BACTERIAL SUCCESSION ON THE LEAVES OF PONDEROSA PINE, PINUS PONDEROSA

Crystal Hostetter and David Bergmann
Black Hills State University
Rapid City, SD 57701

ABSTRACT

We examined bacterial succession on Ponderosa pine needles in the Black Hills of South Dakota. Samples of living, dead, and decomposing pine needles were collected in 2004-2005. Total genomic DNA was extracted and the 16S rRNA gene was amplified using polymerase chain reaction (PCR), and PCR products separated by denaturing gradient gel electrophoresis (DGGE). We identified the different bacterial 16S rRNA genes collected in the bands of the DGGE gel, each with the potential of being a different bacterial species. The bands were sliced out and each was re-amplified again with PCR, cloned into a plasmid, and transformed into Escherichia coli. Transformed colonies were cultured and DNA extracted for sequencing. Sequences were BLAST searched and results were collected into a tree showing relationships.

New samples were collected in summer from mature and young pine needles. Samples were either homogenized or sonicated and then plated out for growth. Samples of phenotypical similarity were isolated, DNA extracted 16S rRNA gene, amplified by PCR, and Restriction Fragment Length Polymorphism (RFLPs) analyzed.

Numbers of bacteria on the surfaces of mature leaves were estimated at 1.3 X 10^4 colony forming units per g from culturing and 5.1 X 10^6 cells per g from fluorescence microscopy. Results showed many more species of bacteria (over 40) could be detected through PCR and DGGE than by culturing. Many DGGE bands were likely Sphingomonas species, and Streptococcus, Acidobacteriaceae, and Friedmanella species were also detected by DGGE. One Sphingomonas species and Pseudomonas species were identified from culturing.