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Cloning and nucleotide sequencing of *catBCA* genes responsible for catechol dissimilation from *Pseudomonas putida* SM25

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Pseudomonas putida SM25 capable of growing on phenol as sole carbon source were isolated from wastewater. To identify catechol catabolic pathway, cell-free extracts from *P. putida* SM25 culture were used to measure catechol 1,2-dioxygenase and catechol 2,3-dioxygenase enzyme activities. Activities of catechol 1,2-dioxygenase was showed more higher than catechol 2,3-dioxygenase. From the result, phenol was converted to catechol, which undergone *ortho* cleavage and further metabolism via the β -keto adipate pathway. *catA* and *catBC* genes were cloned from chromosomal DNA of *P. putida* SM25 using Polymerase Chain Reaction (PCR). *catA* and *catBC* genes which obtained from PCR products were cloned in pT7Blue(R), named pCAA4 (1.3kb) and pCAB6 (1.9kb), respectively. The nucleotide sequence of *catA* was determined and analyzed. An open reading frame corresponding to *catA* encoding catechol 1,2-dioxygenase was composed 936 nucleotides with ATG initiation codon and TGA termination codon. Calculation of the G+C content *catA* gene gave values of 63% and 65.3%, respectively. Homology between *catA* of pCAA4 and *P. putida* mt-2 in both the amino acid (95%) and the DNA sequences (91%) was high. Sequencing of insert DNA (1911 bp) of the plasmid pCAB6 was carried out and it was found the presence of two open reading frames (ORFs) : the larger 1122 bp ORF corresponds to the *catB* gene and the smaller 294 bp ORF corresponds to the *catC* gene. The G+C contents of *catB* gene encoding *cis,cis*-muconate lactonizing enzyme and *catC* gene encoding muconolactonizing isomerase were 63.5% and 62.2%, respectively. It assumed that the *catBC* genes were tightly linked and were transcribed from a single promoter located on upstream of *catB* gene. The deduced amino acid sequences of *catB* and *catC* were consisted 374 and 97 amino acid, respectively, and showed the homologies of 94% and 91% with those of *P. putida* RB1, respectively.