A COMPARISION OF THE PREDOMINATE SOIL MICROFLORA IN EIGHT VEGETATIVE COMMUNITIES IN OKLAHOMA

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Soil samples from eight distinct Oklahoma vegetative communities were assayed for their microbial composition. Gram-positive bacteria were more prominent in grassland communities than forest communities. *Streptomyces* was the predominant genus in all grassland communities. The dye adsorption method of differentiating Gram-positive bacteria was tried and found to lack specificity for the bacterial communities tested.

INTRODUCTION

The study of correlations between microflora and their physical and biological environment is the essence of microbial ecology. Several investigators have studied such relationships between microflora and grassland communities in central Oklahoma (1, 2, 3, 4, 5). Mallik and Rice (6) studied the relationships between microflora and forest communities in central Oklahoma.

In these studies a correlation is sought between the qualitative and quantitative composition of the microbial population and the biotic and abiotic characteristics of the ecosystem. In the past, obtaining of qualitative and quantitative data on bacterial populations has been hampered by the tedium of available techniques. A new dye plate method has been developed by Hagedorn and Holt (7) which promises to simplify the identification of Gram-positive soil bacteria at the generic level.

The purposes of this study were to: a) extend the investigation of microflora—plant community relationships in Oklahoma and b) test the differentiation capabilities of dye-containing media in an actual field survey. In the study of microflora—plant community relationships, the emphasis was placed upon the characterization of the predominant, aerobic components of the microflora. The plant communities studied were representative of those found in the State at the 35th parallel and are described in Table 1.

MATERIALS AND METHODS

Site Location and Description

Sampling sites were chosen in the vicinity of an E-W transect across the State of Oklahoma north of the 35° parallel. Each site represented a different plant community and the locations and information pertinent to the sites are given in Table 1. Six cores from each site were collected. The cores were collected within a radius of 2 m at depths as depicted in Table 1. All cores from a site were combined and transported in a sterile plastic bag at a temp. of 4 C-10 C.

Bacterial Analysis

Bacterial analysis commenced within 14 hr of collection. The methods of Hagedorn and Holt were used with modifications (8). A composite 5-g soil sample from each site was diluted in 495 ml 0.5% peptone broth and agitated aseptically for 5 min with a magnetic stirrer. Serial dilutions were made in 0.5% peptone broth and 0.1-ml aliquots of the appropriate dilutions were spread over the surface of Difco plate count agar (PCA) in petri plates. The plates were incubated at 30 C for 48 hr before counting. All plating was done in triplicate and counts were corrected to dry weight.

The higher dilutions contained few or no fungi. For each site 20 representative procaryotic colonies from the higher dilution plates were selected and replica plated onto BBL phenylethyl alcohol agar (PEA), crystal violet agar (CV), and PCA with sterile tooth picks. After 48 hr incubation at 30 C the colonies were scored according to the following scheme: Gram-positive,

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PEA colony 1.5 × diameter of CV colony; Gram-negative, CV colony > PEA colony; Streptomycete, colony morphology and/or pigment; unclassified, no growth on PEA or CV and not a Streptomycete.

Colonies identified as Gram-positive were replicated to dye plates (Table 2) and identified to the generic level (7).

Soil Analysis

Soil samples were pulverized, mixed, and sifted through a No. 60 screen. Moisture and pH were determined by AOAC methods (9). Soil organic carbon was determined by the potassium dichromate—*s*-diphenyl-carbazide spectrophotometric method (10). Total phosphate, nitrite-nitrate, and chloride were determined with a HACH kit model DR-EL. A 50-g soil sample was mixed with 50 ml distilled water and the mixture centrifuged at low speed. The water layer was decanted, filtered through ALOE coarse filter paper and centrifuged at 3,400 × g of a Sorvall model GLC-1 centrifuge. Inorganics are reported as μg/g of soil (dry wt.).

Statistical Analysis

Statistical analysis of the data in Table 4 utilized the Mann-Whitney U nonparametric test with an *a* of 0.05 (11).

RESULTS

No distinction between grassland communities (sites 1-5) and forest communities (sites 6-8) could be made based upon the physical and mineral soil test conducted (Table 3). No correlation was found between total microbial populations and any single soil parameter or combination of such.

When the sites were ranked by total microbial populations, site eight (postoak-blackjack forest) had the lowest population and site six (oak-pine forest) had the highest population (Table 4). The total population of site six was the only population which differed significantly (one standard deviation) from the mean of the total populations for all sites. The total population of site six was two standard deviations greater than the mean.

TABLE 1. *Location and description of sampling sites.*

Site No.	Date of collection	Location	Core depth	Plant community	Representative flora
1	7/7/76	Beckham County 5 mi W of Sayre, 4 mi on OK 152, 1 mi on dirt road; 1 mi E. of N. Fork Red River	7 cm	Sand-sage grassland	*Artemisia filifolia* *Schizachyrium scoparium* *Ambrosia psilostachya*
2	7/7/76	Beckham County 1 mi E of Exit 11 on I 40	3-8 cm	Shinnery oak-grassland	*Quercus havardii* *Sporobolus sryptandrus*
3	7/7/76	Beckham County 5 mi S of OK 30—US 66 intersection on OK 30	5-10 cm	Mesquite grassland	*Prosopis juliflora* var. *glandulosa* *Bromus japonicus* *Aristida* sp.
4	7/7/76	Washita County ¼ mi S of OK 44—I 40 intersection on OK 44	5-8 cm	Mixed grass eroded plains	*Andropogon saccharoides* *Aristida fendleriana* *Ambrosia psilostachya*
5	7/7/76	Canadian County ½ mi N of OK 270 W—I 40 intersection on OK 270 W	10-15 cm	Tall grass prairie (cultivated)	*Triticum* sp.
6	7/27/76	Latimer County Sun Oil Co. Bratton W.A. No. 1, OK 2 3½ mi S of OK 31 intersection	7-12 cm	Oak-Pine forest	*Pinus echinata* *Quercus* sp.
7	7/27/76	Latimer County SW 10th St., ⅓ mi W of 10th St.—OK 2 intersection	2-5 cm	Oak-Hickory forest	*Quercus* sp.
8	7/27/76	Hughes County US 270 5 mi E of Calvin	7-12 cm	Postoak-Blackjack forest	*Quercus marilandica*

TABLE 2. *Dyes utilized, source, batch number and final concentration in medium.*

Dye	Source	Batch No.	Dye Concentration in medium, (μg/ml)
Crystal (Gentian) Violet[a]	Matheson, Coleman and Bell	14	10.0
Bromthymol Blue	Nutritional Biochemical	6937	30.0
Congo Red	Matheson, Coleman and Bell	NB 272	30.0
Coomassie Brilliant Blue	Bio-RAD	12796	50.0
Eosin Y	Matheson, Coleman and Bell	B286	50.0
Evans Blue	Fisher Sci. Co.	752550	100.0
Safranin O	Allied Chem.	NS 30	0.5
Methyl Red	Fisher Sci. Co.	793016	160.0

[a] Difco PCA used as base for CV plates
Difco tryptic soy agar used as base for all other dye plates

TABLE 3. *Results of physical and mineral analysis of soil for each sampling site.*

Site No.	1	2	3	4	5	6	7	8
pH	7.1	7.0	7.9	5.6	6.1	7.0	7.2	7.2
Moisture, %	0.89	0.99	7.81	1.96	6.01	2.55	2.49	1.09
Organic carbon (%)	0.55	1.69	3.60	1.93	1.24	2.15	2.15	1.18
Chloride[a]	12.6	7.6	24.3	7.7	23.9	25.6	25.6	15.2
Nitrite, Nitrate[a]	1.0	0.6	5.2	0.2	12.7	0.6	1.4	0.8
Total Phosphate[a]	21.2	55.6	34.5	29.6	23.3	30.8	51.3	30.3

[a] In μg/g soil (dry wt.)

TABLE 4. *Populations and relative distributions of major aerobic bacterial types for each sampling site.*

Site No.	Total Microbial count (10^6)	Gram+ bact. and Nocardia (10^6)	Gram− bact. (10^6)	Streptomycetes (10^6)	Unclassified (10^6)
1	1.0[a] (100)[b]	0.37 (36.8)	0.11 (10.5)	0.47 (47.4)	0.053 (5.3)
2	1.4 (100)	0.44 (31.6)	0.44 (31.6)	0.52 (36.8)	0 (0)
3	3.2 (100)	1.1 (35)	1.1 (35)	0.80 (25)	0.16 (5)
4	0.92 (100)	0.48 (52.6)	0.19 (21)	0.24 (26.3)	0 (0)
5	6.8 (100)	2.7 (40)	2.0 (30)	2.0 (30)	0 (0)
6	13 (100)	3.9 (30)	3.3 (35)	0.65 (5)	5.2 (40)
7	1.1 (100)	0.17 (15)	0.44 (40)	0.28 (25)	0.22 (20)
8	0.72 (100)	0.11 (15)	0.40 (55)	0.072 (10)	0.14 (20)

[a] Colonies/g of soil (dry wt.)
[b] Percent of total

TABLE 5. *Percent composition of Gram+ bacteria and* Nocardia *for each sampling site.*

Site No.	*Arthrobacter*	*Bacillus*	*Corynebacterium*	*Micrococcus*	*Nocardia*
1	14.3	28.6	0	28.6	28.6
2	50	0	0	50	0
3	0	0	57	0	42.9
4	50	10	20	0	20
5	25	50	12.5	12.5	0
6	50	16.7	16.7	16.7	0
7	33.3	33.3	0	33.3	0
8	33.3	0	66.6	0	0

In Table 4 the total microbial populations have been divided into four arbitrary units (Gram-positive bacteria, Gram-negative bacteria, Streptomycetes, and unclassified). When the unit compositions of the total microflora populations were statistically analyzed differences were noted between the compositions of grassland communities and forest communities. The Gram-positive bacteria comprised a significantly greater percentage of the total population in the grassland communities than in the forest communities and the unclassified microorganisms comprised a significantly greater percentage of the total population in the forest communities than in the grassland communities. The Gram-negative bacteria and the Streptomycetes did not differ significantly between grassland and forest communities.

The Gram-positive component of Table 4 was subdivided into four bacterial genera and the genus *Nocardia* by the dye adsorption method (Table 5). When the genus *Streptomyces* was compared with these five other Gram-positive genera, this genus was found to be the most prominent Gram-positive one in each of the grassland communities and the oak-hickory forest community.

When the percents shown for each genus in Table 5 are added and divided by eight, the average for the genus in all sampled communities is obtained. The five genera then rank in order of prevalence: *Arthrobacter* 32%, *Corynebacterium* 21.6%, *Micrococcus* 17.6%, *Bacillus* 17.3%, and *Nocardia* 11.4%

DISCUSSION

There were qualitative and quantitative microbial differences between sites, but these differences in microbial populations did not correlate with differences in the measured physical characteristics of the soils. This lack of correlation between microbial populations and physical soil data could have been due to the selection of inappropriate data or an insufficient quantity of data. An attempt to correlate microbial populations with soil moisture content is a good example. In the field an increase in soil moisture, to a point, would correlate with increased microbial activity. In the present study, however, the microbial populations represent not just the metabolically active microorganisms but the total population of both active and dormant microorganisms. If annual precipitation is considered, the grassland sites received less moisture than the forest sites. The Gram-positive bacteria were more prevalent in the grassland sites than the forest sites and the genus *Streptomyces* was the numerically predominant genus in all grassland communities. The genus *Streptomyces* (12) and several of the Gram-positive genera (12, 13) are characterized by an increased resistance to desiccation and would have a selective advantage in the more arid grassland communities. Therefore, annual precipitation but not soil moisture would be causally related to the type of microbial community which developed.

Site 5 was the only site sampled while under active cultivation. Its total microbial population was significantly higher than that of the other grassland sites but was not the highest of all sites sampled. The microbial composition of site 5 (Table 4) did not differ significantly from that of other grassland sites. This indicates that cultivation did not alter the procaryotic microbial community as extensively as it did the botanical and zoological communities.

In the present study the dye adsorption method of Gram-positive bacterial identification was not as definitive as previously described (7). Two possible reasons for the imperfect differentiation of genera in this study are a) the dyes possibly differed in composition, or b) the greater diversity of

soil environments tested in this study exceeded the differential capabilities of the method. If (b) is true, it would add credence to an assumption, in the original report (7), that the dye adsorption method of bacterial differentiation requires standardization for each soil environment tested.

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