RESEARCH ARTICLE

Global biogeography of *Streptomyces* antibiotic inhibition, resistance, and resource use

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Abstract

Although recent molecular techniques have greatly expanded our knowledge of microbial biogeography, the functional biogeography of soil microorganisms remains poorly understood. In this work, we explore geographic variation in *Streptomyces* phenotypes that are critical to species interactions. Specifically, we characterize *Streptomyces* from different locations from multiple continents for antibiotic inhibition, resistance, and resource use phenotypes. *Streptomyces* from different locations varied significantly in antibiotic inhibition, resistance, and resource use indicating that communities vary in functional potential. Among all isolates, there were substantial differences in antibiotic inhibition, resistance, and resource use within and among and within *Streptomyces* species. Moreover, *Streptomyces* with near-identical 16S rRNA gene sequences from different locations sometimes differed significantly in inhibition, resistance, and resource use phenotypes, suggesting that these phenotypes may be locally adapted. Thus, in addition to a likely role of environmental filtering, variation in *Streptomyces* inhibitory, resistance, and resource use phenotypes among locations is likely to be a consequence of local selection mediated by species interactions.

Introduction

Microbial biogeography, the study of the spatial distribution of microbial diversity, has engaged microbial ecologists for almost a century (Beijerinck, 1913; Baas-Becking, 1934; Cho & Tiedje, 2000). Recent studies of global patterns of soil microbial diversity have concluded that the taxonomic structure and diversity of soil microbial communities are variously organized by soil edaphic characteristics (e.g. pH), soil history, climatic variables (moisture, temperature), and plant communities (Garbeva *et al.*, 2004, 2008; Fierer & Jackson, 2006; Lauber *et al.*, 2009; Griffiths *et al.*, 2011). Although valuable to our understanding of microbial community ecology and distribution, these studies have relied predominately on highly conserved marker genes (e.g. 16S rRNA genes) to infer bacterial community composition, structure, and diversity. As a result, these studies fail to capture important variation in bacterial phenotypes or functions that are critical to microbial populations. Many phenotypic traits that are critical to microbial fitness and expected to play important roles structuring in microbial communities are highly variable among closely related microbial taxa (Oda *et al.*, 2002; Davelos *et al.*, 2004a; Al-Dahouk *et al.*, 2012; Schlatter *et al.*, 2013). Moreover, it has been argued that phenotypic traits, rather than phylogenetic units, are more accurately considered the units of microbial biodiversity (Green *et al.*, 2008). However, there have been few efforts to explore the biogeography of microbial traits. A trait-based approach for studying microbial biogeography is likely to provide unique insight into how functional traits are organized among distinct microbial populations and into the factors that structure the distribution of traits in space.

Biogeographic patterns in microbial traits may be generated by multiple processes. Environmental or niche filtering may occur where environmental parameters 'select' for taxa with traits that are best adapted to a given habitat (Langenheder & Székely, 2011). Alternatively, taxa may rapidly adapt to maximize fitness in local conditions via adaptive radiation (Craig MacLean & Bell, 2003; Brockhurst *et al.*, 2007), potentially resulting in similar traits among phylogenetically distinct taxa in the same locations. Although biogeographic patterns may emerge

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from niche filtering, local adaptation of traits, or a combination thereof, the role of local adaptation in the diversification and organization of microbial traits across space and evolutionary time in natural habitats is especially poorly understood. Efforts to link functional traits of microbial taxa from distinct geographic locations with phylogeny are needed to shed light on the role of functional trait adaptation in generating geographic patterns in microbial diversity.

Streptomyces are Gram-positive, filamentous bacteria that are ubiquitous in soils and sediments across the globe (Seipke *et al.*, 2011; Kinkel *et al.*, 2012). As a genus, *Streptomyces* harbors a remarkable diversity of traits important for human health and ecosystem function. Specifically, *Streptomyces* are well known for their production of a large number of diverse antibiotic compounds, with an estimated 5–10% of a typical *Streptomyces* genome associated with antibiotic biosynthetic pathways (Omura *et al.*, 2001; Watve *et al.*, 2001; Bentley *et al.*, 2002; Challis & Hopwood, 2003). Additionally, *Streptomyces* are an important reservoir of antibiotic resistance genes in soil and may carry resistance to clinical antibiotics even in the absence of anthropogenic contamination (D'Costa *et al.*, 2006; Bhullar *et al.*, 2012). Finally, *Streptomyces* are capable of utilizing a diverse array of carbon compounds, including cellulose, chitin, and lignin, as resources for growth (Williamson *et al.*, 2000; Schrempf *et al.*, 2011; Schlatter *et al.*, 2013), thus contributing to nutrient cycling in the environment. Insight into how variation in antibiotic inhibition, resistance, and resource use traits among *Streptomyces* populations is organized across diverse geographic locations will begin to shed light on the functional biogeography of this important genus.

Antibiotic inhibition, resistance, and resource use are believed to be crucial for competitive interactions among *Streptomyces* (Williams & Vickers, 1986; Davelos *et al.*, 2004a; Hibbing *et al.*, 2010; Schlatter *et al.*, 2013). Because *Streptomyces* have linear chromosomes that are prone to recombination in regions that contain genes for antibiotic production and resistance (Egan *et al.*, 1998; Wiener *et al.*, 1998; Hopwood, 2007), as well as multiple plasmids, *Streptomyces* have the potential to evolve rapidly in response to selection and specifically to interactions with coexisting community members. In particular, natural selection is expected to favor those antibiotic inhibitory traits that effectively inhibit resource competitors and resistance traits that protect individuals from inhibition (Kinkel *et al.*, 2014). Thus, local selection for antibiotic inhibition, resistance, and resource use traits will depend on specific interactions that occur within the context of the microbial community. Variation in species interactions, and thus selection on traits involved in species interactions, across different communities may generate biogeographic patterns in these traits and thus contribute to the extensive global diversity of antibiotic inhibition, resistance, and resource use traits among *Streptomyces*.

The objectives of this work were to (1) characterize a global collection of *Streptomyces* for antibiotic inhibition, resistance, and resource use traits; (2) explore variation in *Streptomyces* antibiotic inhibition, resistance, and resource use traits among distinct geographic locations; and (3) determine relationships between *Streptomyces* traits and phylogeny among locations. These data shed light on the biogeography of antibiotic inhibition, resistance, and resource use traits among *Streptomyces* and the potential role of adaptation in structuring trait characteristics among distinct geographic locations.

Methods

Streptomyces isolation

Soil samples from 15 different locations around the globe were collected using a standard soil corer (10 × 2.5 cm). Sampling locations and their abbreviations are described in Table 1. In Minnesota (MN), soil sampling was performed differently, and three adjacent small (30 × 1 cm) soil cores were taken from three locations as described in Davelos *et al.* (2004a). Soil samples were homogenized and subsamples of each soil (1 g) were shaken in 10 mL sterile deionized H_2O in an orbital shaker at 4 °C for 1 h. Samples were dilution-plated on starch-casein agar (SCA), and after 5–7 days of growth at 28 °C, colonies exhibiting characteristic *Streptomyces* morphology were isolated and streaked to obtain pure cultures. A subset of isolates (n = 10–12) from each location was randomly selected for in-depth phenotypic and genetic characterization for a total of n = 153 isolates.

Antibiotic inhibition

Antibiotic inhibitory traits of *Streptomyces* isolates were evaluated against five standard *Streptomyces* isolates that vary in their antibiotic resistances (DL87, LK4-2, LK2-12, LK4-16, and LK6-14; Davelos *et al.*, 2004b) using an agar-overlay method as described in Davelos *et al.* (2004a). Briefly, 10 μL of spore suspension of each isolate (~10^8 CFU mL^{-1}) was dotted onto 15 mL SCA, grown for 3 days, and killed by inverting plates over 4 mL chloroform in a watchglass. After residual chloroform was left to evaporate in a laminar flow hood for 30 min, 15 mL of 1% water agar was pipetted onto each plate and 100 μL of spore stock of each test standard was overlayed by spread plating. After 3 days of growth at 28 °C,

Table 1. Sites from which soil was sampled for *Streptomyces* isolation

Location of origin	Abbreviation	Number of isolates	Habitat type
Antarctica	Ant	$n = 10$	Antarctic peninsula
La Cima, California, USA	CALC	$n = 10$	Hot desert
Cevennes National Park, France	Cev	$n = 10$	Temperate forest
Hawaii, USA	Haw	$n = 10$	Montane forest
Kansas, USA	KS	$n = 10$	Native tallgrass prairie
Morocco	MC	$n = 10$	Agricultural field
Minnesota, USA	MN1	$n = 10$	Native tallgrass prairie
Minnesota, USA	MN3	$n = 10$	Native tallgrass prairie
Minnesota, USA	MN5	$n = 10$	Native tallgrass prairie
Montseny, Spain	Mont	$n = 11$	Montane forest
New Zealand	NZ	$n = 10$	Temperate beech forest
Fort Sherman, Panama	PanFS	$n = 10$	Tropical rain forest
Santa Clara, Panama	PanSC	$n = 10$	Tropical sand beach
Volcan Baru, Panama	PanVB	$n = 10$	Tropical cloud forest
Witzenhausen, Germany	Witz	$n = 12$	Temperate forest

inhibition zone sizes were measured as the radius from the edge of the colony growth to the edge of any clear inhibition zone. Each isolate–standard interaction was replicated 2–3 times, and mean inhibition zone sizes were determined. Interactions were considered inhibitory where inhibition zones were > 2 mm.

Antibiotic resistance

Streptomyces isolates were characterized for resistance to common, *Streptomyces*-produced, clinical antibiotics using a disk diffusion assay as described in Otto-Hanson *et al.* (2013). Briefly, filter paper disks containing streptomycin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), vancomycin (30 μg), rifampicin (10 μg), amoxicillin/clavulanic acid (30 μg), and tetracycline (30 μg) (BD BBL Sensi-Disc; Becton, Dickinson and Company, Sparks, MD) were placed on SCA plates immediately after 100 μL of spore suspension of an isolate was spread-plated and dried. After 3 days of growth at 28 °C, inhibition zones were measured as described above. Isolate–antibiotic interactions where inhibition zones < 2 mm were considered resistant.

Resource use characterization

Resource use was evaluated on 95 sole carbon sources for each isolate using Biolog SF-P2 plates (Biolog, Inc. Hayward, CA) as described previously (Schlatter *et al.*, 2009). Briefly, fresh spore suspensions of each *Streptomyces* isolate were adjusted to an optical density of 0.22 at $OD_{590\ nm}$, diluted according to the manufacturer's instructions (1.5 mL spore suspension in 13.5 mL 0.2% carrageenan), and inoculated into 96-well Biolog plates. The absorbance (au) of each well was determined after 3 days of incubation at 28 °C using a Multiskan EX microplate reader (Labsystems, Helsinki, Finland) at 590 nm. For each plate, the absorbance of the water control well was subtracted from the absorbance of all other wells before analysis. We considered used resources to be those on which a *Streptomyces* isolate grew to an absorbance greater than 0.005 above the water control well. Using this definition, niche width, resource use efficiency, and efficiency on preferred resources were determined for each isolate. We defined the niche width of an isolate as the number of used resources, total growth as the sum of absorbances across all resources, and resource use efficiency as the mean absorbance value for used resources. Niche overlap, a measure of shared resource use among isolates, was calculated as described previously (Kinkel *et al.*, 2014). Briefly, niche overlap = $\{(\sum$ Min absorbance a, b)/total absorbance a + ($\sum$Min absorbance a, b)/total absorbance b$\}/2$ where (Min absorbance a, b) is the minimum absorbance value for a pair of isolates a and b on a given carbon source; values are summarized over $n = 95$ carbon sources. The total absorbance for an isolate a or b is the sum of absorbance values for that isolate over all $n = 95$ carbon sources.

16S rRNA gene sequencing

Sequences for 16S rRNA genes were obtained as described previously (Davelos *et al.*, 2004c). Briefly, genomic DNA was extracted using the Wizard genomic DNA purification kit (Promega, Madison, WI) according to the manufacturer's instructions with minor modification, and 16S rRNA genes were amplified using the universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1541R (5′-AAGGAGGTGATCCAGCCGCA-3′) in a 50 μL reaction volume using PCR Supermix High Fidelity master mix (Invitrogen, Carlsbad, CA) with 10 pM each primer and 100 ng template DNA following the thermocycling protocol of Takeuchi *et al.* (1996). Amplicons were sequenced using the forward primer (27F) at the University of Minnesota Biomedical Genomics Center (Saint Paul, MN). Sequences were edited manually, aligned, and trimmed to 685 bp of good-quality sequence

using MEGA (v5.2; Tamura *et al.*, 2011). A neighbor-joining phylogenetic tree was constructed in MEGA with 1000 bootstraps (Supporting information, Fig. S1). A matrix of pairwise distances was constructed, and sequences were binned into operational taxonomic units (OTUs) based on a 99% similarity cutoff in mothur (v1.31; Schloss *et al.*, 2009). Finally, sequences were classified using the Ribosomal Database Project Naïve Bayesian Classifier (Wang *et al.*, 2007).

Statistical analyses

All statistical tests assessing phenotypic differences among *Streptomyces* from different locations and OTUs were performed in R (version 3.0.2; R Core Team, 2013) unless noted otherwise. Similarity in antibiotic inhibition, resistance, and resource use profiles among isolate pairs was calculated as the Euclidean distance among quantitative profiles (inhibition and resistance zone sizes, resource use absorbance) using the vegan package in R (Oksanen *et al.*, 2011). Relationships between 16S distance and antibiotic inhibition, resistance, and resource use patterns were assessed using Mantel tests with 999 permutations. Finally, AMOVA and UniFrac analyses of 16S rRNA gene sequences were performed in mothur to test for differences in community structure among locations.

Results

Diversity of antibiotic inhibition, resistance, and resource use among a global collection of *Streptomyces*

Among all isolates ($n = 153$), there were 19 distinct profiles (+/−) of antibiotic inhibition of the five test standards out of a total of $n = 32$ possible profiles. On average, isolates inhibited 0.9 standards although most *Streptomyces* did not inhibit any standards (63%, or 97/153; Fig. 1). Of these, 42.9% (25/56 inhibitory isolates) inhibited only one standard, while 23.2% (13/56 inhibitory isolates) inhibited all five standards. This indicates a bimodal distribution of inhibitory capacities among *Streptomyces* isolates.

When all *Streptomyces* isolates were screened for resistance against seven antibiotics, there were 27 unique resistance profiles out of $n = 128$ possible profiles. Although some isolates (21.6%, or 33/153) did not resist any antibiotics, most isolates (78.4%, or 120/153) resisted at least one of the antibiotics tested. The proportion of resistant *Streptomyces* varied among antibiotics ($\chi^2 = 216$, d.f. = 6, $P < 0.0001$; Fig. 2). Resistance to tetracycline or rifampicin was most common (49% of isolates were resistant to one or both of these antibiotics), whereas

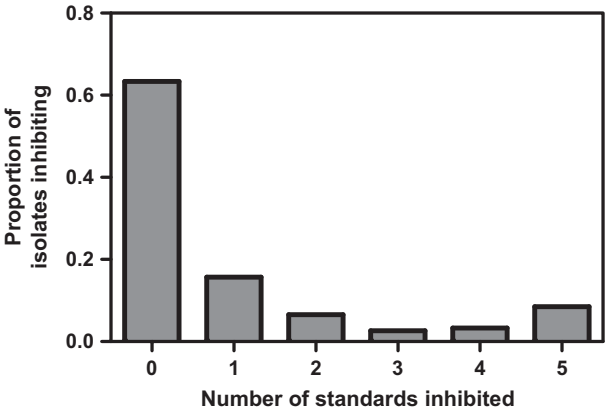

Fig. 1. Distribution of antibiotic inhibitory capacities against five standards among a global collection of *Streptomyces*.

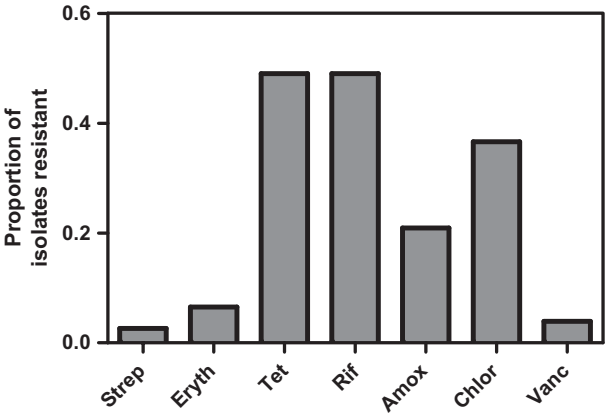

Fig. 2. Proportion of all *Streptomyces* resistant to clinically relevant, *Streptomyces*-produced antibiotic compounds. Compounds include streptomycin (Strep), erythromycin (Eryth), tetracycline (Tet), rifampicin (Rif), amoxicillin (Amox), chloramphenicol (Chlor), and vancomycin (Vanc).

resistance to streptomycin and vancomycin was rare (2.6% and 3.9% of isolates were resistant, respectively). Thus, although antibiotic resistance is common among *Streptomyces*, the likelihood of resistance varies among antibiotics.

Resource use patterns among the global collection of *Streptomyces* were diverse. Considering patterns of utilized resources (+/−), there were 148 unique patterns among the 153 *Streptomyces* isolates. On average, individual *Streptomyces* isolates used 63.8 resources, although use ranged from 9 to 95 of 95 possible resources among all isolates. Total growth of *Streptomyces* on all resources ranged from 0.39 to 33.23 au with an average of 7.32 au. Growth efficiency, or mean growth on utilized resources, ranged from 0.033 to 0.44 au with an average of 0.11 au. Thus, *Streptomyces* isolates exhibited considerable variation

in the numbers and identities of compounds on which they grew as well as in growth efficiency.

Streptomyces communities vary among locations in antibiotic inhibition, resistance, and resource use

The breadth and intensity of antibiotic inhibitory capacities differed among *Streptomyces* from distinct locations. Specifically, *Streptomyces* isolates from different locations varied in the mean number of standards that they inhibited (Fig. 3a; ANOVA, d.f. = 14, $F = 2.39$, $P = 0.005$). On average, *Streptomyces* from PanFS and MN5 inhibited the most standards (2.1 and 2.0 standards, respectively), whereas those from Cev and CALC inhibited the fewest (0 and 0.2, respectively). Thus, some locations supported *Streptomyces* with broader antibiotic inhibitory capacities than others. In contrast, mean inhibition zone size among *Streptomyces* from different locations varied only marginally (Table 1; ANOVA; d.f. = 14, $F = 1.69$, $P = 0.064$). However, when considering *Streptomyces* inhibition of individual standards, mean zone sizes varied significantly among locations for three of the five standards (Table S1). Thus, although the overall intensity of inhibition against all standards varied only marginally among *Streptomyces* from different locations, *Streptomyces* from some locations were significantly more effective at inhibiting specific standards than those from other locations.

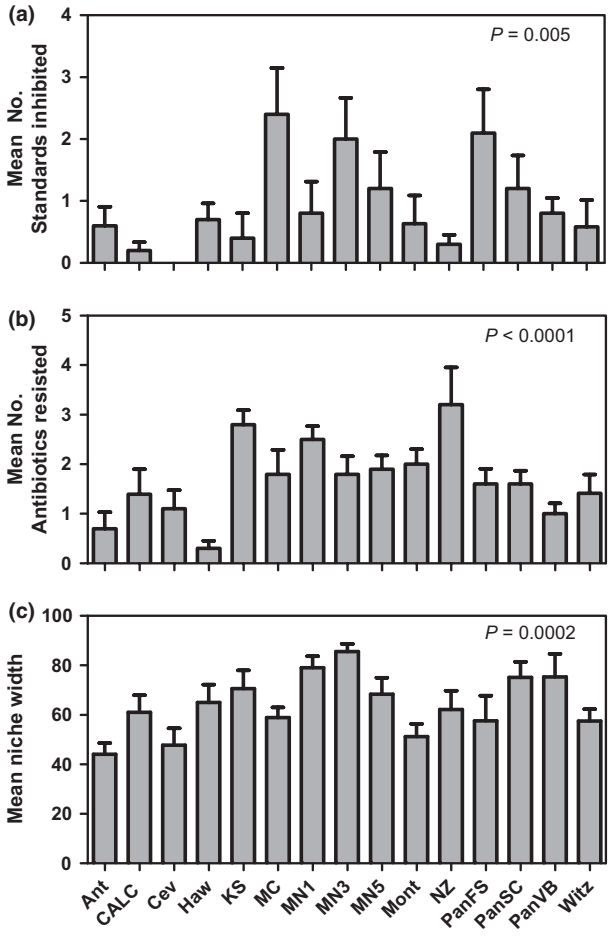

Fig. 3. Variation in the mean number of standards inhibited (a), antibiotics resisted (b), and niche width (c) among *Streptomyces* populations from different locations. *P*-values of ANOVAS are presented.

Antibiotic resistance also varied among *Streptomyces* from different locations. Isolates from different locations varied in the mean number of antibiotics that they resisted (Fig. 3b; ANOVA; d.f. = 14, $F = 4.06$, $P < 0.0001$). Populations from NZ and KS were the most resistant on average (resisting 3.2 and 2.8 of seven antibiotics, respectively), whereas those from Haw and Ant were the least resistant (0.3 and 0.7 resistances, respectively). Thus, some geographic locations were more likely to harbor *Streptomyces* populations possessing resistance to a broad suite of antibiotics. *Streptomyces* also varied among locations in their susceptibility to each of the individual antibiotics tested (Table 2); populations from some locations had greater susceptibility/resistance to inhibition by specific antibiotics.

In addition to antibiotic inhibition and resistance, *Streptomyces* from different locations also varied in their capacities to utilize resources. Specifically, total growth, mean efficiency, and niche widths of *Streptomyces* differed among locations (Fig. 3c; Table S2). *Streptomyces* populations from Mont and CA-LC had the highest total growth overall and on average grew more efficiently on used resources than those from other locations. In contrast, populations from MN1 and Cev had the lowest total growth and grew less efficiently than other locations. Similarly, *Streptomyces* populations from some locations tended to be niche generalists and had large resource use niches (e.g. *Streptomyces* from MN1 and MN3 used an average of 79 and 85.6 of 95 possible resources, respectively) compared to populations from other locations that tended to support niche specialists (e.g. *Streptomyces* from Ant and Cev used on average 44 and 47.8 of 95 possible resources, respectively).

Patterns of *Streptomyces* trait variation among locations

Streptomyces inhibition phenotypes were only marginally more similar among isolates from the same vs. different locations (Euclidean distance = 7.76 and 8.43, respectively; Welch's *t*-test, d.f. = 805; $t = 1.71$, $P = 0.09$). When considering individual locations, *Streptomyces* from six of 15 locations (CALC, Cev, Haw, NZ, PanVB, and

Table 2. Mean susceptibility (mm zone size) of *Streptomyces* to antibiotics (μg per disk) among locations and ANOVAS assessing significant variation in resistance among locations

Location	Strep (10 μg)	Eryth (15 μg)	Tet (30 μg)	Rif (10 μg)	Amox (30 μg)	Chlor (30 μg)	Vanc (30 μg)
Ant	17.4	16.0	10.9	5.3	8.3	10.5	12.8
CALC	21.2	12.6	5.0	4.6	9.8	10.1	16.2
Cev	14.2	10.9	5.4	10.5	5.6	2.0	15.1
Haw	10.9	14.6	6.2	7.1	11.9	8.6	12.1
KS	14.5	9.7	1.4	1.3	4.0	2.8	9.6
MC	12.8	5.9	5.9	2.9	7.5	5.8	11.3
MN1	9.2	10.7	0.4	1.9	4.0	4.3	8.5
MN3	15.3	12.0	1.0	3.0	10.1	7.8	12.8
MN5	17.0	13.5	2.1	2.3	5.9	7.2	11.8
Mont	12.1	9.8	3.2	1.8	6.7	7.4	10.6
NZ	15.9	6.7	2.2	2.3	3.7	4.0	6.2
PanFS	12.7	8.0	3.8	4.3	5.7	7.4	13.0
PanSC	17.3	15.2	2.5	3.2	10.9	10.8	11.9
PanVB	9.1	18.0	6.7	5.1	13.6	3.7	10.8
Witz	9.4	5.4	5.5	5.5	5.4	6.7	8.6
d.f.	14	14	14	14	14	14	14
F	5.06	4.07	5.1	4.56	3.27	2.68	4.99
P-val	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0002	0.002	< 0.0001

Witz) had significantly more similar inhibitory phenotypes within vs. among locations. One location (PanFS) supported isolates a greater diversity of inhibitory phenotypes among isolates within the same location than among locations (Euclidean distance = 18.81 and 14.42, respectively; Welch's *t*-test, d.f. = 48, $t = 2.18$, $P = 0.035$). Among the remaining locations, there was no significant difference in similarity in inhibitory phenotypes among isolates from within or among locations. More similar antibiotic inhibitory phenotypes within vs. among locations may reflect local spread of antibiotic genes within communities as a result of local selection, dispersal, or genetic drift. In contrast, the lack of significant difference in similarity in inhibitory phenotypes within vs. among communities for many locations suggests that there is little selection for specific antibiotic inhibitory phenotypes among geographically distinct *Streptomyces* communities.

Streptomyces from the same location had more similar resistance phenotypes than those from different locations overall (Euclidean distance = 16.13 and 19.18, respectively; Welch's *t*-test, d.f. = 784, $t = 10.39$, $P < 0.0001$). Differences were significant in 10 of the 15 locations (data not shown). This suggests that selection for antibiotic resistance may favor similar antibiotic resistance traits among *Streptomyces* from the same location, perhaps reflecting high frequencies of within-community gene exchange, or that gene flow or genetic drift homogenize resistance phenotypes within *Streptomyces* communities.

Streptomyces isolates from the same locations in soil tended to utilize similar resources compared to those from different locations. *Streptomyces* resource use patterns were more similar among isolates from the same location vs. different locations (mean Euclidean distance = 1.08 and 1.26, respectively; Welch's *t*-test, d.f. = 795, $t = 6.32$, $P < 0.0001$). This pattern was significant for 10 of the 15 locations (data not shown). In addition to resource use patterns, average niche overlap among *Streptomyces* pairs was greater among isolates from the same vs. different locations (mean niche overlap = 0.60 and 0.52, respectively; Welch's *t*-test, d.f. = 772, $t = 15.24$, $P < 0.0001$). However, within-location niche overlap differed among locations (Fig. 4; ANOVA; d.f. = 14, $F = 11.38$, $P < 0.0001$). Together, these data suggest that resource competition is greater between coexisting *Streptomyces* but that the strength of resource competition may vary among *Streptomyces* communities. Moreover, within-location niche overlap was marginally

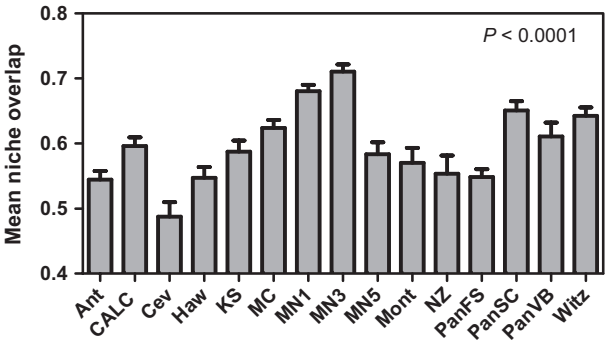

Fig. 4. Mean niche overlap within *Streptomyces* communities among different locations. The *P*-value from an ANOVA across locations is presented.

correlated with mean inhibitory capacity (number of standards inhibited) among *Streptomyces* populations ($R = 0.47$, $P = 0.07$), suggesting that locations with stronger resource competition support somewhat more inhibitory *Streptomyces*. However, mean resistance capacity (number of antibiotics resisted) was not significantly related to niche overlap ($R = 0.25$, $P = 0.37$) or to mean inhibition capacity (number of standards inhibited; $R = -0.006$, $P = 0.98$).

Streptomyces phylogeny

All 153 isolates belonged to *Streptomyces* or closely related groups (*Kitasatospora* and *Streptacidiphilus*; Table S3). Isolates clustered into 60 OTUs at 99% similarity. Two-thirds (20/30) of nonunique OTUs were found in multiple locations, whereas the remaining one-third (10/30 OTUs) of OTUs with multiple representatives clustered to a single geographic location (Table S4). Although we evaluated only a small number of isolates from each location, *Streptomyces* populations were phylogenetically more similar within vs. among locations (AMOVA; Fs = 4.48, $P < 0.001$; unweighted UniFrac = 0.53, $P < 0.001$). These data suggest that although most OTUs are globally distributed, some *Streptomyces* may be more common within or endemic to specific geographic areas.

Relationships between *Streptomyces* phylogeny and phenotype

Streptomyces belonging to the largest OTUs (18 OTUs with $n = 3$–20 isolates) varied in aggregate antibiotic inhibition, resistance, and resource use phenotypes. While *Streptomyces* OTUs did not vary significantly in the number of standard isolates that they could inhibit (ANOVA; d.f. = 17, $F = 1.41$, $P = 0.15$), there were differences among OTUs in mean inhibition zone sizes (ANOVA; d.f. = 17, $F = 2.03$, $P = 0.019$). Further, OTUs varied in intensity of inhibition against two of the five individual standards (data not shown). Overall, this suggests that some OTUs tend to be stronger inhibitors than others. Finally, *Streptomyces* OTUs also varied in the number of antibiotics that they could resist (ANOVA; d.f. = 17, $F = 5.79$, $P < 0.0001$) and their susceptibility (mean inhibition zone size) to antibiotics (ANOVA; d.f. = 17, $F = 5.01$, $P < 0.0001$). OTUs also differed in their susceptibility to each individual antibiotic tested (data not shown). Thus, *Streptomyces* OTUs differed in aggregate antibiotic resistance traits and resistance to specific antibiotics, suggesting that antibiotic resistance traits may be conserved among *Streptomyces* phylogenetic groups. Considering resource use, *Streptomyces* OTUs differed significantly in total growth and growth efficiency (ANOVA; d.f. = 17, $F = 3.20$ and 4.29, $P = 0.0002$ and < 0.0001, respectively), but not niche width (ANOVA; d.f. = 17, $F = 1.34$, $P = 0.19$). Thus, some OTUs were better at utilizing resources for growth than others. In total, these data demonstrate that although antibiotic inhibitory phenotypes are highly diverse among isolates from the same OTU, *Streptomyces* OTUs have distinct strategies for resisting inhibition by antibiotics and for utilizing resources.

Despite some significant trait variation among *Streptomyces* OTUs, relationships between 16S rRNA gene sequences and specific inhibition, resistance, and resource use phenotypes were modest. Similarity in 16S sequence and inhibition profiles was marginally related (Mantel $r = 0.10$, $P = 0.05$). There was a significant correlation between 16S sequences and antibiotic resistance profiles among *Streptomyces* isolates (Mantel $r = 0.16$, $P < 0.001$), although it explained only a small proportion of total variation in resistance profiles. Among all isolates, there was no significant correlation between 16S similarity and resource use profile (Mantel $r = -0.03$, $P = 0.66$) or niche overlap (Mantel $r = -0.17$, $P = 0.99$) among isolates. Thus, 16S rRNA gene sequences have a limited capacity to inform specific inhibition, resistance, and resource use phenotypes among *Streptomyces*. This suggests that local adaptation and horizontal gene transfer may generate substantial trait diversity within closely related phylogenetic groups and that phylogenetic markers with greater resolution than 16S rRNA gene sequences are needed to understand the evolutionary history of *Streptomyces* traits.

Trait variation within phylogenetic groups

When comparing *Streptomyces* belonging to the same OTUs, those originating from distinct locations sometimes differed significantly in antibiotic inhibition, resistance, and resource use. Two OTUs (OTU1 and OTU2) that had at least three isolates from each of multiple locations are considered here. *Streptomyces* belonging to OTU1 varied significantly among locations (MN1, $n = 6$; PanFS, $n = 3$; Ant, $n = 5$) in the number of standards that they could inhibit (Fig. 5; ANOVA; d.f. = 2, $F = 5.851$, $P = 0.019$) and in mean inhibition zone sizes (ANOVA; d.f. = 2, $F = 4.40$, $P = 0.04$). Specifically, OTU1 *Streptomyces* from PanFS inhibited significantly more standards and had larger inhibition zone sizes than those from MN1 or Ant (TukeyHSD, $P < 0.03$ and $P < 0.06$ for number of standards inhibited and inhibition zone size, respectively). Furthermore, these isolates varied significantly among locations in inhibition zone sizes against four of the five individual standards (data not shown). However, isolates from OTU2 (MN5, $n = 3$; Haw, $n = 3$; Mont, $n = 3$) originating from Haw and Mont did not

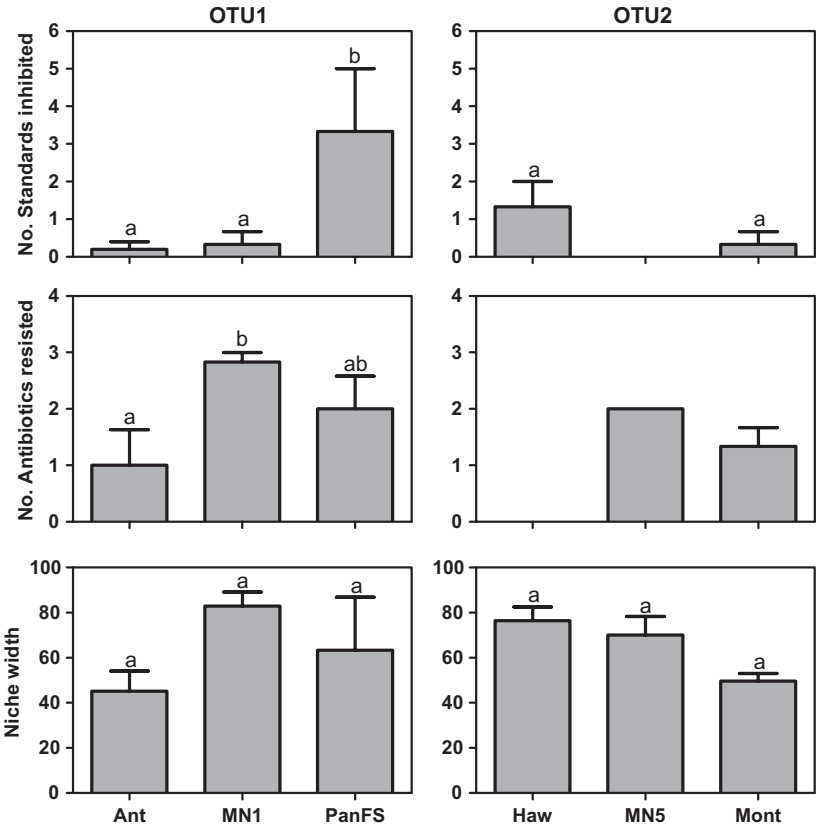

Fig. 5. Variation in the number of standards inhibited (top row), number of antibiotic resisted (middle row), and niche width (bottom row) among *Streptomyces* isolates from the same OTU but originating from different locations. Error bars represent standard error. The same letters above bars indicate no statistical difference among bars, as determined by Tukey's post hoc honest significance tests. For OTU2, MN5 was excluded from analyses of the number of standards inhibited among locations, and *t*-test was used instead of ANOVA. Due to lack of variation in the number of antibiotics resisted among isolates within locations for OTU2, we were unable to test for statistical differences.

differ significantly in number of standards inhibited (*t*-test; d.f. = 2.9, $t = 1.34$, $P = 0.27$), mean inhibition zone sizes (*t*-test; d.f. = 3.7, $t = 0.36$, $P = 0.74$), or in their inhibition zones against individual standards (data not shown). *Streptomyces* belonging to OTU2 originating from MN5 consistently lacked inhibitory activity against any standards. Consequently, although we were unable to statistically differentiate these from Haw or Mont isolates due to the lack of phenotypic variation, there were clear differences in inhibitory capacities of these populations.

Streptomyces isolates from OTU1 but originating from different locations also differed in the number of antibiotics they were able to resist (ANOVA; d.f. = 2, $F = 4.65$, $P = 0.03$). Isolates from MN1 resisted a significantly greater number of antibiotics on average than those from PanFS or Ant (mean of 2.8 vs 2 and 1 antibiotics resisted, respectively). Isolates from MN1 were significantly more resistant to streptomycin and vancomycin than those from Ant (TukeyHSD, $P = 0.04$) and PanFS (TukeyHSD, $P = 0.02$). OTU2 *Streptomyces* from Haw did not resist any antibiotics, whereas those from MN5 and Mont resisted 2 and 1.3 antibiotic on average, respectively. *Streptomyces* from Haw were significantly more susceptible to rifampicin than those from MN5 (TukeyHSD, $P = 0.02$) and Mont (TukeyHSD, $P = 0.01$).

Within OTU1, *Streptomyces* from different locations did not vary significantly in total growth or growth efficiency (ANOVA; d.f. = 2, $F = 0.57$ and 1.94, $P = 0.58$ and 0.19, respectively), and variation in niche width was marginally significant (ANOVA; d.f. = 2, $F = 3.55$, $P = 0.06$). Similarly, *Streptomyces* belonging to OTU2 but from different locations varied in niche width (Mont < HW and MN5; ANOVA; d.f. = 2, $F = 4.93$, $P = 0.05$), but not total growth or mean growth efficiency (ANOVA; d.f. = 2, $F = 0.40$ and 2.72, $P = 0.69$ and 0.14, respectively). These data suggest that although OTU1 and OTU2 *Streptomyces* from different locations have comparable growth efficiencies, niche widths are variable and have a greater capacity to respond to selection. In general, the data presented here show that antibiotic production, antibiotic resistance, and resource use can vary within unique OTUs across locations, suggesting that local selective pressures may be crucial for generating biogeographic patterns in *Streptomyces* traits.

Discussion

Recent studies of soil microbial biogeography typically highlight the importance of soil physical or chemical characteristics, climatic variables, or plant communities

for determining the phylogenetic structure and biodiversity of soil communities (Garbeva *et al.*, 2004, 2008; Fierer *et al.*, 2007; Griffiths *et al.*, 2011). However, a trait-based view of microbial biogeography can offer insight into patterns in functional traits crucial to microbial fitness and the ecological and evolutionary forces that structure and generate functional diversity in soil communities (Green *et al.*, 2008). Trait-based microbial biogeography may also shed light on roles of microbial species interactions in structuring microbial populations. As a first step toward a trait-based understanding of *Streptomyces* biogeography, we document here significant patterns in antibiotic inhibitory, resistance, and resource use capacities among *Streptomyces* populations from distinct locations on multiple continents. Although immigration/dispersal dynamics, environmental filtering, and environmental gradients are all likely to be important in structuring microbial traits (Martiny *et al.*, 2006; Green *et al.*, 2008), competitive species interactions are argued to be important drivers of antibiotic inhibition, resistance, and resource use among *Streptomyces* (Wiener *et al.*, 1998; Davelos *et al.*, 2004a; Schlatter *et al.*, 2013; Kinkel *et al.*, 2014). The great diversity of *Streptomyces* inhibitory, resistance, and resource use phenotypes observed here suggests that the selective forces that shape *Streptomyces* phenotypes vary substantially across locations and that *Streptomyces* phenotypes are highly plastic in response to selection. This suggests that variation in the significance of competitive interactions for *Streptomyces* fitness among distinct communities is likely to be crucial in generating biogeographic patterns in these traits among distinct locations in soil.

Streptomyces populations varied in their antibiotic inhibitory capacities among locations. Locations that support *Streptomyces* with broad and highly potent inhibitory phenotypes may be competitive 'hot spots' that have selected for *Streptomyces* populations that are effective inhibitors of resource competitors. In contrast, in locations where populations have little inhibitory capacity, resource competition may be less important to *Streptomyces* fitness or populations may be niche differentiated (Kinkel *et al.*, 2011). Alternatively, *Streptomyces* populations exhibiting little inhibitory capacity may require specific conditions for expression of antibiotics or produce antibiotics that have inhibitory activities against organisms other than the indicator overlays used in this work. The positive relationship between mean niche overlap and the inhibitory capacity among *Streptomyces* populations from different locations provides some support for the idea that resource competition imposes selection for antibiotic inhibitory phenotypes within local *Streptomyces* populations. However, alternative mechanisms for mediating competition such as signaling (Yim *et al.*, 2007; Vaz Jauri *et al.*, 2013) or strong selection by forces unrelated to resource competition (e.g. abiotic stress, parasitism, or predation; Weekers *et al.*, 1993; Ashelford *et al.*, 2003) may confound the relationship between niche overlap and antibiotic inhibition. Because competitive dynamics and subsequent selection will vary among locations (Thompson, 2005; Kinkel *et al.*, 2014), resource competition is likely to contribute to the large diversity of *Streptomyces* antibiotic phenotypes at a global scale (Czárán *et al.*, 2002; Kinkel *et al.*, 2014).

The poor correspondence between phylogeny and inhibitory phenotype and the high variability of inhibitory phenotypes within and among locations suggest that geographic patterns of inhibitory phenotypes are not likely to be structured by the colonization of specific OTUs. Rather, adaptation of inhibitory phenotypes to inhibit locally coexisting resource competitors may play a major role in structuring *Streptomyces* inhibition (Kinkel *et al.*, 2014). Indeed, OTU 1 *Streptomyces* from PanFS were better inhibitors than those from other locations, suggesting that *Streptomyces* in this phylogenetic group have been selected for stronger antagonistic capacities at PanFS. Alternatively, OTU1 *Streptomyces* may have lost antagonistic capacities in other locations (MN1 and Ant) if antagonistic phenotypes incur a net fitness cost rather than a benefit in those locations (Schlatter and Kinkel, in review). In contrast, *Streptomyces* belonging to OTU2 did not differ in inhibitory phenotypes among locations, suggesting that not all phylogenetic groups are locally adapted, perhaps reflecting a smaller role of antibiotic inhibition in the competitive strategy for this OTU. Alternatively, limitations of our antibiotic assay or small sample size ($n = 3$ isolates/location) may have inhibited our ability to detect a signal of adaptation.

Consistent with previous work (D'Costa *et al.*, 2006), we found that *Streptomyces* were commonly resistant to many clinical antibiotics. Selection for antibiotic resistance in natural communities is thought to be imposed by interactions with antibiotic-producing competitors (Wiener *et al.*, 1998; D'Costa *et al.*, 2007; Kinkel *et al.*, 2014). Thus, variation in resistance to antibiotics among *Streptomyces* from different locations may reflect a history of production of specific antibiotics within communities. Alternatively, antibiotic resistance may also be due to 'intrinsic' resistance mutations or the activity of broad-spectrum efflux pumps (D'Costa *et al.*, 2006; Martinez *et al.*, 2009). The lack of significant correlation between antibiotic resistance capacity and niche overlap among locations suggests that the dynamics of antibiotic resistance among *Streptomyces* populations are distinct from those of resource competition. Moreover, the significant phylogenetic signal in resistance phenotypes suggests that phylogenetic constraints may limit the acquisition of

resistance or that, once acquired, resistance is retained in lineages despite the absence of strong selection. Thus, dispersal dynamics of resistant OTUs may play an important role in structuring antibiotic resistance phenotypes within *Streptomyces* communities in soil. Despite the phylogenetic signal of antibiotic resistance, greater similarity in resistance phenotypes among *Streptomyces* from the same vs. different locations suggests that there may be local spread of antibiotic resistance genes within *Streptomyces* communities, likely facilitated by horizontal gene transfer (Wiener *et al.*, 1998). Indeed, *Streptomyces* belonging to OTU1 originating from MN1 were more resistant to rifampicin and streptomycin than those from other locations (Ant and PanFS), suggesting distinct selective pressures for resistance among these locations. Adaptation of taxa from the same phylogenetic groups in response to distinct selection pressures across the landscape (adaptive radiation) may help generate substantial diversity in antibiotic resistance phenotypes.

Soilborne *Streptomyces* are saprotrophs that acquire carbon by degrading soil organic matter (Schrempf *et al.*, 2011). As a result of their saprotrophic lifestyle, variation in resource use among *Streptomyces* populations from different geographic locations is expected to reflect adaptation to local organic carbon pools (Antony-Babu *et al.*, 2008; Schlatter *et al.*, 2009, 2013). *Streptomyces* from the same locations had more similar resources and greater niche overlap than those from different locations, consistent with local selection for resource use phenotypes. Considering aggregate resource use phenotypes, rather than specific patterns, locations that supported *Streptomyces* with larger niche widths (resource generalists), such as MN1 and MN3, may have more diverse pools of available carbon compounds than locations that support *Streptomyces* with narrower niche widths (resource specialists), such as Ant and Cev. Although conditions that support *Streptomyces* with faster or more efficient growth are less clear, they are likely to depend on resource availability (Schlatter *et al.*, 2013). The availability and diversity of soil resources are thought to be strongly linked to plant community composition and diversity as well as soil type, age, and history (Batjes, 1996; Tilman *et al.*, 1997; Conant *et al.*, 2001). Thus, the nested factors of soil and management history, and plant community characteristics are likely to be broad drivers of *Streptomyces* resource use phenotypes in soil.

Variation in total growth and growth efficiency among *Streptomyces* OTUs suggests that some OTUs tend to be r- or K-selected and may have physiologies that are 'hard-wired' to grow optimally in high- or low-resource environments (Fierer *et al.*, 2007). Due to these physiological constraints, total growth and growth efficiency among *Streptomyces* may be less responsive to selection. In contrast, the large variation in niche width within and among OTUs suggests that niche widths may be more likely to adapt to local resource pools than the overall growth strategy of a streptomycete. Consistent with this idea, *Streptomyces* belonging to the same OTU but originating from different locations did not differ in total growth or growth efficiency, while niche widths for individuals within the same OTU often varied widely among locations, suggesting that niche widths may be more adaptable than growth strategy among *Streptomyces*.

This work explores the biogeography of *Streptomyces* species interaction traits. Variation in antibiotic inhibition, resistance, and resource use traits among locations demonstrates significant geographic differences in phenotypes that are critical to *Streptomyces* species interactions and fitness. Furthermore, our results suggest that local adaptation is likely to play a role in shaping geographic differences in *Streptomyces* antibiotic inhibition, resistance, and resources use. Moreover, local adaptation of species interaction phenotypes to unique selection pressures within distinct microbial communities and abiotic environments across the landscape is likely to play an important role in generating trait diversity within *Streptomyces* and other microbial groups. Further study of the geographic structure of microbial species interactions that include careful measures of fitness will be essential to understand the ecological and evolutionary forces that structure and generate microbial functional diversity.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Phylogenetic tree of *Streptomyces* used in this work.

Table S1. Mean inhibition strength (mm zone size) of *Streptomyces* among locations against five individual test standards.

Table S2. Mean resource use phenotypes (total growth, growth efficiency, and niche width) of *Streptomyces* among locations.

Table S3. Classification of isolates used in this work as determined using the RDP Naïve Bayesian Classifier.

Table S4. Abundance of operational taxonomic units (99% similarity) for each location.