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Alternative primer sets for PCR detection of genotypes involved in bacterial aerobic BTEX degradation: Distribution of the genes in BTEX degrading isolates and in subsurface soils of a BTEX contaminated industrial site

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Abstract

Eight new primer sets were designed for PCR detection of (i) mono-oxygenase and dioxygenase gene sequences involved in initial attack of bacterial aerobic BTEX degradation and of (ii) catechol 2,3-dioxygenase gene sequences responsible for meta-cleavage of the aromatic ring. The new primer sets allowed detection of the corresponding genotypes in soil with a detection limit of 10^3–10^4 or 10^5–10^6 gene copies g^{-1} soil, assuming one copy of the gene per cell. The primer sets were used in PCR to assess the distribution of the catabolic genes in BTEX degrading bacterial strains and DNA extracts isolated from soils sampled from different locations and depths (vadose, capillary fringe and saturated zone) within a BTEX contaminated site. In both soil DNA and the isolates, *tmoA*-, *xylM*- and *xylE1*-like genes were the most frequently recovered BTEX catabolic genes. *xylM* and

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xylE1 were only recovered from material from the contaminated samples while *tmoA* was detected in material from both the contaminated and non-contaminated samples. The isolates, mainly obtained from the contaminated locations, belonged to the *Actinobacteria* or *Proteobacteria* (mainly *Pseudomonas*). The ability to degrade benzene was the most common BTEX degradation phenotype among them and its distribution was largely congruent with the distribution of the *tmoA*-like genotype. The presence of *tmoA* and *xylM* genes in phylogenetically distant strains indicated the occurrence of horizontal transfer of BTEX catabolic genes in the aquifer. Overall, these results show spatial variation in the composition of the BTEX degradation genes and hence in the type of BTEX degradation activity and pathway, at the examined site. They indicate that bacteria carrying specific pathways and primarily carrying *tmoA*/*xylM*/*xylE1* genotypes, are being selected upon BTEX contamination.

Keywords: PCR detection; Aerobic BTEX biodegradation; Catabolic gene distribution; BTEX degrading isolates; BTEX contaminated site

1. Introduction

BTEX (benzene, toluene, ethylbenzene and xylenes) are frequently occurring groundwater contaminants. BTEX can be biodegraded under both aerobic and anaerobic conditions and in situ bioremediation, either passive or active, is increasingly applied for the elimination of BTEX in groundwater (Lovley, 2001; Barker et al., 1987). Decisions as to whether a site should be contained and monitored or actively treated are largely made on an empirical basis. Basic knowledge about the distribution, population densities and activities of BTEX degrading organisms at the polluted sites can contribute to rational decision-making (Baldwin et al., 2003). The ability to rapidly and accurately detect BTEX biodegrading bacteria and their activity in the environment is therefore of major interest. This can be done by demonstrating the occurrence of catabolic genotypes involved in BTEX degradation or their corresponding mRNA in the aquifer by employing sensitive PCR and RT-PCR detection methods (Baldwin et al., 2003).

Initial oxidative attack of BTEX converting the compound into a catechol structure and the cleavage of the catechol structure are key steps in aerobic BTEX degradation. As such, both activities are of direct interest as monitoring objects. Initial oxidative attack consists of direct oxidation of the aromatic ring via a mono-oxygenase (Kahng et al., 2001) or a dioxygenase attack (Zylstra and Gibson, 1989; Furukawa et al., 1993) or oxidation of the alkyl side chain which is catalyzed by mono-oxygenases (Burlage et al., 1989). Ring cleavage occurs by catechol 2,3-dioxygenases (C23O) after which the structure is further degraded into Krebs cycle intermediates (Harayama and Rekik, 1993). Phylogenetic studies of amino acid sequences of the proteins involved show that they can be divided into specific families and subfamilies showing significant sequence homology and indicating a common ancestry which allowed the design of group-specific primer sets for detection by PCR (Baldwin et al., 2003; Eltis and Bolin, 1996). In the past, several studies have reported PCR primers to detect and quantify the presence of specific genotypes encoding those key steps in BTEX biodegradation and their mRNA in environmental samples (Baldwin et al., 2003; Hallier-Soulier et al., 1996; Junca and Pieper, 2003; Mesarch et al., 2000; Meyer et al., 1999; Ogram et al., 1995; Okuta et al., 1998; Ringelberg et al., 2001). However, the recent availability of a lot of new sequence information on BTEX degradation genes indicates that previously published primer sets are not always suitable (Baldwin et al., 2003; Junca and Pieper, 2003). In addition, for some BTEX catabolic protein families there are no primers reported yet (Baldwin et al., 2003). Therefore, design and/or redesign of primers for PCR detection of BTEX catabolic genes is required.

Previously, we reported the design of a degenerate primer set for the detection of *tmoA*-like genes, encoding α-subunit of subfamily 2 of the hydroxylase component of bacterial multi-component mono-oxygenases involved in BTEX degradation (Hendrickx et al., submitted). In this study, we report (i) an alternative primer set for detection of genes encoding subgroup I of subfamily 1 α-subunit of the hydroxylase component of bacterial multi-component mono-oxygenases, (ii) a new primer set for detecting genes