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## **The Variation in Antagonistic *Streptomyces* Populations in Soils from Different Vegetation Types in Western Oregon**

### **Abstract**

Grassland, red alder, and Douglas-fir soils were examined for total number of microorganisms recovered from dilution plating, the number of *Streptomyces*, and the percentage of *Streptomyces* isolates antagonistic to the test fungus *Phellinus weirrii* (Murr.) Gilb. The Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) site with no apparent or obvious *P. weirrii* activity had the highest percentage of antagonistic *Streptomyces*. Plant communities had distinctively different populations of antagonistic *Streptomyces* species and communities with the greatest diversity of vascular plant species supported the greatest diversity of antagonistic *Streptomyces* species.

### **Introduction**

Investigators have shown that both the number and species composition of soil microbes reflect the species diversity and composition of vascular plant communities (Kuster 1976, Wicklow *et al.* 1974). Wicklow *et al.* (1974) found more fungal species in forest soil with a diverse tree community than in a more homogeneous forest. The number and antibiotic activity of *Streptomyces* species are also thought to be influenced by the composition of vascular plant communities. Abraham and Herr (1964) suggested that every plant species fosters a particular *Streptomyces* flora. Both the number of individual *Streptomyces* species and species diversity of the *Streptomyces* population are generally considered to be higher in grassland soils and pastures than in forested sites (Alexander 1977). Hutchins and Li (1981) reported that soils under conifer and alder were strikingly different in the number and species of *Streptomyces* antagonistic to *Phellinus weirrii* (Murr.) Gilb., a pathogenic fungus inducing a serious root rot of conifers in Western North American forests. Rangaswami and Ethiraj (1962) found that three primary factors influenced *Streptomyces* antibiotic production in soil: (1) number and composition of the microbial population, (2) soil pH, and (3) the organic matter content.

In this study we examined the *Streptomyces* species we recovered by dilution-plate techniques on selective media from soil samples from six plant communities in western Oregon to determine if populations were different and whether any such differences were correlated with the type of vascular plant cover. We also compared the percentage

of soil *Streptomyces* antagonistic to *P. weirii* in grassland, alder and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) forest soils to determine if the presence of a coniferous host of *P. weirii* influenced the species composition and number of native *Streptomyces* antagonistic towards this fungal pathogen. Antagonistic *Streptomyces* colonies were isolated and identified as to species in an attempt to qualify and quantify differences in the species composition of *Streptomyces* antagonistic to *P. weirii*.

#### Methods and Materials

Six areas in western Oregon were selected representing three vegetative communities: grasslands, red alder (*Alnus rubra* Bong.), and Douglas-fir.

One grassland site at 62 m elevation was part of a 100-year-old cemetery 10 miles south of Corvallis, Oregon, in the Willamette Valley, on a Woodburn-Willamette silt loam soil. The vegetative community is composed primarily of four grass species: *Festuca rubra* L., *Agropyron repens* (L.) Beauv., *Lolium perenne* L., and *Sorghum halepense* (L.) Pers. This site has been in grassland vegetation for over 100 years and is surrounded by oak and a Douglas-fir forest.

The other grassland site was a natural meadow at 1249 m elevation on Mary's Peak in the Oregon Coast Range on an Apt-Honeygrove-Bohannon association of silty clay loam soils. The vegetation is predominantly red fescue (*Festuca rubra* L.) and sedge (*Carex californica* Bail.). The forest adjacent to the meadow is comprised of Douglas-fir and grand fir (*Abies grandis* (Dougl. ex D. Don) Lindl.).

Red alder is an aggressive pioneer species in the coastal regions and two red alder communities were chosen for sampling. One was a 40-year-old stand growing on a gravelly clay loam at 199 m in the Oregon Coast Range with an understory of vine maple (*Acer circinatum* Pursh), thimbleberry (*R. parviflorus* Nutt.), salmon-berry (*Rubus spectabilis* Pursh), western sword fern (*Polystichum munitum* Kaulf.) Presl), and various cryptogams. A Douglas-fir forest surrounds this alder site. The other alder stand was from a sand dune community 8 km north of Waldport on the Oregon coast. Individual alder trees with associated grass species were growing on stable dunes. The vegetation changes abruptly to shore pine (*Pinus contorta* Dougl. ex Loud.) a short distance from the alder stand.

A 50-year-old Douglas-fir stand at 100 m elevation on a Slickrock gravelly clay loam soil was sampled in the Oregon Coast Range, 10 miles west of Corvallis, Oregon. We chose two sites from this stand. In one, *Phellinus weirii* was causing windthrow and mortality in a large portion of the stand while in the second site there did not appear to be any *P. weirii* activity and no tree mortality. The understories in both sites consisted mainly of long-leaved Oregon grape (*Berberis nervosa* Pursh), western sword fern, wood sorrel (*Oxalis trilliifolia* Hook), and various cryptogams. About 5 cm of litter, mostly Douglas-fir needles and twigs, covered the soil.

Each site was sampled at three locations (plots) in November and May. We ran a transect across each location and collected a soil subsample using a 2 cm soil auger from 12 positions spaced at 5 m intervals across the transect at each of the three plots. The twelve samples, from the surface of mineral soil to 25 cm depth, were combined and mixed to give a composite sample of about 1000 g soil/plot. The soil was sifted through a 2 mm screen and the moisture content was determined by drying at 60° C until a constant weight was reached. Soil was analyzed by the Soil Testing Laboratory,

Oregon State University (Table 1). Organic matter was determined by the Walkley-Black method (Alexander 1977); nitrogen by Kjeldahl Method (Isizawa and Araragi 1976); extractable K, Mg, and Ca were determined by atomic absorption spectrophotometric techniques; available phosphorus was determined by the sodium bicarbonate extraction technique (Watanabe and Olsen 1965); soil reaction (pH) was measured from a water saturation paste on a pH meter.

Soil dilutions using 20 gm/soil plot at the following dilutions 1/100, 1/1000, 1/10,000 were prepared and 3 replications of 1 ml of each dilution were plated on sodium albuminate agar (pH 6.8), a selective medium for *Streptomyces* and non-filamentous or true bacteria (Johnson *et al.* 1965). Colonies were counted after incubation at 28° C for eight days. Colonies of true bacteria and *Streptomyces* were counted under aseptic conditions and colonies of *Streptomyces* isolated onto selective media. Individual colonies of true bacteria were distinguishable from *Streptomyces* by colony characteristics as observed under 10 and 100 × magnification. Hyphal tips from individual *Streptomyces* colonies were transferred to tubes containing sodium albuminate agar, and incubated for two weeks at 28° C. Each *Streptomyces* isolate growing in pure culture was assessed for antagonism against *P. weirii* by using the cross-inoculation assay method of Johnson and Carl (1972). *Streptomyces* isolates in pure culture were transferred to a buffered malt-yeast-peptone agar plate (Hutchins 1980) opposite a culture of *P. weirii*. The buffered malt-yeast-peptone (B-MYP) medium facilitated good growth of both *Streptomyces* and *P. weirii* while the pH remained unchanged. Plates were incubated at 26° C in the dark for two weeks and inspected for a clear zone around the *Streptomyces* colony, indicative of antibiosis. Approximately 250 *Streptomyces* colonies isolated from each site in May, and again in November, were tested for antagonism to *P. weirii*.

The dominant antagonistic *Streptomyces* colonies isolated were identified to species by methods described by Shirling (1968), Shirling and Gottlieb (1966, 1968, and 1969), and modified by Kuster (1976). Color of the aerial mycelium and of the reverse, color of soluble pigment, melanin production, and the morphology of sporophores and spores were noted on pure cultures of the isolated *Streptomyces*. Carbohydrate utilization and growth rates were determined with sucrose, D-mannitol, L-arabinose, D-xylose, D-fructose, rhamnose, i-inositol, and raffinose. Glucose added to B-MYP medium was used as the positive control and B-MYP media without carbohydrate as a negative control. Electron micrographs were used to determine sporophore and spore morphology.

TABLE 1. Chemical analyses of soils sampled.

Site	pH	P (ppm)	K (ppm)	Determinations					
				Ca (meq/100g)	Mg (meq/100g)	CEC	% C	% N	C/N
Grass cemetery	6.1	44	468	10.2	3.1	20.64	3.24	.212	15.2
Grass meadow	5.0	27	343	1.5	0.8	45.24	11.80	.924	12.8
Alder forest	5.0	14	636	22.3	8.3	46.90	5.40	.304	17.8
Alder sand	5.9	11	62	0.3	0.2	0.86	0.11	.004	27.5
Conifer ( <i>P. weirii</i> uninfested)	5.8	60	390	7.2	2.4	26.06	4.61	.205	22.5
Conifer ( <i>P. weirii</i> infested)	5.6	46	421	10.7	3.6	40.48	7.15	.300	23.8

Differences among total bacterial numbers, number of *Streptomyces* isolates, and percentages of antagonistic *Streptomyces* at each of the six sites were tested for statistical significance by one-way analysis of variance (ANOVA).

### Results and Discussion

We found the least diverse populations of antagonistic *Streptomyces* on sites with the least diverse vascular plant communities (Table 2). The antagonistic *Streptomyces* isolates from the grass cemetery site were dominated by one species which accounted for 96 percent of the isolated *Streptomyces*. The *Streptomyces* colonies isolated from the grass meadow site were dominated by one species which accounted for 71 percent of the total isolates. In the sites with more vascular plant diversity and heterogeneity, the alder forest and the two conifer stands, the antagonistic *Streptomyces* were composed of many co-dominant species. These results parallel those of Wicklow *et al.* (1974), who reported an increase in the number of fungal species in forest stands that had a comparatively higher number of vascular plant species.

Several authors have suggested that similar vegetative communities would have similar microbial populations (Alexander 1977, Kuster 1976, Weste and Vithanage 1978). *Streptomyces atroolivaceus*, the dominant species in the alder sand dune site, and *S. gelaticus*, a co-dominant species from the alder forest site, have previously been reported from alder soils (Hutchins and Li 1981). Also, *S. longisporus* and *S. parvulus*, the dominant and co-dominant species from the conifer site, with *P. weirii* mortality have been isolated from conifer rhizospheres (Hutchins and Li 1981, Knutson *et al.*

<sup>3</sup>Cation exchange capacity.

TABLE 2. Antagonistic *Streptomyces* populations isolated from six communities in November.

Site	Total number of antagonistic <i>Streptomyces</i> species per site	<i>Streptomyces</i> species isolated	<i>Streptomyces</i> Percent of total number
Grassland cemetery	72	<i>S. lydicus</i> DeBoer, Dietz, Silver and Savage	96
Grassland meadow	28	<i>S. longisporus</i> Krasilnikov	71
		<i>S. aerocolonigenes</i> Shinobu and Kawato	7
		<i>S. griseoruber</i> Yamaguchi and Saburi	7
Alder forest	19	<i>S. orientalis</i> Pittenger and Brigham	21
		<i>S. filipinensis</i> Ammann, Gottlieb, Brock, Carter, and Whitfield	21
		<i>S. gelaticus</i> (Waksman) Waksman and Henrici	16
Alder sand	21	<i>S. atroolivaceus</i> Preobrazhenskaya, Blinov, and Ryabova	38
		<i>S. albofaciens</i> Thirumalacher and Bhatt	24
		<i>S. pseudogriseolus</i> Okami and Umezawa	10
Conifer ( <i>P. weirii</i> uninfested)	57	<i>S. longisporus</i> Krasilnikov	40
		<i>S. sp. 90</i>	11
		<i>S. parvulus</i> Waksman and Gregory	7
		<i>S. fasciculatus</i> Pittenger and Nelms	5
		<i>S. sp. 98</i>	4
		<i>S. sp. 133</i>	4
		<i>S. sp. 135</i>	4
Conifer ( <i>P. weirii</i> infested)	28	<i>S. siyoensis</i> Nishimura, Okamoto, Mayama, Ohtsuka, Nakajima, Tawara, Shimohira, Shimaoka	10
		<i>S. albiflavus</i> Waksman and Henrici	7
		<i>S. violaceus</i> Preobrazhenskaya and Sueshnikova	7
		<i>S. albus</i> (Rossi-Doria) Waksman and Henrici	7
		<i>S. chibaensis</i> Suzuki, Nakamura, Okuma, and Tomiyama	7
		<i>S. sp. 147</i>	7

1980). *Streptomyces longisporus*, however, was also isolated from the grass meadow site, suggesting that some species of *Streptomyces* have a less host-vegetation-specific distribution. Our finding of little species overlap between communities suggests that a specific and definable microbial community related to dominant vascular plant vegetation may exist in each habitat.

Soil factors also influence the soil microbial community. Soils high in humus and rich in nitrogenous materials are reported to have more non-antibiotic-producing *Streptomyces* than antibiotic-producing *Streptomyces* (Isizawa and Araragi 1976, McGahen 1951, Thaysen 1950). This was also true in our study; the *P. weirii* infested conifer soil with higher levels of C and N had a low percentage of antagonistic *Streptomyces*, while the soil from the conifer site without *P. weirii* activity, with lower C and N, had a high percentage of antagonists (Tables 1 and 2). The grass meadow site, however, with the highest C and N content, and a C/N ratio of 12.8, had an intermediate percentage of antagonistic *Streptomyces* species. Whether high levels of nitrogen influence the number of antibiotic producing *Streptomyces* is problematic at best since increasing levels of nitrogen have not slowed the spread of *Phellinus weirii* in soil (Wallis and Reynolds 1974). In addition, antibiosis could be influenced by other soil characteristics or to aspects of general soil microbial populations.

Fifty-seven antagonistic *Streptomyces* isolates were recovered from soil taken from the uninfested Douglas-fir stand where no *P. weirii* activity was evident. In the *P. weirii* infested site, only 28 antagonistic *Streptomyces* isolates were recovered. These results suggest a possible relationship between a more species diverse antibiotic *Streptomyces* populations and the absence of the pathogen. Weste and Vithanage (1978) reported that *Phytophthora cinnamomi* Rands, a true soil borne pathogen, is most abundant where competitive microorganisms are few. A reduction in actinomycetes in the diseased rhizosphere probably means a reduction in antibiosis (Weste and Vinghange 1978). Our results suggest possible suppression of *P. weirii* in the soils by endogenous *Streptomyces* species.

The highest number of total bacterial was found in the grass cemetery and grassland meadow sites (Table 3). The alder sand dune site, on the most nutritionally deficient soil, supported the lowest population of true bacteria and *Streptomyces*. Although there was no significant difference between the numbers of total bacteria at the two

TABLE 3. Microbial populations and percentages of *Streptomyces* isolates antagonistic to *P. weirii* from soil sampled during November and May from six vegetative communities in western Oregon.

Site	Mean Total Bacterial Counts <sup>1</sup>		Means <i>Streptomyces</i> Counts <sup>1</sup>		% Antagonistic <i>Streptomyces</i>	
	November	May	November	May	November	May
Grass cemetery	13.87 <sup>a</sup>	11.84 <sup>a</sup>	9.16 <sup>a</sup>	6.68 <sup>a</sup>	28 <sup>a</sup>	34 <sup>a</sup>
Grass meadow	7.59 <sup>b</sup>	11.42 <sup>a</sup>	2.93 <sup>b</sup>	5.30 <sup>a</sup>	29 <sup>a</sup>	46 <sup>a</sup>
Alder forest	8.34 <sup>b</sup>	13.66 <sup>a</sup>	5.81 <sup>c</sup>	6.10 <sup>a</sup>	12 <sup>a</sup>	10 <sup>b</sup>
Alder sand dune	0.20 <sup>c</sup>	0.15 <sup>b</sup>	0.05 <sup>d</sup>	0.06 <sup>b</sup>	21 <sup>a</sup>	14 <sup>b</sup>
Conifer ( <i>P. weirii</i> uninfested)	2.19 <sup>c</sup>	9.63 <sup>a</sup>	1.28 <sup>b,c</sup>	6.52 <sup>a</sup>	67 <sup>b</sup>	49 <sup>a</sup>
Conifer ( <i>P. weirii</i> infested)	2.07 <sup>c</sup>	8.73 <sup>a</sup>	0.62 <sup>c</sup>	5.26 <sup>c</sup>	31 <sup>a</sup>	17 <sup>b</sup>

<sup>1</sup>Colonies x 10<sup>6</sup> per gram of soil.

<sup>a</sup>a-e: Numbers with the same superscript are not significantly different at the P = .01 level.

conifer sites, there was a significantly lower number of *Streptomyces* colonies in the soil from the *P. weirii* infested stand than in soil samples from the apparently uninfested stand (Table 3).

The areas we sampled are fundamentally different from one another and we were able to observe significantly different levels of antagonism against *P. weirii* among our sites. Assuming that antagonism against *P. weirii* is an indirect measure of the incidence of a general antibiotic-producing *Streptomyces* population, we suggest that the population of antibiotic-producing *Streptomyces* responds to the vascular plant community in general, and to the presence of a potential pathogen and host in particular. However, our work, limited to sites and soils of western Oregon, should not be extrapolated to other regions and to other plant communities. The determination of the absolute presence or absence of *P. weirii* from a site is a prerequisite for future studies on suppression of *P. weirii* by the soil microflora. The development of *P. weirii* selective medium will enable us to assay the soil for this organism. When developed, more intensive sampling in infested vs. uninfested stands in this and in other regions must be done to determine whether the differences in *Streptomyces* populations and the percent antagonism are related to the presence and behavior of *P. weirii*, and whether this phenomenon is general and widespread.

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