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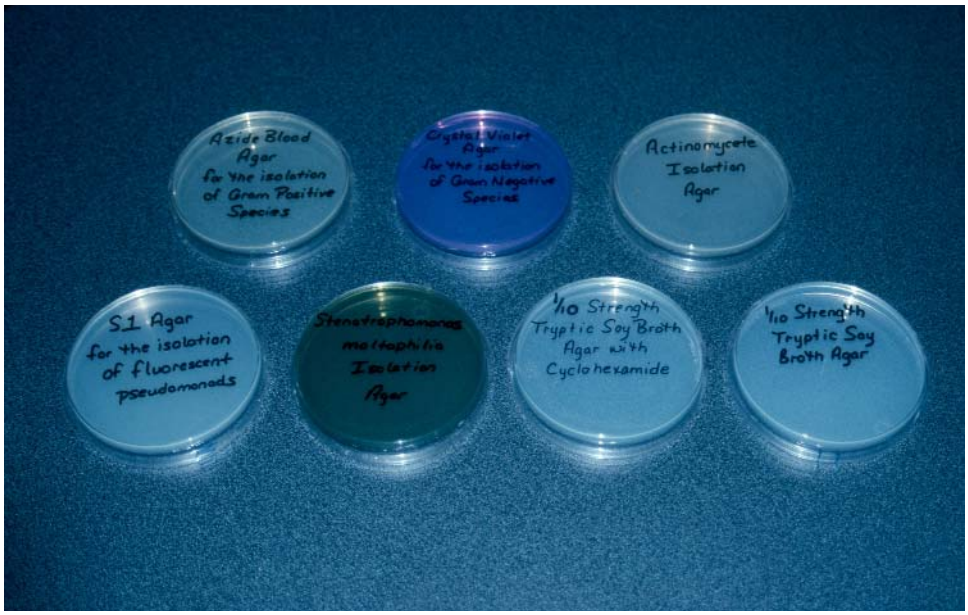


Taxonomic diversity of bacteria associated with turfgrass roots has not been widely explored. Scientists at the University of Florida, Auburn University, and Clemson University initiated a project to isolate and identify culturable bacteria from the rhizosphere of creeping bentgrass and hybrid bermudagrass in the southeastern U.S. Their results demonstrate that there is considerable taxonomic diversity of bacteria present in the rhizosphere of putting greens.

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## PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 350 projects at a cost of $29 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.


### Editor

Jeff Nus, Ph.D.
1032 Rogers Place
Lawrence, KS 66049
jnus@usga.org
(785) 832-2300
(785) 832-9265 (fax)

### Research Director

Michael P. Kenna, Ph.D.
P.O. Box 2227
Stillwater, OK 74076
mkenna@usga.org
(405) 743-3900
(405) 743-3910 (fax)

# Diversity of Rhizosphere Bacteria in USGA Putting Greens

M. L. Elliott, J. A. McInroy, K. Xiong, J. H. Kim, H. D. Skipper, and E. A. Guertal


## SUMMARY

Taxonomic diversity of bacteria associated with turfgrass roots has not been widely explored. The purpose of this project was to isolate and identify culturable bacteria from the rhizosphere of creeping bentgrass and hybrid bermudagrass in the southeastern U.S. Almost 10,000 randomly selected bacterial isolates were analyzed using gas chromatography fatty acid methyl ester (GC-FAME).

- The two dominant genera in both bentgrass and bermudagrass rhizospheres were *Bacillus* and *Pseudomonas*, with *Bacillus* dominant in bermudagrass and *Pseudomonas* dominant or equal to *Bacillus* in bentgrass.
- Other genera that composed at least 1% of the isolates at all four sites were *Clavibacter*, *Flavobacterium*, and *Microbacterium*.
- *Arthrobacter* also composed a significant portion of the bacterial isolates in the bentgrass rhizosphere, but not the bermudagrass rhizosphere. Overall, there were 40 genera common to all four sites.
- At the species level, there were five that composed at least 1% of the isolates at each location - *B. cereus*, *B. megaterium*, *C. michiganensis*, *F. johnsoniae*, and *P. putida*.
- This project demonstrates that there is considerable taxonomic diversity of bacteria present in the rhizosphere of putting greens.


The soil environment immediately around the root frequently has a larger number of microorganisms than soil just a few millimeters away from the root. This zone of influence is called the rhizosphere (20). The rhizosphere is composed of many groups of organisms that are capable of affecting plant health, both beneficially and deleteriously (22).

M. L. ELLIOTT, Ph.D., Professor and Associate Center Director, Department of Plant Pathology, University of Florida, Fort Lauderdale, FL; J. A. MCINROY, Research Associate III, Department of Entomology and Plant Pathology, Auburn University, Auburn, AL; K. XIONG, J. H. KIM, and H. D. SKIPPER, Ph.D., Professor, Department of Entomology, Soil, and Plant Science, Clemson University, Clemson, SC; and E. A. GUERTAL, Ph.D., Professor, Turfgrass Management, Department of Agronomy and Soils, Auburn University, Auburn, AL

Today, putting greens are artificially constructed soils, built from a predetermined mixture that is usually composed of sand and organic matter (25). In the southeastern U.S., newly built putting greens are often fumigated prior to being planted. However, previous research has shown that microbial populations present prior to fumigation rebound quickly after fumigation (5, 7). In addition, as the putting greens mature, thatch, root, and shoot production will cause significant increases in organic matter (10), which will promote microbial growth.

Natural materials, organic materials, and microbial inoculants are used by the golf course industry because there is an assumption that either few microbes are present in the turfgrass system or the "wrong" microbes are present. However, recent studies would indicate that turfgrass systems do have extensive microbial populations (3, 4, 7, 9, 17) and diverse microbial communities (18, 24, 27). Also, it is still unclear whether introduced bacteria can influence bacterial populations currently present in the phyllosphere, thatch, rhizosphere soil, or bulk soil (13, 15, 17, 18, 24).

The emphasis of the project described herein was on culturable bacteria since it is culturable bacteria that are being exploited by the golf course industry. In other words, the implication is that if you can't grow them in large quantities, either by a company or directly on the golf course in fermentation tanks, the bacteria are not useful as products. While we know there is a diverse microbial community present in turfgrass root systems, it is still not known which culturable fluorescent pseudomonad species or which culturable bacilli species are present.

A joint project was undertaken by Auburn University, Clemson University, and the University of Florida to examine bacterial populations and diversity in USGA putting greens over a three-year period after the greens were estab-

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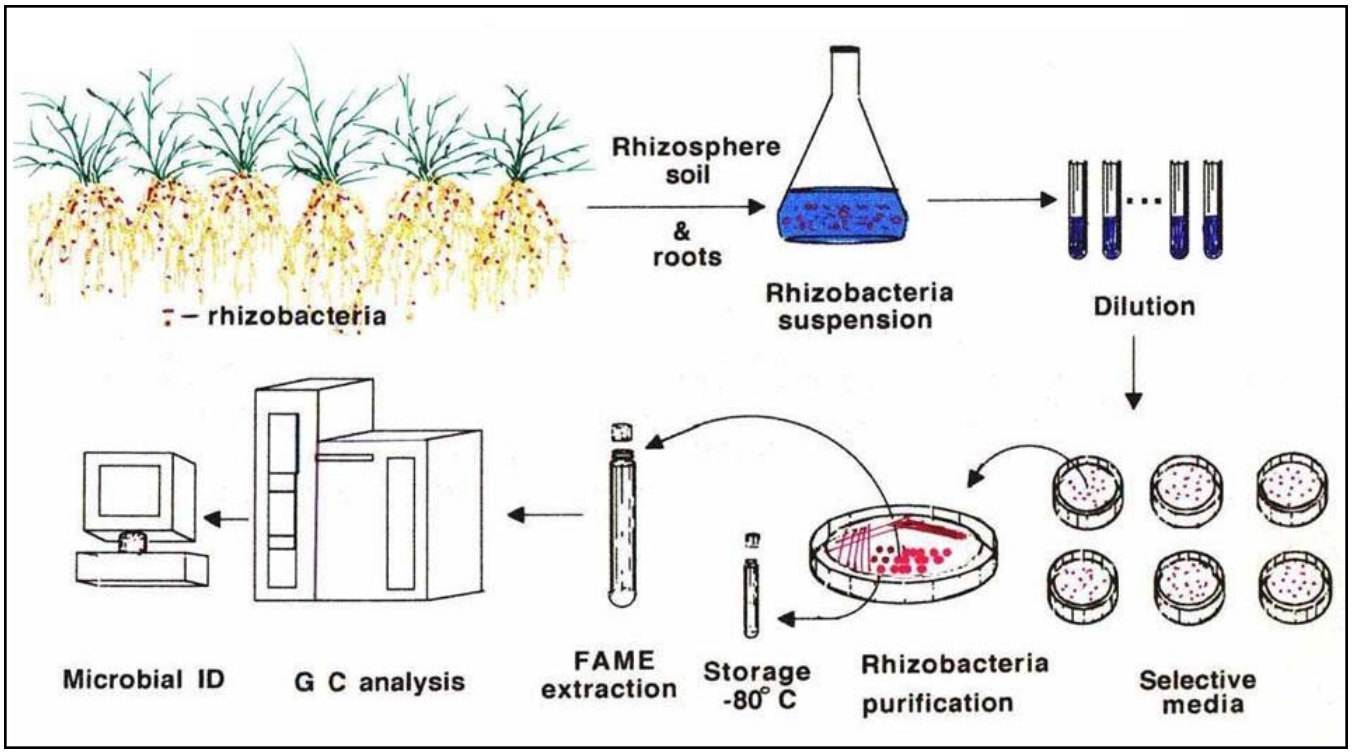


**Figure 1.** Graphic representation of the process of identifying rootzone bacteria

lished. We have previously reported on the flux in the extensive bacterial populations present in putting greens (7), the effect of nitrogen rate and rootzone mix on rhizosphere bacterial populations (6), and the identification of a diverse group of denitrifying bacteria from putting greens (26). This report summarizes which culturable bacterial genera and species were present and dominant in bentgrass and bermudagrass putting greens in the southeastern U.S. (8).

**Study Sites**

The bentgrass putting greens ('Crenshaw') were located at the Charlotte Country Club Golf Course, Charlotte, NC and Auburn University, Auburn, AL. The hybrid bermudagrass putting greens ('Tifdwarf') were located at the Cougar Point Golf Course, Kiawah Island, SC and the University of Florida, Fort Lauderdale, FL. All four sites were fumigated with methyl bromide prior to planting. Putting greens located at university sites (AL and FL) were miniature versions of those found on golf courses. All greens were managed in a manner typical for the region.

**Rhizosphere Sample Collection and Processing**

Four putting greens from each location were sampled four times per year (approximately every three months) for a minimum of three years in 1997-2000. Ten cores (1 cm by 10 cm) were collected per putting green to constitute a sample. Green tissue was removed from each core with a sterile razor blade. For each sample, turfgrass roots were separated from the rootzone mix, and all root material and rhizosphere soil was subjected to shaking in a sterile diluent. Aliquots of dilutions were plated onto duplicate plates of selective and non-selective media (7).

For enumeration of total aerobic bacteria and selection of bacteria for identification with GC-FAME, solidified 10% tryptic soy broth (10% TSBA), amended with 100 µg $mL^{-1}$ cycloheximide to inhibit fungi, was used. For each sampling date, 40 bacterial isolates per green sampled were randomly selected from the 10% TSBA for future identification. An estimated 10,000 bacterial isolates were selected for identification over the course of this study.

| | | | |
|---|---|---|---|
| *Acidovorax* | *Cellulomonas* | *Hydrogenophaga* | *Pedobacter* |
| *Acinetobacter* | *Chryseobacterium* | *Janthinobacterium* | *Phyllobacterium* |
| *Agrobacterium* | *Clavibacter* | *Kocuria* | *Pseudomonas* |
| *Alcaligenes* | *Comamonas* | *Methylobacterium* | *Sphingobacterium* |
| *Arthrobacter* | *Corynebacterium* | *Microbacterium* | *Sphingomonas* |
| *Bacillus* | *Curtobacterium* | *Micrococcus* | *Stenotrophomonas* |
| *Bradyrhizobium* | *Enterobacter* | *Nesterenkonia* | *Tsukamurella* |
| *Brevibacterium* | *Enterococcus* | *Ochrobactrum* | *Variovorax* |
| *Brevundimonas* | *Exiguobacterium* | *Paenibacillus* | *Xanthobacter* |
| *Burkholderia* | *Flavobacterium* | *Pantoea* | *Xanthomonas* |

**Table 1.** All bacterial genera, with similarity index of 0.300 or greater, common to golf course putting greens at all four locations (Alabama, Florida, North Carolina, and South Carolina).

## Identification of Bacteria Isolates

Analysis of the bacterial isolates was conducted using the GC-FAME/Microbial Identification System (MIDI, Inc., Newark, DE, USA) at Auburn University or, as needed, at the Multi-user Laboratory at Clemson University. Isolates were processed according to the protocol for aerobic bacteria of environmental origin (21). Fatty acid peak profiles were analyzed using the Sherlock Standard Aerobe Libraries (MIS version 4.0, Microbial ID, http://www.midi-inc.com). According to literature provided by MIDI, Inc., strains with a similarity index (SI) of 0.500 or greater are considered a good match at the species level, whereas strains with a SI between 0.300 and 0.499 are considered a good match at the species level but indicates an atypical strain (1). Since the bacterial species present in putting greens were largely unknown when this study was initiated, a SI of 0.300 or greater was used as the basis for identifying bacterial isolates.

## Bacterial Genera Present in Putting Greens

A total of 9,216 bacterial isolates were successfully analyzed using the GC-FAME/Microbial Identification System. Overall,

| | % of Total Isolates[y] | | | |
|---|---|---|---|---|
| | Bentgrass | | Bermudagrass | |
| Genera | Alabama | North Carolina | South Carolina | Florida |
| *Bacillus* | 13.9 | 12.5 | 19.4 | 10.5 |
| *Clavibacter* | 2.0 | 2.4 | 1.1 | 1.3 |
| *Flavobacterium* | 1.5 | 1.9 | 1.4 | 1.7 |
| *Microbacterium* | 1.2 | 1.7 | 1.1 | 3.1 |
| *Pseudomonas* | 13.6 | 18.7 | 9.1 | 5.8 |
| No match[z] | 34.3 | 32.0 | 38.0 | 50.1 |

[y] Total isolates analyzed is 1,896 for Alabama, 2,832 for North Carolina, 2,617 for South Carolina, and 1,871 for Florida.

[z] No isolate for that site had a match to a genus in the FAME database.

**Table 2.** Top five bacterial genera isolated and identified from bentgrass or bermudagrass putting greens

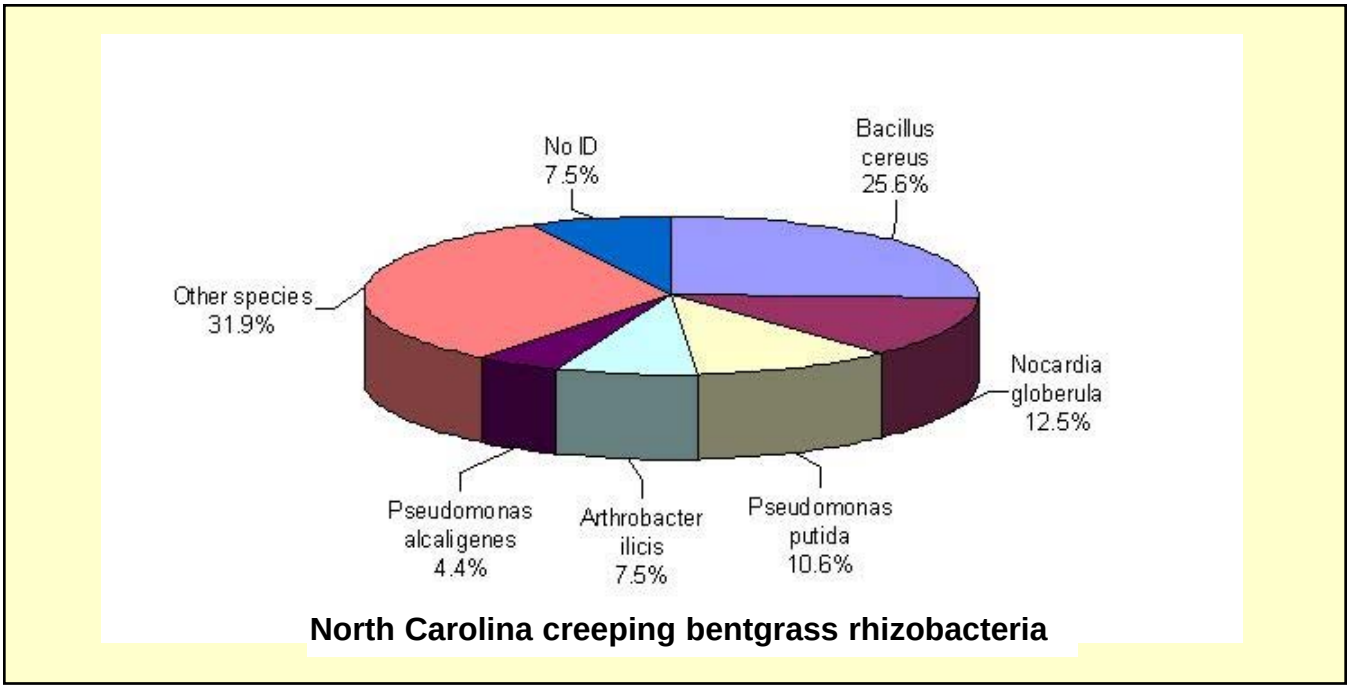


**Figure 2.** Distribution of rhizobacteria by species from NC bentgrass greens in September 2000

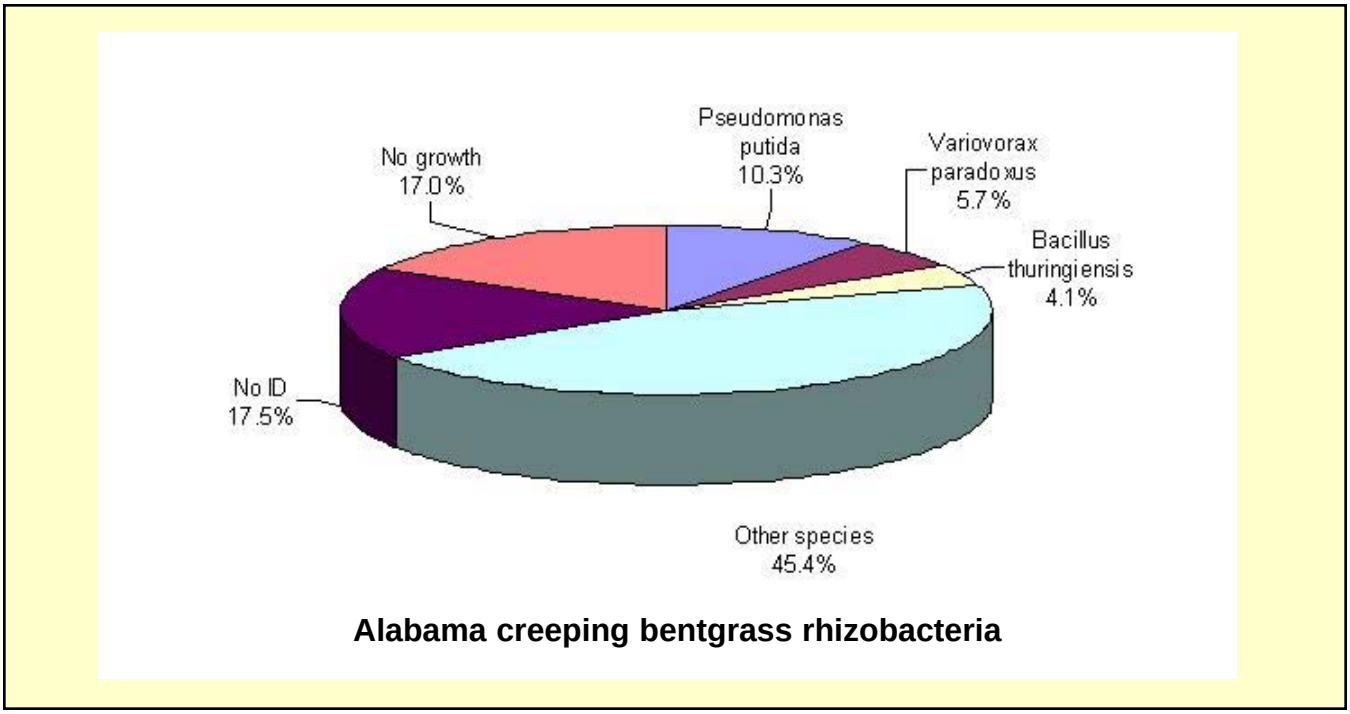


**Figure 3.** Distribution of rhizobacteria by species from AL bentgrass greens in August 2000

there were 50, 57, 64, and 64 bacterial genera identified in AL bentgrass, NC bentgrass, FL bermudagrass, and SC bermudagrass, respectively. There were 76 genera identified at both bermudagrass sites, with 13 unique to FL, 13 unique to SC, and 50 common to both. There were 59 genera identified at both bentgrass sites, with 3 unique to AL, 9 unique to NC, and 47 common to both. Forty genera were common to all four sites (Table 1).

There were five genera that composed at least 1% of the isolates at all four sites (Table 2): *Bacillus*, *Clavibacter*, *Flavobacterium*, *Microbacterium*, and *Pseudomonas*, with *Bacillus* and

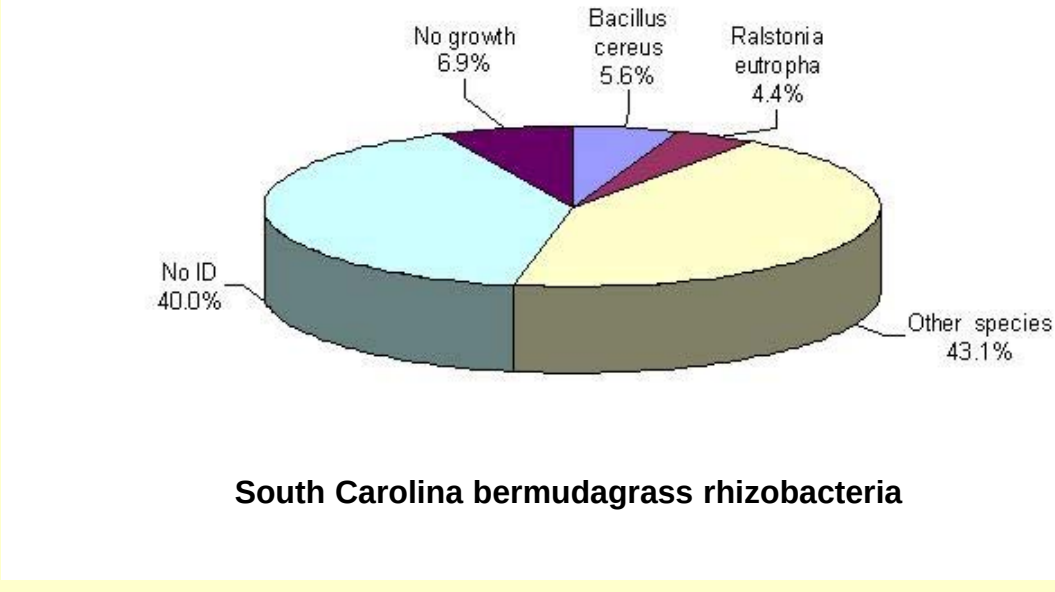


**Figure 4.** Distribution of rhizobacteria by species from SC bermudagrass greens in September 2000

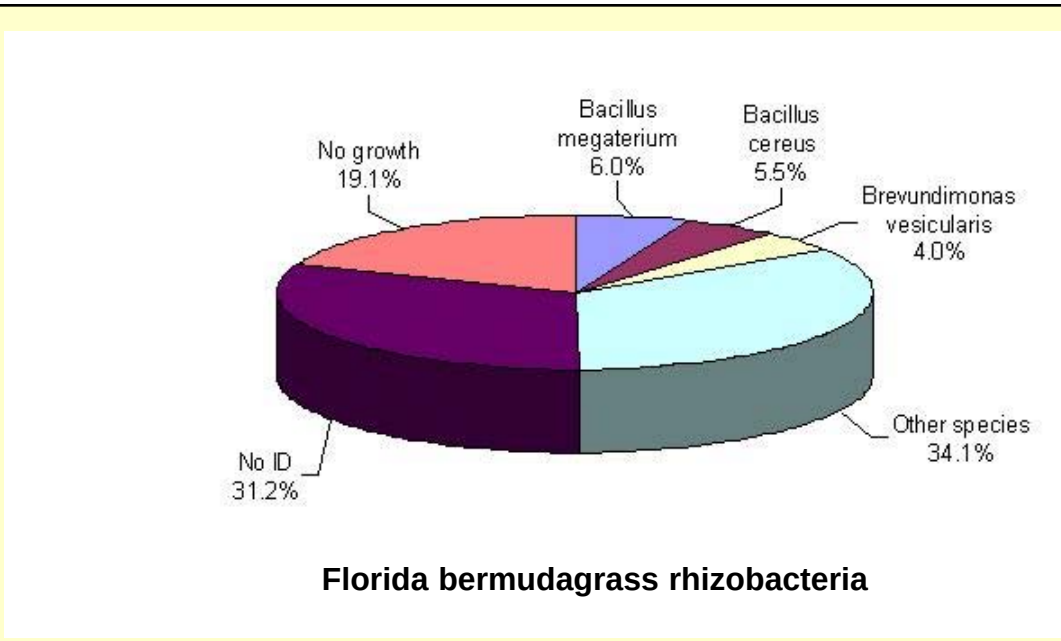


**Figure 5.** Distribution of rhizobacteria by species from FL bermudagrass greens in August 2000

*Pseudomonas* the dominant bacterial genera at each location. However, the percentage of isolates identified as *Bacillus* in the bermudagrass sites (FL and SC) was almost twice the number of isolates identified as *Pseudomonas*. At the bentgrass sites, *Pseudomonas* was either the dominant genus (NC) or was equal to *Bacillus* (AL). This is consistent with the previously reported enumeration data that *Bacillus* is the dominant genus over *Pseudomonas* in the bermudagrass rhizosphere, and that significantly greater numbers of fluorescent pseudomonads are found in the bentgrass rhi-

zosphere than in the bermudagrass rhizosphere (7).

*Arthrobacter* composed a significant portion of the bacterial isolates at the bentgrass sites (9.1% at AL and 7.5% at NC), with only *Bacillus* and *Pseudomonas* composing a greater percentage of the isolates identified. While *Stenotrophomonas* was identified from all four sites, it composed at least 1% of the isolates only at the FL and SC bermudagrass sites.

**Bacterial Species Present in Putting Greens**

There were five species that composed at least 1% of the isolates at all four sites - *Bacillus cereus, B. megaterium, Clavibacter michiganensis, Flavobacterium johnsoniae,* and *Pseudomonas putida*. Another three species, *Agrobacterium radiobacter, B. pumilus,* and *B. thuringiensis*, composed at least 1% of the isolates at the AL, FL, and SC sites, but not the NC site. A fourth species, *Comamonas acidovorans*, composed at least 1% of the isolates at the NC, AL, and FL sites, but not the SC site. One species was common at the 1% level only to the bermudagrass locations, *Stenotrophomonas maltophilia*. Four species were common at the 1% level only to the bentgrass locations, *Arthrobacter ilicis, P. chlororaphis, P. fluorescens,* and *P. syringae*. Figures 2-5 illustrate examples of species composition for single dates at each study site.

**Why Are There So Many Unidentifiable Isolates?**

The number of unidentifiable isolates (SI<0.300) was 50.1% for FL bermudagrass, 38.0% for SC bermudagrass, 34.3% for AL bentgrass, and 32.0% for NC bentgrass (Table 2). These values fall within the range of unidentifiable isolates obtained in other studies using GC-FAME for identification purposes (11, 12, 14, 16, 19, 23). Thus, the number of unidentified isolates in this study, obtained from an artificially constructed soil, would appear to be similar to the number from field soils in the same states using the same identification system.

Why are some bacterial isolates not identified? The MIDI aerobe bacteria library includes fatty acid profiles for 695 environmental species, with usually 20 or more strains representing each species or subspecies (2, 21). Our results and those of others illustrate that a significant number of bacteria isolated from bulk or rhizosphere soils are not part of the bacterial collection that is the basis of the MIDI environmental species library. Any database is only as good as the data (in this case, fatty acid methyl ester profiles of bacterial isolates) accumulated within it. The unidentifiable isolates are not necessarily new species *per se*, but may simply be species not represented in the MIDI database.

**Taxonomic Diversity Exists in Putting Greens**

This is the first study to survey for a portion of the culturable, aerobic bacterial genera and species common to golf course putting greens in the southeastern U.S. It demonstrates that there is considerable taxonomic diversity present in the rhizosphere of putting greens, despite their intense management. Obviously, while we have identified some of the bacteria genera and species present in golf course putting greens, there are still many unidentified bacteria. Even less information is known regarding what these bacteria do in the turfgrass system.

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