Rapid Mass Spectrometric DNA Diagnostics for Assessing Microbial Community Activity During Bioremediation

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Research Objective

The use of DNA-based procedures for the detection of biodegrading organisms or genes that code for pollutant-degrading enzymes constitutes a critical technology for following biochemical transformation and substantiating the impact of bioremediation. In previous studies, DNA-based technology has been demonstrated to be a sensitive technique for tracking micro-organism activity at the molecular level. These procedures can be tuned to identify groups of organisms, specific organisms, and to detect signals that measure microbial community activity. This proposal describes the evaluation of a monitoring strategy that relies on the combined use of DNA diagnostics with mass spectrometry as the detection scheme. The intent of this work is a two-fold evaluation of: 1) the feasibility of replacing the use of gel separations for identifying polymerase chain reaction (PCR) products with a rapid and automatable form of electrospray mass spectrometry and 2) the use of matrix-assisted-laser-desorption-ionization mass spectrometry (MALDI-MS) as a tool to score oligonucleotide ligation assays (OLA).

Mass spectrometry is an attractive detection alternative for PCR and OLA procedures because it is a sensitive analytical technique capable of providing high sample throughput performance and the introduction of samples into a mass spectrometer is automatable. Mass spectra are generated rapidly, in the order of minutes, thus eliminating a time bottleneck caused by time-consuming gel electrophoresis separations. The adaptation of OLA for mass spectrometric analysis will cut costs by eliminating expensive reagents. The techniques are automatable which translates into a decreased human error rate when numerous samples are analyzed as a component of large scale bioremediation studies. The successful conclusion of the work will be the development of a mass spectrometry capability for sizing PCR products and scoring OLA tests in formats that provide high throughput and automation for bioremediation studies.