Bacterial Diversity in Rhizospheres of Nontransgenic and Transgenic Corn

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Bacterial diversity in transgenic and nontransgenic corn rhizospheres was determined. In greenhouse and field studies, metabolic profiling and molecular analysis of 16S rRNAs differentiated bacterial communities among soil textures but not between corn varieties. We conclude that bacteria in corn rhizospheres are affected more by soil texture than by cultivation of transgenic varieties.

Genetically modified or transgenic crops may improve agricultural productivity. Thus, the global agricultural area cultivated to transgenic crops continues to increase despite public concern about environmental effects (13). Possible impacts on rhizosphere microbial communities are of concern because little is known of diversity-function relationships of microorganisms with transgenic plants in the soil environment (1, 3).

Structural and functional diversities of microorganisms in the rhizosphere differ among plant species due to differences in root exudation (2). Abiotic factors (soil physicochemical properties), agronomic practices, and the plant growth stage may also influence rhizosphere microbial composition (2, 15). Microbial communities associated with rhizospheres of transgenic cotton and alfalfa significantly differed from those of the nontransgenic isogenic lines (7, 8). In contrast, *Bacillus thuringiensis* toxins present in transgenic corn rhizospheres did not change culturable microbial communities (16). The diversity of rhizosphere bacteria of transgenic, herbicide (glufosinate)-resistant corn was not different from that of the corresponding nontransgenic variety (17). These studies illustrate the various impacts of transgenic crops on soil microorganisms.

We examined two transgenic corn varieties, *Bacillus thuringiensis*-protected and Roundup Ready (RR), which comprised 25% and 7% of the United States corn acreage, respectively, in 2000 (5). The goal of our study was to examine effects of transgenic corn on bacterial diversity by using denaturing gradient gel electrophoresis (DGGE) and carbon substrate utilization. These methods yield both a molecular assessment and a physiological assessment of impacts of transgenic corn on rhizosphere bacterial communities.

Soils (silt loam, silty clay, and sandy loam) were collected in April 2002 from central Missouri sites, where 8 to 10 samples (approximately 4 kg soil) were collected along 30-m transects at three equidistant points. Each sample was composited and stored in a plastic container at 15 to 20°C and processed within 3 days of collection. Silt loam was from the 0- to 8-cm depth of a Mexico silt loam (fine, smectitic, mesic Aeric Vertic Epia-

qualfs) under grass vegetation at the Bradford Agronomy Research Center (38°54′N, 92°12′W). Silty clay was removed from the B (argillic) horizon (8- to 14-cm depth) of the Mexico silt loam. We collected sandy loam (0- to 10-cm depth) from Sarpy fine sand (mixed, mesic Typic Udipsamments) of a Missouri River flood plain field (38°53′N, 92°13′W) planted to soybeans. Each soil was air dried, sieved (2-mm mesh), fertilized (13-13-13, N-P-K content [percent]) and limed sufficient for optimum corn production based on soil recommendations, and amended with a suspension of original field-moist soil (1:2 soil-to-water ratio) to assure viable microbial populations. Amended soils (10 kg) were packed to a bulk density of 1.20 g · cm^{-3} and placed in 11.3-liter plastic containers, with 12 containers for each soil. Soils (100 g) collected from each container before planting were preplant samples. Transgenic corn varieties Pioneer 33P67 (*Bacillus thuringiensis*-protected) and Asgrow 730 (RR) and nontransgenic isogenic lines (Pioneer 33P66 and Asgrow 740) were planted; soils were watered to 80% water-holding capacity. Nonplanted bulk soils were included. Soil-plant treatments were arranged in a randomized complete block design with four replications. At the 10th-leaf growth stage, intact plants were removed, with soil tightly adhering to roots removed by vigorous shaking, to provide rhizosphere soil samples.

The four corn varieties were planted on a Mexico silt loam in May 2002 at the Bradford Agronomy Research Center in a randomized complete block design with four replications. Rhizosphere and bulk soil (0- to 15-cm depth) samples were collected in May, July, and September. Procedures for processing field-collected plants and soil were in accordance with the greenhouse protocol.

Carbon substrate utilization was determined by incubating 125-μl aliquots of soil suspensions (1:1,000 in 0.85% NaCl) dispensed into each well of BIOLOG GN2 plates. Plates were incubated at 25°C for 72 h, and the optical density in each well measured with a Dynatech microplate reader at 575 nm. Overall color development in BIOLOG plates was expressed as average well color development (AWCD) (9).

Molecular analyses of community structure involved DNA extraction from soil as described elsewhere (18). A universal bacterial primer pair (F984GC-R1378) targeting 16S rDNA at

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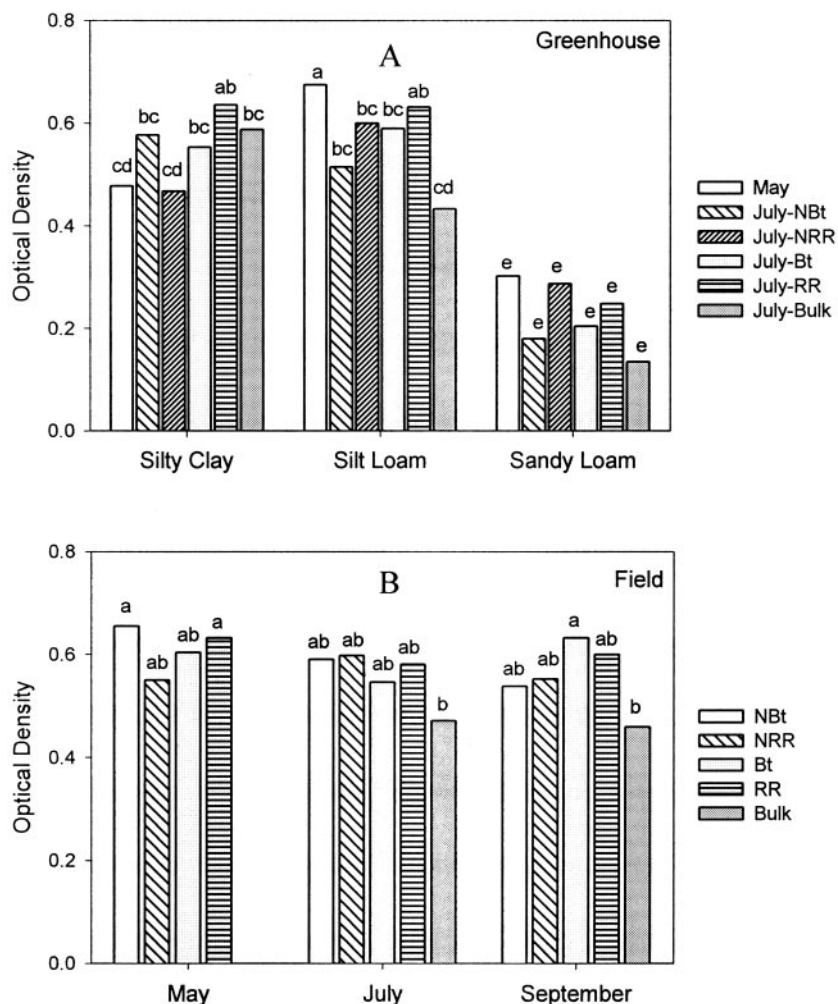
FIG. 1. AWCD in BIOLOG microplates after incubation at 28°C for 72 h. (A) Soils from the greenhouse study (silty clay, silt loam, and sandy loam) were sampled in May (preplanting) and July (10-leaf corn growth stage). (B) Soils from the field study were sampled in May (before planting), July (10-leaf growth stage), and September (harvest). NBt, non-*Bacillus thuringiensis*-protected isogenic lines; NRR, non-Roundup Ready isogenic lines; Bt, *Bacillus thuringiensis*-protected corn; RR, Roundup Ready corn; Bulk, bulk (nonplanted) soil. For each study, bars with the same letter are not significantly different according to Fisher's protected least significant difference test ($P = 0.05$).

968 to 1,401 bp was used to amplify DNA fragments by PCR (12). PCR amplification and DGGE analyses were performed (18). Gels were stained with SYBR green I (Molecular Probes) and visualized under UV illumination (Syngene GeneGenius gel documentation system). The intensities and positions of DNA bands were digitized by Genetool software (Syngene).

The BIOLOG data were analyzed by principle component (PC) analysis using AWCD as a covariable. The principle component scores were subjected to the PC test of Läuter (14). Absorbance values were truncated to lie within the interval of 0 to 2 to eliminate the influence of biased measurements or outliers (11). The analysis of PCR-DGGE molecular profiles was based on DNA band position by use of binary values (1 for visible bands and 0 for absent bands). Similarity indices of DGGE patterns were determined by Group Average using PROC CLUSTER (SAS Institute).

Carbon substrate utilization (AWCD) for sandy loam was significantly lower than that for other soils at both greenhouse sampling dates (Fig. 1A). Rhizospheres sampled at the 10-leaf

growth stage differed in AWCD only for RR corn compared to non-RR corn in silty clay and in bulk silt loam. For field samples, only *Bacillus thuringiensis*-protected corn rhizospheres from the September sampling differed in AWCD from bulk soil (Fig. 1B). We consistently observed no significant differences in optical density between rhizospheres of either nontransgenic or transgenic corn varieties under field conditions.

PC analysis and the PC test analyses illustrated that carbon substrate utilization patterns of bacterial communities between soils were highly significant ($P = 0.001$) (Table 1). Significant temporal variation (preplant and 10-leaf stages) was noted based on three principle components in the greenhouse and in the field (season effect) based on 13 principle components. Bacterial communities were not different between corn varieties in either greenhouse ($P = 0.34$) or field environments ($P = 0.59$).

About 35 different bands of various intensities appeared in DGGE profiles of DNA extracted from all soils and rhizospheres. Sandy loam showed fewer bands compared to other

TABLE 1. Comparisons of bacterial communities based on the PC test of principle components derived from the BIOLOG assays

Soil sample comparison or effect	No. of PCs (% cumulative contribution)	F value (degrees of freedom)	P value
Greenhouse			
Transgenic vs. nontransgenic	7 (81)	1.18 (7–39)	0.34
Silt loam vs. silty clay	9 (81)	17.9 (9–29)	<0.001
Silt loam vs. sandy loam	6 (80)	15.8 (6–32)	<0.001
Silty clay vs. sandy loam	5 (80)	15.2 (5–34)	<0.001
Preplant vs. 10-leaf stage	3 (80)	76.3 (3–20)	<0.001
Rhizosphere vs. bulk soil	8 (81)	1.00 (8–50)	0.45
Field			
Corn growth stage effect	13 (80)	2.62 (26–82)	0.0005
Transgenic vs. nontransgenic	9 (82)	0.83 (9–22)	0.59

soils. In the greenhouse, cluster analyses revealed bacterial communities from sandy loam were about 50% similar with silty clay and silt loam (Fig. 2). Bacterial communities from silty clay and silt loam shared 25 bands and reached 65% similarity. DGGE patterns for silty clay and silt loam sampled at the 10-leaf stage clustered together, indicating a temporal shift in bacterial communities. The similarities of DGGE patterns for transgenic and nontransgenic corn in silt loam and silty clay were >90%. Transgenic corn rhizospheres from sandy loam formed separate subclusters and were highly similar to those of nontransgenic corn in sandy loam (>87%).

DGGE patterns of field samples shared dark bands formed on the gels, suggesting that dominant members of the bacterial community were ubiquitous within all field treatments. A few low-intensity bands differed among the treatments. Cluster analysis (Fig. 3) revealed highly similar (92%) banding profiles for May soils; July and September samples, which were 86% similar to May samples, grouped together. Qualitatively, a rhizosphere effect was detected because July and September bulk soils separated from planted soils. No differences in bacterial community patterns between transgenic and nontransgenic corn rhizospheres were found, confirming greenhouse results.

This study showed that rhizosphere bacterial diversity did not differ between transgenic or nontransgenic corn. Other studies assessing culturable soil microbial communities indicated that bacterial communities in rhizospheres were not affected by varieties of either nontransgenic (4) or *Bacillus thuringiensis*-protected (16) corn. Glufosinate-resistant corn was previously shown not to affect the bacterial community (17); similarly, we found that RR corn had no effect on rhizosphere bacterial communities, and this is the first such report to our knowledge.

Of significant interest is the demonstration that bacterial diversity, characterized by physiological and molecular approaches, differed between soils in which corn was grown. This supports Girvan et al. (10), who concluded that soil texture was the overriding factor in controlling soil bacterial diversity, regardless of the cropping system. Others confirmed the important role of soil texture on bacterial diversity in rhizospheres of field-grown corn at the community (4) and genus (6) levels.

Differences in bacterial diversity increased with time after planting for isolated cases only in both studies. Bacterial com-
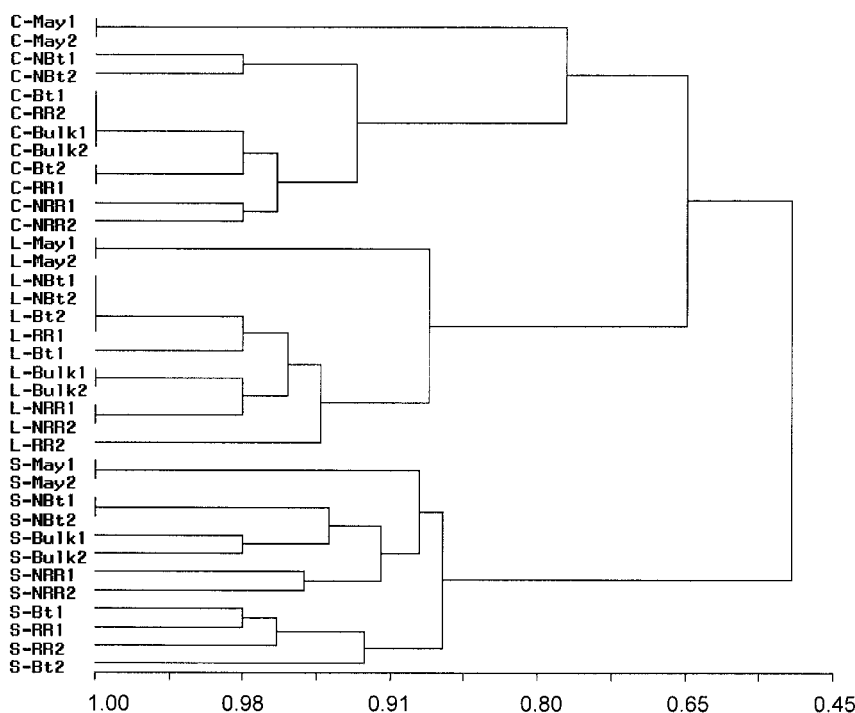

FIG. 2. Cluster analysis of DGGE banding profiles of DNA extracted from soils in the greenhouse study using unweighted pair group with mathematical averages analysis. C, silty clay; L, silt loam; S, sandy loam; NBt, non-*Bacillus thuringiensis*-protected isogenic lines; Bt, *Bacillus thuringiensis*-protected corn; NRR, non-Roundup Ready isogenic lines; RR, Roundup Ready corn; Bulk, bulk soil (nonplanted). Samples were collected before planting (May) and at 10-leaf stage of corn growth. Cluster analyses were conducted for bands developed using DGGE conducted on duplicate soil extracts (i.e., C-May1 and C-May2).
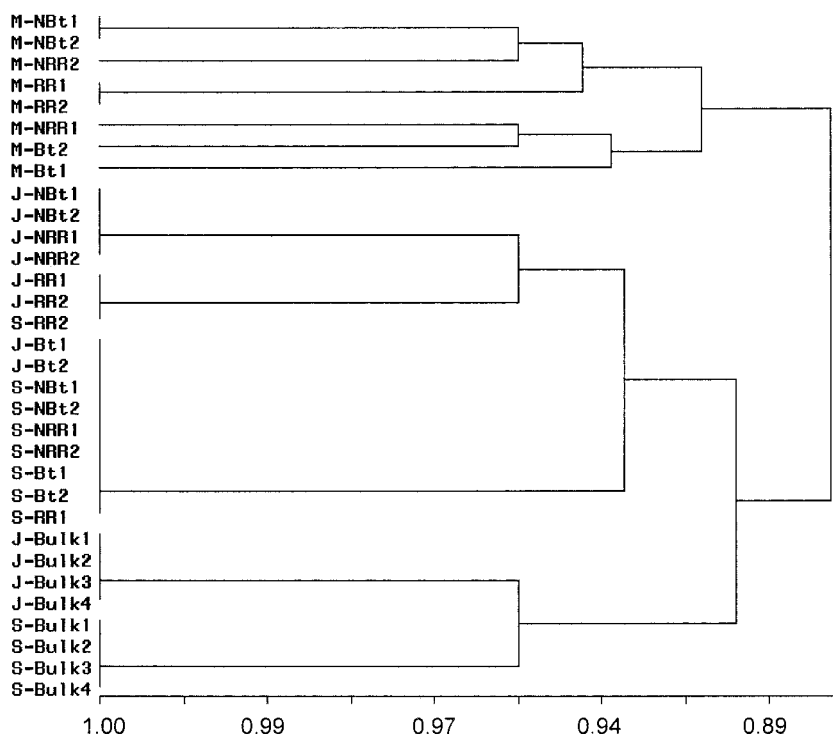
FIG. 3. Cluster analysis of DGGE banding profiles of DNA extracted from soils in the field study using unweighted pair group with mathematical averages analysis. Collection times were May (M), July (J), and September (S). NBt, non-*Bacillus thuringiensis*-protected isogenic lines; Bt, *Bacillus thuringiensis*-protected corn; NRR, non-Roundup Ready isogenic lines; RR, Roundup Ready corn; Bulk, bulk soil (nonplanted). Cluster analyses were conducted for bands developed using DGGE conducted on duplicate soil extracts (i.e., M-NBt1 and M-NBt2).

munities differed due to changes in root exudate quantity and composition by the developing corn plant, which select different bacterial groups during root colonization (2).

In summary, soil texture, rather than presence of plants, significantly affected diversity of rhizosphere microbial communities. Results are from only two transgenic corn varieties; thus, more detailed studies on diversity of rhizosphere microorganisms are necessary to confirm these effects. Even though soil has the capacity for buffering effects of management before these practices affect the microbial community (10), long-term impacts of management that includes frequent cropping to transgenic crops may be significant; thus, further attention on this aspect is needed.

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