisms (Kloepper and Schroth, 1981; Yuen and Schroth, 1986). Treatments that enhance plant health can also increase the frequency of manganese-reducing bacteria in the rhizosphere community, thereby increasing manganese availability to the plant, which may in turn enhance resistance to disease (Huber and Wilhelm, 1988; Elmer, 1995).

Introduction of the biocontrol agent *B. cereus* strain UW85 can induce dramatic changes in the composition of culturable bacterial communities on soybean roots in the field (Gilbert et al., 1993). The change results in a community that more closely resembles a bacterial community from nonrhizosphere soil than does a nontreated root community. This finding, coupled with substantial support from previous work on the effects of host resistance and soil amendments on rhizosphere communities, suggested the "camouflage" hypothesis, which proposes that a mechanism for protecting plant roots from attack by pathogens is to make the roots "look" more like soil by enhancing populations of typical soil inhabitants and reduc-

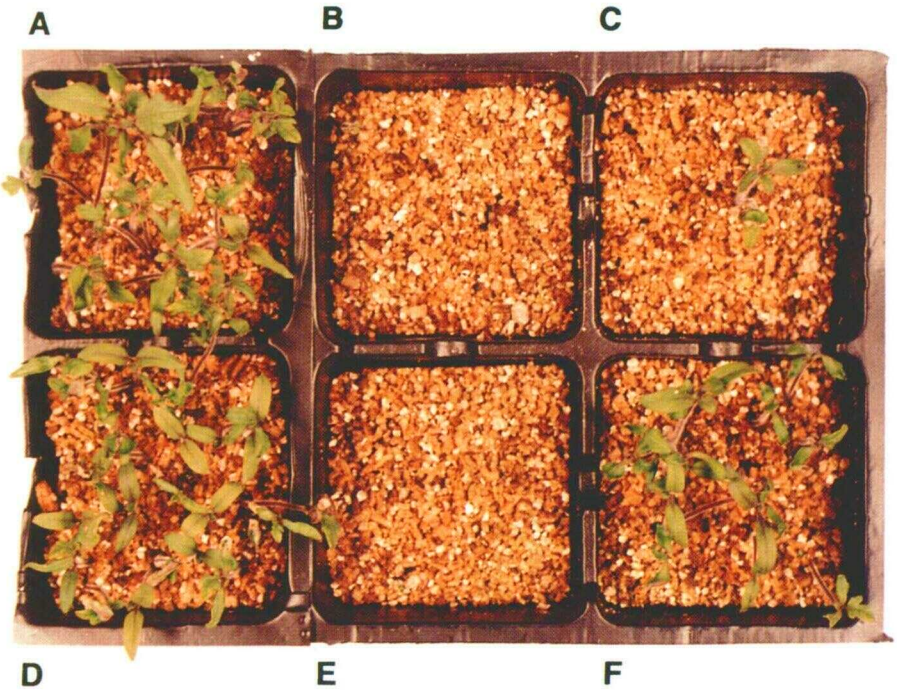

Figure 3. Inbred Lines of Tomato Differing in "Hospitality" to the Biocontrol Agent *Bacillus cereus* UW85.

Genetic variation for this trait will provide the basis for directed breeding to enhance the supportiveness of a plant population to the biocontrol agent. The inbred lines are the result of repeated inbreeding of individuals from a backcross population derived from backcrossing the hybrid of *Lycopersicon esculentum* and *L. pennellii* to *L. esculentum*.

(A) to **(C)** Tomato genotype NIJ18.

(D) to **(F)** Tomato genotype NIJ56.

(A) and **(D)** Noninoculated.

(B) and **(E)** Inoculated with *Pythium torulosum*.

(C) and **(F)** Inoculated with *P. torulosum* and *B. cereus* UW85.

ing populations of typical root-associated microorganisms (Gilbert et al., 1994). The camouflage hypothesis has not been tested directly, but it may provide a useful basis for the study of community processes that affect biocontrol of root-associated pathogens.

There are three challenges associated with studying microbial changes resulting from the introduction of biocontrol agents. First, it is difficult to determine whether a community change plays a role in disease suppression or whether it is simply an unrelated outcome of altering the rhizosphere microflora. Second, the data generated in community analysis require new mathematical tools to deal with the complex communities and their multiple levels of interaction. Caution must be used in interpreting results from purely descriptive studies. The power of such studies can be enhanced by application of multivariate statistical modeling (Pfender and Wootke, 1988; Gilbert et al., 1996). Finally, all of the work addressing the effects of biocontrol organisms on microbial communities has relied on culturing to describe communities. Because <1% (and perhaps far less than that) of the bacteria in soil are culturable (Faegri et al., 1977; Torsvik et al., 1990a, 1990b; S. Bintrim, R.M. Goodman, and J. Handelsman, unpublished results), studies based on culturing undoubtedly ignore some key organisms and interactions. The recent application of molecular analyses to describe nonculturable communities in both extreme and familiar environments (Ward et al., 1990; Weisburg et al., 1991; Amann et al., 1994, 1995) provides a powerful set of tools for the study of microbial interactions that influence biocontrol (O'Connell et al., 1996).

CONCLUSION AND FUTURE DIRECTIONS

Successful biocontrol of plant disease requires an intricate array of interactions. Understanding these interactions at the molecular and ecological levels 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 metabolites

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 three 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 agent and host plant. 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 needs to be paid to nonculturable members of the root-associated 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.

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REFERENCES

Ahmad, J.S., and Baker, R. (1987). Rhizosphere competence of *Trichoderma harzianum*. Phytopathology **77,** 182–189.

Ahmad, J.S., and Baker, R. (1988a). Implications of rhizosphere competence of *Trichoderma harzianum*. Can. J. Microbiol. **34,** 229–234.

Ahmad, J.S., and Baker, R. (1988b). Rhizosphere competence of benomyl-tolerant mutants of *Trichoderma* spp. Can. J. Microbiol. **34,** 694–696.

Amann, R.I., Ludwig, W., and Schleifer, K.-H. (1994). Identification of uncultured bacteria: A challenging task for molecular taxonomists. ASM News **60,** 360–365.

Amann, R.I., Ludwig, W., and Schleifer, K.-H. (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol. Rev. **59,** 143–169.

Anderson, A.J., Habibzadegah-Tari, P., and Tepper, C.S. (1988). Molecular studies on the role of a root surface agglutinin in adherence and colonization by *Pseudomonas putida*. Appl. Environ. Microbiol. **54,** 375–380.

Azad, H.R., Davis, J.R., and Kado, C.I. (1985). Relationships between rhizoplane and rhizosphere bacteria and Verticillium wilt resistance in potato. Arch. Microbiol. **140,** 347–351.

Baldani, V.L.D., and Dobereiner, J. (1980). Host-plant specificity in the infection of cereals with *Azospirillum* spp. Soil Biol. Biochem. **12,** 433–439.

Baldani, V.L.D., Alvarez, M.A.B., Baldani, J.I., and Dobereiner, J. (1986). Establishment of inoculated *Azospirillum* spp. in the rhizosphere and in roots of field grown wheat and sorghum. Plant Soil **90,** 35–46.

Bangera, M.G., and Thomashow, L.S. (1996). Characterization of a genomic locus required for synthesis of the antibiotic 2,4-diacetylphloroglucinol by the biological control agent *Pseudomonas fluorescens* Q2-87. Mol. Plant-Microbe Interact. **9,** 83–90.

Bliss, F.A. (1991). Breeding plants for enhanced beneficial interactions with soil microorganisms. In Plant Breeding for the 1990s, H.T. Stalker and J.P. Murphy, eds (Wallingford, UK: CAB International), pp. 251–277.

Boehm, M.J., and Hoitink, H.A.J. (1992). Sustenance of microbial activity in potting mixes and its impact on severity of Pythium root rot of poinsettia. Phytopathology **82,** 259–264.

Bowers, J.H., and Parke, J.L. (1993). Colonization of pea (*Pisum sativum* L.) taproots by *Pseudomonas fluorescens*: Effect of soil temperature and bacterial motility. Soil Biol. Biochem. **25,** 1693–1701.

Brisbane, P.G., and Rovira, A.D. (1988). Mechanisms of inhibition of *Gaeumannomyces graminis* var. *tritici* by fluorescent pseudomonads. Plant Pathol. **37,** 104–111.

Bull, C.T., Weller, D.M., and Thomashow, L.S. (1991). Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2-79. Phytopathology **81,** 954–959.

Buysens, S., Heungens, K., Poppe, J., and Hofte, M. (1996). Involvement of pyochelin and pyoverdin in suppression of *Pythium*-induced damping-off of tomato by *Pseudomonas aeruginosa* 7NSK2. Appl. Environ. Microbiol. **62,** 865–871.

Carlton, B.C. (1993). Development of improved bioinsecticides based on *Bacillus thuringiensis*. In Pest Control with Enhanced Environ-

mental Safety, S.O. Duke, J.J. Menn, and J.R. Plimmer, eds (Washington, DC: American Chemical Society), pp. 258–266.

Carroll, H., Moënne-Loccoz, Y., Dowling, D.N., and O'Gara, F. (1995). Mutational disruption of the biosynthesis genes coding for the antifungal metabolite 2,4-diacetylphloroglucinol does not influence the ecological fitness of *Pseudomonas fluorescens* F113 in the rhizosphere of sugarbeets. Appl. Environ. Microbiol. **61,** 3002–3007.

Chanway, C.P., Holl, F.B., and Turkington, R. (1988a). Genotypic coadaptation in plant growth promotion of forage species by *Bacillus polymyxa.* Plant Soil **106,** 281–284.

Chanway, C.P., Nelson, L.M., and Holl, F.B. (1988b). Cultivar-specific growth promotion of spring wheat (*Triticum aestivum* L.) by coexistent *Bacillus* species. Can. J. Microbiol. **34,** 925–929.

Chen, J., Jacobson, L.M., Handelsman, J., and Goodman, R.M. (1996). Compatibility of systemic acquired resistance and microbial biocontrol for suppression of plant disease in a laboratory assay. Mol. Ecol. **5,** 73–80.

Chet, I. (1987). *Trichoderma*—Application, mode of action, and potential as a biocontrol agent of soilborne plant pathogenic fungi. In Innovative Approaches to Plant Disease Control, I. Chet, ed (New York: John Wiley and Sons), pp. 137–156.

Chet, I., Harman, G.E., and Baker, R. (1981). *Trichoderma hamatum:* Its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. Microb. Ecol. **7,** 29–38.

Cohen, S.P., Hachler, H., and Levy, S.B. (1993). Genetic and functional analysis of the multiple antibiotic resistance (*mar*) locus in *Escherichia coli.* J. Bacteriol. **175,** 1484–1492.

Cook, R.J. (1993). Making greater use of introduced microorganisms for biological control of plant pathogens. Phytopathology **31,** 53–80.

Cook, R.J., and Baker, K.F. (1983). The Nature and Practice of Biological Control of Plant Pathogens. (St. Paul, MN: APS Press).

Cook, R.J., Thomashow, L.S., Weller, D.M., Fujimoto, D., Mazzola, M., Bangera, G., and Kim, D. (1995). Molecular mechanisms of defense by rhizobacteria against root disease. Proc. Natl. Acad. Sci. USA **92,** 4197–4201.

Corbell, N., and Loper, J.E. (1995). A global regulator of secondary metabolite production in *Pseudomonas fluorescens* Pf-5. J. Bacteriol. **177,** 6230–6236.

Cundliffe, E. (1989). How antibiotic-producing organisms avoid suicide. Annu. Rev. Microbiol. **43,** 207–233.

Davies, J. (1994). Inactivation of antibiotics and the disseminaton of resistance genes. Science **264,** 375–382.

Deacon, J.W. (1983). Microbial Control of Plant Pests and Diseases. (Washington, DC: American Society for Microbiology).

de la Cruz, J., Hidalgo-Gallego, A., Lora, J.M., Benitez, T., Pintor-Toro, J.A., and Llobell, A. (1992). Isolation and characterization of three chitinases from *Trichoderma harzianum.* Eur. J. Biochem. **206,** 859–867.

Dennis, C., and Webster, J. (1971). Antagonistic properties of species-groups of *Trichoderma.* Trans. Br. Mycol. Soc. **57,** 363–369.

de Weger, L.A., van der Vlugt, C.I.M., Wijfjes, A.H.M., Bakker, P.A.H.M., Schippers, B., and Lugtenberg, B. (1987). Flagella of a plant-growth–stimulating *Pseudomonas fluorescens* strain are required for colonization of potato roots. J. Bacteriol. **169,** 2769–2773.

Di Pietro, A., Lorito, M., Hayes, C.K., Broadway, R.M., and Harman, G.E. (1993). Endochitinase from *Gliocladium virens:* Isolation, characterization, and synergistic antifungal activity in combination with gliotoxin. Phytopathology **83,** 308–313.

Duffy, B.K., and Weller, D.M. (1995). Use of *Gaeumannomyces graminis* var. *graminis* alone and in combination with fluorescent *Pseudomonas* spp. to suppress take-all of wheat. Plant Dis. **79,** 907–911.

Duffy, B.K., Simon, A., and Weller, D.M. (1996). Combination of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. Phytopathology **86,** 188–194.

Duijff, B.J., Bakker, P.A.H.M., and Schippers, B. (1994a). Suppression of Fusarium wilt of carnation by *Pseudomonas putida* WCS358 at different levels of disease incidence and iron availability. Biocontrol Sci. Technol. **4,** 279–288.

Duijff, B.J., de Kogel, W.J., Bakker, P.A.H.M., and Schippers, B. (1994b). Influence of pseudobactin 358 on the iron nutrition of barley. Soil Biol. Biochem. **26,** 1681–1688.

Elmer, W.H. (1995). Association between Mn-reducing root bacteria and NaCl applications in suppression of Fusarium crown and root rot of asparagus. Phytopathology **85,** 1461–1467.

English, J.T., and Mitchell, D.J. (1988). Influence of an introduced composite of microorganisms on infection of tobacco by *Phytophthora parasitica* var. *nicotianae.* Phytopathology **78,** 1484–1490.

Faegri, A., Torsvik, V.L., and Goksoyr, J. (1977). Bacterial and fungal activities in soil: Separation of bacteria and fungi by rapid fractionated centrifugation techniques. Soil Biol. Biochem. **9,** 105–112.

Feitelson, J.S., Payne, J., and Kim, L. (1992). *Bacillus thuringiensis*: Insects and beyond. Bio/Technology **10,** 271–275.

Gaffney, T.D., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., and Ryals, J. (1993). Requirement of salicylic acid for the induction of systemic acquired resistance. Science **261,** 754–756.

Gaffney, T.D., Lam, S.T., Ligon, J., Gates, K., Frazelle, A., Di Maio, J., Hill, S., Goodwin, S., Torkewitz, N., Allshouse, A.M., Kempf, H.J., and Becker, O.J. (1994). Global regulation of expression of antifungal factors by a *Pseudomonas fluorescens* biological control strain. Mol. Plant-Microbe Interact. **7,** 455–463.

Gilbert, G.S., Parke, J.L., Clayton, M.K., and Handelsman, J. (1993). Effects of an introduced bacterium on bacterial communities on roots. Ecology **74,** 840–854.

Gilbert, G.S., Handelsman, J., and Parke, J.L. (1994). Root camouflage and disease control. Phytopathology **84,** 222–225.

Gilbert, G.S., Clayton, M.K., Handelsman, J., and Parke, J.L. (1996). Use of cluster and discriminant analysis to compare rhizosphere bacterial communities following biological perturbation. Microb. Ecol. **32,** 123–147.

Goldman, G.H., Hayes, C., and Harman, G.E. (1994). Molecular and cellular biology of biocontrol by *Trichoderma* spp. Trends Biotechnol. **12,** 478–482.

Hachler, H., Cohen, S.P., and Levy, S.B. (1991). *mar*A, a regulated locus which controls expression of chromosomal multiple antibiotic resistance in *Escherichia coli.* J. Bacteriol. **173,** 5532–5538.

Halverson, L.J., Clayton, M.K., and Handelsman, J. (1993). Population biology of *Bacillus cereus* UW85 in the rhizosphere of field-grown soybeans. Soil Biol. Biochem. **25,** 485–493.

Hawes, M.C. (1991). Living plant cells released from the root cap: A regulator of microbial populations in the rhizosphere? In The Rhizosphere and Plant Growth, D.L. Keister and P.B. Cregan, eds (Boston, MA: Kluwer Academic Publishers), pp. 51–59.

Hayes, C.K., Klemsdal, S., Lorito, M., Di Pietro, A., Peterbauer, C., Nakas, J.P., Tronsmo, A., and Harman, G.E. (1994). Isolation and sequence of an endochitinase-encoding gene from a cDNA library of *Trichoderma harzianum.* Gene **138,** 143–148.

Hoefte, H., and Whitely, H.R. (1989). Insecticidal crystal proteins of *Bacillus thuringiensis.* Microbiol. Rev. **53,** 242–255.

Hoffland, E., Pieterse, C.M.J., Bik, L., and van Pelt, J.A. (1995). Induced systemic resistance in radish is not associated with accumulation of pathogenesis-related proteins. Physiol. Mol. Plant Pathol. **46,** 309–320.

Howell, C.R., and Stipanovic, R.D. (1983). Gliovirin, a new antibiotic from *Gliocladium virens,* and its role in the biological control of *Pythium ultimum.* Can. J. Microbiol. **29,** 321–324.

Howell, C.R., Stipanovic, R.D., and Lumsden, R.D. (1993). Antibiotic production by strains of *Gliocladium virens* and its relation to the biocontrol of cotton seedling diseases. Biocontrol Sci. Technol. **3,** 435–441.

Howie, W.J., and Echandi, E. (1983). Rhizobacteria: Influence of cultivar and soil type on plant growth and yield of potato. Soil Biol. Biochem. **15,** 127–132.

Huber, D.M., and Wilhelm, N.S. (1988). The role of manganese in resistance to plant diseases. In Manganese in Soils and Plants, R.D. Graham, R.J. Hannam, and N.C. Uren, eds (Boston, MA: Kluwer Academic Publishers), pp. 155–173.

Johnson, K.B. (1994). Dose–response relationships and inundative biological control. Phytopathology **84,** 780–784.

Jones, D.A., Ryder, M.H., Clare, B.G., Farrand, S.K., and Kerr, A. (1988). Construction of a Tra-deletion mutant of pAgK84 to safeguard the biological control of crown gall. Mol. Gen. Genet. **212,** 207–214.

Keel, C., Wirthner, P., Oberhansli, T., Voisard, C., Burger, U., Haas, D., and Defago, G. (1990). Pseudomonads as antagonists of plant pathogens in the rhizosphere: Role of the antibiotic 2,4-diacetylphloroglucinol in the suppression of black root rot of tobacco. Symbiosis **9,** 327–341.

Keel, C., Schnider, U., Maurhofer, M., Voisard, C., Laville, J., Burger, U., Wirthner, P., Haas, D., and Defago, G. (1992). Suppression of root diseases by *Pseudomonas fluorescens* CHA0: Importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. Mol. Plant-Microbe Interact. **5,** 4–13.

Keel, C., Weller, D.M., Natsch, A., Defago, G., Cook, R.J., and Thomashow, L. (1996). Conservation of the 2,4-diacetylphloroglucinol biosynthesis locus among fluorescent *Pseudomonas* strains from diverse geographic locations. Appl. Environ. Microbiol. **62,** 552–563.

Kempe, J., and Sequeira, L. (1983). Biological control of bacterial wilt of potatoes: Attempts to induce resistance by treating tubers with bacteria. Plant Dis. **67,** 499–503.

Kerr, A., and Htay, K. (1974). Biological control of crown gall through bacteriocin production. Physiol. Plant Pathol. **4,** 37–44.

King, E.B., and Parke, J.L. (1996). Population density of the biocontrol agent *Burkholderia cepacia* AMMDR1 on four pea cultivars. Soil Biol. Biochem. **28,** 307–312.

Kloepper, J.W., and Schroth, M.N. (1981). Relationship of in vitro antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. Phytopathology **71,** 1020–1024.

Kolter, R., Siegele, D.A., and Tormo, A. (1993). The stationary phase of the bacterial life cycle. Annu. Rev. Microbiol. **47,** 855–874.

Koster, M., van de Vossenberg, J., Leong, J., and Weisbeek, P.J. (1993). Identification and characterization of the *pup*B gene encoding an inducible ferric-pseudobactin receptor of *Pseudomonas putida* WCS358. Mol. Microbiol. **8,** 591–601.

Kraus, J., and Loper, J.E. (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.

Lam, S., Ellis, D.M., and Ligon, J.M. (1991). Genetic approaches for studying rhizosphere colonization. In The Rhizosphere and Plant Growth, D.L. Keister and P.B. Cregan, eds (Boston, MA: Kluwer Academic Publishers), pp. 43–50.

Laville, J., Voisard, C., Keel, C., Maurhofer, M., Defago, G., and Haas, D. (1992). Global control in *Pseudomonas fluorescens* mediating antibiotic synthesis and suppression of black root rot of tobacco. Proc. Natl. Acad. Sci. USA **89,** 1562–1566.

Leeman, M., van Pelt, J.A., Hendreckx, M.J., Scheffer, R.J., Bakker, P.A.H.M., and Schippers, B. (1995a). Biocontrol of Fusarium wilt of radish in commercial greenhouse trials by seed treatment with *Pseudomonas fluorescens* WCS374. Phytopathology **85,** 1301–1305.

Leeman, M., van Pelt, J.A., den Ouden, F.M., Heinsbroek, M., Bakker, P.A.H.M., and Schippers, B. (1995b). Induction of systemic resistance against Fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens.* Phytopathology **85,** 1021–1027.

Leeman, M., van Pelt, J.A., den Puden, F.M., Heinsbroek, M., Bakker, P.A.H.M., and Schippers, B. (1995c). Induction of systemic resistance by *Pseudomonas fluorescens* in radish cultivars differing in susceptibility to Fusarium wilt, using a novel bioassay. Eur. J. Plant Pathol. **101,** 655–664.

Levy, E., Eyal, Z., Chet, I., and Hochman, A. (1992). Resistance mechanisms of *Septoria tritici* to antifungal products of *Pseudomonas.* Physiol. Mol. Plant Pathol. **40,** 163–171.

Liddell, C.M., and Parke, J.L. (1989). Enhanced colonization of pea taproots by a fluorescent pseudomonad biocontrol agent by water infiltration into soil. Phytopathology **79,** 1327–1332.

Liu, L., Kloepper, J.W., and Tuzun, S. (1995). Induction of systemic resistance in cucumber by plant growth–promoting rhizobacteria: Duration of protection and effect of host resistance on protection and root colonization. Phytopathology **85,** 1064–1068.

Long, S.R. (1996). *Rhizobium* symbiosis: Nod factors in perspective. Plant Cell **8,** 1885–1898.

Loper, J.E., Haack, C., and Schroth, M.N. (1985). Population dynamics of soil pseudomonads in the rhizosphere of potato (*Solanum tuberosum* L.). Appl. Environ. Microbiol. **49,** 416–422.

Lorito, M., Harman, G.E., Hayes, C.K., Broadway, R.M., Tronsmo, A., Woo, S.L., and Di Pietro, A. (1993a). Chitinolytic enzymes produced by *Trichoderma harzianum:* Antifungal activity of purified endochitinase and chitobiosidase. Phytopathology **83,** 302–307.

Lorito, M., Hayes, C.K., Di Pietro, A., and Harman, G.E. (1993b). Biolistic transformation of *Trichoderma harzianum* and *Gliocladium virens* using plasmid and genomic DNA. Curr. Genet. **24,** 349–356.

Lorito, M., Peterbauer, C., Hayes, C.K., and Harman, G.E. (1994). Synergistic interaction between fungal cell wall degrading enzymes and different antifungal compounds enhances inhibition of spore germination. Microbiology **140,** 623–629.

Lugtenberg, B., van der Bij, A., Bloemberg, G., Dekkers, L., Chin-A-Woeng, T., Mulders, I., Simons, M., de Weger, L.A., and

Wijffelman, C.A. (1996). Mechanisms of rhizosphere colonization by *Pseudomonas* bacteria. In Molecular Plant-Microbe Interactions: Proceedings of the 8th International Congress, G. Stacey, B. Mullin, and P. Gresshoff, eds, in press.

Lumsden, R.D., Ridout, C.J., Vendemia, M.E, Harrison, D.J., Waters, R.M., and Walter, J.F. (1992). Characterization of major secondary metabolites produced in soilless mix by a formulated strain of the biocontrol fungus *Gliocladium virens*. Can. J. Microbiol. **38,** 1274–1280.

Lumsden, R.D., Lewis, J.A., and Fravel, D.R. (1995). Formulation and delivery of biocontrol agents for use against soilborne plant pathogens. In Biorational Pest Control Agents, F.R. Hall and J.W. Barry, eds (Washington, DC: American Chemical Society), pp. 166–182.

Maurhofer, M., Keel, C., Schnider, U., Voisard, C., Haas, D., and Defago, G. (1992). Influence of enhanced antibiotic production in *Pseudomonas fluorescens* strain CHA0 on its disease suppressive capacity. Phytopathology **82,** 190–195.

Maurhofer, M., Hase, C., Meuwly, P., Metraux, J.P., and Defago, G. (1994a). Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: Influence of the *gacA* gene and pyoverdine production. Phytopathology **84,** 139–146.

Maurhofer, M., Keel, C., Haas, D., and Defago, G. (1994b). Pyoluteorin production by *Pseudomonas fluorescens* strain CHA0 is involved in the suppression of *Pythium* damping-off of cress but not of cucumber. Eur. J. Plant Pathol. **100,** 221–232.

Maurhofer, M., Keel, C., Haas, D., and Defago, G. (1995). Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHA0 with enhanced antibiotic production. Plant Pathol. **44,** 40–50.

Mazzola, M., Cook, R.J., Thomashow, L.S., Weller, D.M., and Pierson III, L.S. (1992). Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. Appl. Environ. Microbiol. **58,** 2616–2624.

Mazzola, M., Fujimoto, D.K., Thomashow, L.S., and Cook, R.J. (1995). Variation in sensitivity of *Gaeumannomyces graminis* to antibiotics produced by fluorescent *Pseudomonas* spp. and effect on biological control of take-all of wheat. Appl. Environ. Microbiol. **61,** 2554–2559.

Milner, J.L., Stohl, E.A., and Handelsman, J. (1996a). Zwittermicin A resistance gene from *Bacillus cereus*. J. Bacteriol. **178,** 4266–4272.

Milner, J.L., Silo-Suh, L.A., Goodman, R.M., and Handelsman, J. (1996b). Antibiosis and beyond: Genetic diversity, microbial communities, and biological control. In Ecological Interactions and Biological Control, D. Andow, ed (Boulder, CO: Westview Press), in press.

Milner, J.L., Silo-Suh, L.A., Lee, J.C., He, H., Clardy, J., and Handelsman, J. (1996c). Production of kanosamine by *Bacillus cereus* UW85. Appl. Environ. Microbiol. **62,** 3061–3065.

Montesinos, E., and Bonaterra, A. (1996). Dose–response models in biological control of plant pathogens: An empirical verification. Phytopathology **86,** 464–472.

Natsch, A., Keel, C., Pfirter, H.A., Haas, D., and Defago, G. (1994). Contribution of the global regulator gene *gacA* to persistence and dissemination of *Pseudomonas fluorescens* biocontrol strain CHA0 introduced into soil microcosms. Appl. Environ. Microbiol. **60,** 2553–2560.

Neal, J.L., Jr., Larson, R.I., and Atkinson, T.G. (1973). Changes in rhizosphere populations of selected physiological groups of bacteria related to substitution of specific pairs of chromosomes in spring wheat. Plant Soil **39,** 209–212.

Neilands, J.B. (1981). Microbial iron compounds. Annu. Rev. Biochem. **50,** 715–731.

Neilands, J.B., and Nakamura, K. (1991). Detection, determination, isolation, characterization and regulation of microbial iron chelates. In CRC Handbook of Microbial Iron Chelates, G. Winkelmann, ed (London: CRC Press), pp. 1–14.

Nelson, E.B. (1990). Exudate molecules initiating fungal responses to seeds and roots. Plant Soil **129,** 61–73.

Nikaido, H. (1994). Prevention of drug access to bacterial targets: Permeability barriers and active efflux. Science **264,** 382–388.

O'Connell, K.P., Goodman, R.M., and Handelsman, J. (1996). Engineering the rhizosphere: Expressing a bias. Trends Biotechnol. **14,** 83–88.

Osbourn, A.E. (1996). Preformed antimicrobial compounds and plant defense against fungal attack. Plant Cell **8,** 1821–1831.

Osburn, R.M., Milner, J.L., Oplinger, E.S., Smith, R.S., and Handelsman, J. (1995). Effect of *Bacillus cereus* UW85 on the yield of soybean at two field sites in Wisconsin. Plant Dis. **79,** 551–556.

O'Sullivan, D.J., and O'Gara, F. (1992). Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol. Rev. **56,** 662–676.

Panagopoulos, C.G., Psallidas, P.G., and Alivizatos, A.S. (1979). Evidence of a breakdown in the effectiveness of biological control of crown gall. In Soil-Borne Plant Pathogens, B. Schippers and W. Gams, eds (New York: Academic Press), pp. 569–578.

Park, C., Paulitz, T.C., and Baker, R. (1988). Biocontrol of Fusarium wilt of cucumber resulting from interactions between *Pseudomonas putida* and nonpathogenic isolates of *Fusarium oxysporum*. Phytopathology **78,** 190–194.

Parke, J.L. (1990). Population dynamics of *Pseudomonas cepacia* in the pea spermosphere in relation to biocontrol of *Pythium*. Phytopathology **80,** 1307–1311.

Parke, J.L. (1991). Root colonization by indigenous and introduced microorganisms. In The Rhizosphere and Plant Growth, D.L. Keister and P.B. Cregan, eds (Boston, MA: Kluwer Academic Publishers), pp. 33–42.

Parke, J.L., Moen, R., Rovira, A.D., and Bowen, G.D. (1986). Soil water flow affects the rhizosphere distribution of a seed-borne biological control agent, *Pseudomonas fluorescens*. Soil Biol. Biochem. **18,** 583–588.

Paulitz, T.C., and Baker, R. (1987). Biological control of Pythium damping-off of cucumbers with *Pythium nunn*: Population dynamics and disease suppression. Phytopathology **77,** 335–340.

Pawlowski, K., and Bisseling, T. (1996). Rhizobial and actinorhizal symbioses: What are the shared features? Plant Cell **8,** 1899–1913.

Peterbauer, C.K., Heidenreich, E., Baker, R., and Kubicek, C. (1992). Effect of benomyl resistance on cellulase formation by *Trichoderma reesei* and *Trichoderma harzianum*. Can. J. Microbiol. **38,** 1292–1297.

Pfender, W.F., and Wootke, S.L. (1988). Microbial communities of *Pyrenophora*-infested wheat straw as examined by multivariate analysis. Microb. Ecol. **15,** 95–113.

Pfender, W.F., Kraus, J., and Loper, J.E. (1993). A genomic region from *Pseudomonas fluorescens* Pf-5 required for pyrrolnitrin produc-

tion and inhibition of *Pyrenophora tritici-repentis* in wheat straw. Phytopathology **83,** 1223–1228.

Pierson, E.A., and Weller, D.M. (1994). Use of mixtures of fluorescent pseudomonads to suppress take-all and improve the growth of wheat. Phytopathology **84,** 940–947.

Pierson III, L.S., and Pierson, E.A. (1996). Phenazine antibiotic production in *Pseudomonas aureofaciens*: Role in rhizosphere ecology and pathogen suppression. FEMS Microbiol. Lett. **136,** 101–108.

Pierson III, L.S., and Thomashow, L.S. (1992). Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens* 30-84. Mol. Plant-Microbe Interact. **5,** 330–339.

Pierson III, L.S., Keppenne, V.D., and Wood, D.W. (1994). Phenazine antibiotic biosynthesis in *Pseudomonas aureofaciens* 30-84 is regulated by PhzR in response to cell density. J. Bacteriol. **176,** 3966–3974.

Pierson III, L.S., Gaffney, T., Lam, S., and Gong, F. (1995). Molecular analysis of genes encoding phenazine biosynthesis in the biological control bacterium *Pseudomonas aureofaciens* 30-84. FEMS Microbiol. Lett. **134,** 299–307.

Raaijmakers, J.M., van der Sluis, I., Koster, M., Bakker, P.A.H.M., Weisbeek, P.J., and Schippers, B. (1995a). Utilization of heterologous siderophores and rhizosphere competence of fluorescent *Pseudomonas* spp. Can. J. Microbiol. **41,** 126–135.

Raaijmakers, J.M., Leeman, M., van Oorschot, M.M.P., van der Sluis, I., Schippers, B., and Bakker, P.A.H.M. (1995b). Dose–response relationships on biological control of Fusarium wilt of radish by *Pseudomonas* spp. Phytopathology **85,** 1075–1081.

Raffel, S., Stabb, E.V., and Handelsman, J. (1996). Genotypic and phenotypic analysis of zwittermicin A-producing strains of *Bacillus cereus*. Microbiology, in press.

Ross, A.F. (1961). Systemic acquired resistance induced by localized virus infections in plants. Virology **14,** 340–358.

Rovira, A.D. (1965). Interactions between plant roots and soil microorganisms. Annu. Rev. Microbiol. **19,** 241–266.

Rovira, A.D. (1969). Plant root exudates. Bot. Rev. **35,** 35–57.

Rovira, A.D. (1991). Rhizosphere research—85 years of progress and frustration. In The Rhizosphere and Plant Growth, D.L. Keister and P.B. Cregan, eds (Boston, MA: Kluwer Academic Publishers), pp. 3–13.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.-Y., and Hunt, M.D. (1996). Systemic acquired resistance. Plant Cell **8,** 1809–1819.

Sarniguet, A., Kraus, J., Henkels, M.D., Muehlchen, A.M., and Loper, J.E. (1995). The sigma factor σ^S affects antibiotic production and biological control activity of *Pseudomonas fluorescens* Pf-5. Proc. Natl. Acad. Sci. USA **92,** 12255–12259.

Schippers, B. (1993). Exploitation of microbial mechanisms to promote plant health and plant growth. Phytoparasitica **21,** 275–279.

Schnider, U., Keel, C., Blumer, C., Troxler, J., Defago, G., and Haas, D. (1995). Amplification of the housekeeping sigma factor in *Pseudomonas fluorescens* CHA0 enhances antibiotic production and improves biocontrol abilities. J. Bacteriol. **177,** 5387–5392.

Silo-Suh, L.A., Lethbridge, B.J., Raffel, S.J., He, H., Clardy, J., and Handelsman, J. (1994). Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. Appl. Environ. Microbiol. **60,** 2023–2030.

Spratt, B.G. (1994). Resistance to antibiotics mediated by target alterations. Science **264,** 388–393.

Stabb, E.V., Jacobson, L.M., and Handelsman, J. (1994). Zwittermicin A-producing strains of *Bacillus cereus* from diverse soils. Appl. Environ. Microbiol. **60,** 4404–4412.

Stockwell, V.O., Kawalek, M.D., Moore, L.W., and Loper, J.E. (1996). Transfer of pAgK84 from the biocontrol agent *Agrobacterium radiobacter* K84 to *A. tumefaciens* under field conditions. Phytopathology **86,** 31–37.

Tabashnik, B.E. (1994). Evolution of resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. **39,** 47–79.

Thomashow, L.S., and Weller, D.M. (1988). Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. J. Bacteriol. **170,** 3499–3508.

Torsvik, V., Goksoyr, J., and Daae, F.L. (1990a). High diversity in DNA of soil bacteria. Appl. Environ. Microbiol. **56,** 782–787.

Torsvik, V., Kare, S., Sorheim, R., and Goksoyr, J. (1990b). Comparison of phenotypic diversity and DNA heterogeneity in a population of soil bacteria. Appl. Environ. Microbiol. **56,** 776–781.

Turner, J.T., and Backman, P.A. (1991). Factors relating to peanut yield increases after seed treatment with *Bacillus subtilis*. Plant Dis. **75,** 347–353.

Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E., and Ryals, J. (1992). Acquired resistance in Arabidopsis. Plant Cell **4,** 645–656.

Vakili, N.G., and Bailey, T.B., Jr. (1989). Yield response of corn hybrids and inbred lines to phylloplane treatment with mycopathogenic fungi. Crop Sci. **29,** 183–190.

Vesper, S.J. (1987). Production of pili (fimbriae) by *Pseudomonas fluorescens* and correlation with attachment to corn roots. Appl. Environ. Microbiol. **53,** 1397–1405.

Vicedo, B., Penalver, R., Asins, M.J., and Lopez, M.M. (1993). Biological control of *Agrobacterium tumefaciens*, colonization, and pAgK84 transfer with *Agrobacterium radiobacter* K84 and the Tra^- mutant strain K1026. Appl. Environ. Microbiol. **59,** 309–315.

Voisard, C., Keel, C., Haas, D., and Defago, G. (1989). Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J. **8,** 351–358.

Waisel, Y., Eshel, A., and Kafkafi, U. (1991). Plant Roots: The Hidden Half. (New York: Marcel Dekker).

Ward, D.M., Weller, R., and Bareson, M.M. (1990). 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature **345,** 63–65.

Wei, G., Kloepper, J.W., and Tuzun, S. (1991). Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology **81,** 1508–1512.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. (1991). 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173,** 697–703.

Weller, D.M. (1983). Colonization of wheat roots by a fluorescent pseudomonad suppressive to take-all. Phytopathology **73,** 1548–1553.

Weller, D.M. (1988). Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. **26,** 379–407.

Weller, D.M., and Cook, R.J. (1983). Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology **73,** 463–469.

Whipps, J.M., and Lumsden, R.D. (1991). Biological control of *Pythium* species. Biocontrol Sci. Technol. **1,** 75–90.

Wilhite, S.E., Lumsden, R.D., and Straney, D.C. (1994). Mutational analysis of gliotoxin production by the biocontrol fungus *Gliocladium virens* in relation to suppression of Pythium damping-off. Phytopathology **84,** 816–821.

Wood, D.W., and Pierson III, L.S. (1996). The *phzI* gene of *Pseudomonas aureofaciens* 30-84 is responsible for the production of a diffusible signal required for phenazine antibiotic production. Gene **168,** 49–53.

Yuen, G.Y., and Schroth, M.N. (1986). Interactions of *Pseudomonas fluorescens* strain E6 with ornamental plants and its effect on the composition of root-colonizing microflora. Phytopathology **76,** 176–180.

Zdor, R.E., and Anderson, A.J. (1992). Influence of root colonizing bacteria on the defense responses of bean. Plant Soil **140,** 99–107.

Zhou, T., and Paulitz, T.C. (1994). Induced resistance in the biocontrol of *Pythium aphanidermatum* by *Pseudomonas* spp. on cucumber. J. Phytopathol. **142,** 51–63.