Microbial Ecology

Competing Factors of Compost Concentration and Proximity to Root Affect the Distribution of Streptomycetes

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Abstract

Streptomycetes are important members of soil microbial communities and are particularly active in the degradation of recalcitrant macromolecules and have been implicated in biological control of plant disease. Using a streptomycetes-specific polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (PCR-DGGE) methodology coupled with band excision and sequence analysis, we examined the effect of grape marc compost amendment to soil on cucumber plant–associated streptomycetes community composition. We observed that both compost amendment and proximity to the root surface influenced the streptomycetes community composition. A strong root selection for a soil-derived Streptomycete, most closely related to *Streptomyces thermotolerans*, *S. iakyrus*, and *S. thermocarboxydus*, was independent of compost amendment rate. However, while the impact of compost amendment was mitigated with increasing proximity to the root, high levels of compost amendment resulted in the detection of compost-derived species on the root surface. Conversely, in rhizosphere and non-rhizosphere soils, the community composition of streptomycetes was affected strongly even by modest compost amendment. The application of a streptomycetes-specific PCR primer set combined with DGGE analysis provided a rapid means of examining the distribution and ecology of streptomycetes in soils and plant-associated environments.

Introduction

The family *Streptomycetaceae* comprises an important group of bacteria, often soil derived, belonging to the class *Actinobacteria*. A great deal of interest in streptomycetes has centered on their vast potential for producing a wide variety of secondary metabolites, including antibiotics and extracellular enzymes [7, 25]. Streptomycetes are capable of decomposition of a variety of macromolecules and are heavily involved in soil nutrient recycling. [23, 25]. Streptomycetes have been implicated in biological control of plant pathogens, both as a result of the production of secondary metabolites and via competition with pathogens for substrate [7, 15, 25, 34, 45, 49]. In addition to disease control, many studies have shown that plants can benefit from close associations with streptomycetes, and that these populations are commonly detected in the rhizosphere and in association with plant roots [9, 11, 37, 41, 43]. Reciprocally, root exudates have been shown to serve as a major nutrient source for some streptomycetes [11, 27, 43].

In addition to soil and rhizosphere, streptomycetes and other members of the *Actinobacteria* are frequently detected in composted materials [4, 13, 24, 25, 30, 35, 38, 42]. The colonization of compost by such populations is a result of their capacity for degrading plant and animal residues such as cellulose, chitin and pectin, and in some cases their thermotolerance [20, 23, 25, 35, 42]. By way of compost amendment to soil and potting mixes, compost-derived streptomycetes populations can be introduced into root environments. Composted materials are common agricultural amendments, and they are introduced into poor soils and potting mixes to serve as sources of organic matter and active microbiota [14]. Furthermore, some composts have been shown to suppress disease development, a phenomenon that in some cases has been correlated with levels of *Actinobacteria* [10, 19, 48]. Previous studies have suggested that soil physical characteristics and organic matter are the main factors affecting the number and type of streptomycetes and *Actinobacteria* in soil environments, and with increasing

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Table 1. Chemical analyses of Rehovot soil and grape marc compost

	pH	*EC (mmho/cm)*	*Percent organic matter (OM)*	*Percent total nitrogen*	*Total organic carbon (ppm)*	*C/N ratio*
Grape marc compost (GMC)	8.3	1.4	79.7	4.7	877	8.7
Rehovot soil (RS)	7.4	0.7	0.4	0.03	2.05	7.5

organic matter their number and variability are thought to increase [2, 23, 30]. Compost applications may, therefore, serve to sustain streptomycetes in soils and can enhance potential disease suppression capability.

Though some streptomycetes are easily isolated from soil and composts, cultivation biases can result in the recovery of populations that are not ecologically relevant [13, 21]. Furthermore, despite the enhancement of streptomycetes in composts and in plant-associated environments, these populations can be difficult to detect with general molecular techniques targeting bacteria [13]. The application of specific molecular tools to the study of the ecology of streptomycetes can enhance the detection and understanding of these populations and assist in the isolation of ecologically relevant organisms [33, 39].

In this study, the effect of compost amendment to plant-soil systems on the composition of streptomycetes populations in soil, rhizosphere, and root was investigated. We characterized the community composition of streptomycetes via a selective polymerase chain reaction (PCR) -denaturing gradient gel electrophoresis (DGGE) methodology developed here, combined with sequence analyses. This study had three primary goals: (1) to examine the effect of compost amendment on plant-associated streptomycetes communities at different application rates, (2) to investigate root effects on plant-associated streptomycetes communities in compost-amended soils, and (3) to identify dominant root-colonizing Streptomycete populations.

Materials and Methods

Composts, Potting Soils, Cucumber Growth, and Sampling. A low organic sandy-soil, Rehovot soil (RS), was amended with varying amounts of grape marc compost (GMC). Chemical analyses of the soil [32] and compost are presented in Table 1. The compost-amended soils contained 0 (control), 1%, 5%, and 10% (w/w) compost. These soils were the basis of four treatments: each treatment consisted of five 250-ml pots containing wetted soil and compost, sown with five cucumber seeds (*Cucumis sativus* L. Cfir- 413). All treatments were incubated under greenhouse conditions (daily temperature of 28° ± 5°C) and watered daily for the duration of the experiment.

After 3 weeks of plant growth, each treatment was sampled in triplicate. Soil not closely associated with plant roots was sampled from each pot (''bulk soil''), and approximately 0.25 g (wet weight) was taken for DNA extraction. Plants were uprooted and shaken to remove loosely adhering soil particles. More tightly root-adhering soil particles (''rhizosphere'') were recovered by washing roots in sterile water. Rhizosphere was concentrated by centrifugation at 6000 × *g* for 30 min, and approximately 0.25 g (wet weight) was used for DNA extraction. The washed roots were homogenized, and approximately 0.1 g (wet weight) of root from each plant was used for DNA extraction. Root samples may be considered to include endorhizosphere, rhizoplane, and tightly adhering rhizosphere. Three pots from each treatment were used as replicates. Soil, rhizosphere, and root DNA samples from each of these pots were extracted individually and analyzed independently. The original compost and soil were sampled for DNA extraction prior to incorporation into potting soils.

DNA Extraction. DNA was extracted from all samples using a modified bead-beating method. Briefly, crude extracts were initially recovered from samples via bead-beating (Fast Prep FP 120, Bio101, Savant Instruments Inc., Holbrook, NY) in an extraction buffer [100 mM Tris HCl, pH 8.0; 100 mM potassium phosphate buffer pH 8.0; 1% cetyltrimethylammonium bromide (CTAB); and 2% sodium dodecyl sulphate (SDS)]. The crude extracts were mixed with KCl to a final concentration of 0.5 M, incubated for 5 min, and centrifuged. DNA present in the supernatant was bound to glassmilk (0.5–10μm silica particles, Sigma Chemical Co., St. Louis, MO) with NaI as described elsewhere [6]. The glassmilk was washed with a 5.5 M guanidine thiocyanate solution until coloration was removed (3–4 washes). The silica was then resuspended in an ethanol-based wash buffer solution [6] and this solution, containing the glassmilk, was transferred to a centrifuge tube filter (0.22 μm cut-off nylon filter, Costar, Corning Inc., Corning, NY). After the glassmilk was dried via centrifugation, DNA bound to the silica and retained on the filter was eluted with Tris-EDTA (TE) into a sterile tube and stored at −20°C prior to use.

Primer Design. Two primers specific to the family *Streptomycetaceae*, S661-F and S1218-R (Table 2), designed for use in DGGE were developed using the ARB phylogenetic software package [28]. Potential primer sequences were checked against the ARB 16S rRNA gene database [28] and against the GenBank DNA database

Table 2. Polymerase chain reaction primer sequences and database search results

						ARB Database Search Results		
Primer set specificity	*Primers*	*Primer specificity*	*Primer sequence (5′ → 3′)*	*16S rRNA gene location (E. coli numbering)*	*Reference*	*Identical matches*	*Identical Streptomyces matches*	*Percent Streptomyces targeted*[a]
Actinobacteria	235-F	*Actinobacteria*	CGC GGC CTA TCA GCT TG	235–251	[39][b]	2174	355	95.2
	1392-R	Bacteria	ACG GGC GGT GTG TAC	1406–1392	[26]	11897	360	99.2
Streptomycetes	661-F	*Streptomycetaceae*	GTA GGG GAG ATC GGA ATT	661–678	This study	378	374	98.4
	1218-R[c]	*Streptomycetaceae*	AGC ACG TGT GCA GCC CAA	1235–1218	This study	380	375	98.4

[a]Percent of *Streptomyces* sequences with complete sequence information at the primer location perfectly matching the primer.
[b]Primer was modified from the published sequence of S-C-Act-0235-a-S-20 to reduce annealing temperature.
[c]A 40-bp GC-clamp was attached to the 5′ end of the primer to enhance separation in DGGE analyses, as follows: CGC CCG CCG CGC GCG CCC GTC CCG CCG CCC CCG CCC G.

using the BLAST tool [1]. Primer sets were also used to successfully amplify DNA from a variety of *Streptomyces* isolates, and the results of primer analyses and primer sequences are presented in Table 2.

PCR Amplification. Portions of 16S rRNA genes were amplified from extracted DNA samples using primer sets A235-F/1392-R and S661-F/S1218-R (Table 2). Each PCR reaction mixture contained (per 50 μL): 1.5 units of *Taq* polymerase (Red Taq, Sigma Chemical Co.), 5 μl Sigma PCR buffer, 0.2 mM PCR nucleotide mixture (Promega, Madison, WI), 6.25 μg bovine serum albumin (BSA—Roche Diagnostics, Mannheim, Germany), and 25 pmol of each primer. Final concentrations of $MgCl_2$ were adjusted to 2 mM for primer set A235-F/1392-R and 4 mM for primer set S661-F/S1218-R. All reactions were conducted in a Tgradient Thermocycler (Whatman Biometra) with the following protocol: initial denaturation at 95°C for 3 min, followed by 35 (A235-F/1392-R) or 30 cycles (S661-F/S1218-R) of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and elongation at 72°C for 60 s. A final elongation step at 72°C for 2 min was conducted. Samples were initially PCR amplified with an *Actinobacteria*-specific primer set (A235-F/1392-R). The PCR yields were diluted 1 to 5 with sterile TE, and used without further modification as templates for second (nested), streptomycetes-specific PCR using primers S661-F/S1218-R. Amplification products were checked for size and yield by agarose gel electrophoresis.

DGGE Analysis. DGGE analyses were performed with a D-Gene system (Bio-Rad, CA) using the following ingredients and conditions: 1 × TAE buffer (40 mM Tris HCl, 20 mM acetic acid, 1 mM ethylene diamine tetraacetic acid (EDTA) [pH 8.3]), and 1-mm-thick polyacrylamide gels (6%). Gels contained a 30% to 60% denaturant gradient, and were electrophoresed for 17 h at 100 volts and 60°C [31]. Gels were stained with ethidium bromide and photographed on an ultraviolet (UV) transillumination table (302 nm) with a Kodak digital camera (Kodak Co., New Haven, CT) with imaging software provided by the supplier.

Sequencing and Phylogenetic Analysis. Band excision, cloning, and sequencing were conducted as described elsewhere [18]. Each of the relevant bands (see *Results*), was isolated from DGGE profiles of several samples and independently cloned and sequenced. Four to eight clones were sequenced from each of these bands. Sequences recovered from excised bands were submitted to the National Center for Biotechonolgy Information (NCBI) for BLAST analysis, and the most closely related sequences are presented in Table 3. Further analyses of the 16S rRNA gene sequences were performed using the program package ARB [28]. Se-

Table 3. Partial sequence analysis of streptomycetes 16S rRNA genes recovered from compost, soil, rhizosphere and root

			Nearest relative (BLAST)		
Band identification[a]	*Sequence accession number*	*Sample [percent compost]*	*Name*	*Accession number*	*Similarity %*
S1	AY538601	Non–rhizosphere soil [0]	Unidentified eubacterium	AJ229209	98
S2	AY538602	Non–rhizosphere soil [0]	*S. ferralitis*	AY262826	97
S3	AY538603	Non–rhizosphere soil [0]	*S. africanus*	AY208912	98
S4	AY538604	Non–rhizosphere soil [0]	*S. resistomycificus*	AJ310926	99
S5	AY538605	Non–rhizosphere soil [0]	*S. albus*	AB045884	100
S6	AY538606	Rhizosphere soil [1]	*S. plemorphus*	AY222323	99
S8	AY538607	Rhizosphere soil [1]	*S. speibonae*	AF452714	99
S9	AY538608	GMC	*S. rutgersensis*	Z76688	100
S10	AY538609	Non–rhizosphere soil [5]	*Streptomyces* isolate	X87316	95
S11	AY538610	Rhizosphere soil [5]	*S. resistomycificus*	AJ310926	99
S13	AY538611	Root [10]	*S. thermotolerans*	AJ399482	99
S14	AY538612	Root [10]	*Streptomyces* isolate EF2	AF112165	99

[a]Bands excised from DGGE gels presented in Figure 1a, b, and c.

quences were aligned to the ARB gene database with the ARB automated alignment tool, and refined manually by visual inspection and secondary-structure analysis. A neighbor-joining tree, based on 593 alignment positions, was produced using a streptomycetes 30% maximum frequency conservation filter (i.e., those positions in which less than 30% of the *Streptomyces* sequences were identical were removed from the analysis). The sequences determined in this study have been deposited in the GenBank database under accession numbers AY538601 to AY538612.

Results

Design of a Streptomycetes-Specific Primer Set for DGGE Analysis. To overcome difficulties in detecting streptomycetes populations with general bacterial analyses and to monitor their community composition in environmental systems, we developed a primer set highly specific for the family *Streptomycetaceae*. Specific primers S661-F and S1218-R (Table 2) were designed using the phylogenetic program ARB and verified by analyses in ARB and with NCBI BLAST. The results of these analyses indicated that each primer was highly specific to the family *Streptomycetaceae*, and that they targeted relatively few non-*Streptomycetaceae* populations. Both primers targeted *in silico* greater than 98% of *Streptomycetaceae* sequences in the ARB database for which there was appropriate sequence information (approximately 380 sequences; Table 2). The outgroup targets of each primer were different, yielding a primer set totally specific to the family. Furthermore, sequence analyses revealed that the primer set had a minimum of three mismatches with all non-*Streptomycetaceae* sequences in the ARB database. The primer sets were checked with PCR analysis of a variety of Streptomycete and non-Streptomycete reference strains and were found to be stringent. Furthermore, sequence analyses of dominant bands excised from DGGE analyses indicated that all 12 recovered bands represented *Streptomyces* by phylogenetic analyses (Table 3, Fig. 2).

Community Composition of Streptomycetes in Bulk Soil and Root Environments. A low organic soil (RS, Table 1) was amended with 1%, 5%, and 10% GMC to examine the impact of compost amendment on plant-associated *Streptomyces* community composition. After 3 weeks of growth, DNA extracted from each of the triplicate samples of root, rhizosphere, and bulk soil of each treatment was analyzed by PCR-DGGE. Although minor variation of faint bands was present between replicates of some of the samples, the patterns of dominant bands in the replicate profiles were highly similar. Therefore, PCR products from triplicates samples were combined and used as a representative composite of each sample. Composite samples from bulk soil, rhizosphere, and root of cucumber plants grown in varying levels of compost amendment were analyzed on a single DGGE gel (Fig. 1). In addition, profiles were generated for the soil (RS) and compost (GMC) prior to incorporation into the potting soils (Fig. 1).

In bulk soil samples, the impact of compost amendment was detectable even at the lowest compost amendment rate (Fig. 1a). The DGGE profile of the control bulk soil was highly similar to that of the original soil prior to greenhouse incubation, while a 1% compost amendment rate was sufficient to significantly alter the profile of detected streptomycetes. Note, that although bands S9, S14 and S1 migrate in a similar manner to bands S3, S5, and S10, respectively, a close inspection of the DGGE analyses reveals that these bands do not migrate to identical positions. This finding is further sup-

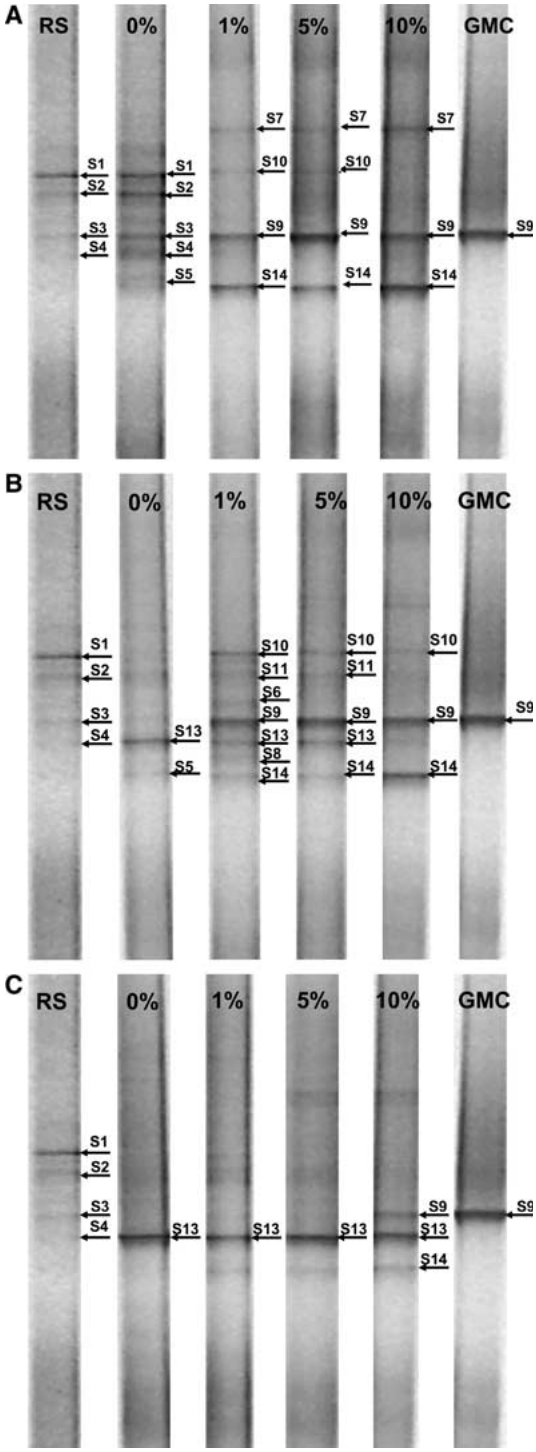

Figure 1. Denaturing gradient get electrophoresis (DGGE) analyses of streptomycetes from **(a)** bulk soil samples, **(b)** rhizosphere soil samples, and **(c)** root samples from cucumber plants grown in soil-based potting mixes amended with compost. Streptomycetes-specific population profiles were generated for bulk soil, rhizosphere soil, and root samples from potting mix treatments amended with 0 (control), 1%, 5%, and 10% compost and incubated for 3 weeks prior to DNA extraction and polymerase chain reaction (PCR-DGGE) analysis, as described in the text. Population profiles of streptomycetes in the original compost (GMC) and soil (RS) are also shown. The generated PCR fragment size is approximately 550 bp.

ported by sequence analyses of these bands. In all compost amended bulk soils, the 3-week profile diverged from that of the original soil toward that of the original compost (Fig. 1a). All compost-amended bulk soil profiles were highly similar to each other and contained two dominant populations (represented by bands S9 and S14), the first of which was detected strongly in the original compost prior to incorporation into the soil (Fig. 1a). Two additional populations, represented by bands S7 and S10, were detected to varying extents in compost-amended bulk soils, but they were not detected in the original compost.

Compost amendment also dramatically affected streptomycetes population profiles in the rhizosphere samples (Fig. 1b). Note that although very close in migration location, bands S13 and S2 do not migrate to the same location as, and have different sequence from, bands S4 and S11 (respectively). The single compost population (band S9) was dominant in all compost-amended rhizospheres and absent from that of the compost-free control. Similarly, the compost-enhanced population (band S14), detected strongly in all compost-amended bulk soils, was weakly detected in the rhizosphere of 1% and 5% treatments, and was dominant only in the rhizosphere of 10% treatment, while absent from the control rhizosphere (Fig. 1b). In the absence of compost amendment, the rhizosphere profile of streptomycetes (Fig. 1b, 0%) also differed significantly from that of the original soil (Fig. 1b, RS) and of the bulk soil sampled at 3 weeks (Fig. 1a, 0%). This profile consisted primarily of the soil-derived population represented by band S13 (Fig. 1b, 0%). However, while band S13 was detected in compost-amended rhizospheres, this population never predominated and was barely detectable in the 10% treatment (Fig. 1b, 1%-10%). Bands S13 and S14 were overshadowed by the dominant presence of the compost-derived population represented by band S9 in all compost-amended rhizospheres.

In contrast to bulk soil and rhizosphere samples, the influence of compost amendment on root-associated streptomycetes population composition was minor (Fig. 1c). Regardless of treatment, the soil-derived Streptomycete population represented by band S13 predominated in all root samples, and in contrast, the dominant compost organism represented by band S9 was absent from all root samples with the exception of the 10% treatment (Fig. 1c). The compost-enhanced population represented by band S14, dominant in the bulk soil and weakly detected in the rhizosphere in all compost treatments, was detectable on the root only in the 10% compost treatment (Fig. 1).

Sequence Analyses. Partial sequencing of 12 bands (approximately 550 bases per band) excised from DGGE profiles was conducted, and the recovered se-

Figure 2. Neighbor-joining tree of streptomycetes 16S rRNA gene sequences recovered from compost-amended plant systems. The phylogenetic analysis was performed on 593 aligned sequence positions for excised and sequenced bands, as described in the text. Band numbers refer to bands isolated from the DGGE analysis in Figure 1. Specific information regarding each sequence is presented in Table 3. Scale bar indicates an estimated 1% sequence divergence.

quences were analyzed by BLAST and with the ARB phylogenetic package [28]. All recovered sequences belonged to the genus *Streptomyces*, and were 95% similar to or greater than published sequences (Table 3). The populations represented by bands S9, S13 and S14 were the most frequently detected and most dominant populations in these analyses. Band S9 was identical, by partial sequence analysis, to *S. rutgerensis, S. griseus* and *S. tendae* (Fig. 2). *S. griseus* have been isolated from rich organic soils as well as composts [23, 24], and *S. tendae* has been reported as a potato pathogen [40].

Bands S13 and S14 were closely related (>99% similar) to each other, and together were most closely related to groups of *Streptomyces*, including *S. thermotolerans* (AJ399482), *S. iakyrus* (AJ399489), *S. thermocarboxydus* (AB098079), *Streptomyces* sp. X9 (AB119009), and EF2 (AF112165). *S. thermocarboxydus* produces a pectate lyase [29], and *Streptomyces* sp. EF2, a non-pathogenic isolate, was previously recovered from scab lesions in potato [16]. *S. thermotolerans* produce macrolide antibiotics such as carbomycin and also have antibiotic resistance [3].

As a single DGGE band can be comprised of more than one sequence [18], multiple clones of bands S9, S13, and S14 were obtained and sequenced from DGGE analyses of different samples. Band S9 was cloned from the GMC sample, from the root at 10% compost, and from the soil at 1% compost (Fig. 1a, 1b, 1c). A total of four clones derived from this band at the different environmental samples were sequenced, and the sequences of these clones were 100% identical. Band S13 was obtained from each of the four cucumber root samples (Fig. 1c). A total of eight clones of this band were sequenced from the different samples and the sequences of these clones were 99.74 ± 0.13% identical. Band S14 was obtained from the soils of 1%, 5%, and 10% compost (Fig. 1a). A total of seven clones obtained from this band recovered from the different samples were sequenced and the sequences of these clones were 99.63 ± 0.13% identical.

Discussion

Compost amendments are frequently used in field and greenhouse plant systems, and they serve as a source of nutrients, soil organic matter, and active microbial biomass [14, 22]. Although compost amendments alter soil physical and chemical conditions as well as microbial population composition and activity, relatively few studies have examined specific shifts in plant-associated populations as a result of such amendments [12, 42]

Furthermore, the effect of compost amendment rate on plant-associated microbial populations has not pre-

viously been clearly delineated. To understand better the dynamics of bacterial populations associated with plants in the context of compost amendment, we examined the development of streptomycetes in bulk soil, rhizosphere, and on the roots of cucumber plants grown in soil amended with varying levels of compost.

Streptomycetes are often present in the rhizospheres of plants as a result of their production of and resistance to antibiotics, as well as their ability to consume a variety of organic carbon sources [25, 37, 45]. In addition, the presence of streptomycetes on root and in the rhizosphere has been correlated, in some cases, to plant growth promotion and disease control [7, 10, 15, 19, 34, 49]. To facilitate the study of streptomycetes in these and other environments, we developed a highly specific PCR-DGGE methodology. Two highly specific PCR primers were designed and applied to pure culture and environmental samples. These primers amplified a fragment of the 16S rRNA gene (approximately 550 bp) exclusively from members of the family *Streptomycetaceae*. These primers are highly specific because of their large number of mismatches with even closely related outgroup populations. In addition, despite the high conservation of the 16S rRNA gene among the streptomycetes [17], the generated DNA fragments were separated effectively by DGGE analysis. This methodology will aid in the analysis of streptomycetes in root and rhizosphere studies, as well as in other environmental systems.

The population shifts after compost amendment suggest that the original soil streptomycetes populations were numerically overwhelmed. Because of the relatively high compost organic content (even at a 1% compost amendment rate, the organic matter in the compost-amended soil was primarily derived from the compost itself) and correspondingly high bacterial load, this dominance was not unexpected and may reflect a simple dilution below detection of soil-derived populations. Because the bulk soil was unaffected by root exudates, amendment of compost, regardless of amount, was the predominant factor influencing the observed population composition.

In the absence of compost, the population composition of the bulk soil was not significantly modified from that of the original soil, a result that may indicate relatively low activity in the control bulk soil streptomycetes population. Although the compost itself was a significant source of streptomycetes to the bulk soil, as indicated by the dominance of the population represented by band S9, we also observed the development of an additional population in the bulk soil of compost treatments that was not detected in the original GMC profile (band S14). Based on these data, the provenance of the population represented by band S14 cannot be determined. Because soil organic matter is a critical factor in determining the level of streptomycetes in soils, the organic matter present in the compost may have enhanced the development of a soil-derived streptomycetes [2, 25]. Boehm et al. [5] suggested that organic matter present in compost amendments can serve to sustain microbial populations unable to subsist on root exudates alone. Alternatively, this population may simply have been below detection in the original GMC.

Despite dramatic shifts in bulk soil population effected by minor amendment of compost, the root streptomycetes community was less influenced by such amendments. All root community profiles were dominated, to the near exclusion of all other populations, by the soil-derived Streptomycete represented by band S13. This population is closely related by sequence analyses to non-pathogenic, plant tissue-associated bacteria and antibiotic producing *S. thermotolerans*. The physiological capabilities of closely related *Streptomyces* spp. suggest the capacity for survival in the rhizosphere and possible colonization of the root and endorhizosphere. The predominance of a single population (i.e., band S13) is consistent with a rhizosphere effect, a strong plant-induced selection via exudation for bacterial communities in close proximity to roots [37, 46]. Only in the 10% treatment was the compost-derived population (band S9) and the compost-enhanced bulk soil population (band S14) appreciably detected on the root surface, demonstrating an amendment rate effect. Curiously, the populations represented by bands S13 and S14 are highly similar by sequence analyses, and their differing root colonization patterns most likely are a result of different physiological characteristics. Overall, these results suggest a significant ''rhizosphere buffering'', in which organisms applied as soil amendments can establish high population levels but because of plant selection, ultimately are not sustained in the rhizosphere or rhizoplane [45]. The apparent insulation of the root streptomycetes community from the effects of compost amendment, as observed in the bulk and rhizosphere soils, must be considered in the employment of compost amendments for modifying plant-associated microbial communities. Nonetheless, as compost organisms were detected in root samples at the highest amendment rate applied here, it is possible that with high enough amendments rates, beneficial populations can be forced into these communities.

The root, rhizosphere, and bulk soil population composition of streptomycetes was influenced both by proximity to the root and by rate of compost amendment, and the interaction of these two factors was most readily visualized in community profiles of rhizosphere, where the two factors overlapped to the greatest extent. In the absence of compost amendment, profiles of streptomycetes in root and rhizosphere samples were highly similar, and they were dominated by the root-colonizing population (band S13). Because the popula-

tion represented by band S13 was never detected in analyses performed on bulk soil samples, its presence in rhizosphere and root samples suggests that it is dependent on root exudates. The community composition of streptomycetes in the rhizosphere, as in the bulk soil, was strongly influenced by the addition of compost. However, the influence of the root on community composition of rhizosphere streptomycetes was modulated by the rate of compost amendment. The rhizosphere of the control treatment is presumably more affected by exudates from the plant roots as a result of lower (relative to compost-amended soils) organic matter and microbial population levels.

These results are consistent with the current understanding of the influence of plant exudates on microbial population dynamics in the root and rhizosphere. Cheng et al. [8] showed that water-soluble carbon concentrations varied inversely with distance from the rhizoplane. Likewise, Wieland et al. [47] found that the influence of soil on microbial population distribution was reduced upon approach to the rhizoplane. This diminishing influence is presumably due to the increasing predominance of exudates closer to root, which in turn yields greater competition among copiotrophic bacteria. However, Semenov et al. [36] observed a decrease in such a rhizosphere effect in wheat plants grown in highly organic soils, relative to a strong rhizosphere effect observed in low organic soils. This may account for the increasing similarity of rhizosphere and root community profiles in compost treatments. Specifically, plants grown in compost-amended soils can be expected to have a less drastic rhizosphere effect because of the higher microbial carrying capacity of the soil itself. Clearly, soil characteristics and proximity to the root surface are critical in determining plant-associated microbial populations, and we observed that streptomycetes were sensitive to both factors. This was most clearly revealed by increasing the concentration of compost in the potting soils, and only at the highest compost concentration did compost organisms become detectable members of the root community. The high compost amendment rates were not sufficiently high to fully displace the soil-derived root colonizing population. These results indicate that the rate of compost amendment to soils and potting mixes must be considered carefully, and potentially higher compost dosages may be needed. Ultimately, the introduction to and maintenance of microorganisms on the root and rhizoplane is critical for potential disease control or plant growth promotion [44].

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