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Monitoring Genetic & Metabolic Potential for In Situ Bioremediation: Mass Spectrometry

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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) to rapidly and accurately detect polymerase chain reaction (PCR) products. In parallel, PCR primers to amplify DNA sequences from microbial genes involved in biodegradation of pollutants are being identified that are short enough to allow MALDI-MS detection. This work will lay the foundation for development of a field-portable MS-based technique for rapid assessment and monitoring of bioremediation processes on site.

Research Progress and Implications

This report summarizes work after 1-1/2 years of a 3-year project. In this time, we have demonstrated MALDI-MS-based detection of signature bacterial PCR products (Hurst *et al.*, 1998). A model system for interfacing MALDI-MS with PCR amplification is based on the *pmoA* gene for the active site subunit of particulate methane monooxygenase, a bacterial enzyme that can oxidize trichloroethylene. PCR primer pairs were designed to amplify relatively short segments (99 bases and 56 bases) of this gene in Type I and Type II methanotrophs. A rapid reverse-phase purification of the resulting PCR products allows MALDI-MS detection from a fraction of one 25-microliter PCR reaction. At this level of sensitivity, MALDI-MS has considerable potential to compete with existing electrophoresis and hybridization methods for detecting PCR products in this size range. To allow increased throughput, the PerSeptive Biosystems MALDI-TOF mass spectrometer allows automated MALDI data acquisition, and we have adapted our purification scheme to a 96-well microtiter plate format that allows parallel treatment of 96 PCR reactions in about ten minutes (Weaver *et al.*, 1998). An in-house-constructed TOF mass spectrometer is being modified to allow more fundamental studies aimed at improving the MS detection of PCR products.

Planned Activities

In FY98/99, we will investigate effects of interferences and estimate lower limits of detection for methanotrophs using MALDI-detected PCR, and compare MALDI-detection of PCR products with accepted methods for detecting methanotrophs in environmental samples. In FY99, we will continue to explore MALDI detection for multiplexed hybridization detection of methanotroph PCR products,

evaluate electron-capture ionization as a means for improving MALDI sensitivity, and pursue more reproducible and convenient methods for combining PCR products with the MALDI matrix.

Other Access To Information

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.

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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.*, in press (1998).

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," to be presented at the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando FL, May 31-June 4, 1998.

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," to be presented at the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando FL, May 31-June 4, 1998.