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Cloning and characterization of catechol 1,2-dioxygenase from *Rhodococcus rhodochrous* EL-GT

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Introduction

Catechol 1,2-dioxygenase (CD) plays a key role in catabolism pathways of many aromatic compounds (Nakai et al., 1995). Catechol are cleaved cis,cis-muconate by the dioxygenase with the incorporation of molecular oxygen (Briganti et al., 1997). It has been reported that variety of bacteria such as *Rhodococcus* sp. AN-22, *Acinetobacter radioresistens* S13, *Acinetobacter calcoaceticus*, *Arthrobacter* sp. mA3, *Alcaligenes eutrophus* CH34, *Pseudomonas putida* C1, *Streptomyces setonii* produced CD protein. *Rhodococcus rhodochrous* EL-GT producing CD protein was isolated and characterized by previous studies (unpublished). In this study, we identified CD sequence from the *R. rhodochrous* EL-GT and induced CD protein with histidine tag using pET vector system.

Materials and Methods

To identify partial CD sequence from *R. rhodochrous* EL-GT, we are carried out PCR with ptCD-F and ptCD-R primer. The PCR product was analyzed sequence after subcloning. LA-PCR was used in order to amplify the unknown CD sequence and primers were designed from known partial sequence. Coding sequence of CD was amplified and ligated into pET16b expression vector including histidine tag after digestion with *Nde* I. The ligated product was transformed into *E. coli* BL21 (DE3) cell and positive clone was selected. The clone was incubated in LB ampicillin broth and induced CD protein by IPTG addition. The expressed protein was identified after lysis with ultra-sonication by SDS-PAGE and checked activity. Expressed CD protein was purified by using His·Bind Kit according to protocol. Protein concentration was determined by BCA™ Protein Assay Kit and specific activity of the expressed protein was assayed.

Results and Summery

To amplify partial sequence of CD, we designed a pair of primer by comparing CD sequences of other bacteria. In order to obtain the full length CD sequence, primers were designed from the partial sequence and amplified forward and reverse sequence of the partial sequence by LA-PCR. The amplified sequence showed 1619 bp nucleotide in length and ORF of 849 bp (283 amino acid). The sequence of CD gene was 96.7% similar to catA2 gene of *Rhodococcus* sp. AN-22. Two primers were designed to amplify the ORF. *Nde*I restriction enzyme site was included to the primers to facilitate the ligation into pET16b vector. CD ORF was amplified from chromosomal DNA of *R. rhodochrous* EL-GT and ligated into pET16b vector after digestion with *Nde*I. The recombinant DNA was transformed into *E. coli* BL21 (DE3) expression cell for overproduction of CD and selected positive clone. The clone was incubated in LB ampicillin broth and inducted after adding IPTG. The expressed protein was identified with SDS-PAGE after lysis. The expressed protein was bound with histidine tag to N-terminal of CD due to the presence of histidine tag sequence in pET16b vector. Theoretical pI and Mw of the fusion protein were 5.55 and 34.1 kDa, respectively. Purification of fusion protein was separated by Ni column of His·Bind Kit and identified by SDS-PAGE.

References

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