Project ID Number: 55108

Project Title: Monitoring Genetic and Metabolic Potential for In-Situ Bioremediation: Mass Spectrometry

Publication Date:

Lead Principal Investigator: Michelle V. Buchanan
Oak Ridge National Laboratory
P.O. Box 2008, MS 6124
Oak Ridge TN 37831-6124
865-574-4521
buchananmv@ornl.gov

Co-investigators: Phillip F. Britt
Oak Ridge National Laboratory
P.O. Box 2008, MS 6197
Oak Ridge TN 37831-6197
865-574-5029
brittpf@ornl.gov

Mitchel J. Doktycz
Oak Ridge National Laboratory
P.O. Box 2008, MS 6123
Oak Ridge TN 37831-6123
865-574-6204
doktyczmj@ornl.gov

Gregory B. Hurst
Oak Ridge National Laboratory
P.O. Box 2008, MS 6365
Oak Ridge TN 37831-6365
865-574-7469
hurstgb@ornl.gov

Mary E. Lidstrom
Departments of Chemical Engineering and Microbiology
University of Washington
Box 351750
Seattle WA 98195-1750
206-616-5282
lidstrom@u.washington.edu

Number of graduate students actively involved in the project: 2

Research Objective: A number of DOE sites are contaminated with dense non-aqueous phase liquids (DNAPLs) such as carbon tetrachloride and trichloroethylene. At many of these sites, microbial bioremediation is an attractive strategy for cleanup, since it has the potential to degrade DNAPLs *in situ*. A rapid screening method to determine the broad range potential of a site's microbial population for contaminant degradation would greatly facilitate assessment for *in situ* bioremediation, as well as for monitoring ongoing bioremediation treatment. Current laboratory-based treatability methods are cumbersome and expensive. In this project, we are developing methods based on matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) for rapid and accurate detection of polymerase chain reaction (PCR) products from microbial genes involved in biodegradation of pollutants. PCR primers are being developed to amplify DNA sequences that are amenable to MALDI-MS detection. This work will lay the foundation for development of a field-portable MS-based technique for rapid on site assessment and monitoring of bioremediation processes.

Research Progress and Implications:
This report summarizes work at the end of a 3-year project, during which we have demonstrated improved MALDI-MS detection of DNA signatures relevant to bacterial bioremediation.

A model system for interfacing PCR amplification with MALDI-MS detection is based on the *pmoA* gene for the active site subunit of particulate methane monooxygenase, a bacterial enzyme that can oxidize trichloroethylene. Andria Costello and Ann Auman, two graduate students in Prof. Lidstrom's laboratory, have developed molecular techniques for analyzing natural populations of methanotrophic bacteria, which are important for *in situ* bioremediation of halogenated solvents such as trichloroethylene, dichloroethylene and vinyl chloride. A DNA sequence database has been developed for methanotrophs in Lake Washington sediment, a habitat studied in detail by the Lidstrom laboratory. This database has been used to design specific PCR primers and hybridization probes that will detect the entire range of these diagnostic genes in known methanotrophs. These molecular tools have been used to analyze the natural populations of methanotrophs in Lake Washington sediments by conventional hybridization techniques. For MALDI-MS detection, PCR primer pairs were designed to amplify relatively short segments (99 bases and 56 bases) of *pmoA* in Type I and Type II methanotrophs.

To determine the range of conditions over which the combined PCR amplification, purification, and MALDI detection methodology is useable, we have varied amounts and types of bacterial DNA added initially to the PCR. MALDI signal can be detected from a PCR preparation that uses as little as 10^5 -10^6 target molecules. The effects of "interfering" DNA from the diverse microbial population found in subsurface environments were explored by performing PCR on mixtures of genomic DNA from the methanotroph *Methylosinus trichosporium* OB3b and *E. coli*. In this initial study, the effect of the "interference" DNA was negligible.

A rapid reverse-phase purification of bacterial PCR products allows MALDI-MS detection from a fraction of one 25-microliter reaction. At this level of sensitivity, MALDI-MS is competitive with conventional methods for detecting PCR products in the <100 bp size range, and methodology improvements should allow larger products to be analyzed. Recovery of the purification is 60% to 75% for PCR products of 50 to 200 bases. We have adapted the purification to a microtiter format for parallel treatment of 96 samples in about 10 minutes.

An important aspect of automated, high throughput MALDI analysis is improving the homogeneity of the dried MALDI matrix/PCR mixture to allow useful signal to be obtained by aiming the MALDI laser anywhere on the spot. Polymeric substrates are proving useful in this regard. A combination of fluorescence microscopy for visualizing the distribution of DNA in the MALDI sample and MALDI measurements of the same spots guides the optimization of the substrate development work. While typical DNA samples for MALDI prepared on a bare metal substrate have a dried "rim" of DNA-doped matrix crystals, samples prepared on polymeric substrates tend to be more evenly distributed across the entire area originally wetted by the sample solution droplet. The mechanism appears to involve increased viscosity in the drying droplet due to partial re-dissolution of the polymer subsrate, as well as partitioning of either the DNA or the matrix between the solvent and the remaining polymeric substrate.

Planned Activities:
A proposal is being prepared for continuation of this project, which was funded through FY 1999, with a no-cost extension through March 2000.

Information Access:
G.B. Hurst, K. Weaver, M.V. Buchanan and M.J. Doktycz, "Analysis of PCR products using delayed-extraction MALDI-TOF," Proceedings of the 45th ASMS Conference on Mass Spectrometry and Allied Topics, Palm Springs CA, June 1-5, 1997, p. 843.

G. B. Hurst, K. Weaver, and M.V. Buchanan, "Improved Mass Spectrometric Resolution for PCR Product Size Measurement," presented at The Sixth Department of Energy Contractor and Grantee Workshop of the Human Genome Program, Santa Fe, NM, November 9-13, 1997.

G.B. Hurst, K. Weaver, M.J. Doktycz, M.V. Buchanan, A.M. Costello, and M.E. Lidstrom, "MALDI-TOF Analysis of Polymerase Chain Reaction Products from Methanotrophic Bacteria," *Anal. Chem.* 1998, *70*, 2693-2698.

G.B. Hurst, K. Weaver, M.J. Doktycz, M.V. Buchanan, A. Costello, and M.E. Lidstrom, "Identification of Methanotrophic Bacteria Using the Polymerase Chain Reaction with MALDI-TOF Detection," Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando FL, May 31-June 4, 1998, p. 1202.

K. Weaver, M.J. Doktycz, P.F. Britt, G.B. Hurst, and M.V. Buchanan, "96-Well Microtiter-Format Purification of DNA for MALDI-TOF Analysis," Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando FL, May 31-June 4, 1998, p. 60.

M.V. Buchanan, G.B. Hurst, M.J. Doktycz, P.F. Britt, K. Weaver, M.E. Lidstrom and A.J. Costello, "Monitoring Genetic and Metabolic Potential for in situ Bioremediation: Mass Spectrometry," Poster presentation at the DOE Environmental Management Science Program Workshop, Chicago, Ill., July 27-30, 1998.

G.B. Hurst, Y. Kim, K. Weaver and M.V. Buchanan, "PCR Product Size Measurement using MALDI Mass Spectrometry," Poster presentation at the 7th DOE Human Genome Contractor-Grantee Workshop, Oakland, California, January 12-16, 1999.

Y. Kim, G.B. Hurst, M.J. Doktycz and M.V. Buchanan, "Improved Spot Homogeneity for DNA MALDI Matrices," to be presented at the 47th ASMS Conference on Mass Spectrometry and Allied Topics, Dallas TX, June 13-18, 1999.