Microbial Ecology

Spatial Variation in *Streptomyces* Genetic Composition and Diversity in a Prairie Soil

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Abstract

Understanding how microbial genotypes are arrayed in space is crucial for identifying local factors that may influence the spatial distribution of genetic diversity. In this study we investigated variation in 16S rDNA sequences and rep-PCR fingerprints of *Streptomyces* stains isolated from prairie soil among three locations and four soil depths. Substantial variation in *Streptomyces* OTU (operational taxonomic unit) and BOX-PCR fingerprint diversity was found among locations within a limited spatial area (1 m^2). Further, phylogenetic lineages at each location were distinct. However, there was little variation in genetic diversity among isolates from different soil depths and similar phylogenetic lineages were found at each depth. Some clones were found at a localized scale while other clones had a relatively widespread distribution. There was poor correspondence between 16S rDNA groupings and rep-PCR fingerprint groupings. The finding of distinct phylogenetic lineages and the variation in spatial distribution of clones suggests that selection pressures may vary over the soil landscape.

Introduction

Soil microbial communities contain substantial phenotypic and genetic diversity [4, 5, 11, 44, 46, 58]. It is hypothesized that the spatially structured habitat, patchiness in nutrient availability, differences among associated plant species, or interactions among members of the soil community may all contribute to this diversity [10, 14, 22, 31, 51, 60, 65, 70, 75]. To identify local factors that may influence the spatial distribution of diversity, including competitive interactions and resource availability, information on how phenotypes and genotypes are arrayed in space is crucial. There is a growing body of empirical studies examining the spatial distribution of genetic diversity in the soil at a range of spatial scales from micrometers to kilometers [3, 8, 20, 25, 48, 56, 69).

Streptomycetes (Order Actinomycetales, Family Streptomycetaceae; [73]) are Gram-positive, filamentous bacteria that are ubiquitous in soil and are prolific producers of antibiotics [64]. Through antibiotic inhibition of a broad range of soil-borne plant pathogens, Streptomycetes can significantly influence plant health [7, 13, 33, 55, 74]. Understanding factors that influence the generation and selection of antibiotic-producing phenotypes may increase our ability to actively manage the soil microbial community to promote plant health. However, despite their significance to plant health and their practical value in human and animal medicine, little is known about the ecology of *Streptomyces* in natural habitats [68]. Antibiotic production is hypothesized to increase microbial fitness by providing a competitive advantage to producing microorganisms [40, 72]. Moreover, recent modeling work shows that antibiotic interactions among microbes can generate and maintain phenotypic diversity when microbial strains are localized in space [10]. This leads to the prediction that phenotypic diversity in antibiotic activities should vary in space. Although there is little empirical evidence for this prediction, recent work documents spatial variation in antibiotic phenotypic diversity for *Streptomyces* [11]; however, information on spatial patterns of genetic diversity in this group is lacking.

Though previous studies have reported on spatial localization of genotypes for a variety of soil bacteria [8, 21, 43, 47–49], the filamentous habit of *Streptomyces* suggests the potential for patterns of spatial localization

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that may differ from those observed for unicellular bacteria in soil. Understanding the spatial scales over which individual *Streptomyces* clones exist in soil is important to identifying the significance of localized selection pressures to *Streptomyces* fitness. That is, determining whether *Streptomyces* clones are arrayed in space over a very fine scale versus more coarsely is critical to discerning factors that are correlated with or generate diversity within this group.

To estimate diversity isolates must be categorized into different genetic groups (e.g., species). A wide range of morphological, cultural, physiological, and biochemical characteristics have been used to delineate species within the *Streptomyces* genus although taxonomic species definitions remain unresolved (reviewed in [2]). However, partial 16S rDNA sequences, in particular those including the variable γ region, have been shown to be useful for distinguishing *Streptomyces* species [2, 32, 62]. Within a species of *Streptomyces*, rep-PCR fingerprints have been shown to differentiate among closely related strains [53]. Examining 16S rDNA sequence diversity may provide insight into factors affecting species distributions and diversity over a broad scale while rep-PCR fingerprints allow the spatial distribution of individual clones to be determined on a local scale.

In this study we investigated variation in 16S rDNA sequences and rep-PCR fingerprints of *Streptomyces* isolated from prairie soil among three locations and four soil depths. Our goal was to document the variation in genetic diversity of the culturable *Streptomyces* community at various spatial scales. Further, the spatial distribution of particular genetic groups (e.g., "species" and clones) was determined in order to gain useful information for examining ecological factors that may structure the observed diversity.

Methods

Streptomyces Isolates. Isolates used in this study were collected at Cedar Creek Natural History Area (CCNHA), a National Science Foundation Long-Term Ecological Research site (http://www.lter.umn.edu). Briefly, within a 1 × 1 m grid three sampling locations were selected randomly. At each sampling location, three soil corers (30 cm × 1 cm) were bundled together and pounded gently into the soil. Soil was left in the corers and transported back to the laboratory for immediate processing. Each core was divided into 2 cm subsections corresponding to soil depths of 0–2 cm, 2–4 cm, 4–6 cm, and 6–8 cm (depths 1–4, respectively). Therefore, there were a total of 36 samples (4 depths × 3 cores × 3 locations). Analyses here focus on locations and depths.

Soil suspensions of each sample were dilution-plated onto oatmeal agar amended with antibiotics [74]. After incubation at 28°C for 7 days, colonies exhibiting characteristic streptomycete colony morphology [6] were selected randomly and pure-cultured. From the 36 soil samples, a total of 153 *Streptomyces* isolates with a maximum of five isolates per sample were purified for further study. Purified spore suspensions of each isolate were stored in 20% glycerol at –80°C.

16S rDNA Sequences. DNA from each isolate was extracted using the Wizard Genomic DNA Purification kit (Promega, Madison, WI) following the manufacturer's instructions. Amplification of 16S rDNA was performed following the protocol of Takeuchi et al. [63] in a final total volume of 50 μL, containing 100 ng of DNA, 10 pmol of each primer, and PCR SuperMix High Fidelity (Invitrogen, Carlsbad, CA) using a PTC-200 DNA Engine (MJ Research, Waltham, MA). All PCR reactions used universal bacterial primers pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and pH (5′-AAGGAGGTGATCCAGCCGCA-3′) that amplify nearly full-length (approximately 1.5 kbp) 16S rDNA products [15]. Partial 16S sequences for each isolate were obtained by automated DNA sequencing using pA as the primer from the Advanced Genetic Analysis Center, University of Minnesota (St. Paul, MN).

Estimates of diversity require definition of operational taxonomic units (OTUs). 16S sequences were placed into OTUs by comparison using the SIMILARITY_MATRIX function of the Ribosomal Database Project [39]. Isolates that had 16S sequences that differed by ≤ 1% were placed into the same OTU. Richness (*S*: the number of OTUs) and the Shannon diversity index [$H = -\sum(x_i/x_0)\ln(x_i/x_0)$, where x_i is the number of isolates in an OTU and x_0 is the total number of isolates] were calculated for each location and depth [38]. The Shannon index provides a moderate weighting to rare and intermediate species in comparison to dominant species, making this index more sensitive to changes in abundance of rare groups than other diversity indices [27]. Diversity indices and standard deviations were calculated using EstimateS [9]. The Basic Alignment Search Tool (BLAST) [1] was used to compare partial 16S rDNA sequences from each isolate with sequences in the GenBank database.

For phylogenetic analyses, a 128-bp region within the 16S gene was used that included the γ region [2, 62], which has been shown to be useful in resolving relationships within the streptomycetes [2, 32]. 16S rDNA sequences were edited using the Wisconsin Package Version 10.2 [Genetics Computer Group (GCG), Madison, WI]. Sequences were aligned with ClustalW (http://www.ebi.ac.uk/clustalw/). Unrooted phylogenetic trees were constructed using a matrix of Jukes–Cantor distances [29] and the neighbor-joining method of Saito and Nei [54] with PHYLIP, version 3.5

[17]. A bootstrap analysis was conducted to test the reliability of the tree [16] using SEQBOOT (PHYLIP, version 3.5).

Statistical analyses examining phylogenetic diversity, in contrast to species richness, do not rely on definition of OTUs or estimations of the frequency of different sequences and can provide additional insight into patterns of microbial diversity [61]. Differentiation among sequences was examined using a nested analysis of molecular variance (AMOVA) with the aid of ARLEQUIN [57]. Three hierarchical levels were defined: among locations, among depths within a location, and among all sequences. Sequence data were transformed into distances using the Tajima and Nei algorithm with $\alpha = 0.5$. Significance was established using 1000 permutations. In addition, the data were partitioned by location and depth, and pairwise F_{ST} and P tests were performed [42]. The parameters for the AMOVA were used for the F_{ST} tests. P tests were based on 1000 random trees. Briefly, the F_{ST} test compares the genetic diversity within each location or depth to the total genetic diversity of the locations and depths combined. The P test examines whether the distribution of unique sequences between locations or depths covaries with phylogeny. If both tests are significant, there is clear differentiation between streptomycete communities at each location or depth. If the P test is significant and the F_{ST} test is not, then each location or depth differs in the phylogenetic lineages present and the lineages are not closely related within each community. If the F_{ST} test is significant and the P test is not, then communities at each location or depth could contain distinct groups of closely related isolates that are distributed throughout the phylogenetic tree. Nonsignificant results for both tests indicate that the *Streptomyces* sequences sampled came from the same community [42].

BOX-PCR Genomic DNA Fingerprints. DNA was extracted from each isolate as described above. DNA amplification followed the procedure of Rademaker et al. [50] using the BOXA1R primer (5′-CTACGGCAAGGC GACGCTGACG-3′) [67]. PCR was performed on a PTC-200 DNA Engine (MJ Research Inc., Waltham, MA) under conditions described in Rademaker et al. [50]. Fluorophore-enhanced rep-PCR (FERP) [50] as modified for agarose gels [28] was used to examine differences among isolates.

Fingerprint images were captured using a Typhoon 8600 Multi-Mode Imager (Amersham Pharmacia Biotech, Piscataway, NJ) and analyzed using BioNumerics (Version 2.5; Applied Maths, Kortrijk, Belgium). Pearson product-moment correlations [26] were calculated for the array of densitometric values formed by the fingerprints using NT-SYS [52]. Cluster analysis was performed using UPGMA (unweighted pair-group method, arithmetic average; [59]) and a dendrogram was constructed (NT-SYS; [52]).

To examine the internal consistency and reproducibility of BOX-PCR fingerprints using the FERP technique, five *Streptomyces* isolates were selected to evaluate variation among DNA isolations from three single spores from an individual isolate, among lanes within a gel for a single DNA isolation, and among gels. DNA isolation and PCR amplification were performed as described above. Gel electrophoresis was conducted as described above. On each gel, a single isolate was represented in five wells: three wells contained the PCR product from one randomly selected single spore DNA isolation and the remaining two wells contained the PCR product from the other two single spore DNA isolations. A total of three gels were run for this experiment.

Based on the results of the internal consistency test (see Results), isolates whose fingerprints were >90% correlated were considered to be clones and were assigned to the same fingerprint group (clone) based on the dendrogram generated above. These groups were used to calculate diversity among locations and depths as described above. The correlation matrix was compared to the 16S rDNA sequence distance matrix using Mantel's test (NT-SYS; [52]). A positive correlation indicates that clustering among isolates is similar for both 16S sequences and genomic fingerprints. Significance of the correlation was determined as described in Lapointe and Legendre [36].

Results

16S rDNA Sequences. Partial 16S rDNA sequences (890–1199 bp) for each of the 153 isolates were compared with each other, and isolates with ≥99% similarity were assigned to the same OTU (Table 1). The 153 isolates were grouped into 34 OTUs; the largest OTU contained 55 isolates and 21 OTUs were represented by a single isolate. Further, the closest bacterial relative of each isolate was determined by comparing the partial sequences to GenBank using BLAST (Table 1). Six isolates did not match a sequence in the database at >96% identity and were considered to be undetermined *Streptomyces* species. Sequences from the largest OTU were most similar to *S. lavendulae* 16S rDNA sequences (Table 1). Total species diversity, as measured by the Shannon index, was 2.44 for all the *Streptomyces* communities sampled.

Four OTUs (1–4) were represented at approximately equal frequency at location 1, which had a total of 49 isolates (Table 1). In contrast, locations 2 and 3 were dominated by OTU 1 (26 out of 55 and 22 out of 49 isolates, respectively). Eight OTUs were represented only once at location 1; four of these OTUs (16–19) were found only in location 1. Location 2 had 11 OTUs found once (nine unique to the sample), whereas location 3 had

Table 1. Spatial distribution of OTUs represented by 153 *Streptomyces* isolates[a]

			Location			*Depth*			
OTU	*Putative species*[b]	*Number of isolates*[c]	*1 (49)*[d]	*2 (55)*	*3 (49)*	*0–2 cm (44)*	*2–4 cm (41)*	*4–6 cm (34)*	*6–8 cm (34)*
1	*S. lavendulae*	55	7	26	22	25	16	7	7
2	*S. olivochromaogenes* *S. mirabilis*	23	10	0	13	2	9	5	7
3	*S. lydicus*	15	9	5	1	2	4	6	3
4	*S. rimosus* subsp. *rimosus*	12	9	1	2	1	0	4	7
5	*S. lavendulae* *S. virginiae*	7	1	6	0	5	0	2	0
6	*S. nodosus* *S. padanus* *S. echinatus*	5	2	3	0	0	0	4	1
7	*S. platensis*	3	3	0	0	2	1	0	0
8	*S. aureofaciens*	2	1	0	1	1	1	0	0
9	*S. galbus*	2	0	1	1	1	1	0	0
10	*S. rimosus* subsp. *rimosus*	2	0	0	2	0	0	2	0
11	*S. tauricus*	2	0	2	0	1	0	0	1
12	*S. galilaeus*	2	0	0	2	1	1	0	0
13	*S. scabies*	2	0	2	0	0	2	0	0
14	*S. flavogriseus*	1	0	1	0	0	0	0	1
15	*S. mirabilis*	1	0	0	1	0	0	0	1
16	Undetermined A	1	l	0	0	0	1	0	0
17	Undetermined B	1	l	0	0	0	1	0	0
18	*S. rimosus* subsp. *rimosus*	1	1	0	0	0	0	0	1
19	Undetermined C	1	1	0	0	0	1	0	0
20	*S. violaceusniger*	1	0	1	0	0	1	0	0
21	*S. galbus*	1	0	1	0	0	1	0	0
22	*S. olivochromaogenes*	1	0	1	0	0	0	1	0
23	Undetermined D	1	0	1	0	0	0	0	1
24	*S. lydicus*	1	0	1	0	0	0	0	1
25	*S. lavendulae*	1	1	0	0	0	1	0	0
26	*S. galilaeus*	1	0	0	1	0	1	0	0
27	Undetermined E	1	1	0	0	1	0	0	0
28	*S. hygroscopicus*	1	1	0	0	1	0	0	0
29	Undetermined F	1	0	1	0	0	0	1	0
30	*S. cyaneus*	1	0	0	1	1	0	0	0
31	*S. olivochromaogenes*	1	0	1	0	0	0	0	1
32	*S. olivochromaogenes*	1	0	0	1	0	0	1	0
33	*S. lavendulae*	1	0	0	1	0	0	0	1
34	*S. lavendulae*	1	0	1	0	0	0	1	0

[a]Operational taxonomic units (OTUs) contain isolates with ≥99% similarity of partial 16S sequences.
[b]Putative species of members of OTU as determined from BLAST searches of GenBank. Species were considered undetermined if partial 16S sequence did not match a named *Streptomyces* species with ≥96% similarity. Some OTUs contain isolates identified as different species.
[c]Number of isolates within OTU.
[d]Number of isolates at each location or depth.

eight OTUs (five unique to the sample) represented by a single isolate. Location 2 had the greatest richness while location 1 had the greatest diversity (Table 2).

Isolates from OTU 1 dominated the community at all depths but the proportion of the community that was represented by the dominant OTU tended to decrease with increasing depth (Table 1). Some OTUs tended to occur deeper in the soil (e.g., OTUs 4 and 6), whereas others were found predominately at shallower depths (e.g., OTUs 5, 7, 12, and 13). The greatest richness was found at depths 2 (2–4 cm) and 4 (6–8 cm) while depth 4 (6–8 cm) had the greatest diversity (Table 2). Although 13 OTUs were found at 0–2 cm, 57% of isolates were from a single OTU (OTU 1). In contrast, at 6–8 cm, no OTU was represented by more than 21% of isolates.

The phylogenetic relatedness of the isolates based on partial 16S rDNA gene sequences (128 bp) was examined (Fig. 1). There was generally good resolution among clusters of OTUs. When a cluster contained multiple OTUs, the cluster always was dominated by a single OTU

Table 2. 16S OTU and BOX-PCR fingerprint diversity among locations and depths

		16S OTU		*Fingerprint*	
	Number of isolates	*Richness*	*Shannon*	*Richness*	*Shannon*
Location 1	49	15	2.24 ± 0.02[a]	44	3.74 ± 0.01
Location 2	55	17	2.02 ± 0.03	34	3.35 ± 0.02
Location 3	49	13	1.74 ± 0.03	28	3.07 ± 0.02
Depth 1 (0–2 cm)	44	13	1.68 ± 0.04	31	3.24 ± 0.03
Depth 2 (2–4 cm)	41	14	1.98 ± 0.04	33	3.41 ± 0.02
Depth 3 (4–6 cm) N	34	11	2.17 ± 0.03	26	3.18 ± 0.02
Depth 4 (6–8 cm)	34	14	2.23 ± 0.04	28	3.27 ± 0.02

[a]Shannon diversity ± standard deviation presented.

but also contained OTUs represented by single isolates (e.g., the cluster containing OTUs 1, 33 and 34; Figure 1).

Variation was compared among locations, among depths, and among all sequences using a hierarchical nested analysis of variance. The results showed significant differentiation at all hierarchical levels ($P < 0.05$ for each level). Most of the variation (81%) was distributed among sequences. Pairwise F_{ST} and P tests for sequences grouped by location revealed that all three locations exhibited significant differentiation ($P < 0.01$ for each pairwise test). In addition, both tests revealed that sequences from depth 1 (0–2 cm) differed significantly from sequences found at other depths ($P < 0.05$) while all sequences from depths >2 cm were similar (N.S.).

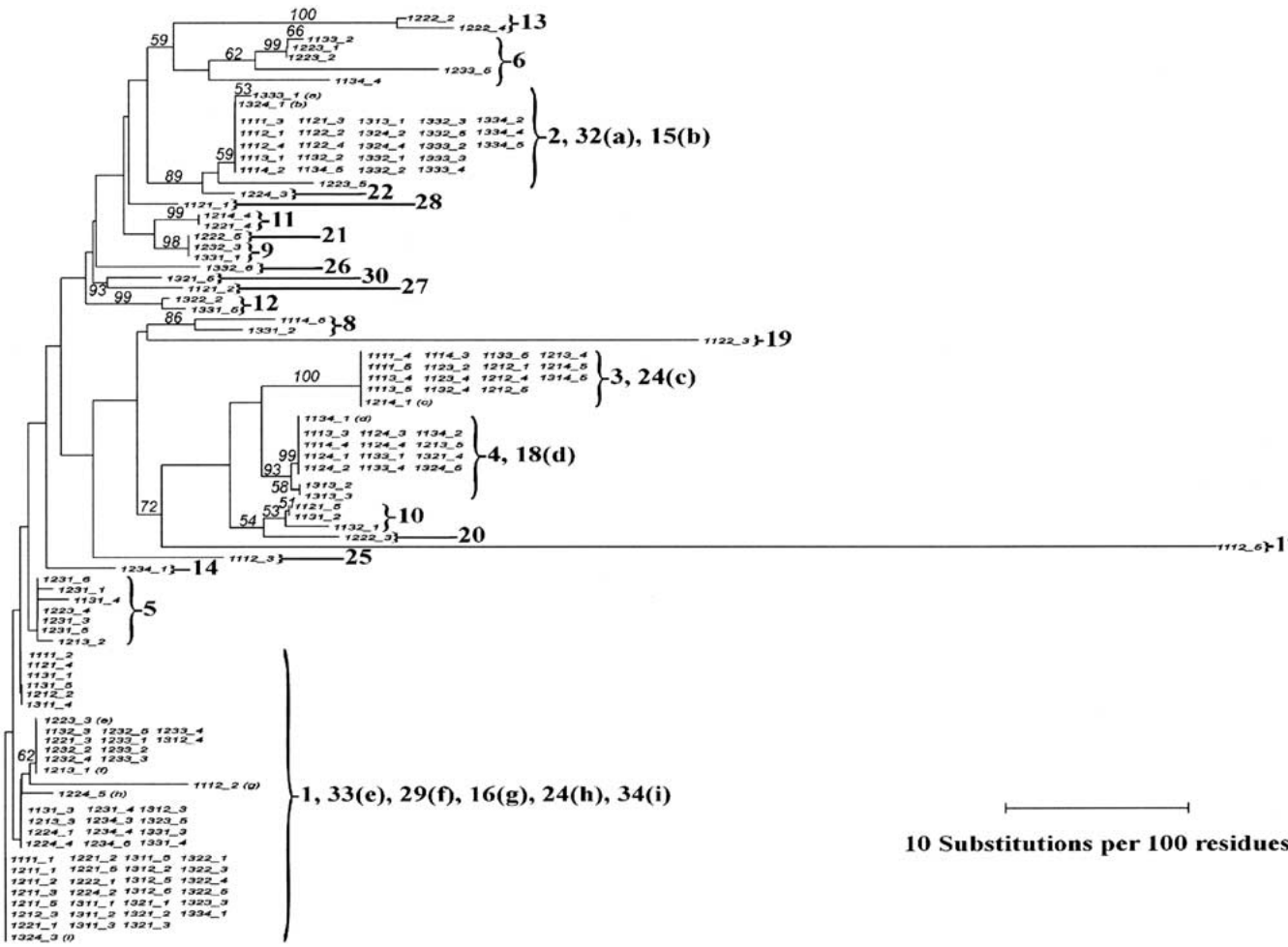

Figure 1. Phylogenetic relationships of 153 *Streptomyces* isolates based on partial 16S rDNA gene sequences (128 bp). Bootstrap values (based on 100 replicates) are given at branch points; only values >50 are presented. Isolate names are derived from their site position and correspond to: plot, location, core, and depth, i.e., isolate 1234_6 is the 6th isolate from plot 1, location 2, core 3, depth 4. OTU group numbers are listed to the right of each group. Minor OTUs within a cluster are indicated with letters (a–i).

Overall, these results show a strong signal for differentiation in *Streptomyces* communities among locations, and some evidence for differentiation in communities among depths.

The 16S rDNA gene sequences of isolates used in this work have been deposited in the GenBank database under accession numbers AY465184–AY465336.

BOX-PCR Genomic DNA Fingerprints. The internal consistency and reproducibility studies revealed that single-spore DNA isolations clustered together for replicates both within and across gels for the five isolates examined (data not shown). Based on these results, we considered isolates clustering together at >90% similarity to be clones.

Using BOX-PCR fingerprints (300–3000 bp), the 153 isolates clustered into 102 different genetic groups based upon 90% similarity (Fig. 2). Seventy-seven of the 153 isolates were represented by unique fingerprints. The remaining isolates clustered into groups of two to seven isolates. The largest cluster consisted of a clone represented by seven isolates from Location 3 sampled from 2–8 cm in depth; six isolates from this cluster were from OTU 1 while the remaining isolate belonged to OTU 33. The fingerprint diversity of the *Streptomyces* community over all samples was 4.42.

In general, clones were localized in space. In 23 of the 25 clusters containing multiple isolates, isolates were sampled from the same or adjacent depths (Fig. 2). However, samples did not simply contain repeated isolations of a single clone. Of 36 samples (three locations, three cores, four depths), only once were all isolates from a sample represented by a single clone (location 3, core 1, depth 1). Further, a single clone dominated (three or more isolates per sample) in <20% of the 36 samples. Few clones were found over a larger area: only five clones out of 102 total clones were found at multiple locations; these five clones belonged to the three largest OTUs. These clones, which occurred across locations, accounted for <12% of the 153 isolates sampled.

The similarity of isolates from the same location is further emphasized by comparing the correlation of BOX-PCR fingerprints of isolates from the same location with the correlation with isolates from other locations. Isolates with similar BOX-PCR fingerprints tended to be spatially aggregated. On average, BOX-PCR fingerprints of isolates within a location were more highly correlated than were fingerprints of isolates from different locations (Fig. 3).

Consistent with diversity estimates based on 16S OTUs, location 1 had the greatest richness and diversity of fingerprint types (Table 2). Among depths, richness and diversity were greatest at depth 2 (2–4 cm).

Isolates within a *Streptomyces* OTU (as defined by 16S sequences above) produced a wide variety of BOX-PCR fingerprint types (data not shown). Indeed, there was no correlation between the fingerprint correlation matrix and the distance matrix based on 16S rDNA sequences (Mantel's test: $r = -0.36$, $P < 0.99$).

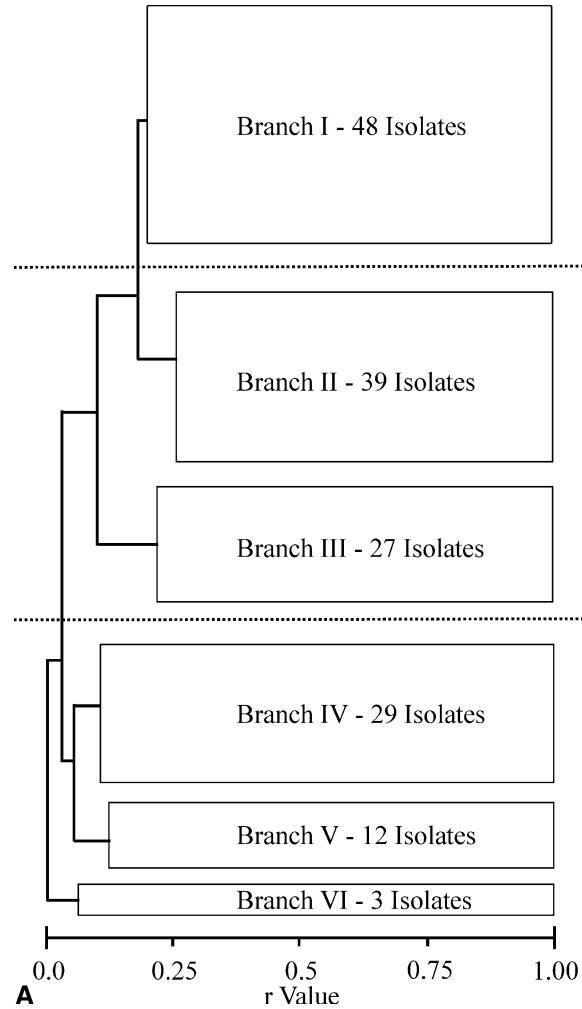

Figure 2. Dendrogram of BOX-PCR genomic DNA fingerprints for 153 *Streptomyces* isolates. Isolates correlated at >90% (*r* Value: Pearson product-moment correlation) were considered to be clones. Clusters were determined using UPGMA (unweighted pair-group method, arithmetic average). Isolate names are derived from their site position and correspond to: plot, location, core, and depth, i.e., isolate 1234.6 is the 6th isolate from plot 1, location 2, core 3, depth 4. Clones are bracketed to the right of the trees.

Discussion

Wide variation in *Streptomyces* OTU and BOX-PCR fingerprint diversity was found among locations within a limited spatial area (1 m^2). A previous study examining the spatial distributions of ecotypes within a single bacterial species (*Rhodopseudomonas palustris*) revealed strong differences in diversity over a scale similar to that used in this study [3]. Further, for the three locations

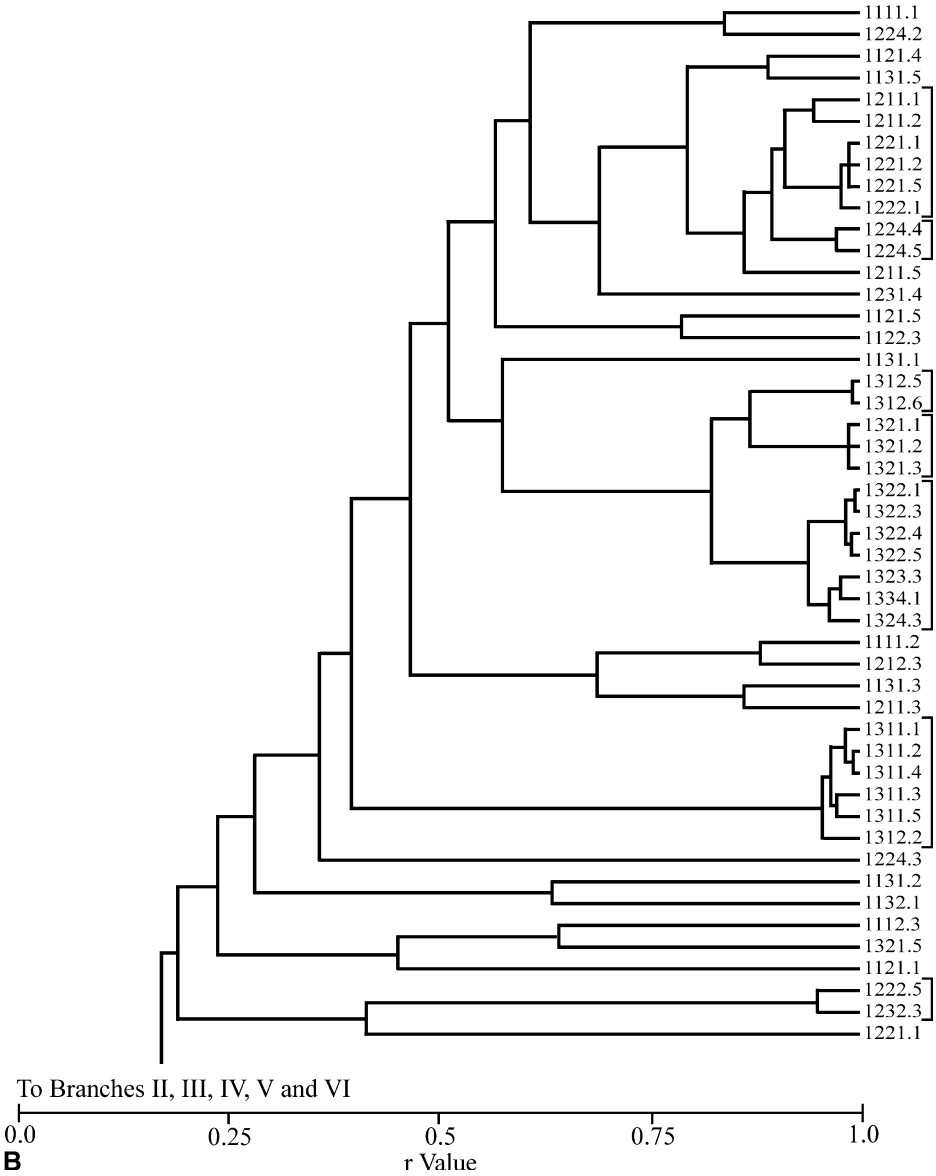

Figure 2b. Continued

studied here, distinct phylogenetic lineages were found at each location. Differences in genetic composition and diversity may suggest distinct selective environments among the locations. Although associated plant species have been shown to alter the structure of the soil microbial community [23, 34, 41], percent cover and plant species composition did not vary significantly among the sampling locations in this study (A.L. Davelos and L.L. Kinkel, unpublished data). Habitat variability for a variety of soil properties was positively correlated with genetic diversity in natural populations of a soil bacterium (*Pseudomonas cepacia*) [45]. Among the locations examined in this study, soil properties (including pH, K, total C, and Fe) did not differ significantly (L.L. Kinkel, unpublished data). Differences in the frequency and intensity of competitive interactions among the locations could also influence genetic composition and diversity. Patchiness in nutrient availability producing localized selection pressures could contribute to spatial structuring of microbial genotypes. Previous research examining differences in antibiotic activity and phenotypes found a relationship between high population density (i.e., high probability of competitive interactions) and phenotypic diversity [11].

In the study presented here, little variation in genetic diversity was found among depths. Over a wide range of depths from tens of centimeters to meters, other researchers have shown that genetic diversity tends to decrease with increasing soil depth [18, 24, 34, 35, 61, 75]. This decrease in diversity has been attributed to a variety of factors, including soil moisture and carbon and nitrogen availability. In contrast, the work presented here

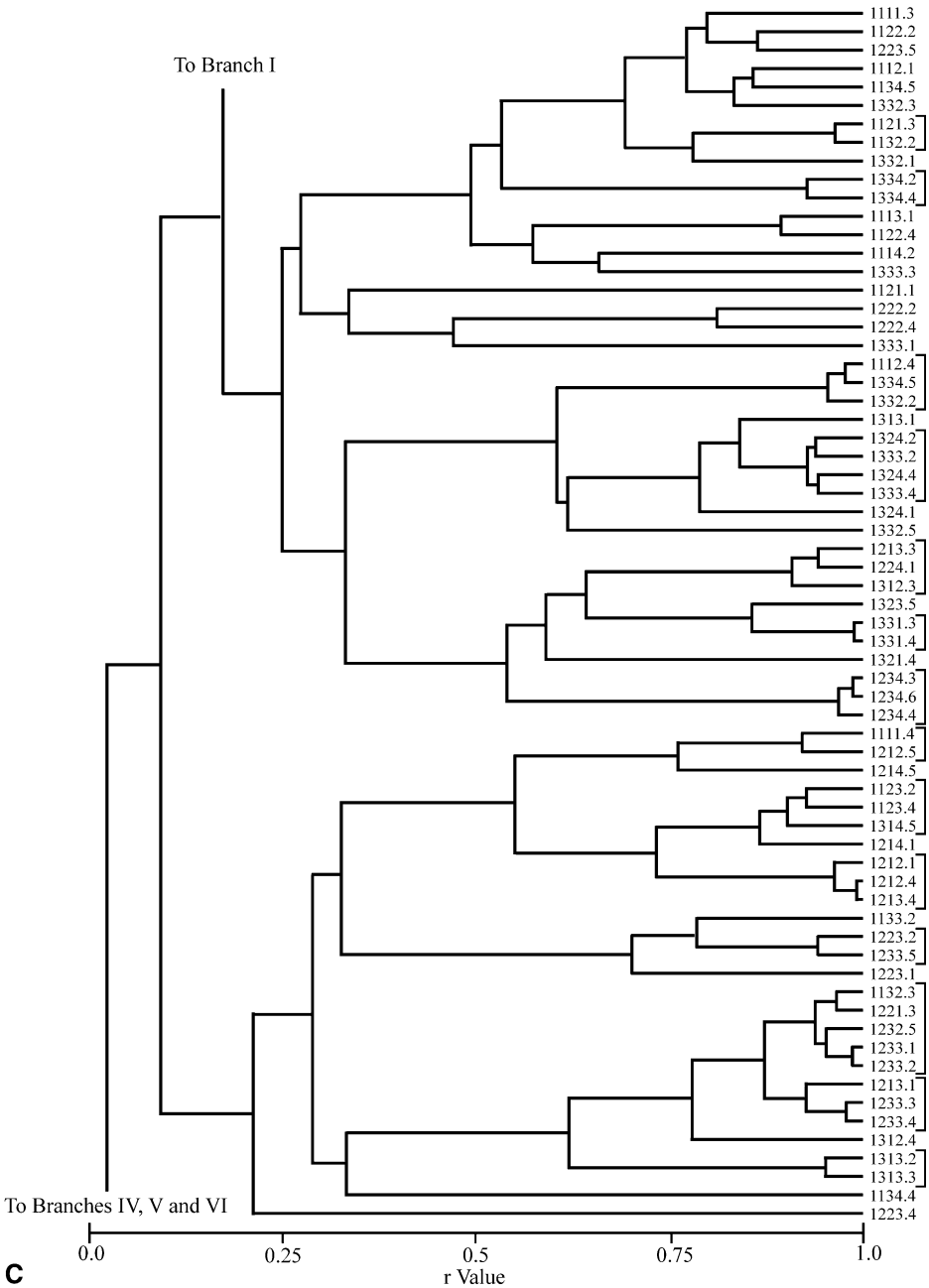

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Figure 2c. Continued

shows a slight trend for diversity to increase with soil depth (Table 2). This may be the result of samples on a smaller scale (8 cm) than in other studies examining the effect of soil depth on genetic diversity.

Almost half of the isolates sampled produced unique BOX-PCR fingerprints. The remaining isolates represented 25 clones. Although these clones generally occurred in the same location and at the same or adjacent depths, these samples did not simply contain repeated isolations of the same clone. In only a single sample out of the 36 total samples were all isolates from the same clone. Therefore, at the smallest scale sampled here, multiple clones co-occurred. Overlapping of clones at small spatial scales has been found for other soil microbes [25, 58, 69]. A recent study focusing on actinomycetes also revealed high diversity among isolates of closely related genera from a single soil sample [44].

In contrast, a small number of clones were found across locations. Clones of other soil microbes (*Pseudomonas* spp.) have also been shown to occur over wide geographic areas [30, 43, 47, 71]. Clones that were identified from 16S sequences as belonging to OTU 1 were found across locations. The finding of both multiple clones at a localized scale and a relatively widespread

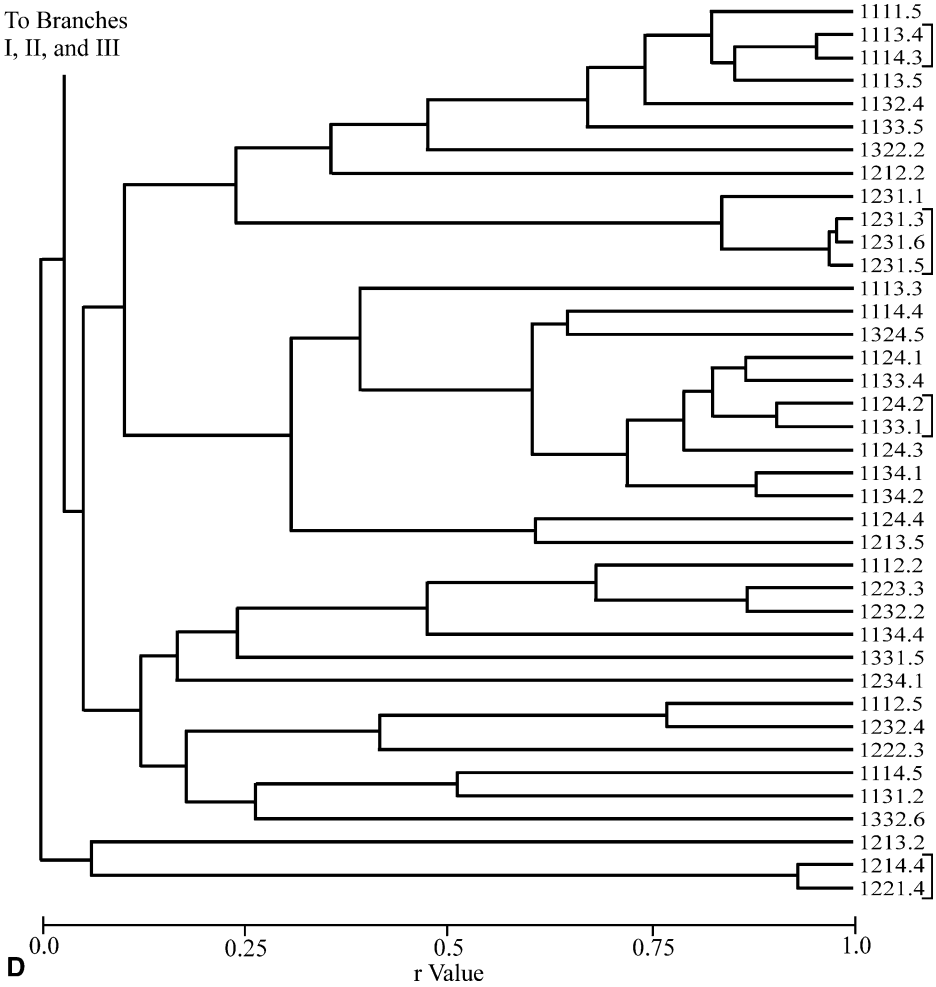

Figure 2d. Continued

distribution for other clones suggests that different clones utilize different ecological strategies for exploiting the heterogeneous matrix of the soil environment.

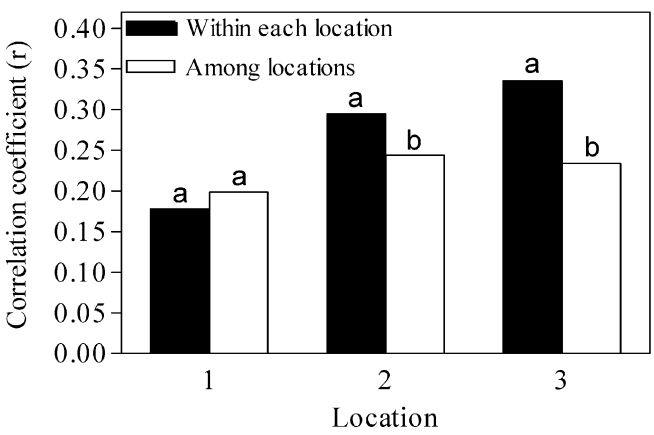

Figure 3. Mean correlation among BOX-PCR fingerprints of isolates within a location and among locations. Different letters indicate significant differences in means (LSD, $P < 0.05$).

There was poor correspondence between 16S groupings and fingerprint groupings. This finding is in contrast with studies of other soil microbes that found consistency between 16S gene sequences and other PCR-based methods used to investigate genetic diversity [3, 44]. The poor correspondence between species groupings and BOX-PCR fingerprints may reflect the instability of the *Streptomyces* genome resulting from chromosomal rearrangements such as deletions and DNA amplifications [12, 19, 37].

Understanding the spatial patterns of genetic diversity for soil microbial communities may provide invaluable insight into abiotic and biotic factors driving the observed heterogeneity and in linking genetic and functional diversity [14, 66]. Information on how genetic composition and diversity are arrayed in space also provides useful guidance for the development of sampling designs to ensure that diversity is adequately sampled. For culturable *Streptomyces*, the finding of clones occurring locally and clones found over a larger spatial scale may indicate that selection pressures and

ecological strategies vary among genotypes within this group.

Acknowledgments

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