Lack of correspondence between genetic and phenotypic groups amongst soil-borne streptomycetes

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Abstract
Correspondence between two distinct genetic traits, 16S rRNA gene sequences and repetitive element-sequence-based BOX-PCR DNA fingerprints, and antibiotic inhibition and resistance phenotypes was explored for a spatially explicit sample of Streptomyces from a prairie soil. There was no correspondence between 16S rRNA gene sequence groups and antibiotic phenotypes. However, 16S rRNA gene sequence groups differed significantly in mean inhibition zone sizes. Specific antibiotic phenotypes may reflect local selection pressures, as suggested by the significant differences in mean inhibition zone sizes against specific test isolates by Streptomyces from the same 16S rRNA gene sequence group but from different locations in soil. Significant correlations between antibiotic phenotypes and BOX-PCR fingerprints were found, but were small (r = 0.19–0.22). Although genetic characterizations alone were not predictive of specific antibiotic phenotypes, 16S rRNA gene sequence analyses may identify isolates that are most or least likely to possess substantial inhibitory potential, providing insight into the broad ecological strategy for individual isolates.

Introduction
Genetic characterization of microbial populations and communities in soil using 16S rRNA gene sequences, repetitive element-sequence-based PCR genomic DNA fingerprints and other molecular techniques has become commonplace. The focus of many of these studies is on contrasting microbial community composition between habitats, locations or plant species (Sikorski et al., 2002; Oda et al., 2003). Spatial patterns of genetic diversity have also been investigated, often with the goal of identifying environmental or phenotypic correlates of genetic diversity or community composition (Zhou et al., 2002; Stach et al., 2003).

A more fundamental goal in measuring genetic diversity is to investigate the strength and effects of natural selection in microbial populations (Doyle & Gaut, 2000). In total, patterns of genetic diversity and composition in space and time reflect the impacts of migration, growth, death (extinction) and evolution within the populations. The challenge to microecologists is to identify the extent to which specific patterns are the result of natural selection vs. stochastic processes. For example, high diversity in local populations of Pseudomonas stutzeri was influenced by migration, mutation and directional selection (Sikorski et al., 2002). Similar to genetic analyses, extensive studies of phenotypic variation, including virulence, denitrification rates, nutrient utilization and antibiotic inhibitory and resistance abilities, in microbial communities between habitats, locations and plant species have been performed (McDonald et al., 1989; Stephan et al., 2000; Rich et al., 2003; Davelos et al., 2004a). Much of this research focuses on the identification of factors (e.g. habitats, locations, plant species, genetic identity) correlated with particular phenotypes or phenotypic diversity. For instance, particular plant functional groups were found to have a significant effect on soil microbial catabolic activity and diversity (Stephan et al., 2000). Numerous studies on microbial populations have demonstrated close correspondence between genetic and phenotypic groups using a variety of genetic markers, including 16S rRNA gene sequences and repetitive element BOX-PCR genomic DNA.
fingerprints, and phenotypic traits, including carbon source utilization, resistance to antibiotics and production of antimicrobial substances (Berg 2000; Stephan et al., 2000; von der Weid et al., 2000; Oda et al., 2003). In particular, multiple studies have considered the correspondence between genetic and phenotypic groups or patterns of diversity for particular taxa within or between locations. For example, consistent genetic and phenotypic differences were found between major genotypes in the purple nonsulfur bacterium *Rhodopseudomonas palustris*, and the similarity between isolates decreased as a function of increasing distance between locations (Oda et al., 2003). However, poor correspondence between genetic and phenotypic groups has also been found for a variety of soil microorganisms, including fluorescent pseudomonads and streptomycetes (Lottmann & Berg, 2001; Davelos et al., 2004b). In these studies, both 16S rRNA gene sequences and repetitive element BOX-PCR genomic DNA fingerprints showed little correspondence with phenotypic traits, such as carbon source utilization, fatty acid methyl ester analysis and antibiotic resistance.

Many complex factors may influence the potential correspondence between presumptively neutral genetic markers and phenotypic traits that are assumed to be important to fitness and under active selection. These factors include the potential for the horizontal transfer of genes between microorganisms that are phylogenetically distinct, the intensity of local selection pressures and the frequencies and spatial scales of microbial movement (Maynard Smith et al., 1993; Metsä-Ketelä et al., 2002; Belotte et al., 2003; Nakamura et al., 2004). In particular, if the frequency of horizontal gene transfer for particular phenotypic traits is high and the intensity of local selection pressures for that phenotype is also high, relatively less correspondence between (neutral) genetic and phenotypic traits may be found than if horizontal transfer is uncommon or localized selection pressures are low. Furthermore, limited dispersal may induce patterns that are location specific: genetic and phenotypic group correspondence may be observed within individual locations, but may be less evident between geographically distinct populations.

**Streptomyces isolates**

The *Streptomyces* isolates used in this study were isolated from prairie soils at the Cedar Creek Natural History Area (CCNHA) in east-central Minnesota (www.cedarcreek.umn.edu). The sampling and isolation methods are detailed in Davelos et al. (2004a). Briefly, within a 1 × 1-m grid, three sampling locations were selected randomly. Plant species composition and soil characteristics, including pH, potassium, total carbon and iron, did not differ significantly between locations (L.L. Kinkel, unpublished data). At each sampling location, three adjacent soil cores (30 × 1 cm) were taken and transported to the laboratory on ice for immediate processing. In the laboratory, each core was divided into 2-cm subsections corresponding to soil depths of 0–2, 2–4, 4–6 and 6–8 cm. Thus, a total of 36 samples (four depths × three cores × three locations) was obtained. The analyses presented here focus on the differences between the three locations. Locations 1 and 2 were 68.6 cm apart, locations 1 and 3 were 91.4 cm apart and locations 2 and 3 were 25.4 cm apart.

Soil suspensions of each sample were dilution plated onto oatmeal agar, and streptomycete colonies were selected randomly and pure cultured (Davelos et al., 2004a). A total of 153 *Streptomyces* isolates, with a maximum of five isolates per sample, was purified. Spore suspensions of each isolate were stored in 20% glycerol at −80 °C.

**Genetic analyses**

**16S rRNA gene sequences**

DNA from each isolate was extracted using the Wizard Genomic DNA Purification Kit (Promega Corp., Madison, WI) following the manufacturer’s instructions. Partial 16S
rRNA gene sequences were obtained for each isolate as described previously (Davelos et al., 2004b). Briefly, amplification of 16S rRNA genes was performed following the protocol of Takeuchi et al. (1996) using the universal bacterial primers pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and pH (5′-AAGGAGGTGATCCAGCCGCA-3′) that amplify nearly full-length (c. 1.5 kbp) 16S rRNA gene products (Edwards et al., 1989). Partial 16S sequences (890–1199 bp) for each isolate were obtained by automated DNA sequencing using pA as the primer at the Advanced Genetic Analysis Center, University of Minnesota (St Paul, MN).

Using the similarity_matrix function of the Ribosomal Database Project (Maidak et al., 2001), isolates that had 16S rRNA gene sequences that differed by ≤1% were placed into the same operational taxonomic unit (OTU), resulting in 34 OTUs for the 153 Streptomyces isolates examined here (Davelos et al., 2004c). This level of similarity has been shown to minimize DNA–DNA reassociation values to <70% [cut-off point for phylogenetic definition of a species (Stackebrandt & Goebel, 1994)] for members of the class Actinobacteria (Stach et al., 2003).

A phylogenetic tree was constructed using phylip, version 3.5 (Felsenstein, 1993) with a matrix of Jukes–Cantor distances (Jukes & Cantor, 1969) and the neighbor-joining method (Saito & Nei, 1987). 16S rRNA gene sequences were edited using the Wisconsin Package Version 10.2 [Genetics Computer Group (GGC), Madison, WI], and were aligned with clustalw (http://www.ebi.ac.uk/clustalw/). This analysis was based on the 128-bp variable γ region within the 16S gene that has been used to resolve relationships within streptomycetes (Anderson & Wellington, 2001).

BOX-PCR genomic DNA fingerprints

DNA fingerprints, based on repetitive intergenic DNA sequences amplified using BOX primers, have been shown to distinguish Streptomyces strains (Sadowsky et al., 1996). Further studies have shown that Streptomyces isolates whose fingerprints are >90% correlated may be considered as clones (Davelos et al., 2004c). To identify clones and closely related isolates, DNA fingerprints for each of the 153 Streptomyces isolates were obtained and analyzed as described previously (Davelos et al., 2004c). Briefly, DNA was extracted from each isolate as described above. DNA was amplified following the procedure of Rademaker et al. (1998) using the BOXA1R primer (5′-CTACGGCAAGGC-GACGCTGACG-3′) (Versalovic et al., 1994). Fluorophore-enhanced repetitive element-sequence-based PCR (FERP) (Rademaker et al., 1998), as modified for agarose gels (Johnson et al., 2004), was used to visualize the fingerprints. Cluster analysis was performed on Pearson product-moment correlations (Häne et al., 1993) calculated for the array of densitometric values formed by the fingerprints, and a dendrogram was constructed (NT-SYS; Rohlf, 1998).

Phenotypic (antibiotic) analyses

All 153 Streptomyces isolates were tested in all possible pairwise combinations for the ability to inhibit and for resistance to members of a collection of 10 Streptomyces test isolates, as detailed in Davelos et al. (2004a, b). The 10 test isolates were isolated from agricultural soils in Minnesota and were selected for their range of inhibitory and resistance abilities (Davelos et al., 2004b). An antibiotic assay modified from Vidaver et al. (1972) was used to determine the inhibition of and resistance to each of the test isolates by the field-collected isolates. Briefly, spore suspensions of individual isolates were dotted onto starch casein agar plates and incubated at 28°C for 3 days. Dotted isolates were sacrificed by inverting the Petri-plates over chloroform. The plates were overlaid with 1% water agar and inoculated with a test isolate. The plates were incubated at 28°C for 3 days. Each field-collected isolate and test isolate were both dotted (to measure inhibition) and overlaid (to measure resistance). The size of any zone of growth inhibition of the overlaid isolate surrounding any dotted isolate was measured in millimeters from the edge of the dotted colony to the edge of the cleared zone. Each interaction was replicated on three separate plates. The mean zone size for each interaction between a field-collected isolate and a test isolate was determined (three replicates for each of 10 inhibition and 10 resistance interactions).

Frequency data summarize the number of test isolates that a field-collected isolate inhibited or was resistant to (presence/absence of an inhibition zone). From the frequency data, similarity matrices were generated using simple matching criteria (Sokal & Michener, 1958), and dendrograms were constructed (NT-SYS; Rohlf, 1998). Phenotypic groups were determined on the basis of 100% similarity of antibiotic phenotypes.

Analyses

Correspondence between genotype and phenotype

Previous analyses revealed a poor correspondence between 16S and DNA fingerprint groupings (Davelos et al., 2004c) and inhibition and resistance phenotypes (Davelos et al., 2004a). Therefore, the genomic DNA fingerprint correlation matrix and the 16S rRNA gene sequence distance matrix were compared with each of the distance matrices based on inhibition and resistance data to determine whether the matrices were correlated using Mantel’s test (NT-SYS; Rohlf, 1998). A positive correlation indicates that clustering amongst isolates is similar for both genetic and phenotypic
data. The significance of the correlation was determined as described in Lapointe & Legendre (1992).

**Patterns of inhibition and resistance amongst genotypes**

To examine the relationship between genetic identity and antibiotic activity, inhibitory and resistance abilities of individual isolates were mapped onto the 16S rRNA gene phylogenetic tree and the repetitive element-sequence-based PCR fingerprint dendrogram. Overall antibiotic capabilities were investigated by examining how the number of test isolates inhibited or resisted were distributed on the genetic trees. Further, the abilities to inhibit or resist each test isolate individually were examined.

**Mean antibiotic activity of OTUs**

Differences in the mean inhibition and resistance zone sizes were evaluated amongst OTUs composed of more than three isolates using ANOVA (PROC GLM; SAS Institute, Inc., 1988). Significant differences between means were determined using least significant differences (LSD). Analyses examined both means over all test isolates and means for each of the 10 test isolates separately.

**Spatial variability of antibiotic activity amongst OTUs**

Within each of the three locations, the mean inhibition and resistance zone sizes overall, and for each of the 10 test isolates individually, were evaluated amongst OTUs using ANOVA (PROC GLM; SAS Institute, Inc., 1988). Further, within each OTU, differences in the mean inhibition and resistance zone sizes between locations were examined overall and for each of the 10 test isolates separately. Significant differences between means were determined using LSD.

**Results**

**Correspondence between genotype and phenotype and patterns of inhibition and resistance amongst genotypes**

There was no correspondence between OTUs based on 16S rRNA gene sequences and antibiotic inhibition \((n = 53)\) phenotypes; Davelos et al., 2004a; \(r = -0.23, P > 0.05\) or resistance \((n = 23)\) phenotypes; Davelos et al., 2004a; \(r = -0.29, P > 0.05\) phenotypes. Within larger OTUs \((n \geq 5)\), multiple inhibition and resistance phenotypes were found (Fig. 1). The distribution of specific antibiotic and resistance phenotypes within the 16S phylogenetic tree revealed little specificity for phenotypes within genetic groups. All isolates of some small OTUs were able to inhibit all 10 test isolates (Fig. 1, OTU 7), but other OTUs included isolates that could inhibit all test isolates and some that could inhibit none (Fig. 1, OTUs 3 and 5).

Inhibition and resistance to each of the 10 test isolates individually were also mapped onto the 16S phylogenetic tree (data not shown). In general, OTUs that had more than five isolates contained streptomycetes that could inhibit and those that could not inhibit individual test isolates. However, all isolates from some small \((n < 5)\) OTUs were unable to inhibit an individual test isolate, suggesting some specificity of inhibitory ability within an OTU. Likewise, resistance to specific test isolates varied between OTUs. For two test isolates \((6–14, 10–3)\), only isolates from OTU 10 (see Fig. 1) were not resistant. For a group of six test isolates \((2–12, 4–16, 4–20, 4–21, 4–24, 93)\), field isolates from specific OTUs also varied in their patterns of resistance. For example, isolates from OTU 3 were resistant to the majority of these six test isolates, whereas isolates from OTU 2 were susceptible to most of them (see Fig. 1).

BOX-PCR genomic DNA fingerprints were statistically significantly correlated with inhibition phenotypes \((r = 0.19, P < 0.0001)\), although the correlation was low (Fig. 2). The association between antibiotic inhibition phenotype and BOX-PCR fingerprints appears to be driven by the largest inhibition phenotype \((n = 61)\) isolates in this group were unable to inhibit any of the test isolates; Davelos et al., 2004a). Over half of these isolates occurred together in a single BOX-PCR fingerprint cluster and represented 77% of the isolates in that cluster \((37 of the 48 isolates in Branch I; Fig. 2a)\). In contrast, isolates that were able to inhibit all 10 test isolates \((n = 14)\) were distributed throughout the BOX-PCR fingerprint dendrogram, although they tended to cluster together in small groups (Fig. 2).

Resistance phenotypes and BOX-PCR fingerprints were also significantly correlated \((r = 0.22, P < 0.0001)\), apparently reflecting the predominance of the largest resistance phenotype \((n = 41)\) isolates able to resist the same five test isolates; Davelos et al., 2004a; Resistance Group A, Fig. 2) within a single fingerprint cluster. Over half of the isolates in the largest phenotype occurred in a single fingerprint cluster \((23 isolates in Branch I; Fig. 2a)\). In contrast, isolates from the next two largest resistance phenotypes \((n = 30)\), able to resist the same four test isolates; \(n = 23\), able to resist the same seven test isolates; Davelos et al., 2004a; Resistance Groups B and C, respectively; Fig. 2) occurred in small clusters throughout the fingerprint dendrogram (Fig. 2).

Finally, inhibition of or resistance to each of the 10 test isolates individually was mapped onto the BOX-PCR fingerprint dendrogram (data not shown). In general, small clusters of fewer than four isolates with similar inhibitory or resistance abilities were distributed throughout the dendrogram.
Mean antibiotic activity of OTUs

Both mean inhibition ($F_{6,1193} = 255, P < 0.0001$; Fig. 3) and resistance ($F_{6,1193} = 28.9, P < 0.0001$; Fig. 3) zone sizes differed significantly between the OTUs that contained more than three isolates (OTUs 1–7). In particular, isolates from OTUs 7 and 5 produced significantly greater mean inhibition zone sizes than isolates in other OTUs.

When the mean inhibition zone sizes were examined for OTUs against each of the 10 test isolates separately, isolates from OTUs 7 and 5 were consistently better at inhibiting test isolates, and produced significantly greater mean inhibition zone sizes than other OTUs against every test isolate. OTUs also varied significantly in their susceptibility to antibiotic inhibition. Isolates from OTUs 2 and 6 were more susceptible to inhibition by the 10 test isolates than isolates from the other OTUs (Fig. 3). When differences in mean resistance zone sizes between OTUs for each test isolate separately were examined, significant differences were found for six of the 10 test isolates (results not presented). In each case,
isolates from OTUs 2 and 6 showed significantly greater inhibition than the other OTUs. Similar to the results found for inhibition, there was no evidence for specificity in resistance ability for field-collected isolates towards particular test isolates across all locations.

Spatial variability of antibiotic activity amongst OTUs

The mean inhibition and resistance of isolates within the seven most abundant OTUs were examined to determine
whether there was evidence for the spatial localization of antibiotic activities (significant differences between locations) within individual OTUs (Fig. 4). In the three OTUs that occurred in all locations (OTUs 1, 3 and 4), there were no significant differences in mean inhibition or resistance activities between locations for these OTUs. However, for two of the smaller OTUs that were each found in two locations, significant differences in mean inhibition and resistance zone sizes were observed between locations (Fig. 4).

To further examine spatial localization in selection pressures, the inhibitory and resistance activities for individual OTUs against specific test isolates were compared between locations. Only two of the OTUs (OTUs 1 and 2) had isolates in sufficient numbers across multiple locations to adequately test for differences in inhibition or resistance activities.
against each of the 10 test isolates. For OTU 1, there were no
significant differences in mean inhibition or resistance zone
sizes across locations for each of the 10 test isolates individ-
ually (Table 1). However, for OTU 2, there were significant
differences in mean inhibition and resistance zone sizes for
different test isolates between the two locations (Table 1),
providing evidence for localized selection. Isolates from
OTU 2 from Location 3 produced significantly larger mean
inhibition zone sizes against two of the test isolates (2–12
and 4–21) than isolates from Location 1. In contrast, isolates
from OTU 2 from Location 1 produced significantly larger
mean inhibition zone sizes against another test isolate
(10–3) than isolates from Location 3. When examining
mean resistance zone sizes, isolates in OTU 2 from Location
1 were significantly more resistant than isolates from Loca-
tion 3 to one of the test isolates (2–12; Table 1).
Discussion

The genetic identity of an isolate, as determined by the 16S rRNA gene sequence, was not predictive of its antibiotic phenotype. Consistent with this finding, very similar antibiotic biosynthesis genes have been found in distantly related Streptomyces species, and diverse antibiotic genes have been found in closely related species (Metsä-Ketelä et al., 2002). Diversity in antibiotic phenotypes and 16S rRNA gene sequences is likely to be generated by different evolutionary processes contributing to this lack of correspondence. For example, amongst streptomycetes, the horizontal transfer of antibiotic resistance and biosynthetic genes has been well documented (Wiener et al., 1998; Egan et al., 2001; Tolba et al., 2002), and should produce a net-like population structure reflecting frequent genetic transfer amongst lineages (Maynard Smith et al., 1993). In contrast, mutations are believed to be the primary generator of variation in 16S sequences between bacteria, although it has been suggested that horizontal transfer of genetic material may also generate 16S sequence diversity (Cooper & Feil, 2004). Further, under positive selection, novel antibiotic phenotypes are more likely to be retained than are selectively neutral 16S rRNA gene sequence variants. This difference in selective advantage is likely to produce streptomycete isolates that may have identical or very similar 16S rRNA gene sequences, but different phenotypes, as a consequence of local selection for enhanced antibiotic activities (Fig. 1; e.g. OTUs 1 and 2). Likewise, horizontal transfer of antibiotic biosynthesis genes between distinct 16S rRNA gene sequence groups, followed by positive selection for those gene functions, will produce isolates having similar phenotypes but different 16S rRNA gene sequences (Fig. 1).

BOX-PCR fingerprints and antibiotic phenotypes were significantly associated. However, the correlations were very low, accounting for 3.6% and 4.8% of the variance for inhibition and resistance, respectively ($r = 0.19$ for inhibition, $r = 0.22$ for resistance), and the relationship appears to be driven by a few groups of isolates with similar BOX-PCR fingerprints that tend to have similar antibiotic phenotypes. However, overall, isolates that show similar BOX-PCR fingerprints sometimes possess widely differing antibiotic phenotypes (Fig. 2). For example, within a large cluster of noninhibiting isolates, there were two isolates which were
able to inhibit all 10 test isolates (topmost cluster in Fig. 2a). These isolates may differ in the presence or absence of specific antibiotic biosynthesis genes or in the regulation of gene expression (Hallet, 2001; Feldgarden et al., 2003). A similar lack of correspondence between genomic fingerprints and phenotypic traits has been found amongst some Pseudomonas species (Lottmann & Berg, 2001). In contrast, work on a range of other soil microorganisms has shown substantial agreement between BOX-PCR genomic DNA fingerprints and a variety of phenotypic traits (e.g. Berg, 2000; Oda et al., 2003; Davelos et al., 2004). For example, clustering of Serratia plymuthica isolates was consistent for BOX-PCR fingerprints, antibiotic resistance and carbon source utilization (Berg, 2000). Variation in the correspondence between BOX-PCR fingerprints and phenotypic traits may be influenced by differences between phenotypic traits in the significance of positive selection for those traits and the frequency of horizontal transfer of genes coding for specific traits.

Despite the lack of correspondence between 16S rRNA gene sequences and specific antibiotic inhibitory or resistance phenotypes in this streptomycete community, there was significant variation amongst OTUs (16S rRNA gene sequence groups) in overall antibiotic activity (Fig. 3). Although isolates within a particular OTU often did not share a specific inhibitory or resistance phenotype, OTUs could be characterized by generally strong or weak antibiotic activities. For example, OTUs that had isolates that tended to be strong inhibitors or resistors may rely upon a life history strategy that emphasizes interference competition, whereas weak inhibitors may possess other potentially selectively advantageous phenotypes, including higher growth rates, more efficient nutrient utilization or greater niche specialization. Further work is needed to distinguish the significance of these possibilities for streptomycete populations in soil.

In previous work, we argued that differences in antibiotic inhibitory activities amongst streptomycete populations from different locations reflected local differences in the selection for antibiotic inhibitory activities and in the significance of antibiotic inhibition to streptomycete fitness (Davelos et al., 2004a). If selection varies significantly between locations, or is localized in space, streptomycete isolates having similar 16S rRNA gene sequences that have been isolated from different locations in soil should, in some cases, have distinct phenotypes. Although only two 16S rRNA gene sequence groups had sample sizes adequate to test this hypothesis, there was clear evidence for such local adaptation. Specifically, for OTU 2, there were significant differences in the inhibitory activity and in resistance to the inhibition of the 10 streptomycete test isolates by isolates from two different locations (Table 1). This finding supports the idea that specific antibiotic phenotypes are a function of local habitat. This local variation in antibiotic activity against a stable genetic background (i.e. within a single OTU) suggests that local selection pressures on antibiotic phenotypes are strong and likely to be important in generating diversity in these communities. Moreover, both the likelihood of horizontal gene transfer (Pennisi, 2004) and similarity in the horizontal gene pool available for transfer are greater for isolates in close spatial proximity. Further data are needed to clarify the significance of these distinct mechanisms in generating spatial variation in antibiotic phenotypes.

Table 1. Mean inhibition and resistance zone sizes of field-collected Streptomyces isolates from two operational taxonomic units (OTUs) against a set of 10 test Streptomyces isolates in three locations (1, 2, 3)*

<table>
<thead>
<tr>
<th>Test isolate</th>
<th>Mean inhibition zone size</th>
<th>Mean resistance zone size</th>
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<tbody>
<tr>
<td></td>
<td>OTU 1</td>
<td>OTU 2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2–12</td>
<td>n = 7</td>
<td>n = 26</td>
</tr>
<tr>
<td>4–2</td>
<td>0.00a</td>
<td>0.12a</td>
</tr>
<tr>
<td>4–16</td>
<td>0.11a</td>
<td>0.04a</td>
</tr>
<tr>
<td>4–20</td>
<td>0.14a</td>
<td>0.05a</td>
</tr>
<tr>
<td>4–21</td>
<td>0.00a</td>
<td>0.01a</td>
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<tr>
<td>4–24</td>
<td>0.00a</td>
<td>0.03a</td>
</tr>
<tr>
<td>6–14</td>
<td>0.00a</td>
<td>0.01a</td>
</tr>
<tr>
<td>10–3</td>
<td>0.00a</td>
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<tr>
<td>87</td>
<td>0.00a</td>
<td>0.05a</td>
</tr>
<tr>
<td>93</td>
<td>0.05a</td>
<td>0.32a</td>
</tr>
</tbody>
</table>

*n is the sample size of streptomycete isolates.

Raw mean zone sizes, measured in millimeters, are reported. Values with the same letter are not significantly different at $P < 0.05$ (least significant difference). Test isolates are described in Davelos et al. (2004b).
Streptomyces populations in prairie soil exhibited substantial spatial variation in both phenotype and 16S rRNA gene sequence, but there was no correspondence between these traits. Thus, genetic characterization alone, focusing on 16S rRNA gene sequence analyses, will provide incomplete information on the specific interaction potential of individual Streptomyces isolates. However, this work suggests that genetic characterization may provide insight into the broad ecological strategy for individual isolates, specifically identifying those isolates that are most or least likely to possess substantial inhibitory potential. Furthermore, although 16S rRNA gene sequence groups may define the broad capacity of an organism to inhibit others, the data suggest that local processes can be important in determining the capacity of an isolate to inhibit other specific target isolates. Thus, variation in local evolutionary and ecological processes across the soil landscape is likely to be an important contributor to the generation and maintenance of phenotypic diversity within individual 16S lineages and to the lack of correspondence between genetic and phenotypic groups within Streptomyces. Further work is needed to resolve the origins of spatial variation in antibiotic phenotypes within individual 16S rRNA gene sequence groups, particularly to determine the relative significance of distinct evolutionary (horizontal transfer, mutation, recombination) and ecological (local selection, dispersal) forces in generating this variation.

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