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 profil ing 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

Over the past recent years, rhizobacteria have been isolated quarterly from roots of bentgrass or bermudagrass at new constructed or reconstructed greens in the USGA-sponsored project entitled "Bacterial Populations and Diversity with New USGA Putting Greens." The goal of this project was to identify these bacterial isolates by fatty acid methyl ester (FAME) analysis for identification (Sherlock System).

The resulting identification data were then used to monitor microbial shifts over the duration of the study in South Carolina, North Carolina, Florida and Alabama. Although 5,000 strains were analyzed, approximately 500 havae been unidentifiable with the Sherlock System. If these 500 unidentified isolates represent one to three species, they could become the dominant rhizobacteria rather than the *Pseudomanas* and *Bacillus* species as identified in the previous study.

The overall goal is to fill in the void in the identificaiton of turf rhizobacteria generated by the FAME analysis. It is essential to determine if the unidentified isolates represent many species of a few genera, many genera of a few species, or perhaps entirely new, unidentified and unnamed turf rhizobacteria.

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

The Sherlock System software for bacterial identification comes with a few additional software tools to help get the most information out of a single analysis. One of these tools is the 2-D plot program. The 2-D plot program is especially useful for finding relationships among large numbers of organisms.

This is done by employing the statistical procedure of principal component analysis to plot results of individual analyses in a three-dimensional array (x, y, and z axes). These 3-dimensional relationships are graphically represented in 2 dimensions by printing on paper.

Each bacterial strain analyzed with the Sherlock System software can be used in a 2-D plot. Each analysis of a strain or different strains is represented by a single point in the 2-D plot graph. The unit of distance between two points is a Euclidean Distance (ED).

In general, a cluster of points within 110 ED (approximately 10.5 on the x-axis and 10.5 on the y-axis) constitutes representatives within the same species. Similarly, data points within 60 ED constitute members of a single subspecies.

Approximately 900 strains, which were unidentifiable with the Sherlock System software, were analyzed with the 2-D plot software program. Although these strains remained without a taxonomic category, the analysis on the Sherlock System could still prove useful if the strains could be put into species groups and representatives of each group could be subjected to further tests to determine their identity.

At Clemson University, Dr. Horace Skipper continues to identify the soil bacteria that naturally inhabit amended sand rootzones. There are more than a billion microorganisms found in one gram of soil.

After analyzing these strains with the 2-D plot software, it was determined that 15 predominant clusters were present. Most of these clusters exceeded the limits for a species cluster, indicating that each cluster represented a genus or at least multiple species of a single genus. When each cluster was analyzed further, it was determined that subclusters existed within each main cluster.

These subclusters are about the size of a 'species' cluster and there are approximately 38 in total. Of the 15 main clusters, 4 appear to be most similar to Gram positive bacteria and 8 appear to be most similar to Gram negative bacteria, with 3 clusters being inconclusive.

Representative isolates from these clusters will be subjected to MicroLogTM System and DNA techniques for identification.

Summary Points

- Out of 900 strains, there are 38 that cluster at the species level.
- For the main 15 clusters, four appear to be gram negative bacteria, three clusters are inconclusive.