Environmental Engineering and Management Journal

March 2012, Vol.11, No. 3, Supplement, S3
http://omicron.ch.tuiasi.ro/EEMJ/

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INDUCTION MECHANISM OF BIPHENYL/PCB-DEGRADATION PATHWAY IN A *RHODOCOCCUS* DEGRADER

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Abstract

Rhodococcus jostii RHA1 is a gram-positive bacterium, and degrades polychlorinated biphenyls (PCBs) during the growth on biphenyl or ethylbenzene. Its biphenyl/PCB degradation pathway enzyme genes are distributed among five gene clusters, and the transcription from respective promoter is induced in the presence of biphenyl or ethylbenzene. These five promoters share an 18-bp consensus sequence, which is deduced to be involved in transcriptional activation in the presence of ethylbenzene by two sets of two-component regulatory systems (BphST systems). The BphST systems are composed of the BphS1/BphS2 sensor kinases and the BphT1/BphT2 response regulators, and are encoded by *bphS1T1* and *bphS2T2* gene sets. In this study mutational analysis of consensus sequence in *bphAa* promoter were performed to elucidate the induction mechanism of biphenyl/PCB degradation pathway.

The consensus sequence of *bphAa* promoter was mutagenized by site-directed mutagenesis and the resulting *bphAa* promoter derivatives were connected to the *luxAB* luciferase gene in a reporter plasmid, which was introduced into *R. erythropolis* IAM1399 containing a *bphS1T1* plasmid. The resulting transformants were subjected to luciferase assay. Some mutations in the consensus sequence significantly altered promoter activity of *bphAa* promoter not only in the presence but in the absence of ethylbenzene, suggesting that the 18-bp consensus sequence is involved in the BphST-dependent induction of transcription from *bphAa* promoter.

A hybrid promoter was also constructed by connecting the 18-bp consensus sequence with the part of *benA* promoter, which is responsible for benzoate dioxygenase gene transcription. This artificial hybrid promoter was subjected to mutagenesis similarly, and the resultant plasmids were introduced into *R. erythropolis* IAM1399 containing a *bphS1T1* plasmid. The resulting transformants were subjected to luciferase assay, and some mutations surrounding the consensus sequence significantly altered promoter activity of the hybrid promoter derivatives not only in the presence but in the absence of ethylbenzene, suggesting that the 24-bp consensus sequence including the 18-bp is required and enough for the BphST-dependent induction of transcription from the hybrid promoter. The 24-bp consensus sequence is conserved among the corresponding degradation gene sequences of other degraders. The results of this study provide the suitable promoter sequence, which is useful for constructing a degrader derivative expressing its degradation pathway constitutively in the absence of an inducing substrate.