Catabolic Genes Dependent Detection of Aerobic BTEX Degraders

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Overview

Aromatic hydrocarbons are common groundwater and soil contaminants due to their frequent usage in industrial process and leakages of petroleum during drilling, transportation and storage. Monoaromatic hydrocarbons also known as BTEX are relatively water soluble compared to other hydrocarbons allowing them to transfer far away from contaminated region. Bioremediation is thought as more advantageous for removing of BTEX from environment due to its cost effectiveness. Biodegradation of BTEX have been well studied and many bacteria have been found as BTEX degrader however distribution of the key genes and exact metabolic pathways in aerobic BTEX degradation, are still unclear. In this study, aerobic BTEX degradation genes *todC1*, *tmoA*, *xylA* and *catA* were screened by polymerase chain reaction (PCR) bacterial strains isolated from petroleum hydrocarbon contaminated river. It was shown that bacteria is frequently harboring *todC1* and *catA* genes encoding the ring deoxygenation enzymes and ortho cleavage enzymes respectively.

Introduction

Monoaromatic hydrocarbons, including benzene, toluene, ethylbenzene, and xylene, collectively called BTEX, are major components of gasoline and are thought to be the most significant contaminants of soil and groundwater due to frequent leakages from underground storage tanks and accidental spills [1]. Degradation of BTEX compounds by bacteria is known to be one of the most efficient ways to remove these compounds from soil and groundwater [2]. There have been extensive studies demonstrating that BTEX degradation is performed by variety of pathways including the oxidation of methyl group, ring monooxigenation at positions 2, 3, or 4 or ring dioxygenation using the toluene as a model hydrocarbon. In lower pathway, ring cleavage is mediated by catechol dioxygenases after which the molecule is further degraded into citric acid cycle [3] (Figure 1.). Therefore, this study aimed at secreening of aerobic BTEX degraders with their corresponding catabolic genes; *xylA* (291 bp), *todC1* (510 bp), *tmoA* (505 bp) and *catA* (282 bp) by ploymerase chain reaction (PCR).



Figure 1. An aerial view of the oil spilled from Deepwater Horizon platform into the Gulf of Mexico in 2010 (Picture: REUTERS).

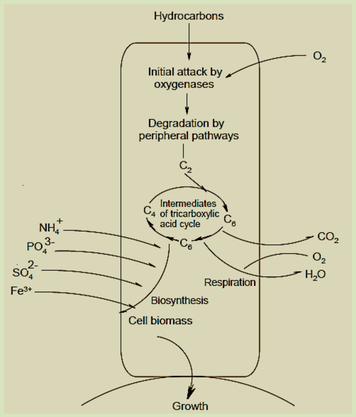


Figure 2. Main principles of aerobic hydrocarbon degradation by microorganisms [4]

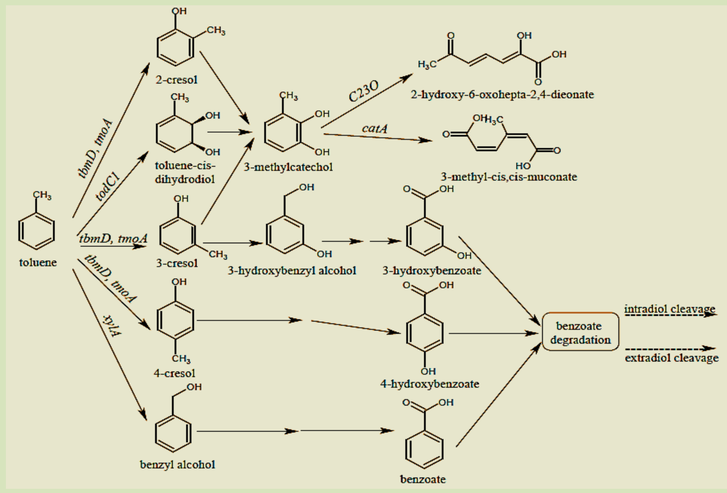


Figure 3. Aerobic routes in toluene degradation as a model (modified from KEGG)

Methods and Materials

20 different bacteria previously isolated and identified by our lab from river water contaminated with petroleum hydrocarbons, including the strains of *Pseudomonas plecoglossicida* Ag10, *Raoultella planticola* Ag11, *Staphylococcus aureus* Al11, *Staphylococcus aureus* Ba01, *Stenotrophomons rhizophila* Ba11, *Delftia acidovora* Cd11, *Staphylococcus warneri* Co11, *Pseudomonas koreensis* Cu12, *Acinetobacter calcoaceticus* Fe10, *Pseudomonas koreensis* Hg10, *Pseudomonas koreensis* Hg11, *Staphylococcus aureus* Li12, *Serratia nematodiphila* Mn11 *Acinetobacter haemolyticus* Mn12, *Comamonas testosteroni* Ni11, *Acinetobacter johnsonii* Sb01, *Pantoea agglomerans* Sn11, *Micrococcus luteus* Sr02, *Micrococcus luteus* Sr11, *Acinetobacter haemolyticus* Zn01, were used in this study [5]. Bacteria were routinely grown in nutrient broth for DNA extraction. Total DNA extraction was done by alkaline lysis method with some modifications [6]. Isolated DNA was used as a template and PCR was carried out by using the xylA (side chain monooxygenase), todC1 (ring hydroxylating dioxygenase), tmoA (ring hydroxylating monooxygenase), and catA (ring cleavage dioxygenase) primer sets in a 25 µl reaction mixture containing 50 ng template DNA, 5 pmol forward and reverse primers, 100 µM of each dNTP, and 2,5 µl NEB 10X Taq reaction buffer and 1,25 U Taq DNA polymerase (NEBM0230). Finally, PCR products were run on %1 agarose gel and visualized under UV light by using Quick-Load 100 bp DNA ladder (100-1517 bp) as marker.

Table 1. Primers used in the study [3]

Primers	Primer Sequence	Amplicon Size	Annealing temp (°C)	$MgCl_2$ (mM)
tmoA	5′-CGAAACCGGCTT(C/T)ACCAA(C/T)ATG-3′ 5′-ACCGGGATATTT(C/T)TCTTC(C/G)AGCCA-3′	505	61.5	2
todC1	5′-CAGTGCCGCCA(C/T)CGTGG(C/T)ATG-3′ 5′-GCCACTTCCATG(C/T)CC(A/G)CCCCA-3′	510	66	2
xylA	5′-CCAGGTGGAATTTTCAGTGGTTGG-3′ 5′-AATTAACTCGAAGCGCCCACCCCA-3′	291	64	2
catA	5′-GCCAACGTCGACGTCTGGCA-3′ 5′-CGCCTTCAAAGTTGATCTGCGTGGT-3	282	57	2

Results

For the detection of genes encoding the initial step enzymes of aerobic BTEX degradation, three different primers were used. *tmoA* primer was used to amplify the gene region encoding the subfamily 2 of a-subunits of hydroxylase component of multi-component monooxygenases but no PCR product was detected. Secondly, *xylA* primer was used to screen the genes encoding the electron transfer component of two-component side chain monooxygenases involved in toluene and xylene degradation. No *xylA* related sequence was found by PCR. Differ from monooxygenation, ring oxidation is catalyzed by deoxygenation enzymes including toluene/benzene/ethylbenzene dioxygenase also know as aromatic ring hydroxylating dioxygenases (RHDO). In order to detect genes producing the subfamilies D.1.B+D.1.C+ D.2.A+D.2.B+D.2.C of a-subunits of type D iron–sulfur multi-component RHDO enzymes. According to PCR results, it was shown that 14 bacteria harbored *todC1* gene (Figure 4.A). *catA* primer was used to amplify the *catA* gene encoding the catechol 1,2 dioxygenase enzyme.(c12o). Ortho cleavage of aromatic ring is mediated by c12o enzyme. PCR results demonstrated that catA gene was deected in 10 bacteria (Figure 4.B).

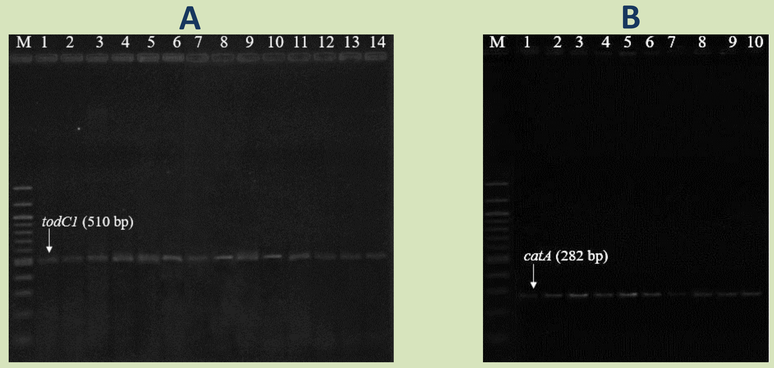


Figure 4. (A), Agarose gel picture of *todC1* gene harboring bacteria; *R. planticola* Ag11 lane 1, *S. rhizophila* Ba11 lane 2, *S. warneri* Co11 lane 3, *P. koreensis* Cu12 lane 4, *A. calcoaceticus* Fe10 lane 5, *P. koreensis* Hg10 lane 6, *P. koreensis* Hg11 lane 7, *S. aureus* Li12 lane 8, *S. nematodiphila* Mn11 lane 9, *A. haemolyticus* Mn12 lane 10, *C. testosteroni* Ni11 lane 11, *A. johnsonii* Sb01 lane 12, *P. agglomerans* Sn11 lane 13, *M. luteus* Sr11 lane 14. (B), Agarose gel picture of *catA* gene harboring bacteria; *R. planticola* Ag11 lane 1, *S. aureus* Al11 lane 2, *S. aureus* Ba01 lane 3, *D. acidovorans* Cd11 lane 4, *P. koreensis* Cu12 lane 5, *A. calcoaceticus* Fe10 lane 6, *P. koreensis* Hg10 lane 7, *P. koreensis* Hg11 lane 8, *S. nematodiphila* Mn11 lane 9, *M. luteus* Sr11 lane 14. M NEB 3231 100 bp DNA Ladder (100 - 1500 bp, 12 fragments from bottom to top; 100-200-300-400-500-600-700-800-900-100-1200-1517 bp)

Conclusions

For successful bioremediation, genotypes of bacteria related with BTEX degradation should be deciphered. The genes encoding the aerobic BTEX degradation enzymes are commonly found in operons (TMO and TOD pathways) and often located on catabolic plasmids (TOL pathway) . In conclusion, catabolic genes in aerobic BTEX degradation were screened among 20 different bacteria by PCR. The study revealed that, in 20 potential BTEX degraders, *todC1* and *catA* genes were harbored in almost all isolates used in the study. According to these results, it can be emphasized that initial step of BTEX degradation is catalyzed by deoxygenation enzymes followed by c12o enzyme responsible for *ortho* cleavage of aromatic ring.

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