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Can We Reliably Predict the Biodegradation Potential of Aromatic Hydrocarbons Using Molecular Tools?

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Abstract

Biodegradation potential of a former manufactured gas plant site was determined using molecular probes specific for dioxygenases of aromatic compounds and radioisotope analyses. Signals of Southern hybridization were positively related to the amounts of aromatic hydrocarbons contaminated in soil samples, but the signal intensity was greatly dependent on the types of probes used and the origin of soil samples. Furthermore, the data were not consistent with the biodegradation results obtained by using ^{14}C-labeled compounds (CO_2 evolution), suggesting that biodegradation potential should be carefully estimated when molecular tools are to be used. Especially, limitations of DNA extraction and appropriate primers should be well considered and deliberate approaches ought to be executed.

Introduction of organic pollutants into the environment especially soil results in changes of the function and structure of indigenous microflora. It is reasonable to assume that microbial species that can degrade contaminants will flourish in a site where contamination occurs, while those that cannot compete with the enriched species or withstand such a pollution will diminish with time. For a successful bioremediation of a site, it is helpful to understand the microbiological characteristics of the area such as biodegrading activity of contaminants and the composition of indigenous microbial population. Recently, methodology mainly comprising of direct extraction of DNA from soil and hybridization of metabolic genes of interests with specific probes is frequently used to characterize biodegradation potential of a site due to its specificity, easiness, and rapidity [1-3]. In a project to develop a remediation technology combining biological and chemical means for a former manufactured gas plant site where contamination with coal tar had occurred for over 100 years, we have determined the biodegradation potential of polycyclic aromatic hydrocarbons (PAHs) and compositional change of indigenous microbial population. The biodegradation potential was determined using molecular techniques and radioisotopes as well.

Soils contaminated by coal tar were collected from four depths (0-2, 3-5, 6-8 and 10-12 m below surface), and uncontaminated soils were collected from three depths (1-2, 4-5, and 6-8 m below surface) at an adjacent location. A total of seven soil samples were used for DNA extraction and further experiments. For the extraction of total DNA from soil, a freeze-and-thaw method [4] was used with some modifications. The biodegradation potential for seven soil samples was estimated by using PCR amplification of dioxygenase genes. To detect and amplify dioxygenase genes from total soil DNA, degenerate oligonucleotide primers were designed for the conserved Rieske iron-sulfur motif from dioxygenases found in many bacterial species capable of degrading neutral aromatic hydrocarbons [4]. These dioxygenases mediate the initial step in conversion of the neutral aromatic hydrocarbons to form

cis-dihydrodiol intermediates. Total DNA was extracted directly from soil samples and dioxygenase genes were amplified using the PCR process and the dioxygenase primers. PCR products (ca. 80 bp) were cloned using pGEM-T vector and over 100 randomly selected clones were sequenced. Clones identified as dioxygenases based on database homology searches using the BLAST algorithm were selected and a phylogenetic tree was generated with the 100 partial dioxygenase gene sequences. Four dioxygenase clones, which were chosen to represent the full range of sequence diversity found in the clonal library, were used as probes in a subsequent round of Southern hybridizations. Total soil DNA isolated from each of the soil samples was used for hybridization with the four dioxygenase genes to estimate the PAH-degrading potential of the soils. The strongest hybridization signal was obtained with DNA extracted from surface soil (0-2 m) from the coal tar-contaminated site and the second strongest signal from the 3-5 m depth at the coal tar-contaminated site. Very weak hybridization was obtained from soils at greater depths and from uncontaminated soils with all three depths. These results correlated positively with the amounts of PAHs extracted from the soil samples.

Degradation of PAHs in the same soil samples by indigenous microorganisms was also tested using radioisotope analyses. ^{14}C-labeled PAHs including phenanthrene, anthracene, pyrene, and benzo(a)pyrene were individually spiked to soil samples and degradation of each compound in individual samples was monitored by determining the evolved CO_2 using a liquid scintillation counter. The biodegradation potential of a farm soil, which had never been exposed to hydrocarbons was also determined. The ability of these soil samples to metabolize ^{14}C-labeled PAHs was compared to the results from the gene probe analyses in order to evaluate these approaches for assessing comparative biodegradative capacities of the soil microbial community. In general, biodegradation of hydrocarbons was the least in the farm soil and more biodegradation was observed in contaminated soils than in uncontaminated soils. The amount of CO_2 evolved was the greatest in contaminated surface soil when phenanthrene was used as a substrate, which was consistent with gene probe analyses. However, for the other hydrocarbons, biodegradation data were not comparable to gene probe analyses.

The fact that soils from higher depths showed higher biodegradation than surface soils while little genetic homology was found by known dioxygenase genes suggests the abundant presence of unknown microorganisms capable of degrading PAHs. It has been proposed that dioxygenases of different genera and substrates specificities are assumed to be more divergent or if independent origin [5]. Therefore, it is plausible to considerably underestimate the true biodegradative potential for the hydrocarbons of a site if the assessment relies on limited known dioxygenase genes and is performed without deliberate considerations on such aspects.

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