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Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases

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Abstract

Plant-associated bacteria reside in the rhizosphere, phyllosphere, and inside tissues of healthy plants. This chapter discusses concepts and examples of how naturally occurring and introduced bacteria may contribute to management of soilborne and foliar diseases. Introduced bacteria which have demonstrated biological control activity against soilborne pathogenic fungi and nematodes include rhizobacteria (root-colonising bacteria) and endophytic bacteria (bacteria isolated from within healthy plant tissues). Recently, some introduced rhizobacteria have been found to enhance plant defences, leading to systemic protection against foliar pathogens upon seed or root-treatments with the rhizobacteria. In these cases, introduction of the rhizobacteria results in reduced damage to multiple pathogens, including viruses, fungi and bacteria. An alternative strategy to the introduction of specific antagonists is the augmentation of existing antagonists in the root environment. This augmentation may result from the use of specific organic amendments, such as chitin, which stimulate populations of antagonists, thereby inducing suppressiveness. Inter-cropping or crop rotation with some tropical legumes, including velvetbean (*Mucuna deeringiana*), lead to management of phytoparasitic nematodes, partly through stimulation of antagonistic microorganisms. Some biorational nematicides, such as specific botanical aromatic compounds, also appear to induce suppressiveness through alterations in the soil microbial community.

Introduction

Plant-associated microorganisms have been extensively examined for their roles in natural and induced suppressiveness of soilborne diseases. Among the many groups of such organisms are root-associated bacteria, which generally represent a subset of soil bacteria. Rhizobacteria are a subset of total rhizosphere bacteria which have the capacity, upon re-introduction to seeds or vegetative plant parts (such as potato seed pieces), to colonise the developing root system in the presence of competing soil microflora. Root colonisation is typically examined by quantifying bacterial populations on root surfaces; however, some rhizobacteria can also enter roots and establish at least a limited endophytic phase. Hence, root colonisation may be viewed as a continuum from the rhizosphere to the rhizoplane to internal tissues of roots. Some endophytic bacteria transcend the endodermis, crossing from the

root cortex to the vascular system and subsequently can be located in stem tissues above ground.

Because rhizobacteria and endophytes are part of the natural microflora of healthy plants, they may be considered to be important contributors to plant health and general soil suppressiveness. Approaches to extending this concept to use bacteria for control of diseases generally involve either application of specific introduced bacterial strains as biological control agents or various cultural and agronomic practices to induce suppressiveness via alterations in the community of rhizosphere microorganisms.

This review will present concepts of using introduced and naturally occurring bacteria for control of soilborne diseases by discussing selected examples. Recent evidence that disease control by this means may extend to systemic and foliar diseases will be discussed.

Plant Growth-Promoting Rhizobacteria (PGPR)

Rhizobacteria which exert a beneficial effect on the plant being colonised are termed 'Plant Growth-Promoting Rhizobacteria'. PGPR may benefit the host by causing plant growth promotion or biological disease control. The same strain of PGPR may cause both growth promotion and biological control. Efforts to select and apply PGPR for control of specific soilborne fungal pathogens have been reviewed (Klopper 1993; Glick and Bashan 1997). Among the soilborne pathogens shown to be negatively affected by PGPR are *Aphanomyces* spp., *Fusarium oxysporum*, *Gaeumannomycesgraminis*, *Phytophthora* spp., *Pythium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Thielaviopsis basicola*, and *Verticillium* spp. In most of these cases, biological control results from bacterial production of metabolites which directly inhibit the pathogen, such as antibiotics, hydrogen cyanide, iron-chelating siderophores, and cell wall-degrading enzymes. Plant growth promotion by PGPR may also be an indirect mechanism of biological control, leading to disease escape when the growth promotion results in shortening the time that a plant is in a susceptible state, e.g. in the case where PGPR cause enhanced seedling emergence rate, thereby reducing the susceptible time for preemergence damping-off. An alternative mechanism for biological control by PGPR is induced systemic resistance which is discussed in the final section of this review.

Endophytes More recently, investigations using endophytic bacteria as potential biological control agents of some fungal diseases have been reported (Chen et al. 1995; Hinton and Bacon 1995). The designation of 'endophyte' can be used restrictively to refer only to those microorganisms which reside in vascular tissues of the plant and hence move freely inside the plant, or it can be used more broadly to refer to any microorganism which resides inside the plant without regard to the specific tissue colonised (Hallmann et al. 1997a). We prefer the broad definition and define endophytic bacteria functionally as those bacteria which can be isolated from inside surface-disinfested plants. It was originally reasoned that endophytic bacteria which could colonise vascular tissues of plants would be potential antagonists of vascular-invading pathogens, such as *Fusarium oxysporum* and *Verticillium* spp. (Hallmann et al. 1997 a). It was also presumed that an

introduced endophytic bacterium would be subjected to much less microbial competition than an introduced rhizobacterium because of less diversity and lower populations of indigenous microorganisms inside plant tissues compared to on roots and in the rhizosphere. However, initial studies on the microbial ecology of bacterial endophytes suggested that bacterial diversity among endophytes was similar to diversity in the rhizosphere, although total population densities were less within plants (Hallmann et al. 1997 a).

To use plant-associated bacteria in practical crop production, practical methods of inoculum delivery must be developed. Methods developed for delivery of rhizobacteria also allow introduction of endophytic bacteria, although the optimum delivery system can be different for different endophytic bacterial strains (reviewed in Hallmann et al. 1997 a).

Biocontrol of nematodes Compared to the substantial volume of work reported with the use of bacteria as biological control agents of soilborne pathogenic fungi, considerably less work has been done examining the potential of plant-associated bacteria to control phytoparasitic nematodes. A few reports indicate that both rhizobacteria and endophytic bacteria have the potential to reduce plant damage to nematodes. Suggested mechanisms include induction of systemic resistance and production of antagonistic compounds, however, definitive mode-of-action studies have not been done with these systems. Some rhizobacteria isolated from a field site suppressive to peach tree short life were suppressive to population increases of the ring nematode, *Criconebella xenoplax* (Kluepfel et al. 1993). In a separate study, some endophytic bacterial strains from cotton significantly reduced galling of cotton roots by the root-knot nematode, *Meloidogyne incognita* (Hallmann et al. 1997 b). In the same study (Hallmann et al. 1997 b), it was reported that nematode populations were correlated with establishment of endophytic bacteria within roots, suggesting that root-penetrating nematodes provide increased entry sites for endophytic bacteria.

Alteration of indigenous bacterial communities

An alternative or supplemental approach to introducing specific PGPR or endophytic bacteria as biological control agents is to manipulate the indigenous bacterial communities of the rhizosphere or

endorhiza in a manner which leads to enhanced soil suppressiveness to nematodes or other soilborne pathogens.

Recent work with several nematode control strategies demonstrates that it is possible to achieve at least a limited induced soil suppressiveness, through shifts in microbial community structure and function, by several cultural practices. Examples of such practices include the use of organic amendments, inclusion of antagonistic plants in cropping systems, and applications of biorational nematicides.

Among the many organic amendments which have been demonstrated to be efficacious in leading to control of plant parasitic nematodes, chitin has been perhaps most extensively studied. Amendment of soil with chitin leads to enhanced activity of chitinolytic microorganisms, and it has been considered that the observed biocontrol results partly from activity of chitinases on nematode eggs and partly from the accumulation of ammonium, which is toxic to nematodes, by microbial deamination of the chitin polymers of acetylglucosamine and chitobiose. However, changes in one component of the microflora in a community often lead to other changes, and it was recently reported that soil amendment with 1% chitin led to alterations in the taxonomic structure of the bacterial communities of the soil, rhizosphere and endorhiza (Hallmann *et al.* 1998). Several bacterial species were found in chitin-amended soils and cotton rhizospheres which were not detected in non-amended soils and rhizospheres. Even more surprising, amendment of soil with chitin selectively influenced the community structure of endophytic bacteria within cotton roots. For example, *Phyllobacterium rubiacearum* was not a common endophyte following chitin amendment, although its populations in soil were stimulated by chitin, while *Burkholderia cepacia* was the dominant endophyte following chitin amendment but was rarely found among the endophytic community of non-amended plants. Hence, alterations in microbial community structure are associated with the control of nematodes which occurs upon soil amendment with chitin.

Monoculture of crops in some geographical areas has been reported to lead to reduced disease severity by induced suppressiveness resulting from population increases of a specific antagonistic or parasitic microbial group, as in the case of take-all decline (Cook and Baker 1983) and cereal cyst nematode decline (Stirling 1991). However, this is not a

universal phenomenon. In many parts of the world, especially in highly eroded soils with poor fertility and low organic matter, such as the southeastern United States of America, crop monoculture leads to continually declining yields, without inducing suppressiveness, even after over 20 years of monoculture (reviewed in Rodríguez-Kábana and Canullo 1992). However, suppressiveness can be induced in these soils through specific crop rotation systems which include planting with some tropical legumes, such as velvetbean (*Mucuna deeringiana*), which has shown nematode control potential as either a rotational crop or an inter-planted crop in diverse geographical areas, including the southeastern United States of America, Puerto Rico, Brazil, Mexico, and Spain (reviewed in Vargas-Ayala *et al.* 1999). Velvetbean produces nematicidal compounds which were initially considered to explain the observed control of nematodes. However, reductions in populations of juveniles of *Meloidogyne arenaria* with velvetbean persisted throughout the following season on a susceptible peanut crop (Rodríguez-Kábana *et al.* 1992), while with chemical nematicides the end-of-season populations usually recover to pre-treatment levels. This unexpected persistence of nematode antagonism with velvetbean suggested that soil suppressiveness had been induced via activity of antagonistic microflora. Recent studies on the effect of velvetbean on soil and rhizosphere microflora support this conclusion. Planting of velvetbean resulted in significantly lower fungal populations at the end of the season and lower bacterial populations the following season, compared to cowpea, another legume without antagonistic properties to nematodes (Vargas-Ayala *et al.* 1999). In the same study, velvetbean also influenced bacterial diversity, generally increasing frequency of bacilli, *Arthrobacter* spp., and *Burkholderia cepacia*, while reducing fluorescent pseudomonads. All of these shifts occurred simultaneously with an increase in antagonism to nematodes and biological control.

Another approach for control of nematodes which may lead to at least a limited induction of suppressiveness through microbial activity is the use of selected 'biorational' compounds. Botanical aromatics include non-phytotoxic, biodegradable compounds of plant origin with nematode control activity, and hence, they act as biorational nematicides. Three such botanical aromatics, furfural, citral and benzaldehyde have shown potential for control of both fungal pathogens and phytoparasitic nema-

todes (reviewed in Bauske *et al.* 1997) and they do not reduce colonisation of cotton roots by PGPR. Application of furfural and benzaldehyde to soil causes both quantitative and qualitative shifts in the composition of the soil bacterial community (reviewed in Bauske *et al.* 1997). After decreasing in the first 24 h after application, bacterial populations increased by 1 week after application and remained higher than in non-treated control soils for 7 weeks. There was a corresponding increase in frequency of *Burkholderia cepacia* in treated soils.

These results with botanical aromatics demonstrate that a biorational compound may have dual activity, causing direct inhibition or kill of the pathogen and also stimulating shifts in the soil or rhizosphere microbial communities which lead to enhanced antagonism. This also appears to be the case with a new biorational nematicide, DiTera, produced from fermentational broth of a fungus by Abbott Labs. Application of the compound to non-autoclaved soil resulted in enhanced parasitism of eggs of *Meloidogyne incognita*, but there was no effect on antagonism in autoclaved soil, indicating that native soil microorganisms are required for the observed development of antagonism (Fernandez *et al.* 1999). Application of DiTera also increased bacterial populations and enzyme activities in soil in the first week and subsequently increased bacterial populations and enzyme activities in rhizospheres of tomato planted into treated soil, compared to non-treated controls.

Extension of biocontrol to systemic and foliar pathogens

Some approaches to the control of soilborne pathogens can also be used to target systemic and foliar pathogens. This occurs through the action of an inducing agent which systemically activates the host plant's chemical or physical defence mechanisms, leading to reduced symptoms to pathogens which infect tissues distal to the inducer, a process referred to as 'induced systemic resistance' (ISR) or 'systemic acquired resistance.' Some strains of PGPR, with limited endophytic colonisation, can act as inducers.

Subsequent research at Auburn University has demonstrated PGPR-ISR against various fungal and bacterial pathogens of cucumber. More recent experiments with PGPR-ISR against bacterial wilt of cucurbits, caused by *Erwinia tracheiphila*, have

shown that feeding by the cucumber beetle, a vector of the pathogen, was reduced on PGPR-treated cucumber plants. Field experiments in cucumber demonstrated that seed treatment with PGPR resulted in significantly lower numbers of cucumber beetles, *Diabrotica undecimpunctata howardi* and *Acalymma vittatum*, and lower incidence of bacterial wilt compared with non-treated control plants and plants sprayed weekly with the insecticide esfenvalerate (Zehnder *et al.* 1997a). Free choice greenhouse experiments with cucumber beetles were conducted in which beetles that had acquired the wilt pathogen were released in screen cages and allowed to feed on PGPR- or non-treated plants. Cucumber beetle feeding damage on cotyledons and stems and incidence of wilt symptoms were significantly lower on PGPR-treated plants than on non-treated control plants (Zehnder *et al.* 1997b). Because the feeding behaviour of cucumber beetles is strongly influenced by cucurbitacins, a group of triterpenoid plant metabolites that occur in the plant family Cucurbitaceae, and previous studies demonstrated a positive correlation between cucurbitacin content and cucumber beetle feeding, we hypothesised that plants induced by PGPR treatment may contain reduced concentrations of cucurbitacin. To test this, we subjected cotyledon leaves of PGPR-treated and untreated cucumber to HPLC analysis for cucurbitacin. This test included two cucumber cultivars; one with high and one with constitutive low levels of cucurbitacin. Results indicated that PGPR-treated plants of both cultivars contained significantly lower levels of cucurbitacin than untreated plants (Zehnder *et al.* 1997b). This suggested that a mechanism for PGPR-induced resistance against cucumber beetle feeding involves a change in the metabolic pathway for cucurbitacin synthesis.

Some of the same PGPR strains used to induce resistance of cucumber against the bacterial pathogen which causes cucurbit wilt were tested for induced protection of tomato against cucumber mosaic cucumovirus (CMV) (Yao *et al.* 1997). Four PGPR strains were selected from among 26 strains tested in the greenhouse, all of which previously demonstrated significant protection of cucumber against anthracnose disease, caused by *Colletotrichum orbiculare*. These four strains, which were identified as *Bacillus pumilus*, *B. amyloliquifaciens*, *B. subtilis* and *Kluyvera cryocrescens*, were tested in two field trials in 1996. PGPR were applied as seed treatments and soil drench at transplanting. In the first trial, CMV was mechanically inoculated onto

plants, whereas in the second trial, plants were naturally infected with CMV by indigenous aphids. In both trials, treatment with all four PGPR strains resulted in significant reductions in symptom development as measured by AUDPC (area under the disease progress curve). In addition, all strains resulted in reduced percent infection as well as in reductions in accumulation of CMV, based on ELISA in both trials. Yield was significantly enhanced by all PGPR strains in trial 1 but by none in trial 2.

Hence, PGPR-mediated biocontrol can be extended to foliar and systemic diseases, even when the PGPR are applied only to seeds and roots, if the mechanism for control involves induction of host defences. Conceptually, it should also be possible to induce resistance via the use of some of the techniques described above to enhance antagonism of indigenous soil bacteria. For example, use of certain organic amendments such as chitin or certain biorational nematocides such as furfural could act as elicitors, and further work should be aimed at testing this hypothesis.

As described briefly in this review, plant associated bacteria can have diverse interactions with plant roots. We are beginning to be able to direct these interactions into practical benefits for crops by the introduction of specific PGPR or endophytic bacteria or by the targeted enhancement of components of the indigenous soil bacterial community. The future challenge will be to integrate these approaches to provide an economically significant level of induced suppressiveness against crop pathogens and pests.

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