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Analysis of cAMP regulation-related genes in *Serratia marcescens*

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Five different clones obtained from *Serratia marcescens* relate cAMP regulation in *E. coli* TP2139 (*Δlac, Δcrp*). pCKB12, 13, and 15. The *crp* gene clone, pCKB12, was confirmed by Southern hybridization and the stimulation of the β-galactosidase activity. The nucleotide sequence of the *crp* region consisting of 1979 bp was determined. The sequencing of the fragment led to the identification of two open reading frames and one of these, the *crp* gene, encoded 210 amino acids, and the other encoded a truncated protein. The *S. marcescens* and *E. coli crp* genes presented a higher degree of divergence in their nucleotide sequence with 120 changes, however, the corresponding amino acid sequences presented only two amino acid differences. Not yet, an analysis of the amino acid divergence, revealed that the catabolite gene activator protein, the *crp* gene product, is the most conserved protein observed so far. Using a *crp-lac* protein fusion, it was demonstrated that *S. marcescens* CRP can repress its own expression, probably *via* a mechanism similar to that previously described for the *E. coli crp* gene. A DNA fragment pCKB13 containing two genes encoding CoA transferase was isolated from a genomic DNA library of *S. marcescens* KCTC2172. The complete nucleotide sequence of pCKB13 consisting of 2081 bp was determined. Sequencing of the fragment led to the identification of two open reading frames showing high homology with Coenzyme A (CoA) transferases, Acetoacetyl CoA transferase (Acot) and Succinyl CoA transferase (Scot), enzymes catalyzing the reversible transfer of CoA from one carboxylic

acid to another. Therefore, we have confirmed that the clone, pCKB13 codes for Coenzyme A transferase gene by partial nucleotide sequencing in the terminal region. So pSCO123 and pSCO989 were designed to get upstream of *scotB, scotA* and putatived promotor region. The truncated protein of ScotA gene is located directly upstream of *scotB*, with a same direction of transcription. Active site and CoA binding site motif of ScotA and B was highly conversed. The enzyme activity of Coenzyme A transferase increased after introduction of the multicopy of the cloned gene in *E. coli*. The recombinant protein overexpressed by multicopy and induction with IPTG, the polypeptide of 42-kDa, was confirmed by SDS-PAGE. The protein was purified to homogeneity through two sequential chromatographic techniques including DEAE-sepharose ion exchange and CM-sepharose.

The nucleotide sequence of the 2.9 kb *Bam*HI fragment of pCKB15 was determined. Sequence analysis revealed the presence of two open reading frames, one of the truncated ORF was identified as the aconitase gene.