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Characterization of the BTEX-degrading pathway genes in *Ralstonia* sp. strain PHS1

Sun Bok Lee[1,2] and Sung-Kuk Lee[2]

[1]Department of Chemical Engineering, [2]School of Environmental Science and Engineering
Pohang University of Science and Technology, Pohang 790-784, Korea

Abstract

A thermotolerant bacterium, designated as PHS1, was isolated from a hot spring in Pohang, Korea, on the basis of its ability to grow on BTEX as a sole carbon source. We cloned and sequenced the entire BTEX-degrading pathway genes of PHS1 and found that two multicomponent mono-oxygenases together with *meta*-pathway genes are responsible for the BTEX biodegradation.

Introduction

Aromatic compounds are common pollutants in many industrial wastewaters, such as those produced from coal-conversion plants, oil refineries, petrochemical plants, and phenolic-resin industries. In particular, the non-oxygenated mono-aromatic hydrocarbons, which include benzene, toluene, ethyl-benzene, and xylenes, are of concern because they are confirmed or suspected carcinogens, even at very low concentration. Of them, the degradation of *o*-xylene is an important issue, since *o*-xylene is a compound of considerable environmental interest owing to its recalcitrancy. Although a large number of pure cultures capable of degrading *m*- and *p*-xylene through the progressive oxidation of a methyl group are known, very few microorganisms have been reported to degrade *o*-xylene through direct oxygenation of the aromatic ring.

In treating contaminated wastes, on the other hand, higher temperatures have the advantages of both increasing the solubility of the substrates and lowering the risk of contamination by pathogenic microorganisms. In addition, thermophilic microorganisms can be used for bioremediation as a polishing step following a thermal gasoline remediation process. Nevertheless, little information is available on thermophilic bacteria capable of degrading aromatic compounds. In this presentation, we report on the isolation and characterization of BTEX-degrading thermotolerant bacteria.

Materials and Methods

The thermotolerant bacterium used in this study was enriched from a water sample from a hot spring in Pohang, Korea (1). The genomic DNA was digested partially with restriction endonuclease *Sau3A1*. After agarose gel electrophoresis, fractions containing DNA enriched fragments of 5 to 15 kb were eluted from the agarose slice. The fragments were ligated into the pBluescript vector, which was digested with *Bam*HI and treated with alkaline phosphatase. Transformants containing oxygenase were identified on LB agar plates containing 100 μM ampicillin and 2 mM indole. After 3 days of growth, the colonies were inspected for the accumulation of blue pigment.

Results and Discussion

Strain PHS1 is a gram-negative, rod-shaped aerobe and grows optimally at 42 and pH 7.2. According to 16S rDNA analysis, strain PHS1 showed highest similarity to *Ralstonia eutropha* (previously named *Alcaligenes eutrophus*). Unlike its closest known *Ralstonia* species, however, strain PHS1 was able to utilize toluene, ethylbenzene, *o*-xylene, and both *m*- and *o*-cresol. The degradation of *o*-xylene by strain PHS1 is particularly important, since *o*-xylene is a compound of considerable environmental interest, owing to its recalcitrance; and very few microorganisms have been reported to utilize *o*-xylene as a sole carbon source (2). It was found that strain PHS1 transformed *o*-xylene to 2,3-dimethylphenol through direct oxygenation of the aromatic ring.

The genes involved in BTEX utilization were clustered in a region of 18.2 kb, comprising twenty-one genes grouped in three functional parts (Fig. 1). The first monooxygenase (Btxm1) shared similarity with polypeptides of toluene-2-monooxygenase (*Burkholderia cepacia* G4, *Pseudomonas* sp. JS150) and those of phenol hydroxylase (*Comamonas* TA441/R5, *P. putida* P35X/CF600/H). The second monooxygenase (Btxm2) was homologous to toluene-3-monooxygenase (*B. pickettii* PK01), toluene/*o*-xylene monooxygenase (*P. stutzeri* OX1), toluene-4-monooxygenase (*P. mendocina* KR1), and benzene/toluene monooxygenase (*B. cepacia* AA1). By using a monooxygenase-catechol 2,3-dioxygenase fusion reporter system and the metabolite analyses, Btxm1 involved in *o*-xylene catabolism was identified as a toluene-2-monooxygenase. In the case of Btxm2, a mixture of *o*-cresol, *m*-cresol, and *p*-cresol was formed from toluene, indicating that Btxm2 is a toluene monooxygenase with low regioselectivity. It was also found that the *meta*-pathway genes of PHS1 have distinctive features present in both á- and ã-subgroup proteobacterial *meta* operons (3).

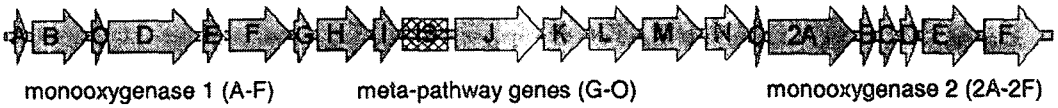

Fig. 1. Genetic map of the *Ralstonia* sp. PHS1 locus coding for BTEX-degrading genes

References

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