P46

Molecular Characterization of the Genes Encoding Acetoacetyl CoA Transferase Operon from Serratia maecescens KCTC2172

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A DNA fragment pCKB13 containing two genes encoding CoA transferase was isolated from a genomic DNA library of S. marcescens KCTC 2172. The complete nucleotide sequence of pCKB13 consisting of 2081 bp contained two open reading frames (Acot and ScotB) and 5'-partial region for ScotA, showing high homology with Coenzyme A (CoA) transferases, Acetoacetyl CoA transferase (Acot) and Succinyl CoA transferase (Scot), which catalyze the reversible transfer of CoA from one carboxylic acid to another. Two different recombinant pasmid DNA, pSCO123 and pSCO989, containing two genes encoding transcriptional regulatory protein (TR) and ScotA were also isolated and their nucleotide sequence was determined. The putative promotor region, very conserved -10 box and -35 box, was located on the upstream of scotA gene, and a putative ribosomal binding site existed 6 bp upstream from ATG start codon, the promoter for the TR and scot operon genes were adjacent to one another and the two genes were transcribed divergently. The binding site for CRP was located at the upstream of a putative promoter region. Compared amino acid sequences of CoA transferases reported previously, DN-GN-[LIVMFA]-G-G-F-X-X-X-G-X-P corresponding to the conserved CoA binding domain and SENG corresponding to the conserved in all homologous transferase were detected in ScotA and ScotB, respectively. The recombinant enzyme expressed in E. coli cells by induction with IPTG exhibited a high Coenzyme A transferase activity, and the corresponding protein was detected by SDS-PAGE. The protein was purified to homogeneity with two sequential chromatography including DEAE-Sepharose and CM-Sepharose ion exchange column.