Development of a protocol for High Molecular Weight DNA extraction from BTEX- and PAH-contaminated sites

Emna Bouhajja, Mark Liles[1], Isabelle George and Spiros Agathos

Unité de Génie Biologique, Université Catholique de Louvain, Place croix de Sud 2/19,1348 Louvain-la-neuve,Belgium, [1] Department of Biological Sciences, Aubern University, Aubern,Alabama.

Introduction

Metagenomic analysis of polluted sites holds great promise in bioremediation. Microbial communities play a fundamental role in the detoxification of contaminated environments, yet many biodegradative pathways and microorganisms remain little characterized, if not totally unknown. Metagenomics gives direct access to genes involved in degradation processes, which may help designing novel specialized strains and enzymes useful for bioremediation.

The successful application of metagenomics to polluted environments requires appropriate DNA extraction and purification protocols for high yield, high-purity DNA recovery. Indeed, humic acids and pollutants can inhibit DNA polymerases, restriction digestion enzymes and/or reduce the efficiency and specificity of DNA hybridization. Moreover, extraction of high molecular weight DNA is needed to recover complete biosynthetic pathways. This step is crucial for function-based and gene based screening of metagenomic libraries, when entire cluster of genes must be cloned for the function of interest to be expressed.

Here, we describe an improved protocol for isolating, purifying and cloning HMW DNA from BTEX- and PAH (Polycyclic Aromatic Hydrocarbons)-contaminated aquifer sediments.

Methods and Results

The extraction method was tested on a sample from a BTEX- and PAH-contaminated site in Düsseldorf, Germany. It was based on a direct lysis of bacterial cells in the sediment using high-salt extraction buffer, lysozyme, proteinase K and SDS. The crude extracted DNA was purified using pulsed-field gel electrophoresis to eliminate humic acids contaminants. The PFGE showed that the DNA size ranges from 48 kb to 23 kb. The metagenomic DNA within the agarose plug was then treated with high concentration of formamide (to denature the DNA) and sodium chloride (to enhance DNA stability) for removing associated nucleases and increasing the cloning efficiency. Finally, the large gel-fractionated DNA was electroeluted. Comparison of Nano Drop spectrophotometer graphs showed that recovered DNA did not contain proteins or aromatic contaminants.

Conclusion

This HMW DNA purification protocol yielded intact and clean DNA from a highly contaminated site. This result is notable for improving the cloning efficiency needed for cosmid metagenomic library construction from such sites.