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Molecular Genetic Mechanism of Aromatic Compound Biodegradation by soil Streptomycetes

Eung-Soo Kim
Dept. of Environmental Science, Hankuk University of Foreign Studies, Korea

Abstract

A Southern-hybridization analysis and size-selected DNA library screening led to the isolation of a 6.3-kbp *S. setonii* DNA fragment, from which the C12O-encoding genetic locus was found to be located within a 1.4-kbp DNA fragment. A complete nucleotide sequencing analysis of the 1.4-kbp DNA fragment revealed a 0.84-kbp ORF, which showed a strong overall amino acid similarity to the known high-G+C gram-positive bacterial mesophilic C12Os. The heterologous expression of the cloned 1.4-kbp DNA fragment in *E. coli* demonstrated that this C12O possessed a thermophilic activity within a broad temperature range and showed a higher activity against 3-methylcatechol than catechol or 4-methy-catechol, but no activity against protocatecuate.

Introduction

Streptomyces setonii (ATCC 39116), originally isolated from vanilate-enriched Idaho soil, is a gram-positive thermophilic actinomycete which degrades various single aromatic compounds including phenol or benzoate at the optimum temperature of 45 ×C through a catechol intermediate via *ortho*-cleavage pathway using catechol 1,2-dioxygenase (C12O) (1). In this manuscript, we present the complete nucleotide sequence of the thermophilic C12O-encoding gene, *catA* isolated from *S. setonii*, and enzyme characteristics of the heterologously-expressed *S. setonii* C12O in *E. coli*.

Materials and Methods

A pair of degenerate PCR primers were designed based on the central conserved regions of the known C12O-encoding genes and the Southern-hybridization was performed (2). The deduced amino acid sequence alignment of *catA*s among gram-positive bacteria were performed with Multiple Sequence Alignment with hierarchical clustering. The *E. coli* containing the plasmid was cultivated, harvested, and disrupted by sonification. The clear supernatant was used as a crude lysate for both C12O enzyme assay and SDS-PAGE (3).

Results and Discussions

The PCR, Southern-hybridization, and library screening led to an isolation of a 6.3-kbp *Pst*I fragment (named pESK002) and the C12O-encoding genetic locus was localized into a 1.4-kbp *BamH* I-*EcoR* I fragment (pESK002-9). The 1.4-kbp DNA insert of pESK002-9 was completely sequenced, revealing two complete open reading frames, the sizes of which were 0.84-kbp and 0.3-kbp, respec-

tively. A DNA database search using these ORFs revealed that the ORF1 showed a significant similarity with the previously reported gram-positive C12O-encoding gene (*catA*).. However, a much less significant similarity was found between the *S. setonii* ORF1 (hereafter named *catA*) and other known gram-negative bacteria *catA* genes. As expected, the characteristic regions conserved in all known C12O genes (e.g. Iron-binding site) were well preserved within the *S. setonii catA*. The crude lysate supernatant of *E. coli* containing pESK002-9 was used for an *in vitro* C12O enzyme assay. Only the crude lysate supernatant from the *E. coli* containing pESK002-9(+) showed a clear catechol to *cis,cis*-muconate conversion activity, but no such activity exhibited by the supernatant containing the plasmid with an oppositely-cloned insert, pESK002-9(-). A putative C12O protein band was also clearly detected on SDS-PAGE only from the *E. coli* containing pESK002-9(+), but not from the *E. coli* containing the pESK002-9(-). Interestingly, this C12O possessed a thermophilic enzyme activity within a broad temperature range from 25°C up to 65°C, and also showed a higher enzyme activity against 3-methylcatechol than catechol or 4-methycatechol, but no activity against protocatecuate.

References

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