Cloning and Sequence Analysis of The Benzaldehyde Dehydrogenase From Rhodococcus ruber UKMP-5M

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Background & Objectives: The benzaldehyde dehydrogenase (BZDH) is encoded by xylC gene, which catalyzes the conversion of benzaldehyde to benzoate. This gene has a role in different pathways such as toluene degradation and mandelate pathway.

Methods: The gene xylC was amplified by polymerase chain reaction (PCR). The purified fragment xylC was ligated into pGEM-T vector and then was transformed in *E. coli* DH5α as a cloning host. The positive transformants were screened by colony PCR and double digestion using NdeI and HindIII. The recombinant plasmid pGEMT-xylC was sent for sequencing. The ability of positive clones to grow in benzaldehyde was assessed.

Results: The xylC gene of *Rhodococcus ruber* UKMP-5M was amplified at 64°C and cloned into E. coli successfully. The positive transformant was able to utilize benzaldehyde at the range of 0.5-2 mM as a carbon source. The xylC gene contains 789 nucleotide acids, encoding a protein of 263 amino acids with one catalytic domain of aldehyde family. The nucleotide sequence shows a high similarity of 90% with xylC sequence of R. aetherivorans strain I24. The amino acid analysis showed the BZDH from R. ruber UKMP-5M has 51% identity with benzaldehyde dehydrogenase in Pseudomonas putida. The amino acid alignments were determined that the most of aldehyde dehydrogenase have four catalytic sites but this protein has only three residues in N-terminal side. This BZDH has just 15 from 24 NAD binding sites, which has role in chemical binding location.

Conclusion: The recombinant BZDH from *R. ruber* UKMP-5M is able to degrade benzaldehyde, which is essential in many biodegradation processes such as toluene degradation.

Keywords: Benzaldehyde Dehydrogenase; Sequence; Cloning; *Rhodococcus ruber*