Diversity of Root Bacteria from Peanut Cropping Systems

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ABSTRACT

Rhizobacteria play an important role in sustainable agriculture via plant growth and biological control of pests in a number of ecosystems. Understanding the interactions of crop rotation and rhizobacteria on peanut production is a critical research need. Development of a database on the rhizobacteria obtained from continuous and rotational fields of peanut was initiated in 1997 and terminated in 2000. Peanut was planted in monoculture for 4 yr. In rotational plots, peanut, cotton, corn, and peanut were planted in sequence. Rhizobacteria were isolated from the roots of crop plants grown in a Norfolk soil near Florence, SC. These isolates were identified by composition of fatty acids from gas chromatography analysis (GC/FAME). *Arthrobacter* and *Bacillus* were the major genera from non-rhizosphere soils. At initiation of this study in July 1997, the plots selected for continuous peanut had more diversity in rhizobacteria than those plots selected for rotation. In July 2000, rhizobacteria diversity was greater from peanut roots in the rotation cropping system than continuous peanut. Even though rhizobacteria diversity was greater in the rotation system, higher peanut yields were recorded in the continuous peanut system in 2000. *Burkholderia* spp. were always isolated from the peanut and other crop rhizospheres at each sampling date.

Key Words: Rhizobacteria, bacterial ecology, crop rotations.

The soil environment immediately around the root system frequently has a larger number of microorganisms than soil just a few millimeters from the root system.

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This zone of influence is called the rhizosphere. Methods for studying the rhizosphere have been established and reviewed (Rovira, 1991; Kloepper and Beauchamp, 1992; Bolton *et al.*, 1993). After approximately 20 yr of intense research on the rhizosphere, Rovira (1991) indicated that there are more than 2,000 publications on the this topic and stated, "prospects are bright for improving our understanding of rhizosphere biology and managing the rhizosphere microflora to increase plant growth." However, he indicated that frustrations would continue unless more thought and effort are put into the microbial ecology of the rhizosphere.

The rhizosphere is composed of many groups of organisms that are capable of affecting plant health, both beneficially (Nelson and Craft, 1991; Hodges *et al.*, 1993) and deleteriously (Suslow and Schroth, 1982; Elliott and Lynch, 1985; Schippers *et al.*, 1987; Frederick *et al.*, 2001; Wang and Skipper, 2004). Four genera isolated from the rhizoplane of canola (*Brassica napus* L.), *Agrobacterium*, *Phyllobacterium*, *Pseudomonas*, and *Variovorax*, induced an increase of root dry weight up to 52% (Bertrand *et al.*, 2001). The cell-free culture filtrate of *Pseudomonas fluorescens*, a plant growth inhibitory bacterium, showed a strong inhibitory effect on wheat (*Triticum aestivum* L.) root elongation and the inhibitory substance(s) was synthesized when the bacterium was grown in wheat root exudates (Astrom *et al.*, 1993). In a soybean [*Glycine max* (L.) Merr.]-corn (*Zea mays* L.) rotation, *Acidovorax avenae*, a weak pathogen, was the dominant rhizobacteria obtained from roots of continuously grown soybean and a significant yield reduction was associated with the presence of this organism (Frederick *et al.*, 2001). However, Shiomi *et al.* (1999) indicated it was difficult for a pathogen to dominate in a diversified rhizobacterial community in contrast to a community dominated by a single species or with few species.

As in many plant-rhizobacteria relationship studies, the information on interactions between peanut roots and their rhizobacteria is limited. Since peanut is an important crop in the U.S., a critical research need for peanut production is to understand the bacterial interactions in the rhizosphere. Some species of fungi are known to be causal agents of peanut diseases. For example, the fungi *Puccinia arachidis* and *Didymella arachidicola* are responsible for rust and web blotch, respectively. *Rhizoctonia solani*, *Pythium myriotylum*, and *Aspergillus niger* are responsible for rhizoctonia limb, pod, and root rot; pythium pod rot; and crown rot, respectively. By inoculating peanut plants with beneficial rhizobacteria where fungal diseases are widespread, peanut may be protected from the diseases caused by fungi. To promote vigorous, healthy plants, a large, diverse population of beneficial bacteria is essential in crop rhizospheres. The objectives of this research were to develop a database for the major root bacteria associated with peanut roots and to monitor ecological shifts of peanut rhizobacteria associated with crop rotations.

Materials and Methods

This study was conducted from 1997 to 2000 at the Pee Dee Res. and Educ. Center near Florence, SC. Roots from peanut/cotton/corn plants and associated non-rhizosphere Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudult) soil samples were obtained in May, June, July, or Aug. of each year. Peanuts were planted for 4 yr in the continuous peanut plots; whereas in the rotational plots, peanut, cotton, corn, and peanut were planted in 1997, 1998, 1999, and 2000, respectively. Root samples were collected during the summers of each year from two replicates arranged in a randomized complete block design. A mixture of root and soil within a 15-cm radius from the plant and 15-cm deep was collected for each crop plant. For non-rhizosphere control soil, samples were collected from soil without vegetation. The samples were kept on ice until processed within 48 hr.

Plant roots were separated from soil, placed in a sterile dilution buffer ($Na_4P_2O_7$, 1.0 g; 6N HCl, 0.69 mL; glycerol, 10 mL; H_2O, 1,000 mL), and shaken for 30 min at 200 rpm on a rotary shaker. For the non-rhizosphere control, soil without roots was used. The resulting suspensions were subjected to serial dilution and plating using standardized techniques and medium (Fig. 1). A 0.1 strength tryptic soy broth agar (TSBA) supplemented with cycloheximide (100 mg/L) to inhibit fungi was used for total bacterial populations. From the 0.1 strength TSBA plates, 40 bacterial isolates/plot or non-rhizosphere control were randomly selected to be identified using gas chromatographic analysis of fatty acid methyl esters (GC-FAME analysis; Fig. 1) (Sasser, 1990; MIDI, 2000). The data represent an average for two replicates or 80 bacterial isolates. Identifications are reported for isolates that accounted for 5% of the total isolates. Peanut yields were subjected to an analysis of variance.

Results and Discussion

The major genera from non-rhizosphere Norfolk soil samples were *Arthrobacter* and *Bacillus* and together they accounted for 46 to 93% of the isolates (Table 1). In addition, more than 70% of the bacterial isolates identified were Gram-positive. The abundance of Gram-positive bacteria that can form spores in the non-rhizosphere could be the result of stress conditions like high temperature and low soil moisture content under summer conditions (Alexander, 1998).

From the continuous peanut fields, a range of nine to 19 genera (Table 2) and 11 to 25 species (Table 3) were identified by GC-FAME. Of the total peanut isolates identified, *Burkholderia* was the dominant genus across

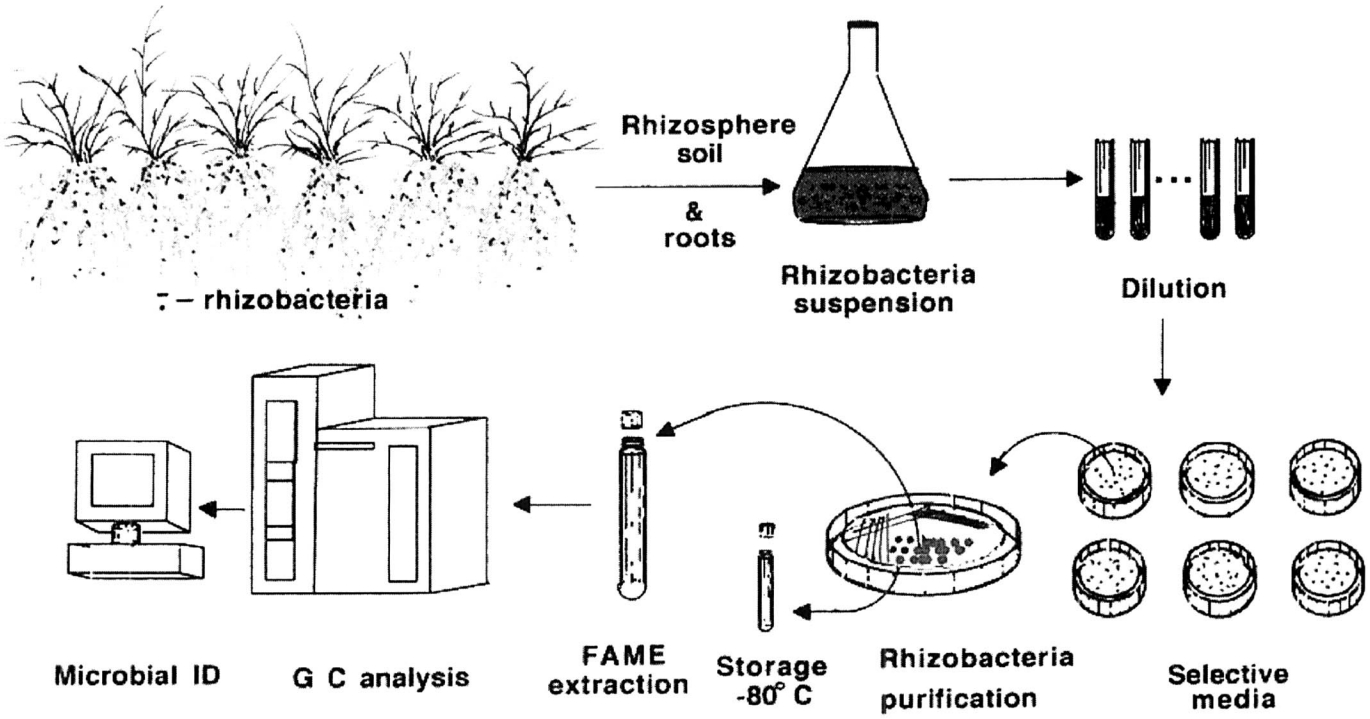

Fig. 1. Flow chart for the enumeration, isolation, and identification of rhizobacteria from plant rhizosphere.

years followed by *Chryseobacterium*, *Bacillus*, and *Phyllobacterium* (Table 2). *Burkholderia cepacia*, *Burkholderia gladioli*, *Chryseobacterium indologenes*, *Phyllobacterium rubiacearum*, and *Bacillus megaterium* were the major species over the 4 yr (Table 3). While *B. cepacia* was the dominant species in 1997 and 1998, *B. gladioli* was the dominant species in 1999 and 2000. Based on GC-FAME identification, 10 to 41% of the bacterial isolates from the continuous peanut plots did not match with any of the approximately 1100 known species contained in the aerobic library (MIDI, 2000).

From the rotational peanut plots, a range of seven to 19 genera (Table 4) and 11 to 30 species (Table 5) were identified. Of the total peanut isolates identified,

Table 1. Percentage of genera present in non-rhizosphere samples collected during June-July in 1998, 1999, and 2000. Blank indicates that the genus was not detected or the percentage was less than 5%.

	Month/Year					
Genus	6/98	7/98	6/99	7/99	6/00	7/00
	%					
Arthrobacter+[a]	30	8	25	14	11	11
Bacillus+	50	85	21	57	46	46
Brevibacillus+				6	8	
Burkholderia	8					
Cellulomonas+						11
Kocuria+					5	6
Micrococcus+			9		10	5
Paenibacillus+	5		15	5		5
Other genera	3(1)[b]	5(2)[b]	18(8)[b]	14(5)[b]	13(8)[b]	8(6)[b]
No match	4	2	12	4	7	8

[a]Plus sign (+) indicates Gram-positive and no sign indicates Gram-negative bacteria.

[b]Numbers in the parentheses indicate the total number of genera with percentages below 5%.

Table 2. Percentage of rhizobacterial genera isolated from continuous peanut fields during June-August in 1997, 1998, 1999, and 2000. Blank indicates that the genus was not detected or the percentage was less than 5%.

	Month/Year					
Genus	7/97	6/98	7/98	8/99	6/00	7/00
	%					
Acidovorax		6				
Bacillus+[a]	8			9		26
Burkholderia	18	24	33	70	54	21
Chryseobacterium		21				
Clavibacter+					9	
Enterobacter						5
Phyllobacterium	15		6			
Ralstonia			5			
Xanthobacter	5					
Other genera	30(15)[b]	21(11)[b]	15(8)[b]	11(7)[b]	21(11)[b]	11(6)[b]
No match	24	28	41	10	16	37

[a]Plus sign (+) indicates Gram-positive and no sign indicates Gram-negative bacteria.

[b]Numbers in the parentheses indicate the total number of genera with percentages below 5%.

Table 3. Percentage of rhizobacterial species isolated from continuous peanut fields during June-August in 1997, 1998, 1999, and 2000. Blank indicates that the species was not detected or the percentage was less than 5%.

Species	Month/Year					
	7/97	6/98	7/98	8/99	6/00	7/00
	%					
Bacillus cereus+[a]				5		6
Bacillus megaterium+	5					11
Bacillus pumilus+						6
Burkholderia cepacia	16	20	29	26		
Burkholderia gladioli				36	44	15
Burkholderia glathei				8	8	
Chryseobacterium indologenes		19				
Clavibacter michiganense+					9	
Enterobacter cancerogenus						5
Phyllobacterium rubiacearum	15					
Ralstonia pickettii			5			
Xanthobacter agilis	5					
Other species	35(21)[b]	33(16)[b]	25(14)[b]	15(7)[b]	23(15)[b]	20(12)[b]
No match	24	28	41	10	16	37

[a]Plus sign (+) indicates Gram-positive and no sign indicates Gram-negative bacteria.

[b]Numbers in the parentheses indicate the total number of species with percentages below 5%.

Burkholderia, *Bacillus*, *Phyllobacterium*, and *Ralstonia* were the major genera and *B. cepacia*, *B. gladioli*, and *P. rubiacearum* were the major species (Tables 4 and 5). The shift from *B. cepacia* to *B. gladioli* over time noted in continuous peanut rhizobacteria (Table 3) was also noted for the rotational plots with different crops (Table 5). Since peanut was planted in both the continuous and rotational peanut plots in 1997, the total number of genera ranged from 14 to 19, respectively, and species identified ranged from 17 to 25, respectively. Based on GC-FAME identification, 8 to 35% of the bacterial isolates from the rotational peanut plots did not match with any of the known species contained in the MIDI library (MIDI, 2000).

Bacillus, *Burkholderia*, and *Phyllobacterium* were the common genera found in both cropping systems. *Burkholderia* spp. were always isolated from the peanut and other crop rhizospheres at each sampling date. To the authors' knowledge, this is the first detailed report of rhizobacteria from peanut roots in cropping systems.

When the Gram-negative and Gram-positive bacteria from the peanut roots were compared, Gram-negatives had a higher percentage in all sampling dates except for July 2000 in the continuous peanut fields (Tables 2 and 4). Peanut roots might create the soil/root environment favorable for Gram-negative rhizobacteria (Rovira, 1991; Kloepper and Beauchamp, 1992). A shift of bacterial genera was observed when the isolates at each sampling date were compared. This fluctuation may be a result of environmental factors, such as different residue nutrient levels due to change of chemical components of peanut root exudates, temperature, and moisture content (Kloepper and Beauchamp, 1992).

Table 4. Percentage of rhizobacterial genera isolated from rotational peanut fields during May-July in 1997, 1998, 1999, and 2000. Blank indicates that the genus was not detected or the percentage was less than 5%.

Genus	Month/Year						
	7/97	6/98	7/98	5/99	6/99	6/00	7/00
	Crop planted						
	Peanut	Cotton	Cotton	Corn	Corn	Peanut	Peanut
	%						
Arthrobacter+[a]				5		6	
Bacillus+	5	5	6		50	5	6
Burkholderia	25	40	36	55	9	16	31
Corynebacterium+							8
Paenibacillus+					5		
Phyllobacterium	14	15	20				
Pseudomonas						14	5
Ralstonia		9		10	14		
Xanthobacter	5						
Other genera	16(10)[b]	6(4)[b]	10(4)[b]	16(9)[b]	14(9)[b]	29(15)[b]	21(14)[b]
No match	35	25	28	14	8	30	29

[a]Plus sign (+) indicates Gram-positive and no sign indicates Gram-negative bacteria.

[b]Numbers in the parentheses indicate the total number of genera with percentages below 5%.

Table 5. Percentage of rhizobacterial species isolated from rotational peanut fields during May-July in 1997, 1998, 1999, and 2000. Blank indicates that the genus was not detected or the percentage was less than 5%.

	Month/Year						
	7/97	6/98	7/98	5/99	6/99	6/00	7/00
	Crop planted						
Species	Peanut	Cotton	Cotton	Corn	Corn	Peanut	Peanut
	%						
Bacillus cereus[+a]					11		
Bacillus megaterium[+]			5		29		
Bacillus pumilus[+]					6		
Burkholderia caryophylli							5
Burkholderia cepacia	20	36	29	36			
Burkholderia gladioli	5		8	6	5	10	23
Burkholderia glathei				13		5	
Corynebacterium aquaticum[+]							8
Phyllobacterium myrsinacearum			10				
Phyllobacterium rubiacearum	11	11	10				
Pseudomonas putida						9	5
Ralstonia pickettii		8			11		
Ralstonia solanacearum				8			
Xanthobacter agilis	5						
Other species	24(13)[b]	20(10)[b]	10(6)[b]	23(14)[b]	30(19)[b]	46(27)[b]	30(19)[b]
No match	35	25	28	14	8	30	29

[a]Plus sign (+) indicates Gram-positive and no sign indicates Gram-negative bacteria.

[b]Numbers in the parentheses indicate the total number of species with percentages below 5%.

Peanut yields in 1997 were 3742 and 4558 kg/ha in rotational and continuous peanut plots, respectively (unpubl. data). In 2000, the yields were 3983 and 4392 kg/ha in rotational and continuous peanut plots, respectively (unpubl. data). Even though the yields for continuous versus rotated plots differed by 800 kg/ha in 1997 and 400 kg/ha in 2000, they were not significantly different at the P = 0.05 level. Thus, the greater diversity in peanut rhizobacteria in 2000 with rotation (Table 6) was not reflected in peanut yields under the conditions of this study. With a continuous peanut cropping system of longer than 4 yr and with continued decrease in diversity of rhizobacteria, a potential pathogen could have less competition and become a problem for the peanut producer. Suppressive soils can develop without crop rotations as noted for monoculture tomatoes (Shiomi *et al.*, 1999), and in a comparable soybean study, soybean yields were reduced 30% in a continuous soybean cropping system in comparison to a soybean-corn rotation system. Since this yield reduction may be linked to a high proportion of *Acidovorax avenae* on the soybean roots, a cause and effect is being investigated (Frederick *et al.*, 2001). Even though *A. avenae* was detected in tobacco rhizobacteria, it was not linked to yield reductions (Kim *et al.*, 2001). It is the authors' recommendation that more efforts be devoted to understand the microbial ecology of rhizobacteria associated with cropping systems.

Since rhizobacteria play an important role in sustainable agriculture via plant growth and biological control of pests in a number of ecosystems, a critical research need in peanut management is to understand the interactions of crop rotation and rhizobacteria on peanut production. The database on rhizobacteria for peanut developed in this study is limited to one location and it needs to be expanded to more locations in the future and with more intense sampling times. Even with its limitations, the database is essential to ecological studies.

Table 6. Diversity of peanut rhizobacteria from continuous peanut and rotated peanut cropping systems in July of 1997 and 2000.

	July 1997		July 2000	
Factor	Continuous	Rotated	Continuous	Rotated
	no.			
Genera	19	14	9	18
Species	14	17	18	23

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