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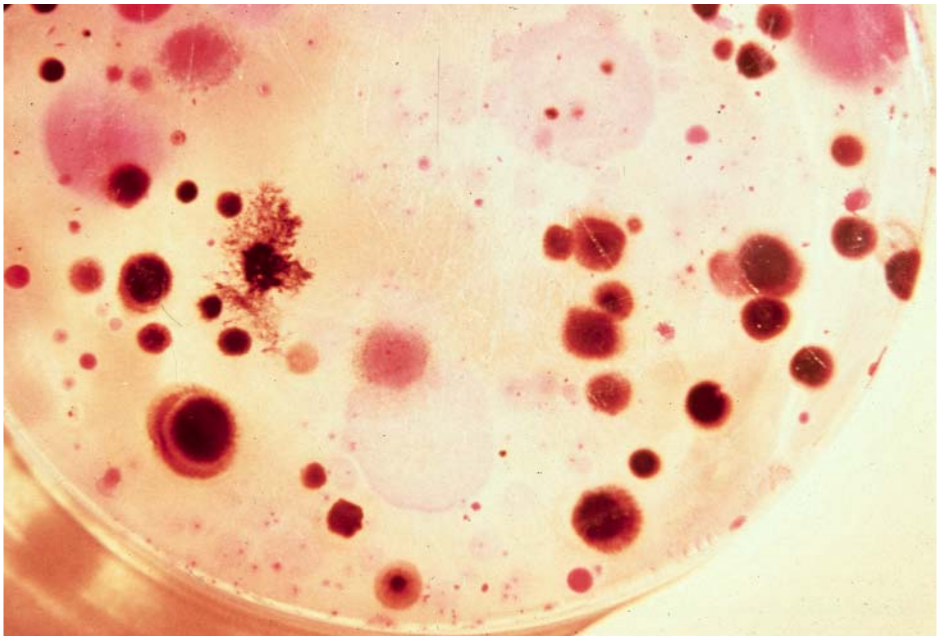

This study conducted at Cornell University was designed to examine bacterial communities associated with the rhizosphere of *Agrostis stolonifera* grown in soil-based and sand-based rootzones. Their aim was to compare the two communities to determine whether bacterial diversity differs between sand-based and soil-based rootzones and whether species composition differs between the two growing media

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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 290 projects at a cost of $25 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***.

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# Bacterial Communities Associated with Creeping Bentgrass in Soil and Sand Rootzones

Mary Ann Karp and Eric B. Nelson

## SUMMARY

The aim of this study conducted at Cornell University was to determine whether *Agrostis stolonifera* grown in sand and soil putting green rootzones selected for different microbial communities. The composition and diversity of bacteria communities associated with the rhizosphere of 'L-93' creeping bentgrass were compared using both conventional culture plating methods and modern molecular methods. The study's findings include:

- Few species were detected in either sand or soil rootzones using culture-based approaches for describing rhizosphere bacterial communities
- Isolate communities were dominated by species from genera such as *Bacillus* and *Arthrobacter*.
- Sand and soil rootzone isolate communities differed in type and number of taxa.
- Soil rootzone communities characterized by direct DNA extractions were significantly more diverse and different from sand rootzone communities; soil communities contained a high frequency of gram positive species, whereas sand communities contained a high frequency of gram negative species.
- Diversity of soil communities was significantly greater than that of sand rootzone communities.
- Results suggest a greater level of bacterial diversity than previously believed and that rootzone substrate may influence the type of microorganisms associated with bentgrass roots.

Golf course putting greens represent a high-value and intensively managed plant production system largely due to the need to maintain a uniform and well manicured playing surface. Historically, putting greens were constructed from soils native to the golf course site. However, over the past several decades, putting green construction has moved to artificial rootzone mixes containing high sand contents (>80-85%) employed to minimize compaction under high traffic and maximize root development (25).

In temperate regions of the world, putting greens are most commonly planted to varieties of creeping bentgrass (*Agrostis stolonifera*). *Agrostis stolonifera* is ideal because of its prostrate growth habit and tolerance to extremely low mowing heights. However, intensive fertility, irrigation, and mowing regimes are required to maintain quality. This is particularly a problem on high sand-content putting greens where cation exchange and water holding capacities are low. Furthermore, the lack of available organic matter in high sand content greens is believed to result in lower microbial populations and diversity, exacerbating maintenance and plant health problems.

Microbial communities are becoming increasingly recognized as important components of soil health (26). However, there have been few efforts to describe and study microbial communities associated with highly managed turfgrasses on putting greens. This is of particular concern on sand-based rootzones because of the lack of physical, chemical, and biological properties that commonly contribute to healthy soils, as well as the environmental load of pesticides and fertilizers required to maintain turfgrasses on sand-based rootzones that may have detrimental effects on soil microbial communities.

Limited work has shown that culturable microbial populations recovered from sand-based rootzones may be initially low, particularly during construction and seedling establishment (1, 4). However, these populations increase during turfgrass establishment, eventually stabilizing within one year at levels not dramatically different from those found in soil-based rootzones (1, 3, 4, 6, 10, 11). Populations of gram negative bacteria and fungi are believed to be the principle microbial components that increase during the growth and development of *Agrostis stolonifera* (20).

While these studies revealed that sand-based rootzones may contain populations rivaling those found in soils, little information is available

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on the diversity of communities associated the rhizospheres of putting green turfgrasses. Recent advances in molecular microbial ecology have changed the way we view microbial diversity in soils and in association with plants because of the ability of these technologies to access a greater proportion of the organisms that are actually present. These approaches have led to the discovery of thousands of new sequences and some entirely new divisions of bacterial life (24). Only limited efforts have been aimed at highly managed turfgrasses.

The use of molecular signatures for microorganisms has opened up new opportunities for understanding the ecology and biodiversity of microorganisms in natural habitats (23, 31). Ribosomal DNA (rDNA) has served as a useful signature for microorganisms in aquatic and terrestrial ecosystems (32). This approach has been used for over a decade for the study of microbial communities in natural and agricultural systems, but has rarely been applied to turfgrass systems. Using PCR amplification of 16S rRNA genes followed by the separation of PCR products by DGGE, Sigler et al. demonstrated a high level of bacterial diversity in rhizosphere communities associated with *Agrostis stolonifera* on soil-based golf course putting greens (18, 19).

Similarly, Sigler and Turco (19) demonstrated a high level of bacterial and fungal diversity in a sand-based rootzone. While the level of bacterial diversity in golf putting greens was much greater than that found in forest soil or agricultural soil, the level of fungal diversity was much less. Disappointingly in both of these studies, no attempt was made to identify the species associated with these turfgrass rhizospheres.

Recently, denitrifying bacterial communities associated with sand-based rootzones on both creeping bentgrass and bermudagrass putting greens was studied using 16S rDNA analysis, as well as by culturing (30). Results revealed a diversity of denitrifying species and a greater level of community resolution when 16S rDNA was amplified from DNA directly extracted from soil than when DNA was extracted from isolated strains. Denitrifying communities were dominated by species of *Pseudomonas*, *Bacillus*, and *Microbacterium*.

Our study was designed to examine bacterial communities associated with the rhizosphere of *Agrostis stolonifera* grown in a soil-based and a sand-based rootzone. Our aim was to compare the two communities to determine whether bacterial diversity differs between sand-based and soil-based rootzones, and whether species composition differs between the two growing media. We further compared a standard culture-based approach for describing communities to that involving direct DNA extractions and 16S rDNA analysis.

## Methods of Microbiological Analysis

### Sampling site

Rhizosphere soil samples were collected on September 11, 2001 from two soil types planted with ‘L93’ creeping bentgrass at the Cornell Turfgrass Research Center. These plots were part of the National Turfgrass Evaluation Program trial being conducted at Cornell. Plots were arranged in a randomized complete block design with five replications. The soil was and Arkport sandy loam soil (pH 6.2), whereas the sand was an acidic sand (pH 5.5). Samples were pulled from replicate plots and pooled for subsequent analysis. Before plating or extracting DNA, soil or sand was shaken from *Agrostis* roots, mixed, then frozen at 20$^o$ C.

### *Isolation and Maintenance of Bacterial Strains*

Soil samples were allowed to thaw overnight. One-gram aliquots were dispensed into 9 ml of phosphate buffered saline (PBS), which contained 8g NaCl, 0.2g KCl, 1.44g $Na_2HPO_4$, 0.24 g $KH_2PO_4$, and 800 ml $H_2O$. The pH was adjusted to 7.4 with HCl. Samples were vortexed for 20 seconds then placed on a shaker at 135 rpm for 10 min at room temperature (~22$^o$ C). Serial dilutions were made to $1 \times 10^{-9}$. One hundred µl of each dilution was spread on 0.1 strength tryptic soy agar (0.1% TSA), prepared with 1.5 g trypticase soy broth, 10 g Bacto Agar, and 500 ml $H_2O$. Each dilution was plated onto 5 petri dishes and cultures were incubated at 25° C. At 24 hours, all of the colonies were marked and transferred to 0.1% trypticase soy broth (0.1% TSB) and incubated overnight at 25° C. The process was repeated at 48 and 76 hours. Cultures were then stored at 4° C until further analysis.

*Initial Screening of Bacteria*

The cultures were transferred to 0.1% TSA and incubated at 25° C overnight. Cultures were then grouped by colony color, size, shape, edge, elevation, and surface morphology. Gram stain tests were performed on all cultures, except those that appeared to be actinobacteria. Several representative cultures from each group were then chosen for DNA extractions and PCR amplifications.

*DNA Extraction, PCR Amplification, and Sequencing of 16S rDNA from Isolates*

DNA from cultured isolates was isolated using the Ultraclean™ Microbial DNA Isolation Kit (MoBio Laboratories, Inc.) according to the manufacturer's instructions then subject to PCR amplification. The PCR reactions contained 10 mM Trizma HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM 8f (8f 5'-CACGGCTCCAGACTTTGATYMTGGCTC -3'), 0.2 µM 1512r (1512r 5'- CTGAAGCTTACGGYTAGCTTGTTACGACTT -3'), 2.5 units of Taq polymerase, and 1.0 µl template DNA per 50 µl reaction. DNA was amplified with a Hybaid PCRExpress thermal cycler using the following program: initial denaturation at 94° C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50° C for 30 seconds, extension at 72° C for 30 seconds, followed by a final extension at 72° C for 5 minutes. PCR reactions were purified with the Ultraclean PCR Purification Kit (MoBio Laboratories, Inc.), then quantified.

Samples were submitted to the Cornell University Biotechnology Resource Center for sequencing as follows: 300 ng + 8 pmole 1512r in 18µl $H_2O$. DNA sequencing was performed with an Applied Biosystems Automated 3700 DNA Analyzer with Big Dye Terminator chemistry and AmpliTaq-FS DNA Polymerase. Sequences were compiled in Sequencher 4.1 (Gene Codes Corp, Ann Arbor USA).

*Extraction and 16S rDNA Amplification of DNA Obtained Directly from* Agrostis *Rhizospheres.*

DNA was extracted directly from rhizosphere rootzones using the Ultraclean™ Soil DNA Isolation Kit (MoBio Laboratories, Inc.) according to the manufacturer's instructions. For DNA extraction, soil or sand was allowed to thaw overnight, then 0.5 grams were used for each prep. DNA was further purified with the Ultraclean PCR Clean-Up Kit (MoBio Laboratories, Inc.)

PCR reactions contained 10 mM Trizma HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM 8f, 0.2 µM 1512r , 2.5 units of Taq polymerase, and 1.0 µl template per 50 µl reaction. DNA was amplified with a Hybaid PCRExpress thermal cycler using the following program: initial denaturation at 94° C for 5 minutes, followed by 35 cycles of denaturation at 94° C for 30 seconds, annealing at 50° C for 30 seconds, extension at 72° C for 30 seconds, followed by a final extension at 72° C for 5 minutes. PCR reactions were purified with the Ultraclean PCR Purification Kit (MoBio Laboratories, Inc.), then quantified.

*Cloning and Sequencing of DNA Directly Extracted from Soil*

PCR products were cloned using INVaF' competent cells with the pCR®2.1 vector from the TA Cloning®Kit (Invitrogen, Carlsbad, CA). All white colonies were cultured from the reaction on LB+Kan (Luria Broth + 50 µg/ml Kanamycin) for 16 hours at 37° C. Clones were mixed with glycerol then stored at -80° C. Clones were screened using PCR to determine if the insert was present. The PCR reactions contained 10 mM Trizma HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl2, 200 µM of each dNTP, 0.2 µM M13f (5'- GTAAAACGACGGCCAG -3'), 0.2 µM M13r (5'-CAGGAAACAGCTATGAC -3'), 1 unit of Taq polymerase, and 1 toothpick tip of cells per 25 µl reaction. DNA was amplified with a Hybaid PCRExpress thermal cycler using the following program: initial denaturation at 94° C for 5 minutes, followed by 35 cycles of denaturation at 94º C for 30 seconds, annealing at 50º C for 30 seconds, extension at 72º C for 30 seconds, followed by a final extension at 72º C for 5 minutes.

Plasmid DNA was purified from the clones that contained the insert with the MoBio Plasmid Purification Kit. DNA was mixed with the M13f primer and submitted to the Cornell University Biotechnology Resource Center. DNA sequencing was performed with an Applied Biosystems Division Automated 3700 DNA Analyzer with Big Dye Terminator chemistry and

| Clone | Closest Identified Strain[1] | Gen Bank Accession No. | Phylogenetic Group |
|---|---|---|---|
| SoB1 | *Bacillus megaterium* strain SAFR-011 | AY167865 | Firmicutes |
| SoC1 | *Bacillus sp.* B90 | AF128875 | Firmicutes |
| SoC2 | *Bacillus sp.* B90 | AF128875 | Firmicutes |
| SoD1 | *Bacillus sphaericus* isolate NP71 | AY291474 | Firmicutes |
| SoD12 | *Bacillus sphaericus* isolate NP71 | AY291474 | Firmicutes |
| SoD2 | *Bacillus sphaericus* isolate NP71 | AY291474 | Firmicutes |
| SoD3 | *Bacillus psychrodurans* strain WED2.3 | AY536564 | Firmicutes |
| SoD4 | *Bacillus sphaericus* isolate NP71 | AY291474 | Firmicutes |
| SoD6 | *Bacillus sphaericus* isolate NP71 | AY291474 | Firmicutes |
| SoD7 | *Bacillus sphaericus* isolate NP71 | AY291474 | Firmicutes |
| SoD8 | *Arthrobacter sp*. LCSAOTU6 | AF506065 | Actinobacteria |
| SoE2 | *Bacillus sphaericus* isolate NP71 | AY291474 | Firmicutes |
| SoE3 | *Arthrobacter sp.* LCSAOTU6 | AF506065 | Actinobacteria |
| SoE4 | *Arthrobacter sp.* LCSAOTU6 | AF506065 | Actinobacteria |
| SoE5 | Low G+C Gram-positive bacterium D-St(1)-3 | AB074690 | Firmicutes |
| SoE6 | *Bacillus megaterium* strain SAFR-011 | AY167865 | Firmicutes |
| SoE7 | *Bacillus sp.* B90 | AF128875 | Firmicutes |
| SoF1 | *Arthrobacter sp.* 'SMCC ZAT056' | AF197052 | Actinobacteria |
| SoG3 | *Bacillus cereus* | AY741719 | Firmicutes |
| SoH1 | *Arthrobacter sp.* LCSAOTU6 | AF506065 | Actinobacteria |
| SoH2 | *Arthrobacter sp.* M4 | AY177360 | Actinobacteria |
| SoH3 | *Arthrobacter sp.* LCSAOTU6 | AF506065 | Actinobacteria |
| SoI1 | Glacial ice bacterium ML-A12 | AY378216 | Unknown |
| SoI2 | Glacial ice bacterium ML-A12 | AY378216 | Unknown |
| SoI3 | *Bacillus megaterium* strain SAFR-011 | AY167865 | Firmicutes |
| SoI5 | *Bacillus sp.* | AB016270 | Firmicutes |
| SoI6 | *Bacillus megaterium* strain SAFR-011 | AY167865 | Firmicutes |
| SoI7 | *Paenibacillus sp.* TRO4 | J251193 | Firmicutes |
| SoI8 | *Bacillus megaterium* strain SAFR-011 | AY167865 | Firmicutes |
| SoI9 | *Actinobacterium* EC5 | AY337600 | Actinobacteria |
| SoJ1 | *Bacillus megaterium* strain SAFR | AY167865 | Firmicutes |
| SoJ4 | *Bacillus megaterium* strain SAFR | AY167865 | Firmicutes |
| SoJ5 | *Bacillus megaterium* strain SAFR | AY167865 | Firmicutes |
| SoJ6 | *Bacillus sp.* | AB016270 | Firmicutes |
| SoJ7 | *Bacillus megaterium* strain SAFR | AY167865 | Firmicutes |
| SoJ8 | *Bacillus megaterium* strain SAFR | AY167865 | Firmicutes |
| SoK2 | *Bacillus megaterium* strain SAFR-011 | AY167865 | Firmicutes |
| SoL11 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SoL2 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SoL3 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SoL4 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SoL5 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SoL6 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SoL7 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SoL9 | *Bacillus sp.* SVM | AF503203 | Firmicutes |

[1] BLAST searches conducted on October 20, 2004

**Table 1.** Identification of isolates recovered from the rhizospheres of *Agrostis stolonifera* plants grown in soil rootzones

AmpliTaq-FS DNA Polymerase. Sequences were compiled in Sequencher 4.1 (Gene Codes Corp, Ann Arbor USA).

### *Sequence Alignments and Phylogenetic Analyses*

Raw sequences were edited using EditSeq to manually remove vector sequences and eliminate poorly resolved regions. These edited sequences were aligned using either MegAlign 5.07 (DNASTAR, Madison, WI) or Clustal X version 1.83(2, 21), both using the Clustal W algorithms (22). Separate alignments were generated for sand and soil rootzone sequences, as well as combined sand and soil sequences. Sequences obtained from *Agrostis* rhizospheres were compared to known 16S rDNA sequences in the National Center for Biotechnology Information database (Gen Bank) using the megablast algorithm (33).

Ribosomal DNA (rDNA) sequences from reference taxa were included in alignments used for subsequent phylogenetic analysis. They were selected to broadly represent the diversity of known bacterial species and to complement (based on BLAST searches) specific groups of our observed sequences. The 16S rDNA sequence from the Archeon *Sulfolobus solfataricus* (GenBank X90478) was used as an outgroup in this study. After initial alignments in MegAlign, sequence alignments were manually edited using BioEdit 6.0.7 (9) to correct misaligned sequences and ambiguous base designations. During this final editing, all sequences were trimmed to a fixed length of xxx bp (gaps included). In initial alignments, a wide range of known 16S rDNA sequences from bacterial species were included. However, for subsequent phylogenetic analyses, only those standards with apparent associations with our unknown sequences were included.

Phylogenetic analyses were conducted using the neighbor-joining (NJ) method (15) as implemented in TreeCon 1.3b (27). Branch support was based on 1000 bootstrap replications. Gaps and missing or ambiguous data were ignored. Nucleotide substitution rates for each alignment were calibrated as described by Van de Peer and De Wachter (28, 29) using TreeCon.

### *Assignment of Operational Taxonomic Units (OTUs) and Calculation of Community Diversity and Species Richness*

Sequences were assigned to operational taxonomic units (OTUs) using the program DOTUR (16) and based on distance matrices constructed from sequence alignments. OTUs are used to essentially designate species when species can only be described from molecular sequences. For the purpose of these analyses, OTUs were defined as any set of sequences that differed by 1% or less. Using the calculated OTU composition data, rarefaction curves were established and community diversity estimated using the Shannon Index, whereas species richness was determined based on the Chao1 richness estimator.

### *Comparative Analysis of Bacterial Communities from Sand and Soil Rootzones*

We used phylogenetic methods described by Martin (13) for comparing *Agrostis* rhizosphere communities from sand and soil rootzones. Various measures of diversity were calculated for each rhizosphere community. Sequence diversity, nucleotide diversity, and $\Theta_\pi$ were calculated using the program Arlequin 2.001 (17).

## Results on the Comparative Diversity of Sandand Soil Rhizosphere Communities: Isolates

Bacteria recovered on culture media from both sand and soil rootzones were dominated largely by gram positive species (e.g., *Arthrobacter*, *Bacillus*, and *Microbacterium* species). Soil rhizosphere communities consisted almost entirely of *Actinobacterium*, *Arthrobacter* and *Bacillus* isolated (Table 1), whereas sand rootzones contained clones of a few gram negative genera such as *Aminobacter*, *Chelatobacter*, *Ensifer*, and *Pseudomonas* (Table 2). The number of OTUs observed did not differ significantly (P=0.05) between sand and soil rootzones (Figure 1A). Clones were defined rather liberally (=99% similar sequence). Despite this, relatively few OTUs (~10) were observed over 45 clones sam-

| Clone | Closest Identified Strain[1] | Gen Bank Accession No. | Phylogenetic Group |
|---|---|---|---|
| SaC13 | *Arthrobacter sp.* 108 | AY238501 | Actinobacteria |
| SaC20 | *Ensifer adhaerens* | AY040360 | α-Proteobacteria |
| SaC22 | *Ensifer adhaerens* | AY040360 | α-Proteobacteria |
| SaD15 | *Arthrobacter sp.* 108 | AY238501 | Actinobacteria |
| SaD22 | *Arthrobacter sp.* 108 | AY238501 | Actinobacteria |
| SaD23 | *Arthrobacter sp.* 108 | AY238501 | Actinobacteria |
| SaD24 | *Actinobacterium* TB4-12-I | AY599737 | Actinobacteria |
| SaD25 | *Arthrobacter sp.* SB | AY327445 | Actinobacteria |
| SaD26 | *Arthrobacter sp.* 108 | AY238501 | Actinobacteria |
| SaD27 | *Arthrobacter sp.* SB | AY327445 | Actinobacteria |
| SaE1 | *Arthrobacter sp.* SB | AY327445 | Actinobacteria |
| SaE10 | Permafrost bacterium DT-IIT23 | AY378262 | Unknown |
| SaE11 | *Arthrobacter sp.* DY12-2 | AY383043 | Actinobacteria |
| SaE12 | *Microbacterium sp.* I33 | AF306542 | Actinobacteria |
| SaE13 | *Arthrobacter sp.* DY12-2 | AY383043 | Actinobacteria |
| SaE15 | Bacterium W-4b | AY345529 | Unknown |
| SaE17 | *Pseudomonas sp.* PILH1 | AY456708 | γ-Proteobacteria |
| SaE19 | *Pseudomonas sp.* 12C | AY689083 | γ-Proteobacteria |
| SaE2 | *Pseudomonas sp.* P97.26 | AY456707 | γ-Proteobacteria |
| SaE5 | *Arthrobacter sp.* DY12-2 | AY383043 | Actinobacteria |
| SaE6 | *Arthrobacter sp.* CPA2 | AY310305 | Actinobacteria |
| SaF1 | *Arthrobacter sp.* DY12-2 | AY383043 | Actinobacteria |
| SaF13 | *Pseudaminobacter sp.* LCSAOTU18 | AF506051 | α-Proteobacteria |
| SaF2 | *Pseudaminobacter sp.* LCSAOTU18 | AF506051 | α-Proteobacteria |
| SaF3 | *Chelatobacter heintzii* strain DSM6450T | AJ011762 | α-Proteobacteria |
| SaG1 | *Microbacterium sp.* I33 | AF306542 | Actinobacteria |
| SaG10 | *Microbacterium sp.* I33 | AF306542 | Actinobacteria |
| SaG14 | *Microbacterium sp.* I33 | AF306542 | Actinobacteria |
| SaG17 | *Microbacterium phyllosphaerae* SAFR-012 | AY167852 | Actinobacteria |
| SaG2 | *Microbacterium sp.* I33 | AF306542 | Actinobacteria |
| SaG3 | *Microbacterium sp.* I33 | AF306542 | Actinobacteria |
| SaG4 | *Microbacterium sp.* I33 | AF306542 | Actinobacteria |
| SaG6 | *Microbacterium phyllosphaerae* SAFR-012 | AY167852 | Actinobacteria |
| SaG7 | *Microbacterium sp.* I33 | AF306542 | Actinobacteria |
| SaG8 | *Pseudomonas sp.* P97.26 | AY456707 | γ-Proteobacteria |
| SaG9 | *Microbacterium sp.* I33 | AF306542 | Actinobacteria |
| SaH1 | *Staphylococcus epidermidis* SAFN-039 | AY167804 | Firmicutes |
| SaH2 | *Ensifer adhaerens* | AY040360 | α-Proteobacteria |
| SaI2 | *Arthrobacter sp.* SB | AY327445 | Actinobacteria |
| SaI4 | *Actinobacterium* EC5 | AY337600 | Actinobacteria |
| SaI5 | *Arthrobacter sp.* DY12 | AY383043 | Actinobacteria |
| SaI6 | *Arthrobacter sp.* DY12 | AY383043 | Actinobacteria |
| SaJ2 | *Arthrobacter sp.* DY12 | AY383043 | Actinobacteria |
| SaK1 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SaK2 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SaL2 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SaL3 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SaL4 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SaL5 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SaL6 | *Bacillus sp.* SVM | AF503203 | Firmicutes |
| SaL7 | *Bacillus sp.* SVM | AF503203 | Firmicutes |

[1] BLAST searches conducted on October 20, 2004

**Table 2.** Identification of isolates recovered from the rhizospheres of *Agrostis stolonifera* plants grown in sand rootzones

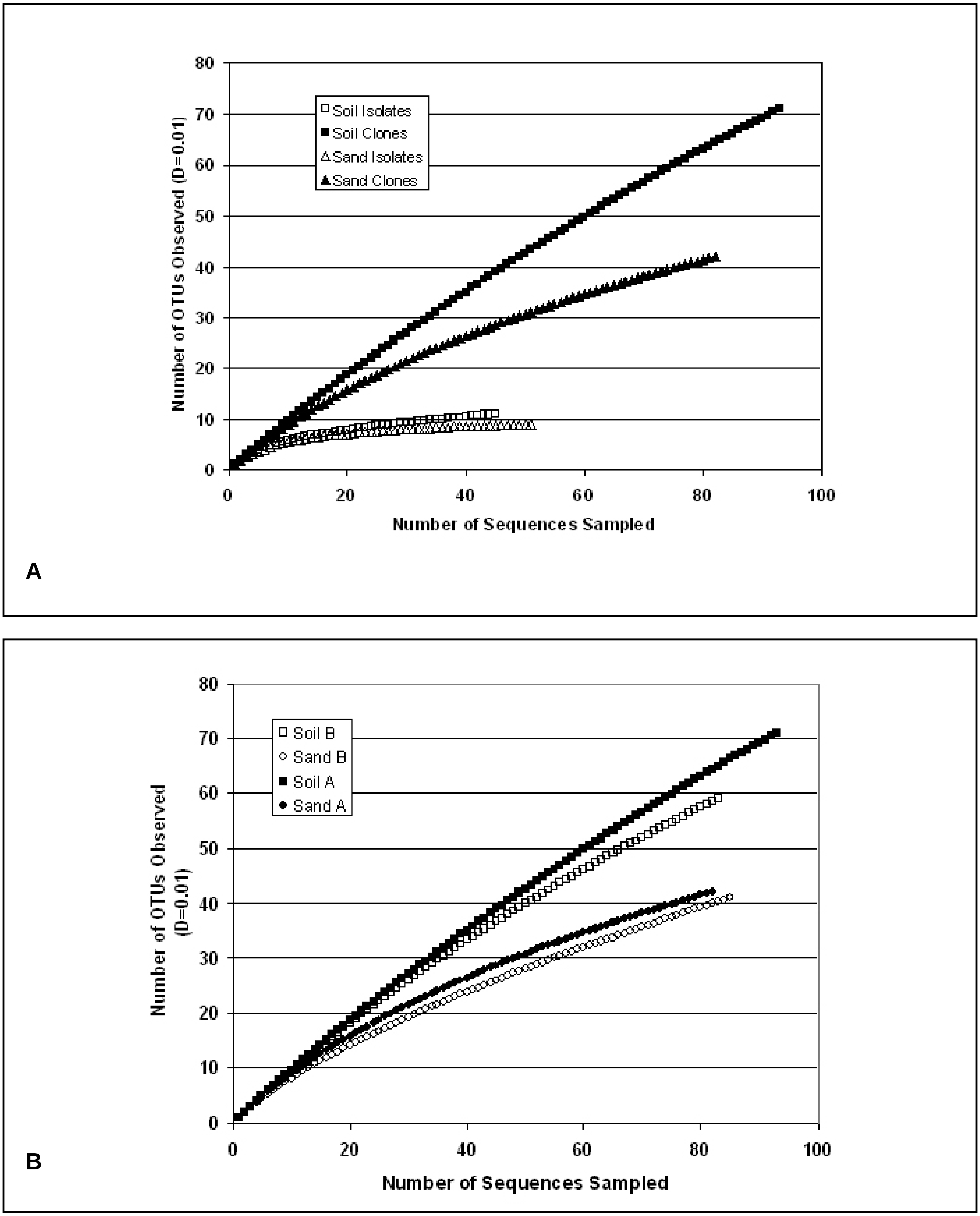

**Figure 1.** Number of unique taxa as defined by operational taxonomic units (OTUs) for (A) two groups of sequences obtained directly from soil and sand rhizospheres and from (B) bacterial isolates recovered from soil and sand rhizospheres. OTUs defined as 99% sequence similarity. Isolate OTUs were significantly less than OTUs obtained from sand or soil rhizospheres ($P=0.05$) (95% confidence limits not shown).

pled and few additional OTUs were obtained by sampling more than 20 to 30 clones.

Although sand and soil isolate communities did not differ in sequence diversity, nucleotide diversity, or mean sequence divergence, a significant level of differentiation was observed (data not shown). In other words, the taxa found in sand rhizospheres were significantly different from those taxa found in soil rhizospheres. However, although few OTUs were shared between the two communities, the bacterial diversity did not differ between the two isolate communities based on the Shannon diversity index (Figure 2).

## Comparative Diversity of Sand and Soil Rhizosphere Communities: 16S rDNA Sequence Directly from the Rhizosphere

Between 150 and 190 clones were characterized from each of the two rootzone rhizospheres. Clones analyzed from each of these rhizospheres could be categorized into two distinct and somewhat divergent groups (designated soil clones A and B).

Clones obtained from sand rhizospheres are shown in Table 3 (Sand Clones A) and Table 4 (Sand Clones B). In both groups of clones, sequences representing bacterial species that have not yet been cultured dominated these communities. A high representation of *Pseudomonas* and other gram negative species were found among Sand A clones, whereas Sand B clones contained higher frequencies of gram positive bacteria in the genera *Bacillus* and *Paenibacillus*.

Clones obtained from soil rhizospheres are shown in Table 5 (Soil Clones A) and Table 6 (Soil Clones B). Again in both groups of clones, sequences representing bacterial species that have not yet been cultured dominated these communities. Similar to Sand A clone communities, there was a predominance of gram negative species, but gram positive species were present. Soil B clones, however, aside from being dominated by previously uncultured species, were clearly dominated by gram positive species largely from the genus *Bacillus*.

Whereas the number of observed OTUs from soil rootzone rhizospheres were significantly (P=0.05) greater than OTUs from sand rootzone rhizospheres, the number of OTUs from A clones did not differ from those of B clones, regardless of the rootzone substrate (Figure 1B).

Results of our community analyses indicate that the taxa found in each of the distinct sand or soil communities were different from each other.  For example, Sand Clone A communities were different from Soil Clone A commuities. Similarly, Sand Clone B communities were different from Soil Clone B communities, and regardless of the rootzone material, communities of A clones differed from communities of B clones. A clones could not be easily aligned with B clones from either rootzone material.  However, Sand and Soil A clones could be easily aligned.

Phylogenetic analysis of Soil A clones and Sand A clones show both sand and soil clones are disbursed throughout the phylogenetic tree with clusters of sequences representing many of the major groups of bacteria commonly found associated with plant rhizospheres. These include the α-Proteobacteria, ß-Proteobacteria, γ-Proteobacteria, δ/ε-Proteobacteria, Fibrobacteres/Acidobacteria group, Planctomycetes, and Actinobacteria. Additionally, clones representing candidate division OP5 were also detected in soil rootzone rhizospheres, but not in sand rootzone rhizospheres. This new division of bacterial has only been described from sequence data. No known culturable members exist. Most interestingly, two phylogenetically-distinct groups of sequences could not be assigned to any taxon or known bacterial division. Clones falling into clusters of unknown affiliation group C came from both sand and soil rootzone rhizospheres, whereas clones falling into unknown affiliation group B came exclusively from sand rootzones.

## Significance of Our Findings

Previous studies of microbial communities associated with turfgrasses in golf course putting greens have relied largely on culture-based approaches with only general descriptions of microbial groups (1, 3-6, 10, 11). While these

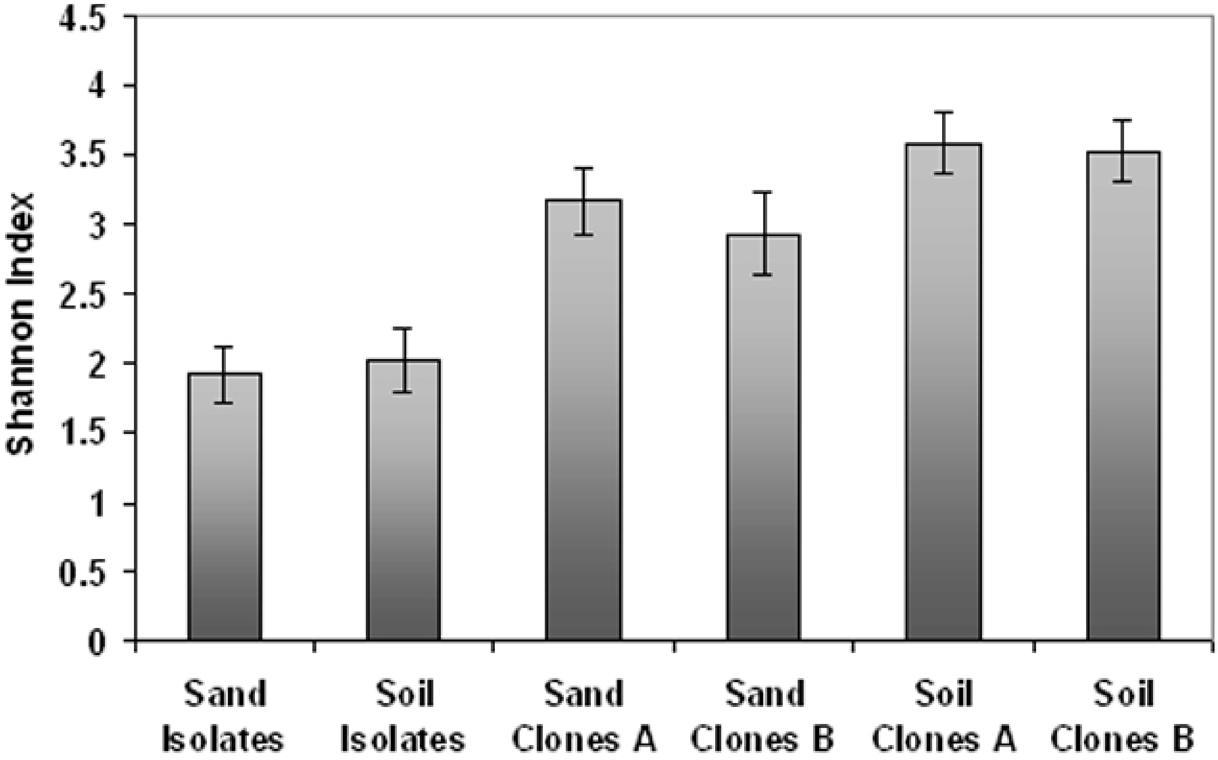

**Figure 2.** Shannon diversity indices among isolates and 16S rDNA clones obtained from the rhizospheres of *Agrostis stolonifera* grown in sand or soil rootzones. Bars represent 95% confidence intervals.

studies have been valuable to our understanding of the gross seasonal dynamics of general microbial populations and the effects of management inputs on these dynamics, they have failed to provide meaningful information on the biodiversity and function of microbial species found in putting greens. While some promising molecular studies have indicated a considerable diversity of taxa associated with *Agrostis* species in putting greens (8, 18), the composition and diversity of microbes associated with golf turf remains virtually unknown.

Our work has shown that both soil-based and sand-based roots contain a relatively high level of bacterial diversity. Furthermore, this diversity is dramatically greater than that observed from cultured microbes. This points to the need for more detailed molecular analyses of microbial communities in golf turf putting greens that might shed light on the seasonal dynamics of microbial communities and the influence of grass species and cultivar on microbial communities. One limitation of our study is that one set of samples were taken at one point in time on one bentgrass cultivar. Since it is becoming quite clear that plant species may dramatically influence the types of microbial communities found in soils (7, 12), further research in this area on golf turf is warranted.

One of the most significant findings from our work is that bacterial communities in soil-based rootzones differ significantly from those in sand-based rootzones. They differ not only in species composition, but also in species diversity. This suggests that the substrate in which bentgrasses are grown may have a significant impact on the microbes with which roots associate. This may be particularly significant to soil and plant health issues, as well as to overall turfgrass performance. At the present time, the significance of these differences cannot be fully appreciated. However, these results lay the groundwork for more detailed studies on the roles of particular species to soil and plant health.

A prominent result from our work is the skewed picture of bentgrass microbial communi-

| Clone | Closest Identified Strain[1] | Gen Bank Accession No. | Phylogenetic Group |
|---|---|---|---|
| 1Sa1 | Uncultured bacterium clone LAE10 | AF392756 | Unknown |
| 1Sa122 | Uncultured ?-proteobacterium NMS8.109WL | AY043801 | γ-Proteobacteria |
| 1Sa15 | Uncultured soil bacterium clone 1180-1 | AY326635 | Unknown |
| 1Sa156 | *Bacillus cereus* strain G8639 | AY138271 | Firmutes |
| 1Sa207 | Uncultured bacterium clone LAE10 | AF392756 | Unknown |
| 1Sa222 | Uncultured soil bacterium clone 1180-1 | AY326635 | Unknown |
| 1Sa227 | Uncultured soil bacterium clone Tc133-98 | AY242700 | OP5 |
| 1Sa231 | Uncultured d-proteobacterium S36.54SM | AF431395 | δ-Proteobacteria |
| 1Sa234 | Uncultured bacterium clone 267ds10 | AY212717 | α-Proteobacteria |
| 1Sa239 | Unidentified eubacterium from anoxic soil | AJ229177 | α-Proteobacteria |
| 1Sa240 | Unidentified eubacterium from anoxic soil | AJ229177 | α-Proteobacteria |
| 1Sa265 | Uncultured sludge bacterium S7 | AF234717 | α-Proteobacteria |
| 1Sa27 | Uncultured bacterium clone FW25 | AF524027 | OP5 |
| 1Sa276 | *Rickettsia montana* | L36215 | α-Proteobacteria |
| 1Sa30 | Uncultured bacterium clone CCD21 | AY221035 | Unknown |
| 1Sa96 | Uncultured bacterium clone SIMO-2407 | AY711773 | Fibrobacteres/Acidobacteria |
| 2Sa503 | *Pseudomonas migulae* | AF074383 | γ-Proteobacteria |
| 2Sa509 | *Pseudomonas sp.* (strain DhA-51) | AJ011507 | γ-Proteobacteria |
| 2Sa510 | *Rhizobium sp.* ORS 1439 | AY500257 | γ-Proteobacteria |
| 2Sa511 | Uncultured soil bacterium clone 1142 | AY493964 | Unknown |
| 2Sa516 | *Sphingomonas sp.* | AB018439 | γ-Proteobacteria |
| 2Sa517 | Uncultured ß-proteobacterium clone B-BH93 | AY622261 | ß-Proteobacteria |
| 2Sa518 | *Pseudomonas sp.* PsG | AF105385 | γ-Proteobacteria |
| 2Sa519 | *Pseudomonas sp.* WG7#1 | AY263469 | γ-Proteobacteria |
| 2Sa520 | *Pseudomonas sp.* Ps 3-1 | AF468453 | γ-Proteobacteria |
| 2Sa523 | *Massilia timonae* strain 99A9205 | AY157761 | ß-Proteobacteria |
| 2Sa524 | *Pseudomonas sp.* Fa24 | AY131219 | γ-Proteobacteria |
| 2Sa526 | *Pseudomonas sp.* NZTK7 | AF408871 | γ-Proteobacteria |
| 2Sa527 | *Brevundimonas alba* | AJ227785 | α-Proteobacteria |
| 2Sa528 | *Pseudomonas putida* strain IA2YCDA | AY512612 | γ-Proteobacteria |
| 2Sa534 | *Pseudomonas sp.* An15 | AJ551153 | γ-Proteobacteria |
| 2Sa536 | *Pseudomonas migulae* | AF074383 | γ-Proteobacteria |
| 2Sa537 | *Pseudomonas sp.* 4/11GCS3#e | AY263481 | γ-Proteobacteria |
| 2Sa538 | Oxalobacteraceae bacterium HTCC315 | AY429715 | ß-Proteobacteria |
| 2Sa539 | *Pseudomonas sp.* Ps 3-1 | AF468453 | γ-Proteobacteria |
| 2Sa540 | ß-proteobacterium RG-52 | AY561611 | ß-Proteobacteria |
| 2Sa542 | *Brevundimonas alba* | AJ227785 | α-Proteobacteria |
| 2Sa608 | *Pseudomonas sp.* MSB2071 | AY275482 | γ-Proteobacteria |
| 2Sa614 | Uncultured Pseudomonas sp. isolate 4f57 | AY177777 | γ-Proteobacteria |
| 2Sa621 | *Bacillus sp.* MSB2029 | AY275494 | Firmicutes |
| 2Sa622 | Uncultured bacterium clone KRA30-19 | AY081990 | γ-Proteobacteria |
| 2Sa624 | *Pseudomonas sp.* MSB2071 | AY275482 | γ-Proteobacteria |
| 2Sa626 | Uncultured a-proteobacterium clone E1B7 | AY632434 | α-Proteobacteria |
| 2Sa628 | ß-proteobacterium ML6 | AB013427 | ß-Proteobacteria |
| 2Sa630 | Uncultured bacterium clone S1-1-CL12 | AY725249 | Unknown |
| 2Sa631 | *Massilia timonae* | U54470 | ß-Proteobacteria |
| 2Sa638 | Uncultured bacterium clone 300I-F12 | AY661946 | ß-Proteobacteria |
| 2Sa639 | Uncultured bacteria clone F2-42 | AY096173 | Unknown |
| 2Sa647 | ß-proteobacterium 4l' | AY216887 | ß-Proteobacteria |
| 2Sa652 | Uncultured bacterium clone Y27 | AF407677 | Unknown |
| 2Sa657 | *Pseudomonas sp.* NZTK7 | AF408871 | γ-Proteobacteria |
| 2Sa659 | *Pseudomonas sp.* Ps 3-1 | AF468453 | γ-Proteobacteria |
| 2Sa663 | Uncultured a-proteobacterium FL14F11 | AF446297 | α-Proteobacteria |

| | | | |
|---|---|---|---|
| 2Sa664 | *Chryseobacterium sp.* ACP12 | AY464462 | Bacteroidetes |
| 2Sa670 | *Pseudomonas cannabina* | AJ492827 | γ-Proteobacteria |
| 2Sa545 | *Pseudomonas sp.* SMCC B0361 | AF500621 | γ-Proteobacteria |
| 2Sa546 | *Pseudomonas sp.* Ps 3-1 | AF468453 | γ-Proteobacteria |
| 2Sa549 | Unidentified bacterium clone W4-B59 | AY345492 | Fibrobacteres/Acidobacteria |
| 2Sa550 | *Pseudomonas sp.* B0326 | AY463202 | γ-Proteobacteria |
| 2Sa558 | Uncultured soil bacterium clone 739-2 | AF423289 | α-Proteobacteria |
| 2Sa561 | Uncultured ß-proteobacterium clone B-BH93 | AY622261 | ß-Proteobacteria |
| 2Sa564 | Uncultured proteobacterium OCS7 | AF001645 | ß-Proteobacteria |
| 2Sa565 | *Agrobacterium tumefaciens* strain UP-3 | AY364329 | α-Proteobacteria |
| 2Sa566 | *Paenibacillus phyllosphaerae* | AY598818 | Firmicutes |
| 2Sa568 | *Brevundimonas alba* | AJ227785 | α-Proteobacteria |
| 2Sa570 | Uncultured bacterium clone 218ds20 | AY212665 | α-Proteobacteria |
| 2Sa571 | *Pseudomonas sp.* Ps 3-1 | AF468453 | γ-Proteobacteria |
| 2Sa572 | Uncultured bacterium | AJ621990 | ß-Proteobacteria |
| 2Sa574 | *Sphingomonas sp.* KT-1 | AB022601 | α-Proteobacteria |
| 2Sa577 | *Bacillus cereus* strain HJ9 | AY176771 | Firmicutes |
| 2Sa578 | Uncultured proteobacterium OCS7 | AF001645 | ß-Proteobacteria |
| 2Sa579 | Uncultured Bacteroidetes bacterium NAB31 | AY395035 | Bacteroidetes |
| 2Sa580 | *Zoogloea sp.* AI-20 | AY437629 | ß-Proteobacteria |
| 2Sa584 | Uncultured ß-proteobacterium WCB190 | AY217460 | ß-Proteobacteria |
| 2Sa589 | *Pseudomonas sp.* SMCC B0628 | AF501878 | γ-Proteobacteria |
| 2Sa595 | Uncultured Brevundimonas sp. | AY177781 | α-Proteobacteria |
| 2Sa597 | *Massilia timonae* | U54470 | ß-Proteobacteria |
| 2Sa598 | *Phenylobacterium sp.* G26 | AY035307 | α-Proteobacteria |
| 2Sa600 | Uncultured ß-proteobacterium B-BH93 | AY622261 | ß-Proteobacteria |
| 2Sa601 | *Pseudomonas sp.* Ps 3-1 | AF468453 | γ-Proteobacteria |
| 2Sa602 | Uncultured proteobacterium OCS7 | AF001645 | ß-Proteobacteria |
| 2Sa606 | Uncultured bacterium clone D14ST | AY395136 | ß-Proteobacteria |

[1] BLAST searches conducted on October 20, 2004

**Table 3.** Identification of clones (Sand Clones A) recovered from the rhizospheres of *Agrostis stolonifera* plants grown in sand rootzones

ties that result from culture-based studies alone. Our cultured isolates were identified using 16S rDNA, which is now a standard means of identifying microorganisms. Based on these isolated strains, sand rootzone communities were composed largely of *Arthrobacter*, *Microbacterium*, and *Bacillus* species, many of these with 100% sequence identity. Furthermore, the soil rootzone community was composed almost exclusively of *Bacillus* species with a few *Arthrobacter* species. This may likely reflect the selective nature of the culturing process, which has been observed with populations of microorganisms in turfgrass (3), but is also known from decades of studies in agricultural soils.

Many of the species of bacteria encountered in our study are commonly found in soils across the globe and are not particularly revealing about possible relationships between rootzone and bentgrass rhizosphere communities. Of the known species that were detected, both rootzones were dominated by *Pseudomonas* and *Bacillus* species. In contrast, Sigler and Turco (19), using molecular methods similar to ours, found a number of sequences in putting greens that could be assigned to species of *Blastomonas*, *Agrobacterium*, *Pseudomonas*, *Lysobacter*, *Flexibacter*, *Leptothrix*, *Polyangium*, and *Nevskia* (19). Many of these were not detected in our study, with the exception of *Pseudomonas* and *Agrobacterium* species. However, recent studies on sand-based greens under ‘Crenshaw’ creeping bentgrass has

| Clone | Closest Identified Strain[1] | Gen Bank Accession No. | Phylogenetic Group |
|---|---|---|---|
| 1Sa105 | Crater Lake bacterium CL500-85 | AF316731 | Unknown |
| 1Sa117 | *Bacillus sp.* C332 | AY756511 | Firmicutes |
| 1Sa148 | Uncultured actinobacterium | AJ575541 | Actinobacteria |
| 1Sa209 | Uncultured Green Bay ferromanganous micronodule bacterium MNE12 | AF293003 | Unknown |
| 1Sa228 | Uncultured bacterium clone 124 | AY391099 | Unknown |
| 1Sa264 | *Rhizobium sp.* ORS 1439 | AY500257 | α-Proteobacteria |
| 1Sa272 | Uncultured soil bacterium clone 1180-1 | AY326635 | Unknown |
| 1Sa281 | *Bacillus thuringiensis* strain 4Q281 | AF155954 | Firmicutes |
| 1Sa34 | Uncultured bacterium clone CCD21 | AY221035 | Unknown |
| 1Sa35 | Uncultured bacterium clone CCD21 | AY221035 | Unknown |
| 1Sa36 | Uncultured sludge bacterium S7 | AF234717 | Unknown |
| 1Sa37 | Uncultured d-proteobacterium clone KY221 | AB116509 | δ-Proteobacteria |
| 1Sa44 | Uncultured bacterium clone 144ds20 | AY212595 | Unknown |
| 1Sa57 | Uncultured bacterium clone CCU23 | AY221079 | Unknown |
| 1Sa67 | *Paenibacillus sp*. JL-02 | AY443389 | Firmicutes |
| 1Sa79 | Bacterial clone RB43 | Z95723 | Unknown |
| 1Sa87 | *Polyangium cellulosum* strain So02007-3 | AY252114 | δ-Proteobacteria |
| 1Sa91 | *Arthrobacter sp.* CAB1 | AB039736 | Actinobacteria |
| 1Sa98 | Uncultured bacterium clone CCD21 | AY221035 | Unknown |
| 2Sa17 | *Pseudomonas veronii* strain UFZ-B547 | AY144583 | γ-Proteobacteria |
| 2Sa505 | Drinking water bacterium MA9 | AY328830 | Unknown |
| 2Sa508 | *Pseudomonas sp.* An22 | AJ551160 | γ-Proteobacteria |
| 2Sa512 | *Paenibacillus chondroitinus* | AB073206 | Firmicutes |
| 2Sa514 | *Pseudomonas sp.* 12C_7 | AY689082 | γ-Proteobacteria |
| 2Sa529 | Uncultured eubacterium WD225 | AJ292592 | Unknown |
| 2Sa530 | Uncultured bacterium clone 29 | AY250094 | Unknown |
| 2Sa531 | *Caulobacter sp*. type IV | AJ227791 | α-Proteobacteria |
| 2Sa532 | Uncultured bacterium clone L-4 | AY625148 | Unknown |
| 2Sa533 | Uncultured bacterium clone 29 | AY250094 | Unknown |
| 2Sa535 | *Paenibacillus borealis* | AJ011321 | Firmicutes |
| 2Sa543 | Uncultured bacterium clone 29 | AY250094 | Unknown |
| 2Sa544 | Uncultured soil bacterium clone NAP2d5 | AY699591 | Unknown |
| 2Sa547 | Uncultured bacterium clone cvf65091 | AY100526 | Unknown |
| 2Sa548 | Uncultured soil bacterium clone NAP7d37 | AY699592 | Unknown |
| 2Sa552 | *Sphingopyxis alaskensis* | AY337601 | α-Proteobacteria |
| 2Sa553 | Uncultured soil bacterium clone NAP7d35 | AY699588 | Unknown |
| 2Sa554 | *Paenibacillus alginolyticus* | AB073362 | Firmicutes |
| 2Sa555 | *Caulobacter sp.* type IV | AJ227791 | α-Proteobacteria |
| 2Sa556 | *Rhizobium sp.* ORS 1439 | AY500257 | α-Proteobacteria |
| 2Sa557 | Uncultured soil bacterium clone NAP7d37 | AY699592 | Unknown |
| 2Sa559 | *Caulobacter sp.* type IV | AJ227791 | α-Proteobacteria |
| 2Sa560 | *Pseudomonas sp.* 3C_6 | AY689033 | γ-Proteobacteria |
| 2Sa563 | Uncultured soil bacterium clone NAP2d21 | AY699601 | Unknown |
| 2Sa567 | Uncultured *Xanthomonas sp.* Cl-106-TB4-II | AY599709 | γ-Proteobacteria |
| 2Sa576 | Uncultured bacterium clone t038 | AF422608 | Unknown |
| 2Sa582 | *Bacillus cibus* strain JG-30 | AY550276 | Firmicutes |
| 2Sa583 | Uncultured bacterium clone MeCl 62 | AY439192 | Unknown |
| 2Sa585 | *Paenibacillus sp.* 436-1 | AY266989 | Firmicutes |
| 2Sa588 | Uncultured soil bacterium clone NAP2d5 | AY699591 | Unknown |
| 2Sa591 | *Caulobacter sp.* type IV | AJ227791 | α-Proteobacteria |
| 2Sa593 | Uncultured bacterium clone MeCl 62 | AY439192 | Unknown |
| 2Sa594 | *Pseudomonas tolaasii* | AF255336 | γ-Proteobacteria |
| 2Sa596 | Uncultured ?-proteobacterium clone C-CL42 | AY622230 | γ-Proteobacteria |
| 2Sa599 | *Pseudomonas sp.* 12C_10 | AY689083 | γ-Proteobacteria |
| 2Sa603 | Uncultured *Chryseobacterium sp.* YJQ-106 | AY569302 | Bacteroidetes |
| 2Sa607 | Uncultured bacterium clone 29 | AY250094 | Unknown |
| 2Sa611 | Uncultured soil bacterium clone NAP2d21 | AY699601 | Unknown |

| | | | |
|---|---|---|---|
| 2Sa612 | Uncultured bacterium clone 29 | AY250094 | Unknown |
| 2Sa613 | Uncultured soil bacterium clone NAP2d5 | AY699591 | Unknown |
| 2Sa616 | Uncultured bacterium | Y07608 | Unknown |
| 2Sa618 | *Arthrobacter pascens* | AJ576068 | Actinobacteria |
| 2Sa620 | Uncultured seep bacterium BPC087 | AF154097 | ß-Proteobacteria |
| 2Sa623 | *Brevundimonas sp.* | AJ227797 | α-Proteobacteria |
| 2Sa627 | Uncultured bacterium clone cvf61008 | AY100542 | Unknown |
| 2Sa629 | *Bacillus cereus* SH 01 | AF522353 | Firmicutes |
| 2Sa636 | Uncultured soil bacterium clone NAP2d21 | AY699601 | Unknown |
| 2Sa637 | *Pseudomonas putida* | AF447394 | γ-Proteobacteria |
| 2Sa640 | Uncultured bacterium clone KRA30-39 | AY081993 | Unknown |
| 2Sa641 | *Paenibacillus agarexedens* | AJ345020 | Firmicutes |
| 2Sa642 | *Ralstonia campinensis* strain WS2 | AF312020 | ß-Proteobacteria |
| 2Sa643 | *Paenibacillus chondroitinus* | AB073206 | Firmicutes |
| 2Sa644 | Uncultured bacterium clone 29 | AY250094 | Unknown |
| 2Sa645 | Uncultured bacterium clone 29 | AY250094 | Unknown |
| Sa646 | Uncultured bacterium clone 29 | AY250094 | Unknown |
| 2Sa648 | *Zoogloea ramigera* ATCC 25935 | X74914 | ß-Proteobacteria |
| 2Sa649 | *Arthrobacter sp*. 'SMCC ZAT056' | AF197052 | Actinobacteria |
| 2Sa650 | *Pseudomonas sp.* 12C_10 | AY689083 | γ-Proteobacteria |
| 2Sa653 | Uncultured proteobacterium OCS7 | AF001645 | ß-Proteobacteria |
| 2Sa655 | Uncultured bacterium clone G13 | AF407700 | Unknown |
| 2Sa660 | *Paenibacillus alginolyticus* | AB073362 | Firmicutes |
| 2Sa668 | Uncultured bacterium clone 29 | AY250094 | Unknown |
| 2Sa669 | *Rhizobium sp*. DCP3 | AY064413 | α-Proteobacteria |
| 2Sa671 | Uncultured ß-proteobacterium Cl-73-TB2-II | AY599723 | ß-Proteobacteria |
| 2Sa674 | *Mycoplana sp.* 6C_11 | AY689051 | α-Proteobacteria |
| 2Sa675 | Uncultured bacterium clone 29 | AY250094 | Unknown |

1 BLAST searches conducted on October 20, 2004

**Table 4.** Identification of divergent clones (Sand Clones B) recovered from the rhizospheres of *Agrostis stolonifera* plants grown in sand rootzones

shown that relatively high frequencies of many of the same genera and species found in our current study (30). Furthermore, other molecular ecology studies of *Agrostis capillaris* (colonial bentgrass) in natural grasslands have shown a bacterial community composition similar to that detected in our study (14). As with *A. stolonifera*, *A capillaris* rhizosphere communities were dominated by α-Proteobacteria, but also significant frequencies of actinobacterium and firmicute clones. The reason for the preponderance of gram positive sequences in our putting green samples is unclear, but may simply represent strains that are most suited to survival under these particular management conditions.

Collectively, these results may indicate the possibility of considerable site selectivity in which the main factors controlling the composition of microbial communities in putting greens is the geographic site and not particularly the rootzone or plant species. Further work is needed to resolve this question.

A conspicuous finding from our work is the high frequency of sequences that belong to bacteria that have been studied in other habitats, but which have not yet been cultured. These clones were designated as uncultured or unidentified. High frequencies of these clones were found both in sand-based rootzones and in soil-based rootzones. Our phylogenetic analyses facilitated broad taxonomic placement into groups such as proteobacteria, actinobacteria, Firmicutes, etc., yet, at the present time, we are unable to definitively identify these taxa. One entire cluster of bacteria (unknown affiliation C) could not be assigned to any known taxa, they most likely rep-

| Clone | Closest Identified Strain[1] | Gen Bank Accession No. | Phylogenetic Group |
|---|---|---|---|
| 1So115 | Uncultured a-proteobacterium | AJ532707 | α-Proteobacteria |
| 1So146 | Actinobacteria from anoxic bulk soil VeSm15 | AJ229243 | Actinobacteria |
| 1So156 | *Bacillus sp.* AH 533 | AF290556 | Firmicutes |
| 1So173 | Unidentified eubacterium | AJ005992 | Unknown |
| 1So175 | Actinobacteria from anoxic bulk soil VeSm15 | AJ229243 | Actinobacteria |
| 1So186 | Uncultured bacterium clone NK231 | AB064703 | Unknown |
| 1So198 | Uncultured bacterium clone C20.13WL | AF432798 | Unknown |
| 1So202 | Uncultured ?-proteobacterium MFC-EB8 | AJ630278 | γ-Proteobacteria |
| 1So254 | Uncultured bacterium clone D110 | AY274120 | γ-Proteobacteria |
| 1So267 | Uncultured soil bacterium clone 1152-2 | AF423211 | Unknown |
| 1So32 | Uncultured Acidobacterium N12.40WL | AF431413 | Fibrobacteres/Acidobacteria |
| 1So33 | Actinobacteria from anoxic bulk soil VeSm15 | AJ229243 | Actinobacteria |
| 1So37 | Uncultured Rubrobacteridae clone EB1130 | AY395449 | Actinobacteria |
| 1So403 | *Rhizobium loti* | U50166 | α-Proteobacteria |
| 1So429 | Uncultured bacterium clone 300A-B12 | AY661979 | Fibrobacteres/Acidobacteria |
| 1So433 | Unidentified Actinomycetes bacterium | D84612 | Actinobacteria |
| 1So443 | Uncultured bacterium clone NMS8.52WL | AF432676 | δ/ε-proteobacteria |
| 1So444 | *Burkholderia sp.* Ellin121 | AF408963 | ß-Proteobacteria |
| 2So505 | *Frateuria sp.* A12 | AF406661 | γ-Proteobacteria |
| 2So508 | Uncultured Acidobacterium group S22.04WL | AF431429 | Fibrobacteres/Acidobacteria |
| 2So509 | Uncultured Rubrobacteridae clone EB1130 | AY395449 | Actinobacteria |
| 2So510 | *Burkholderia sp.* Ellin121 | AF408963 | ß-Proteobacteria |
| 2So513 | Uncultured Sphingomonadaceae M10Ba57 | AY360645 | α-Proteobacteria |
| 2So518 | *Paenibacillus sp*. DSM | AJ345019 | Firmicutes |
| 2So520 | *Burkholderia phytofirmans* strain PsJN | AY497470 | ß-Proteobacteria |
| 2So521 | *Stigmatella aurantiaca* strain Sg a15 | AJ233936 | δ/ε-Proteobacteria |
| 2So524 | Uncultured soil bacterium clone 768-2 | AF423293 | α-Proteobacteria |
| 2So527 | Uncultured α-proteobacterium JG36-TzT-193 | AJ534619 | α-Proteobacteria |
| 2So533 | Uncultured bacterium clone MSC49 | AJ830735 | OP5 |
| 2So534 | Unidentified eubacterium from anoxic bulk soil clone BSV39 | AJ229195 | Unknown |
| 2So535 | Uncultured soil bacterium clone Tc132-51 | AY242664 | Unknown |
| 2So536 | Uncultured soil bacterium clone Tc121-E05 | AY242746 | Unknown |
| 2So542 | Uncultured *Bacilli bacterium* clone X9Ba87 | AY607217 | Firmicutes |
| 2So545 | *Frateuria sp.* A12 | AF406661 | γ-Proteobacteria |
| 2So547 | *Bacillus sp.* 19498 | AJ315066 | Firmicutes |
| 2So551 | 2,4-D-degrading bacterium TFD6 | AF184931 | OP5 |
| 2So553 | Uncultured Acidobacterium group S22.04WL | AF431429 | Fibrobacteres/Acidobacteria |
| 2So554 | Uncultured eubacterium clone M10 | AF495417 | γ-Proteobacteria |
| 2So556 | Uncultured Acidobacteria clone JAB FS 04 | AY734285 | Fibrobacteres/Acidobacteria |
| 2So557 | Uncultured Acidobacteriales clone WS107 | AY174201 | Fibrobacteres/Acidobacteria |
| 2So559 | Bacterium Ellin423 | AF432247 | Actinobacteria |
| 2So562 | Uncultured Bacilli bacterium clone M3Ba39 | AY360703 | Firmicutes |
| 2So565 | Uncultured Bacilli bacterium clone H5Ba27 | AY360579 | Firmicutes |
| 2So566 | Unidentified bacterium clone Qui4P2-31 | AJ518575 | Firmicutes |
| 2So568 | Actinobacterium EC5 | AY337600 | Actinobacteria |
| 2So571 | *Bacillus sp*. IDA5367 | AY289508 | Firmicutes |
| 2So575 | Uncultured earthworm cast bacterium C005 | AY037664 | α-Proteobacteria |
| 2So577 | *Bacillus cereus* strain G8639 | AY138271 | Firmicutes |
| 2So580 | Uncultured Bacilli bacterium clone M3Ba39 | AY360703 | Firmicutes |
| 2So581 | *Swingsiella fulva* | AB100608 | γ-Proteobacteria |
| 2So582 | Uncultured *Holophaga sp.* JG37-AG-67 | AJ519378 | Fibrobacteres/Acidobacteria |
| 2So583 | Uncultured Acidobacterium UA3 | AF200699 | Fibrobacteres/Acidobacteria |
| 2So584 | Uncultured Bacilli bacterium clone M3Ba39 | AY360703 | Firmicutes |
| 2So585 | *Bacillus sp.* 19498 | AJ315066 | Firmicutes |
| 2So587 | *Sphingomonas sp.* 8b-1 | AY561541 | α-Proteobacteria |
| 2So589 | Uncultured a-proteobacterium SMW4.4WL | AY043755 | α-Proteobacteria |
| 2So590 | Uncultured Bacilli bacterium clone M3Ba39 | AY360703 | Firmicutes |

|  |  |  |  |
|---|---|---|---|
| 2So591 | *Bacillus sp.* LMG 20241 | AJ316313 | Firmicutes |
| 2So593 | Unknown organism SBR2094) | X84624 | α-Proteobacteria |
| 2So595 | Uncultured Bacilli bacterium clone M3Ba39 | AY360703 | Firmicutes |
| 2So599 | Uncultured bacterium clone C20.13WL | AF432798 | δ/ε-Proteobacteria |
| 2So600 | Uncultured soil bacterium clone 1142 | AY493964 | Firmicutes |
| 2So602 | Uncultured Bacilli bacterium clone X9Ba25 | AY607169 | Firmicutes |
| 2So610 | Uncultured Acidobacterium group S51.05PG | AF431507 | Fibrobacteres/Acidobacteria |
| 2So611 | *Frateuria aurantia* | AJ010481 | γ-Proteobacteria |
| 2So614 | Uncultured bacterium DUN1_63-1387_+C11 | AY723979 | Fibrobacteres/Acidobacteria |
| 2So615 | Uncultured Bacilli bacterium clone M10Ba75 | AY360662 | Firmicutes |
| 2So616 | Uncultured soil bacterium clone 768-2 | AF423293 | α-Proteobacteria |
| 2So619 | Uncultured bacterium clone BItii37 | AJ318189 | OP5 |
| 2So623 | Uncultured eubacterium WR897 | AJ292868 | OP5 |
| 2So631 | Uncultured earthworm cast bacterium C037 | AY037690 | Firmicutes |
| 2So632 | Uncultured ß-proteobacterium WCB190 | AY217460 | ß-Proteobacteria |
| 2So633 | Uncultured Bacilli bacterium clone X9Ba87 | AY607217 | Firmicutes |
| 2So634 | *Hyphomicrobium sp.* HM | AF156711 | α-Proteobacteria |
| 2So636 | Uncultured CFB group bacterium N12.23WL | AF431557 | α-Proteobacteria |
| 2So637 | Uncultured bacterium DUN1_63-1387_+C11 | AY723979 | Fibrobacteres/Acidobacteria |
| 2So638 | Unidentified eubacterium | AF010036 | Fibrobacteres/Acidobacteria |
| 2So639 | *Bacillus sp.* NK13 | AY654897 | Firmicutes |
| 2So642 | Uncultured eubacterium WCHB1-08 | AF050573 | Firmicutes |
| 2So645 | Uncultured eubacterium WR876 | AJ292843 | α-Proteobacteria |
| 2So647 | Uncultured bacterium clone BCM3S-34B | AY102909 | α-Proteobacteria |
| 2So651 | *Bacillus sp.* 19498 | AJ315066 | Firmicutes |
| 2So654 | Uncultured ß-proteobacterium S15B-MN115 | AJ583171 | ß-Proteobacteria |
| 2So660 | Uncultured ß-proteobacterium S15B-MN115 | AY360659 | ß-Proteobacteria |
| 2So664 | *Bacillus sp.* D-39-25-3 | AB190127 | Firmicutes |
| 2So665 | *Herbaspirillum sp.* PIV-34-1 | AJ505863 | ß-Proteobacteria |
| 2So671 | Uncultured bacterium clone AF1.5 | AF143839 | Unknown |
| 2So676 | Bacterium Ellin6007 | AY234659 | Actinobacteria |
| 2So677 | *Paenibacillus alginolyticus* | AB073362 | Firmicutes |
| 2So678 | Uncultured Clostridia bacterium X3Ba44 | AY607135 | Firmicutes |
| 2So679 | Soil bacterium is01 | AF128754 | Unknown |
| 2So680 | Uncultured bacterium clone BIfci40 | AJ318117 | Unknown |

[1] BLAST searches conducted on October 20, 2004

**Table 5.** Identification of clones (Soil Clones A) recovered from the rhizospheres of *Agrostis stolonifera* plants grown in sand rootzones

resent Firmicutes, which includes species of *Bacillus* and *Paenibacillus* since many of the clones had their nearest relative based on BLAST searches within this group.

While our inabilitiy to fully identify taxa from our study presents obstacles to understanding the full nature of rhizosphere microbial communities, it allows us to get an appreciation of the breadth of diversity present in golf course putting greens that has previously not been recognized. As we consider issues of soil and plant health as well as overall performance of bentgrass in golf course setting, this diversity should be appreciated so that interpretations of microbial function and turfgrass responses that are based on culture-based assessments can be placed in a proper perspective. Furthermore, as our efforts to develop effective culturing methods for some of these strains improves, our understanding of the distribution and roles of these taxa in golf turf will be facilitated.

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| Clone | Closest Identified Strain[1] | Gen Bank Accession No. | Phylogenetic Group |
|---|---|---|---|
| 1So105 | Uncultured bacterium clone 210ds10 | AY212657 | Unknown |
| 1So139 | Uncultured bacterium clone CCM9a | AY221072 | Unknown |
| 1So183 | Uncultured bacterium clone 2-20 | AY548946 | Unknown |
| 1So217 | Uncultured bacterium clone DA113 | Y07608 | Unknown |
| 1So220 | Uncultured soil bacterium clone 594-1 | AY326605 | Unknown |
| 1So231 | Uncultured d-proteobacterium clone KU12 | AB074949 | δ/ε-Proteobacteria |
| 1So275 | Uncultured bacterium clone 015C-F01 | AY661996 | Unknown |
| 1So322 | Uncultured bacterium clone 300A-B12 | AY661979 | Unknown |
| 1So334 | Uncultured d-proteobacterium clone KU12 | AB074949 | δ/ε-Proteobacteria |
| 1So419 | Uncultured soil bacterium clone 178-2 | AY326611 | Unknown |
| 1So451 | Uncultured bacterium clone 1790-3 | AY425771 | Unknown |
| 1So485 | Uncultured bacterium clone KD6-86 | AY188323 | Unknown |
| 1So494 | Uncultured bacterium clone DA113 | Y07608 | Unknown |
| 1So499 | *Janthinobacterium sp.* WSH04-01 | AY753304 | ß-Proteobacteria |
| 1So501 | *Janthinobacterium sp.* WSH04-01 | AY753304 | ß-Proteobacteria |
| 1So513 | Uncultured bacterium clone KD6-86 | AY188323 | Unknown |
| 2So502 | Uncultured bacterium clone 9 | AY235583 | Unknown |
| 2So503 | Uncultured bacterium clone 216ds20 | AY212663 | Unknown |
| 2So504 | Uncultured bacterium clone 145 | AY391120 | Unknown |
| 2So506 | Uncultured soil bacterium clone HS9-25 | AY221608 | Unknown |
| 2So507 | Uncultured bacterium clone CCU24 | AY221080 | Unknown |
| 2So514 | *Myxococcus fulvus* 128-7 | AY032879 | δ/ε-Proteobacteria |
| 2So515 | Uncultured bacterium clone KD5-51 | AY188317 | Unknown |
| 2So516 | Uncultured bacterium clone DA113 | Y07608 | Unknown |
| 2So517 | Uncultured bacterium clone KD6-86 | AY188323 | Unknown |
| 2So519 | Uncultured soil bacterium clone HS9-25 | AY221608 | Unknown |
| 2So523 | Uncultured soil bacterium clone 345-2 | AY326528 | Unknown |
| 2So525 | *Zoogloea sp.* 'Mali 147-01' | AY211165 | ß-Proteobacteria |
| 2So526 | *Bacillus sp.* VAN23 | AF286484 | Firmicutes |
| 2So528 | Uncultured soil bacterium clone 1299 | AY493914 | Unknown |
| 2So529 | Uncultured soil bacterium clone HC-D | AY437993 | Unknown |
| 2So530 | *Dyemonas todaii* strain XD10 | AB110496 | γ-Proteobacteria |
| 2So531 | *Bacillus niacini* strain Mali 148 | AY211166 | Firmicutes |
| 2So537 | Uncultured bacterium clone 1790-3 | AY425771 | Unknown |
| 2So538 | Uncultured soil bacterium clone NAP7d37 | AY699592 | Unknown |
| 2So539 | *Nocardioides aquiterrae* strain GW-9 | AF529063 | Actinobacteria |
| 2So540 | *Bacillus bataviensis* | AJ542508 | Firmicutes |
| 2So544 | *Bacillus pichinotyi* strain RS2 | AF519464 | Firmicutes |
| 2So546 | *Bacillus cereus* | AY741719 | Firmicutes |
| 2So549 | *Bacillus bataviensis* | AJ542508 | Firmicutes |
| 2So555 | Uncultured bacterium clone KD6-86 | AY188323 | Unknown |
| 2So558 | *Bacillus sp.* KJ2C12 | AY514023 | Firmicutes |
| 2So560 | Uncultured soil bacterium clone HC-D | AY437993 | Unknown |
| 2So561 | *Bacillus cereus* strain G8639 | AY138271 | Firmicutes |
| 2So563 | Uncultured Comamonadaceae bacterium B-23 | AF523053 | ß-Proteobacteria |
| 2So564 | Uncultured bacterium clone 300E2-D11 | AY662016 | Unknown |
| 2So567 | Uncultured bacterium clone 1959-1 | AY425785 | Unknown |
| 2So569 | Unidentified bacterium clone W4-B20 | AY345503 | Unknown |
| 2So570 | Uncultured soil bacterium clone 991-1 | AY326567 | Unknown |
| 2So572 | Microbacterium sp. VKM Ac-1389 | AB042070 | Actinobacteria |
| 2So576 | Uncultured soil bacterium clone 1289 | AY493950 | Unknown |
| 2So578 | Uncultured Bacteroidetes bacterium | AJ567587 | Bacteroidetes |
| 2So586 | Uncultured soil bacterium clone HS9-25 | AY221608 | Unknown |
| 2So594 | *Bacillus pichinotyi* strain RS2 | AF519464 | Firmicutes |
| 2So601 | *Variovorax sp*. WDL1 | AF538929 | ß-Proteobacteria |
| 2So613 | *Bacillus bataviensis* | AJ542508 | Firmicutes |
| 2So617 | Uncultured bacterium clone DA113 | Y07608 | Unknown |
| 2So621 | *Actinoplanes digitatis* | AB037000 | Actinobacteria |
| 2So624 | *Bacillus senegalensis* strain RS8 | AF519468 | Firmicutes |

| | | | |
|---|---|---|---|
| 2So625 | *Bacillus cereus* ZK | CP000001 | Firmicutes |
| 2So627 | *Bacillus megaterium* strain g1 | AY373360 | Firmicutes |
| 2So629 | Cultured CFB group bacterium | AJ581624 | Bacteroidetes |
| 2So630 | *Bacillus sp.* KJ2C12 | AY514023 | Firmicutes |
| 2So635 | Uncultured soil bacterium clone HS9-25 | AY221608 | Unknown |
| 2So641 | *Burkholderia sp.* LMG 19076 | AF215704 | ß-Proteobacteria |
| 2So643 | Uncultured bacterium clone 177up | AY212628 | Unknown |
| 2So644 | Uncultured soil bacterium clone HS9-25 | AY221608 | Unknown |
| 2So646 | Uncultured Bacteroidetes clone S1-4-CL9 | AY728066 | Bacteroidetes |
| 2So649 | Uncultured bacterium clone KD6-86 | AY188323 | Unknown |
| 2So650 | Uncultured bacterium clone 9 | AY235583 | Unknown |
| 2So652 | *Caulobacter sp.* SE2-103 | AY568471 | α-Proteobacteria |
| 2So653 | Uncultured Xiphinematobacteriaceae EB1104 | AY395423 | Verrucomicrobia |
| 2So656 | Uncultured soil bacterium clone HS9-25 | AY221608 | Unknown |
| 2So661 | *Paenibacillus sp.* SG | AB179866 | Firmicutes |
| 2So666 | Uncultured soil bacterium clone HS9-25 | AY221608 | Unknown |
| 2So667 | *Bacteroides cellulosolvens* (ATCC 35603) | L35517 | Bacteroidetes |
| 2So668 | *Bacillus bataviensis* | AJ542508 | Firmicutes |
| 2So669 | Uncultured Bacteroidetes clone S1-4-CL9 | AY728066 | Bacteroidetes |
| 2So672 | Bacterium Ellin5001 | AY234418 | Fibrobacteres/Acidobacteria |
| 2So673 | Uncultured soil bacterium clone 1289 | AY493950 | Unknown |
| 2So674 | Uncultured soil bacterium clone HS9-25 | AY221608 | Unknown |
| 2So675 | Uncultured bacterium clone KD6-86 | AY188323 | Unknown |

[1] BLAST searches conducted on October 20, 2004

**Table 6.** Identification of divergent clones (Soil Clones B) recovered from the rhizospheres of *Agrostis stolonifera* plants grown in soil rootzones

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