

The Nature and Application of Biocontrol Microbes: *Bacillus* spp.Ecology of *Bacillus* and *Paenibacillus* spp. in Agricultural Systems

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ABSTRACT

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Diverse populations of aerobic endospore-forming bacteria occur in agricultural fields and may directly and indirectly contribute to crop productivity. This paper describes recent advances in our understanding of the ecology of *Bacillus* and *Paenibacillus* spp. and how different subpopulations of these two genera can promote crop health. The abundance, diversity, and distribution of native populations and inoculant strains in agricultural fields have been characterized using a variety of methods. While native populations of these two genera occur abundantly in most agricultural soils, plant tissues are differentially colonized by distinct

subpopulations. Multiple *Bacillus* and *Paenibacillus* spp. can promote crop health in a variety of ways. Some populations suppress plant pathogens and pests by producing antibiotic metabolites, while others may directly stimulate plant host defenses prior to infection. Some strains can also stimulate nutrient uptake by plants, either by promoting rhizobial and mycorrhizal symbioses or by fixing atmospheric nitrogen directly. Despite a wealth of new information on the genetics and physiology of *Bacillus* and related species, a better understanding of the microbial ecology of these two genera must be developed. To this end, several important, but unanswered, questions related to the ecological significance and potential for managing the beneficial activities of these bacteria are discussed.

Aerobic endospore-forming bacteria (AEFB) are essentially ubiquitous in agricultural systems. Common physiological traits important to their survival include production of a multilayered cell wall structure, formation of stress-resistant endospores, and secretion of peptide antibiotics, peptide signal molecules, and extracellular enzymes. However, significant variation exists in other key traits, including nutrient utilization, motility, and physiochemical growth optima. Quantitative and qualitative variations in these traits allow for these bacteria to inhabit diverse niches in agroecosystems. Their microscopic size and omnipresence in soils facilitates their colonization of plants and animals, but the degree of niche localization of most species has not been thoroughly studied. Indeed, the ecological significance of the genotypic and phenotypic diversity of named species of *Bacillus* and related genera remains largely a mystery. Here we review recent advances in our understanding about the diversity, distribution, and activities of these bacteria and how they relate to plant health management.

Diversity and distribution in agricultural fields. Multiple *Bacillus* and *Paenibacillus* spp. can be readily cultured from both bulk and rhizosphere soils. Culturable counts of these bacteria generally range from log 3 to log 6 cells per gram fresh weight, with soil counts typically exceeding those obtained from the rhizosphere (25,44,65,75). Standard isolations on complex media typically yield multiple isolates of phylogenetically and phenotypically similar species related to *B. subtilis* and *B. cereus*. Most distinctive among these morphologically is *B. mycoides*, which often confound attempts to accurately enumerate cultured populations by virtue of their rapid mycelial-like growth patterns on agar media. *B. megaterium* has been reported to be one of the most abundant in some soils (42), but it seems unlikely that a single

species will dominate numerically in most soils. While multiple species of *Paenibacillus* can be detected in the soils and rhizosphere (65), less work has been done to indicate which might be the most commonly isolated species.

Culture-independent analyses of soil DNA have confirmed the presence of the easily cultured species and revealed additional, uncultured diversity in both the *Bacillus* and *Paenibacillus* rRNA lineages (7,18,21,70). However, contradictory evidence exists on the relative abundance of cultured and uncultured representatives of these genera in different soils. Some reports indicated that the large majority of *Bacillus*-like sequences cloned from soils (21, 70) were highly similar to known species. But, others report that the dominant *Bacillus* sequences present in a different soil are not the same as those present in easily cultured isolates (7,18). Interestingly, the substantial effort leading to the isolation of this previously uncultured lineage (referred to as DA001) also led to the isolation of even more microdiversity that had not been previously directly detected in DNA clone banks of sequences obtained from the same soil (20).

Genetic microdiversity within different *Bacillus* and *Paenibacillus* species is known to exist, though to varying degrees. Probably the most studied topic has been the intra- and interspecies variation among *B. anthracis*, *B. cereus*, and *B. thuringiensis* strains. The phylogenetic relatedness of these three was initially established by both phenotypic and genotypic characterizations (59). More recently, the degree of homogeneity of this group has been further established by analyses of multiple genetic loci (29) and amplified fragment length polymorphisms (73). Nonetheless, numerous genomic differences have been found and may be useful to distinguish the three named species (31,60). Different isolates of *B. subtilis* could be distinguished using a variety of genotypic and phenotypic tests, but biocontrol functions have not been strictly correlated with any of these (46). In *B. thuringiensis*, variation in *cry* genes (which encode the crystal proteins that are toxic to various invertebrate species) is well known (72) and continues to be investigated (9,47). This plasmid-

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encoded diversity is in part responsible for the variation in virulence and host range of different *B. thuringiensis* isolates (72). In contrast, most plasmids in *B. subtilis* are cryptic and appear to be unusually homologous (82). In *B. cereus*, the gene for production of and resistance to the antibiotic zwittermicin A was successfully used to identify strains with similar capacities to suppress plant pathogens (61,71). In one study, zwittermicin A-producing strains with differing membrane composition (as determined by fatty acid methyl ester profiles) were observed to occur in different soils (61). Different strains of *P. azotofixans* (62,65) and *P. polymyxa* (17) have also been identified, but no functional distinction was made among the isolates analyzed. Heterogeneity in the DNA sequences of the dinitrogenase reductase gene, *nifH*, has been used to distinguish *P. azotofixans* from *P. macerans* and *P. polymyxa* strains (62). Genetic exchange among *Bacillus* spp. can occur, but recent studies indicate that such exchange is relatively modest in soils (77). Such investigations represent the first steps in linking genetic markers to niche specificity and the ecological activities of functionally distinct subpopulations in these two genera.

Phenotypic characterizations of culture isolates have revealed a tremendous degree of ecologically relevant diversity at the species level in the AEFB (59). The most obvious distinction exists for invertebrate pathogens, such as *P. larvae*, *P. lentimorbus*, *P. popilliae*, which are relatively fastidious, and *B. sphaericus* and *B. thuringiensis*, both of which are more capable of saprophytic growth (72). Nitrogen fixation occurs in *P. azotofixans*, *P. macerans*, and *P. polymyxa*, but does not seem to occur in *Bacillus* spp. (1). Some species were initially defined based on the extreme physical or chemical conditions under which they were first isolated (e.g., *B. psychrophilus*), but few examples obligate extremophiles exist (e.g., *B. stearomophilus*, which are typically isolated from thermophilic composts) (59). Instead, niche specificity and important ecological activities in *Bacillus* and *Paenibacillus* spp. appear to span phylogenetic boundaries. Most species can survive as saprophytes in soils, which are considered the primary reservoirs of these bacteria; however, most viable cells probably occur as inactive spores at any given time (55). Furthermore, multiple species can be recovered as epiphytes and endophytes of plants and animals, as well as foodstuffs and composts derived from them (59,69,72). The rich variety of organic substrates and micro-niches present in those environments support a complex milieu of microbial species, so it is perhaps not surprising that multiple species of *Bacillus* and *Paenibacillus* inhabit them. Thus, it is reasonable to infer that functionally distinct isolates occur within and among the phylogenetically distinct species of these two genera. This is clearly the case for isolates functionally defined as “beneficial” to plant health because only a fraction of isolates of any given species can be shown to inhibit the activities of a pathogen under a given set of conditions. For example, we have observed that just under half of the *B. subtilis*-like ribotypes and fewer than 10% of the *B. cereus*-like ribotypes cultured from soybean rhizospheres inhibited the growth of damping-off pathogens of soybeans *in vitro* (data not shown).

Recently, ribosomal sequences amplified from environmental samples (41) have been used to characterize the relative distribution of *Bacillus* and *Paenibacillus* spp. between soils and plant tissues. Overall, the structure of soil bacterial communities is known to vary with soil type more than with management regime (23 and references therein); however the magnitude of such variation may be relatively small for *Bacillus* and *Paenibacillus* spp. We have performed terminal restriction fragment length polymorphism analyses of these populations using the novel, group-specific primers Ba1F and Ba2R. This approach allows us to characterize multiple populations of AEFB that may promote plant health (Table 1). In those assays, we observed only minor quantitative differences in the relative abundance of different *Bacillus*-like ribotypes occurring at multiple sites throughout Ohio

(data not shown). Previously, DNA-based studies of bacteria, including AEFB, revealed relatively little variation in the abundance of soil microbes, even though distinct differences in community structure could be observed in adjacent sites differing in land management practices (19,21). At the species level, most *Bacillus* and *Paenibacillus* are globally distributed (59), and such widespread occurrence of more defined subspecies of *B. subtilis* and *B. cereus* with the capacity to suppress plant pathogens has also been reported (58,71). Other studies have reported only a limited degree of geographic endemicity in *B. thuringiensis* (9,13) and *P. azotofixans* (65) over spatial scales similar to our studies in Ohio. In contrast, highly significant differences were observed in the total and relative abundance of *Bacillus*-like sequences amplified from bulk soil and crop roots. This is indicated by the large difference in the relative abundance of terminal restriction fragments (TRFs) (measured as total fluorescent peak areas) amplified from bulk soil compared with similar quantities of root tissue on a fresh-weight basis (Fig. 1). This is consistent with analyses of whole bacterial communities that showed similar distinctions between soil and rhizosphere communities (70). Previously, the diversity of *P. azotofixans* in bulk soil was observed to be greater than that in the rhizosphere (62). Furthermore, the genotypes of soil populations of *P. azotofixans* and *P. polymyxa* have been reported to differ to some extent from rhizosphere populations colonizing corn and wheat, respectively (48,65). Culture-based studies on bacterial endophytes indicated that *Bacillus* spp. were present inside various plant tissues; however, their population size is generally quite low (24). At the level of ribotype, we did not

TABLE 1. Terminal restriction fragments (TRFs) predicted to correspond to well-studied *Bacillus* and *Paenibacillus* spp., as well as nontarget species of *Staphylococcus* and *Lactobacillus* containing homologous priming sites for the group-specific primers Ba1F and Ba2R^a

TRF	<i>Bacillus</i> spp.	<i>Paenibacillus</i> spp.	Other genera
M37	<i>caldolyticus</i> <i>stearomophilus</i> <i>thermodenitrificans</i>	<u><i>larvae</i></u> <i>macerans</i> <u><i>popilliae</i></u>	
M48	<i>acidovorans</i> <i>amyloliquefaciens</i> <i>anthracis</i> <i>ceruus</i> <i>coagulans</i> <i>firmus</i> <i>globisporus</i> <i>licheniformis</i> <i>mycoides</i> <i>pumilus</i> <u><i>sphaericus</i></u> <u><i>subtilis</i></u> <u><i>thuringiensis</i></u>	<u><i>lentimorbus</i></u> <u><i>popilliae</i></u>	<i>S. piscifermentans</i> <i>S. saprophyticus</i> <i>S. schleiferi</i>
M49		<i>azotofixans</i> <i>polymyxa</i>	
M55	<u><i>sphaericus</i></u>		
M56	<i>benzoevorans</i> <i>circulans</i> <u><i>lentus</i></u> <i>macroides</i>		<i>S. aureus</i> <i>S. haemolyticus</i> <i>S. saprophyticus</i>
M68	<i>alcalophilus</i> <i>fastidiosus</i> <i>megaterium</i>		<i>L. acidophilus</i> <i>L. acetotolerans</i>

^a The predicted lengths of TRFs were determined using the TRFLP-TAP tool (45) based on user-supplied information of primer sequences and restriction enzymes to be used for analysis. In this instance, primer Ba1F and the restriction enzyme *MspI* were used for discrimination of various taxa. TRFs are designated by a one-letter abbreviation for the restriction enzyme used and the length in base pairs. The names of species previously identified as having the potential to promote plant growth or suppress plant pathogens are indicated in bold, and the names of insect pathogens are underlined. The named *Staphylococcus* and *Lactobacillus* species are known animal epiphytes and pathogens.

observe any significant variation in population structure of *Bacillus*-like bacteria between roots of corn and soybeans planted in the same field (data not shown), but significant variation was observed among the different microenvironments of bulk soil, roots, and leaves (Fig. 2). *B. megaterium* has been reported to be one of the most abundant populations of culturable AEFB present in the soybean rhizosphere (42), and *B. pumilus* and *B. subtilis* were reported to be the most abundant bacteria cultured from the phyllosphere of soybeans (2). Interestingly, TRFs of the size predicted for those three species (Table 1) were among the most abundant signals observed in our profiles of root- and leaf-inhabiting *Bacillus* populations on soybeans (Fig. 2). Such observations indicate that different subsets of AEFB predominate in different soil and plant compartments. Better characterization of such niche differentiation could aid in the identification and application of *Bacillus* and *Paenibacillus* inoculants for plant disease control.

Impact on plant health. Numerous *Bacillus* and *Paenibacillus* strains express activities that suppress pests and pathogens or otherwise promote plant growth. A number of these strains already have been developed commercially as biological fungicides, insecticides, and nematicides or generic plant growth promoters, and their use in agriculture has recently been reviewed (36,51,57,66, Jacobsen et al. [this volume]). Improvements in plant health and productivity are mediated by three different ecological mechanisms: antagonism of pests and pathogens, promotion of host nutrition and growth, and stimulation of plant host defenses. The last of these is reviewed in this volume by Kloepper et al. (this volume) and will not be discussed extensively here.

Antagonism of pest and pathogen populations by *Bacillus* sp. and closely related AEFB takes many forms. Some species are pathogens of insects (72) or nematodes (66). Perhaps the most studied of the insect pathogens are those classified as *B. thuringiensis*.

This species is distinguished from the common saprophytic species *B. cereus* by the occurrence of plasmids that encode pathogenicity factors that make the strains pathogenic to various invertebrates. The production of the crystalline inclusion bodies within their spores allow for opportunistic growth when consumed by soil invertebrates. While the crystalline proteins are widely known to be disruptive to the digestive tracts of numerous Lepidoptera and Diptera larvae, evidence also exists for their toxicity to nematodes (78). The wide variation in *cry* gene structure and the known occurrence of tolerance to the protein toxins produced by various isolates indicates that a range of virulence exists in nature. *B. sphaericus* are pathogenic to various Diptera species, but the species appears to be more effective at controlling insects that bite animals and humans rather than those that damage crops. *B. sphaericus* also produce protein toxins, but these are deposited outside the spore coat by the mother cell. *P. popillae* and *P. lentimorbus* cause milky disease in the larvae of some beetles (order Coleoptera) including those that can damage crops. However, not all insect pathogens are beneficial to crop growth. *P. larvae* causes American foulbrood disease of honeybees (*Apis mellifera*), a disease that can significantly reduce pollination activity in fruit and vegetable crops. As a rule, the pathogens belonging to *Paenibacillus* spp. appear to be more fastidious in their growth habits than those of *Bacillus*, indicating that pathogenic species of the latter genera are more likely to be widely distributed in agricultural soils.

Similarly, strong antagonism to plant pathogens is known to occur, but is not strictly correlated with named species. Several species of *Bacillus* are known to produce toxins that are inhibitory to the growth and/or activities of fungal and nematode pathogens of plants. The most thoroughly studied of these include *B. subtilis* (3,8,28,34,38,40,58). Additionally, a number of studies

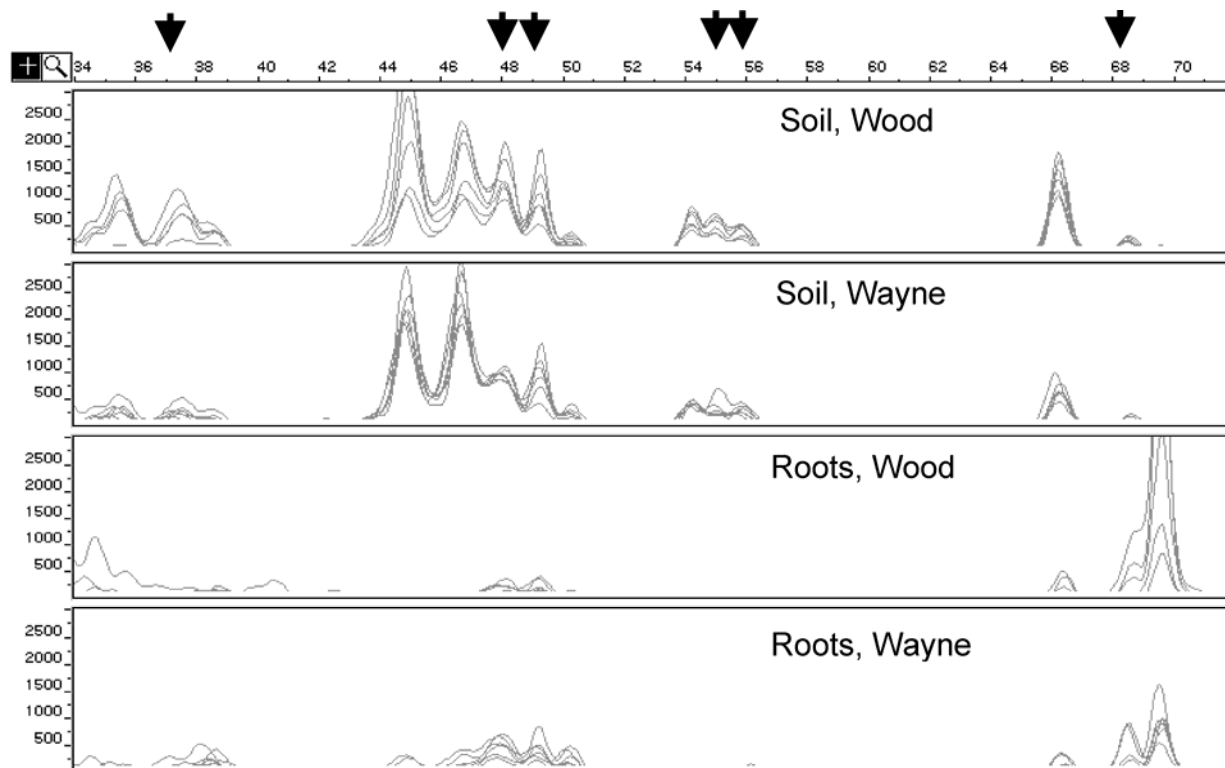


Fig. 1. Terminal restriction fragment (TRF) length polymorphism profiles of *Bacillus*-like sequences amplified from two different cornfields. Template DNA was isolated from bulk soil and the roots of corn plants sampled from two different Ohio counties ($n = 6$, $N = 24$). 16S sequences were amplified using Ba1F-HEX and Ba2R and digested with *Msp*I. Digested products were loaded onto an ABI 377 DNA sequencer for separation, detection of labeled fragments, and subsequent analyses with GeneScan 3.0. Each panel contains overlaid profile traces from six independent samples to indicate the degree of natural variation present across the area sampled (3 m \times 10 m) at each location. The size of TRFs in base pairs is shown on the x axis at the top of the figure, and the relative abundance of each ribotype is indicated by fluorescence intensity measured in arbitrary units on the y axis. Arrows point to TRFs indicative of *Bacillus* and *Paenibacillus* spp. with the capacity to promote plant health.

have reported direct antagonism by other species including *B. amyloliquefaciens*, *B. cereus*, *B. licheniformis*, *B. megaterium*, *B. mycoides*, and *B. pumilus* as well as isolates of unidentified species from the genus (27,37,38,42,53,81). Although less frequently reported in the literature, some isolates of *P. macerans* and *P. polymyxa* may also be antagonistic to plant pathogens (37). Most studies focus on control of fungal and oomycete pathogens, but other isolates of these same species have been reported to antagonize pathogenic nematodes belonging to the genera *Heterodera* and *Meloidogyne*. Spore-forming *Pasteuria* spp. are well-known parasites of nematodes, but some studies indicate that other AEFB may sometimes contribute to suppression of plant-parasitic nematodes (54,66). Catabolic enzymes (e.g., proteases, chitinases, and glucanases), peptide antibiotics, and small molecules can be secreted by various species (59) and may all contribute to pathogen suppression. Peptide antibiotics and other compounds toxic to plant pathogens have been isolated from several *Bacillus* strains (3,28,34,38,40,58,67,81). The importance of antibiotic production to plant disease suppression by *Bacillus* spp. has been demonstrated. *B. subtilis* strains that produce the lipopeptide antibiotics iturin A and surfactin could suppress damping-off in tomato while mutants could not (3). And, in *B. cereus*, production and resistance to zwittermicin A have been correlated to suppression of damping-off in alfalfa (61). Antagonism may also involve competition for niche space and nutrients with other chemoheterotrophs in the phytosphere. Motile and chemotactic strains of *B. megaterium* were shown to better colonize roots and suppress *Rhizoctonia solani* better than nonmotile derivatives (83).

Bacillus and *Paenibacillus* populations may also promote plant health by stimulating the plant host or mutualistic symbionts. Induction of host resistance pathways, locally and systemically, has been reported for some isolates of *B. amyloliquefaciens*, *B. cereus*, *B. mycoides*, *B. pumilus*, *B. sphaericus*, and *B. subtilis* (Klopper et al., [this volume]). Induced resistance to viral, bacterial, oomycete, fungal, and nematode pathogens as well as insect pests may be conferred to varying degrees, depending on a variety of biotic and abiotic factors. Plant hosts may also be affected by hormones known to be produced by various microbial species including *B. subtilis* (59). Such compounds (i.e., auxins, gibberellins, and cytokinins) mediate processes such as plant cell enlargement, division, and extension in symbiotic as well as

nonsymbiotic roots. Some isolates of *B. subtilis*, *B. cereus*, and *B. thuringiensis* have been reported to stimulate symbioses between *Bradyrhizobium japonicum* and soybeans, though such synergistic interactions are not consistently expressed in the field (4,10,16,26). Additionally, such stimulation by *Bacillus* strains is likely constrained by the genetic variation of the nitrogen-fixing symbiont and the plant host (12,76). Where they occur, *Bacillus*-mediated increases in nodulation may result from the production of compounds similar to lipo-chitooligosaccharide Nod factors (39). Phosphate-solubilizing *B. subtilis* strains have been reported to synergistically increase plant nitrogen and phosphate accumulation when co-inoculated with *Glomus intraradices* (74). Other studies have shown that some *Bacillus* strains can increase infection by endo- and ecto-mycorrhizae, but such synergies do not always stimulate plant growth (52 and references therein). Additional compounds of symbiotic importance are likely to be discovered. Recent work has shown that volatile compound 2,3-butanediol can be released by some strains of *B. subtilis* and *B. amyloliquefaciens* and stimulate plant tissue growth (63).

Colonization of the phytosphere is required for a microbe to directly influence plant health. Most studies of inoculant strains include analyses of colonization and survival. Seed factors set the stage for early colonization of germinating seedlings by diverse soil microbes (11,49), and they have been shown to be important determinants for root colonization by *B. subtilis* GB03 (43) and *B. cereus* UW85 (68). Root colonization by inoculant strains has been associated with disease suppression, enhanced mutualisms plant growth promotion, and yield increases in several systems (6,8,26,27,32,33). Though less frequently reported, spray applications of *Bacillus* inoculant strains to above-ground plant parts can lead to colonization and also have beneficial effects on plant health (14,28,64). Native phytosphere-colonizing populations of *Bacillus* and *Paenibacillus* spp. have been shown to vary with time (2,25,42), soil type (65), crop cultivar (17), and cropping pattern (20,21,75). However, no ecologically significant pattern of variation in these natural populations has been discerned. The abundance of *Bacillus* inoculant strains can also fluctuate, but they typically decrease over time, especially when applied at concentrations higher than those of native populations (14,25,33,42). Nonetheless, inoculant populations can persist in excess of log 4 cells per gram fresh weight throughout the growing season (25,33) and recolonize crops grown in subsequent years (42). The

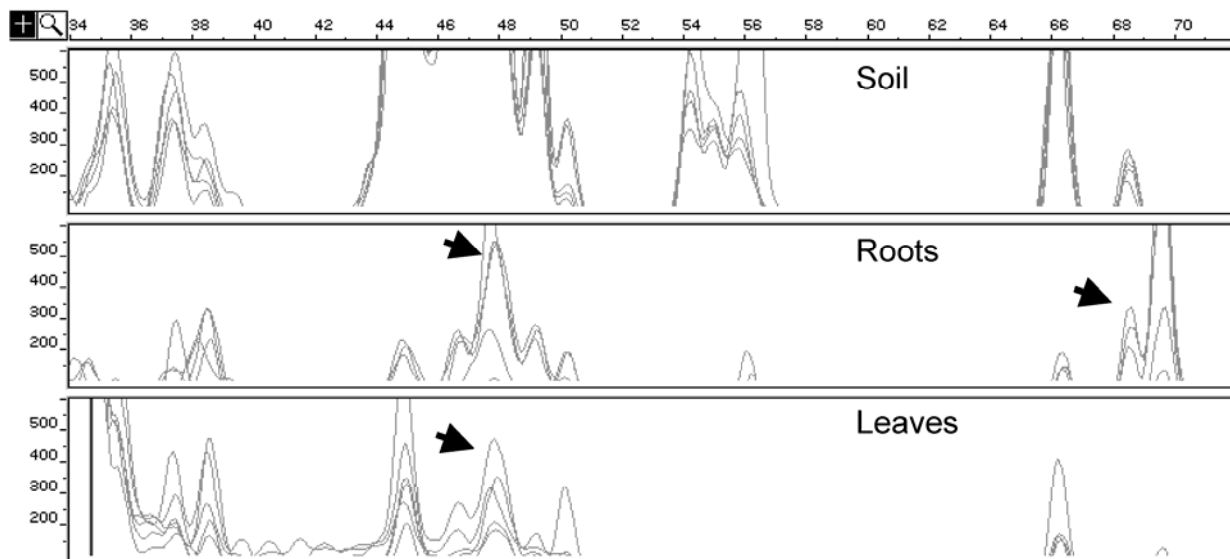


Fig. 2. Terminal restriction fragment (TRF) length polymorphism analysis of *Bacillus* and *Paenibacillus* populations inhabiting a single soybean field located in Wayne County, OH. Template DNA was isolated from soil, root, and leaf samples ($n = 6$, $N = 18$). Amplification, restriction digestion, electrophoretic separation, and analyses of labeled fragments were conducted as described in Figure 1. Here, arrows point to TRFs indicative of *Bacillus* spp. previously reported to be abundant in the rhizosphere (43) and phyllosphere (2) of soybeans by culture-based methods.

occurrence of phytosphere-colonizing populations of AEFB that can inhibit plant pathogens raises the question of their individual and collective contributions to plant health.

Some unanswered questions. Multiple populations of *Bacillus* and *Paenibacillus* can contribute to plant health in a variety of ways. Numerous isolates of these genera have been developed as biological control agents of plant pests and pathogens (36,51,66). In order to more successfully apply such agents, a greater understanding of their ecology is needed. Indeed, the safety and efficacy of inoculants will be determined in a large part by the ecological success of the applied strains in the environments into which they are introduced. Greater knowledge of the diversity, distribution, and activities of *Bacillus* and *Paenibacillus* spp. will be useful for identification of new inoculant strains and the cropping systems into which they can be most profitably applied.

On a fundamental level, the contributions of inoculant and indigenous bacteria to plant health, food quality, and crop yield need to be further characterized. On the cellular level, more work needs to be done to characterize the multiple factors expressed by phytosphere-colonizing microbes that suppress plant disease and the environmental variables that affect their expression. Which bacterial genes are involved in the production of antibiotics, the stimulation of host defenses, and the facilitation of nitrogen-fixing symbioses? And, how is their expression affected by various biotic and abiotic variables? Molecular techniques have been used to characterize genes for antibiotic production in *Bacillus* spp. (3,22,40,61), but the genetic basis of other biocontrol mechanisms has yet to be characterized. Clearly the taxonomic diversity of *Bacillus* and *Paenibacillus* spp. shown capable of reducing plant diseases indicates that much work remains to be done on the mechanisms by which these bacteria promote plant health. On the ecological level, quantitative associations between specific populations and different measures of crop health need to be established. While plant health may benefit from the application of one or a few strains, do populations of *Bacillus* and *Paenibacillus* spp. native to any given soil substantially contribute to plant health in agricultural fields? If so, which species or subspecies contribute most to plant health in different cropping systems? And, can those contributions be supplemented or enhanced more effectively with specific management strategies? Molecular profiling of mixed populations can be used to screen for specific populations present in the phytosphere that contribute most to disease suppressiveness (79). However, it is currently not known whether broadly conserved phylogenetic markers (e.g., ribosomal sequences) or more narrowly present functional markers (e.g., those related antibiotic synthesis) will be more useful for such characterizations. On one hand, the secretion of specific inhibitory compounds (e.g., peptide antibiotics and catabolic enzymes) by most species of AEFB (59) indicates that multiple species and subspecies may contribute to disease suppression. On the other hand, the apparently infrequent occurrence of effective biocontrol strains within named species of *Bacillus* and *Paenibacillus* indicates that functional markers such as those proposed for use in *B. subtilis* (22) and *B. cereus* (61) may be more useful. In either case, the recent characterization of whole genome sequences from different *Bacillus* species (31,35) should greatly accelerate the discovery of useful markers. We are currently investigating the associations of specific taxonomic and functional markers present in *Bacillus* spp. and related genera to the suppression of plant diseases and improvements in crop quality and productivity. By identifying TRFs indicative of those multiple species known to contain biocontrol strains, such as those described in Table 1, we expect to quantitatively assess the contributions of individual and defined groups of bacterial species to plant health. Lastly, more work needs to be done on determining the extent to which specific and general suppression of plant diseases can improve crop health. Extensive field studies of *Bacillus* seed treatments (5,15,50,56) have indicated that yield increases may be small on average but can sometimes exceed

110% of untreated controls in some locations in some years. This patchy responsiveness most likely reflects the complex interactions among biotic and abiotic factors known to affect plant-microbe interactions related to plant health (30,80). Despite this complexity, can assays be developed to better predict when and where microbial inoculants can be most effective? And, can such assays be used to determine the spatial scales to which individual treatments might most profitably be applied? Such determinations would allow for more selective applications of microbial products, an approach that would be more sound ecologically and economically.

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