Identification and Metabolic Diversity of Rhizobacteria from Bentgrass and Bermudagrass Greens

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Objectives:

1. Complete the identification of approximately 500 turf rhizobacteria using a combination of biochemical profiling and DNA techniques.
2. Determine the metabolic diversity of turf rhizobacteria by carbon substrate utilization patterns and potential for denitrification.

Start Date: 2000
Project Duration: 2 years
Total Funding: $36,656

Varying the growing medium allows researchers to identify the kinds of soil bacteria that occur in amended sand rootzones.

The biological process of denitrification releases nitrogen to the atmosphere that causes major nitrogen losses in agricultural soils. The terminal products of NO and N_2O contribute to global warming and the destruction of the ozone layer. Bioremediation of environmental pollution can also be performed by the denitrification process in certain bacteria.

In our laboratory over the past four years, rhizobacteria have been isolated from roots of new USGA bentgrass and bermudagrass putting greens. Approximately 9,000 rhizobacteria isolates were identified by Gas Chromatography-Fatty Acid Methyl Ester technology. Previous studies showed that only a small range of bacteria were capable of denitrification. *Pseudomonas*, *Bacillus*, *Paracoccus*, *Alcaligenes* and *Enterobacter* were the dominant denitrifying bacteria in many environments. Research on denitrifying rhizobacteria associated with turfgrass has been limited. Our results will provide information of these key microorganisms that determine the fate of nitrogen in the turfgrass ecosystem.


At Clemson University, Dr. Horace Skipper continues to identify soil bacteria that inhabit amended sand rootzones.

A total of thirty-two (16%) denitrifying rhizobacteria were identified in bentgrass and bermudagrass by biochemical analysis. Another 97 rhizobacteria (49%) were able to reduce nitrate to nitrite and were classified as nitrate-reducing bacteria. The other 71 rhizobacteria (35%) were non-denitrifiers.

The identified denitrifiers include seven species of genus *Pseudomonas*, four of *Bacillus*, two of *Acidovorax*, two of *Methylobacterium*, two of *Microbacterium*, two unidentified isolates and thirteen of other genera. *Bacillus pumilus* and *Pseudomona pickettii* were identified only in bermudagrass. Even though the isolates were obtained from either bentgrass or bermudagrass, 30 of 32 isolates identified as denitrifiers from one turfgrass were also found in the master list of species from the other turfgrass.

The nirK gene fragments were amplified in PCR from 24 rhizobacteria (75%) in at least one of the six primer combinations, and not amplified from any of the non-denitrifiers evaluated in this study. Products of the expected size could be obtained from all the six primer combinations for *Methylobacterium organophilum*, *Paracoccus denitrificans* and *Pseudomonas aeruginosa*. The non-nirK amplified denitrifiers may contain other kinds of the nir gene, or their nirK gene sequences are less homologous with the regions that the primers could amplify.

The denitrifying rhizobacteria constituted 16% of the 200 rhizobacteria selected from USGA putting greens. *Pseudomonas* and *Bacillus* were the dominant genera in the denitrifying rhizobacteria in both bentgrass and bermudagrass.

Summary Points

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- The denitrifying rhizobacteria constituted 16% of the 200 rhizobacteria selected from USGA putting greens. *Pseudomonas* and *Bacillus* were the dominant genera in the denitrifying rhizobacteria in both bentgrass and bermudagrass.