J. Microbiol. Biotechnol. (2001), **11**(5), 870–875

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

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Molecular Characterization of the Genes Encoding Acetoacetyl-Coenzyme A Transferase from *Serratia marcescens* KCTC 2172

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Received: May 24, 2001
Accepted: August 24, 2001

Abstract A DNA fragment, pCKB13, containing two genes encoding Coenzyme A transferase, was isolated from a genomic DNA library of *S. marcescens* KCTC 2172. The complete nucleotide sequence of the 2,081-bp *Bam*HI fragment on pCKB13 was determined. Sequencing of the fragment led to the identification of two open reading frames showing high homology with two 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. The enzyme activity of Coenzyme A transferase increased after introducing the multicopy of the cloned gene in *E. coli*. The recombinant protein, overexpressed by multicopy and induction with IPTG, was a polypeptide of 42 kDa, as confirmed by SDS-PAGE. The protein was purified to homogeneity through three sequential chromatographic procedures including ion-exchanged DEAE-sepharose, CM-sepharose, and Mono Q.

Key words: *Serratia marcescens*, acetoacetyl- and succinyl-CoA transferase genes, gene cloning and expression

Serratia marcescens is an enteric bacterium closely related to *E. coli*, and secretes several enzymes through the membrane into the culture broth, including nucleases, lipase, protease, and chitinases [7]. In a recent study, Gyaneshwar *et al.* [10] reported that *S. marcescens* IRBG500 acts as a plant growth-promoting bacteria under field conditions.

Coenzyme A (CoA)-transferases are enzymes catalyzing reversible transfer of CoA from one carboxylic acid to another, and have been identified in many prokaryotes. Although the CoA-transferases appear to be mechanistically and functionally very similar, their substrate specificities and activities are quite different [22].

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The acetate (succinate)-acetoacetate-CoA transferase (EC 2.8.3.9) found among *Clostridia* acts mainly to detoxify the medium by removing the acetate and butyrate excreted earlier in the fermentation. Therefore, this enzyme has a fundamentally different role from other CoA-transferases, and is usually involved in the uptake of substrate for energy and structural use [4, 6, 8].

The succinyl CoA:3-oxoacid-CoA transferase (SCOT, EC 2.8.3.5) is responsible for the formation of acetoacetyl-CoA by undergoing a transfer of a CoA moiety from succinyl-CoA to a 3-oxoacid, usually acetoacetate. This enzyme has been found to have the highest activity in hearts and kidneys of various mammals [5].

The best characterized CoA-transferases are the β-ketoadipate-CoA transferases of *Pseudomonas putida* and *Acinetobacter calcoaceticus*, the butyrate-acetoacetate-CoA transferase from *Clostridium acetobutylicum*, and the succinyl-CoA:3-oxoacid-CoA transferase found in mammalian mitochondria. The β-ketoadipate-CoA transferase (β-ketoadipate:succinyl-CoA transferase, EC 2.8.3.6) carries out the penultimate step in the conversion of benzoate and 4-hydroxybenzoate to tricarboxylic acid cycle intermediates in bacteria utilizing the β-ketoadipate pathway, and several genes of the CoA transferase in bacteria were well characterized [5, 6, 8, 17, 20, 22, 24].

In this study, we report on the cloning and identification of the genes, designated *acot*, encoding the subunits of acetoacetate-CoA transferase from *S. marcescens*.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Culture Conditions

S. marcescens KCTC 2172 strain was purchased from the Korea Culture Type Collection [7]. The *E. coli* JM109 and TP2139 were used for host strains [12]. pBluescript KS(+) (Promega, Madision, U.S.A.) and pKK223-4 (Pharmacia

P-L Biochemical Inc., Wiscosin, U.S.A.) were used for cloning vectors. Bacterial strains were routinely grown and maintained at 37°C. The antibiotic concentration used was 50 µg/ml ampicillin (USB, Buckinghamshire, U.K.). Cultures for enzyme assay were grown in M9 minimal medium with 10 mM potassium acetate and 0.1% casamino acids.

DNA Isolation and Construction of the Recombinant DNA from *S. marcescens*

Total cellular DNA from *S. marcescens* was prepared as previously described [25, 26]. Short-gun library was constructed in the cloning vector pBluscript KS (pBS). Briefly, total DNA from *S. marcescens* KCTC 2172 was partially digested with *Bam*HI to yield various size fragments. The digested DNA was ligated with *Bam*HI-cleaved, dephosphorylated pBS by using T_4 DNA ligase. The short-gun library was determined by introducing *E. coli* TP2139 cells with red colony, followed by plating on MacConkey agar (Difco, Michigan, U.S.A.) medium with maltose (Sigma, Missouri, U.S.A.) as the carbon source. Rapid, small-scale plasmid DNA isolation was performed by the method of Birnbaum and Doly [2] with a Wizard miniprep kit of Promega Biotech, U.S.A. All restriction enzymes, T4 DNA ligase, DNA ligation kit ver. 2, and polynucleotide kinase were obtained from Takara Shuzo Co., Japan, and were used according to the recommendation of the suppliers.

Nucleotide Sequencing

Various restriction fragments derived from the pCKB13 plasmid DNA were subcloned into the same restriction enzyme site of pBluescript (KS+) [18]. Double-stranded DNA was used as templates for sequencing reactions. Both strands of the DNA were sequenced by the dideoxy-chain termination method by using an AutoRead DNA sequencing kit and A.L.F. DNA sequencer (Amersham Pharmacia Biotec., Piscataway, U.S.A.) [19].

Preparation of Cell Extracts and Enzyme Assays

Cultures grown in M9 minimal medium with 10 mM potassium acetate and 0.1% casamino acids were assayed for enzyme activity. The cultured cells were harvested and then suspended in a cell suspension buffer (50 mM MOPS, pH 7.0, 0.5 M $(NH_4)_2SO_4$, 20% [vol/vol] glycerol, 1 mM EDTA). The suspension was sonicated and cell debris was removed by centrifugation. The protein content of the cell extracts was determined by the method of Bradford [1] with bovine serum albumin as the standard (Bio-Rad, California, U.S.A.).

CoA-transferase activity was analyzed aerobically at room temperature for carboxylic acid conversion by monitoring the decreased absorbance in A310 as an indication of the disappearance of the enolate from acetoacetyl-CoA [4, 23]. The assay mixture contained in a final volume of 1.0 ml: 100 mM Tris HCl (pH 7.5), 150 mM carboxylic acid (potassium salt of acetate), pH 7.5, 40 mM $MgCl_2$, 0.1 mM acetoacetyl-CoA, 5% (vol/vol) glycerol, and 50 µl of enzyme solution appropriately diluted in cell suspension buffer. The enzyme unit was defined as the amount of enzyme that can convert 1 µmol of acetoacetyl-CoA per min under the conditions. Crude enzyme extracts were prepared from cells grown overnight in L-broth. The harvested cells were sonicated. The proteins were separated by SDS-polyacrylamide gel electrophoresis by the method of Laemmli [13].

Purification of Acetoacetyl-CoA Transferase

E. coli transformants harboring pCKB13P were cultured to OD_{610} 0.5 at 37°C with vigorous shaking in 1 liter of M9 broth and induced with 0.1 mM IPTG for several hours. The cultured cells were collected by centrifugation. The harvested cells were washed once in 5 ml of 100 mM Tris-HCl, pH 7.5, and suspended in 2 ml of the same buffer. After sonication for 2 min with intermediate cooling, the cell debris was removed by centrifugation at 30,000 ×g for 20 min at 4°C. The protein solution was applied to the columns of DEAE-Sepharose and CM-Sepharose (0.7× 5 cm, Pharmacia). The proteins were then eluted with a linear gradient of KCl (50 to 300 mM). Active fractions from the columns were concentrated with a microconcentrator tube, and were loaded onto a Mono-Q FPLC column (1 ml, Pharmacia) setting up AKTA design system (Amersham Pharmacia Biotec., U.S.A.), and proteins were eluted by increasing the concentration of 50 mM potassium phosphate buffer (pH 7.0). Fractions with high activity were collected.

RESULTS AND DISCUSSION

Identification of the *S. marcescens acot* Gene

In a previous study, we reported several clones from *Serratia marcescens*, which stimulate the cells to use maltose as a carbon source in *E. coli* TP2139 (*Δlac*, *Δcrp*) [25]. One of the cloned genes, pCKB13, was further analyzed. The putative CoA-transferase gene region of pCKB13 was cut with *Eco*RI-*Bam*HI and ligated into the same site of pKK223-4 to construct pACO13, and restriction endonuclease mapping was used to confirm the correct orientation of insert. For construction of pACO13P and pCKB13P, the *Bam*HI-*Pst*I fragment of pACO13 was used to ligate with each vector of pKK223-4 and pBS treated with the same restriction enzyme. We constructed a restriction map of the cloned DNA fragment and several recombinant subclones (Fig. 1). The subclone, pACO13, was positive upon stimulating TP2139 cells to use maltose as a sole carbon source, and pCKB13P was negative (data not shown). The nucleotide sequence of the end region of pCKB13 was determined. During analysis of the nucleotide sequence of the region with a gene data

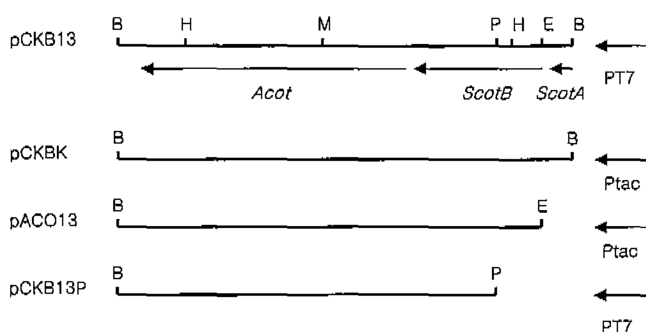

Fig. 1. Physical map of the plasmid pCKB13 of *S. marcescens* and derivative plasmids.
The transcriptional directions of CoA transferase genes are shown by the thick arrows. B, *Bam*HI; E, *Eco*RI; H, *Hinc*II; M, *Mlu*I; P, *Pst*I.

bank, we identified two truncated ORFs. The ORFs were homologous to several bacterial genes encoding acetate and butyrate-acetoacetate-Coenzyme A transferase. Therefore, we have confirmed that the clone, pCKB13, coded for the Coenzyme A-transferase gene, by partial nucleotide sequencing in the terminal region.

Sequencing of a pCKB13 DNA Region

The nucleotide sequences of the complete structural genes as well as that of the untranslated region of the *scotB* gene are shown in Fig. 2. The nucleotide sequence has been submitted to the GenBank Data Bank with accession number AF276244. This fragment encoded two ORFs. Both ORFs were oriented in the same direction, which were separated by only 14 bp, suggesting that the two genes may constitute an operon. ORF1 (*scotB*) encodes a polypeptide of 217 amino acids in length and starts at nucleotide 107 with AUG as the initiation codon. ORF2 (*acot*) encodes a polypeptide of 398 amino acids in length and begins at nucleotide 775, with the unusual initiation codon GUG [8], which normally codes for valine. The gene products of *acot* and *scotB* as deduced from the nucleotide sequence are composed of 394 and 217 amino acids, respectively, with a molecular mass of 41,900 and 23,200 Da, respectively. With respect to the sequence data presented, the 3'-end of the insert contained the end of the *acot* gene, which is known to form a monocistronic operon. Furthermore, the truncated hydroxybutyrate dehydrogenase

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GGATCCGGCGGCGATCCACACCCCGGCATTTACGTCGATCGGCTGATCCAGGGCACCTTC   60
GAGAAGCGTATCGAACAACGCACCTGCGGGCATAAGGAGAATTCGCATGCTTACCCGTGA  120
                                  SD   SCOTB ↑ M  L  T  R  E
ACAAATGGCGCAGCGCGTGGCGCAAGAGCTGAGGGACGGTTACTACGTCAACCTGGGCAT  180
 Q  M  A  Q  R  V  A  Q  E  L  R  D  G  Y  Y  V  N  L  G  I
CGGCATCCCGACGCTGGTGGCCAACTACGTGCCGGCGGGCATGGACGTGATGCTGCAGTC  240
 G  I  P  T  L  V  A  N  Y  V  P  A  G  M  D  V  M  L  Q  S
GGAAAACGGCCTGCTGGGGATGGGCGAGTTCCCGACCGAGCAGCAGCTGGACGCCGACAT  300
 E  N  G  L  L  G  M  G  E  F  P  T  E  Q  Q  L  D  A  D  M
GATCAACGCCGGCAAGCAGACGGTCACCGCGCGCCTCGGCGCGTCGATCTTCGATTCGGC  360
 I  N  A  G  K  Q  T  V  T  A  R  L  G  A  S  I  F  D  S  A
GCAGTCGTTCGCTATGATCCGCGGCGGCCATGTCGATCTGACCGTGCTGGGGGCGTTTGA  420
 Q  S  F  A  M  I  R  G  G  H  V  D  L  T  V  L  G  A  F  E
AGTGGACGTCGAGGGCAACATCGCCTCGTGGATGATCCCCGGCAAGATGGTCAAGGGCAT  480
 V  D  V  E  G  N  I  A  S  W  M  I  P  G  K  M  V  K  G  M
GGGCGGCGCGATGGATCTGGTGGCGGGGGCGGAGAACATCATCGTGGTGATGACCCACGC  540
 G  G  A  M  D  L  V  A  G  A  E  N  I  I  V  V  M  T  H  A
CGCGAAGGGCGGCGAGTCCAAGCTGCTGCCGCGCTGTACGCTGCCGCTGACCGGCGCCCG  600
 A  K  G  G  E  S  K  L  L  P  R  C  T  L  P  L  T  G  A  R
CTGCATTCGCAAGGTATTGACCGATCTGGCCTACCTGGAAATTGAAGACGGCGCCTTCGT  660
 C  I  R  K  V  L  T  D  L  A  Y  L  E  I  E  D  G  A  F  V
GCTGCGCGAACGCGGGCCGGGCGTCAGCGTGGCGGAGATTGTCGCCAAAACCGCCGGCCG  720
 L  R  E  R  G  P  G  V  S  V  A  E  I  V  A  K  T  A  G  R
GCTGATCGTGCCGGAGCAAGTGCCGGAAATGCGTTTCTGAAACGGGAGGTGAATGTGCAA  780
 L  I  V  P  E  Q  V  P  E  M  R  F ***    SD ACOT ↑  M  Q
CAACGTGAAGTGGTGATCGTGGCGGCGACGCGCACGCCGGTCGGCAGTTTTCACGGCGCG  840
 Q  R  E  V  V  I  V  A  A  T  R  T  P  V  G  S  F  H  G  A
CTGGCGCCGCTGACGGCGGTGGAACTGGGCGCGGCGGGTGTGCAGGGGCTGCTGGCGCAA  900
 L  A  P  L  T  A  V  E  L  G  A  A  G  V  Q  G  L  L  A  Q
AGCGGCGTGCCGCCGCAGCAGATCGATGAAGTGATCCTCGGCCAGGTGCTGACCGCCGGC  960
 S  G  V  P  P  Q  Q  I  D  E  V  I  L  G  Q  V  L  T  A  G
TGCGGGCAGAATCCGGCGCGCCAGACTGCGCTCAACGCCGGGCTGCCCGTTACGACGCCG 1020
 C  G  Q  N  P  A  R  Q  T  A  L  N  A  G  L  P  V  T  T  P
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TCGCCTTACCTGATGGCCGGCGCGCGCGCCGGTCTGCGCCTCGGTCACGCGCAGATGGTG 1200
 S  P  Y  L  M  A  G  A  R  A  G  L  R  L  G  H  A  Q  M  V
GACAGCGTGATCCACGACGGCCTGTGGGACGCGTTCAACGACTACCACATGGGCATCACC 1260
 D  S  V  I  H  D  G  L  W  D  A  F  N  D  Y  H  M  G  I  T
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CGTTCGCAGCAGAAGGCGCAGGCGGCGCAGAGGGCCGGGCGTTTCGCGCAGGAGATCACG 1380
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CCGGTGACGGTGCCGCAGCCGAAGGGGGAGGCGCTGAGAGTTGAGCGCGACGAACAGCCG 1440
 P  V  T  V  P  Q  P  K  G  E  A  L  R  V  E  R  D  E  Q  P
CGCGACACCAGCCTGGAGGCGCTGGCGCGGCTGCGTCCGGCGTTTCGCAAGGAGGGCTCG 1500
 R  D  T  S  L  E  A  L  A  R  L  R  P  A  F  R  K  E  G  S
GTGACCGCCGGCAACGCCTCGTCGCTCAACGACGGCGCCGCCGTGGTGTTGCTGATGAGC 1560
 V  T  A  G  N  A  S  S  L  N  D  G  A  A  V  V  L  L  M  S
GCGGAAAAAGCCGCTCGAGTGCGCTTGCCGGTGCTGGCGCGCATTGCCGGTTACGCCTCG 1620
 A  E  K  A  A  R  V  R  L  P  V  L  A  R  I  A  G  Y  A  S
GGCGTCGATCCGGCGATCATGGGCATCGGCCCGGCGCCGGCCGCGCGCCGCTGCCTGGAA 1680
 G  V  D  P  A  I  M  G  I  G  P  A  P  A  A  R  R  C  L  E
AAAGCCGGTTGGCGGCTGGAGGAAGTCGATCTGATCGAAGCCAACGAAGCCTTCGCCGCG 1740
 K  A  G  W  R  L  E  E  V  D  L  I  E  A  N  E  A  F  A  A
CAGGCGCTGGCGGTGGGTAAAGAACTGGGCTGGGAGGCGGAGCGGGTCAACGTCAACGGC 1800
 Q  A  L  A  V  G  K  E  L  G  W  E  A  E  R  V  N  V  N  G
GGAGCGATCGCGCTGGGGCATCCGATCGGCGCATCCGGCTGTCGCATTTTGGTGTCGCTG 1860
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CTGTACGAAATGCAGCGCCGCGGGGTGAACAAGGGGCTGGCGATGCTGTGCATCGGCCGA 1920
 L  Y  E  M  Q  R  R  G  V  N  K  G  L  A  M  L  C  I  G  R
AAACAGGGGGTGGCGCTGGCGGTAGAGCGCCCGTAAACAACCGAGGAGGGCACCATGCAG 1980
 K  Q  G  V  A  L  A  V  E  R  P ***
TCATCTGACTATGCGGTATTGGGCGCCGGGCTGATGGGGGTGGGCATCGCCACCCACTTT 2040
ATTCGCCACGGCCATGAGGTGCTGCTGTATGACCCGGATCC-2081
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Fig. 2. Nucleotide sequence of the Coenzyme A (CoA) transferase gene region from *S. marcescens*.
The initiation sites of the translation are indicated by the arrows. The nucleotide sequence reported in this paper has been submitted to the GenBank Data Bank with accession number AF276244.

gene has been shown to be continuous to the *acot* gene. The truncated protein of the *scotA* gene is located directly upstream of the *scotB* gene, with the same direction of transcription. These results indicated that the two genes encoding succinyl-CoA, acetoacetyl-CoA transferase, and hydroxybutyrate dehydrogenase were closely linked and may constitute an operon. This was the first detailed report that adjacent bacterial genes, the succinyl-CoA and acetoacetyl-CoA transferases, were analyzed. The putative ribosomal binding sites (GGAGA and GGAGG) of the *acot* and *scotB* genes were located 12 bp and 6 bp upstream of the start codon, respectively.

Sequence Comparison with Known Proteins

Using the Blast program from the University of Kyoto Genome Net WWW server, the predicted amino acid sequences were searched through the database. Significant homology of the amino acid sequence could be found between acetoacetyl-CoA transferase and the β-subunit of succinyl-CoA transferase, which interact specifically with the same catabolic pathway. The sequences of the β-subunit of succinyl-CoA transferase were highly homologous with known proteins (data not shown). The protein most closely related to *S. marcescens* ORFs was an acetoacetyl-CoA transferase protein. Amino acid sequence comparisons, combined with measurements of enzyme activities using several different CoA donors and acceptors, identified the *S. marcescens* CoA transferase as an acetoacetyl-CoA: acetoacetate-CoA transferase. Figure 3 shows an alignment of the *acot* gene from *S. marcescens* with the analogous proteins from other organisms. Acetoacetyl-CoA transferases from highly diverse organisms show remarkable amino acid conservation (Fig. 3). By analyzing our sequence together with the recently published CoA transferases, such as *Pseudomonas putida* [16], *C. acetobutylicum* [17], *H. pylori* [5], and other sequences, several important features can be seen about the structure-function relationships of CoA transferases during evolution. The conserved active site glutamate (E) is the probable site of CoA esterification in the *E. coli*, *P. putida*, and pig heart CoA transferase enzymes [14]. In the amino acid sequence of ScotB, the residue SENG, which were conserved in all homologous transferases, were detected (data not shown). Parales and Harwood [17] reported that, in these residues, glutamate was the active site of the ketoadipate:succinyl-CoA transferase. Homology in the amino acid sequence between *S. marcescens* Acot and the *Azotobacter* sp. β-ketothiolase, *Pseudomonas* sp. 61-3 β-ketothiolase (*phbA*) [15], *E. coli* CoA transferase [3], and *Acinetobacter* sp. β-ketothiolase [21] genes was 62.7, 62.2, 57, and 54.5%, respectively.

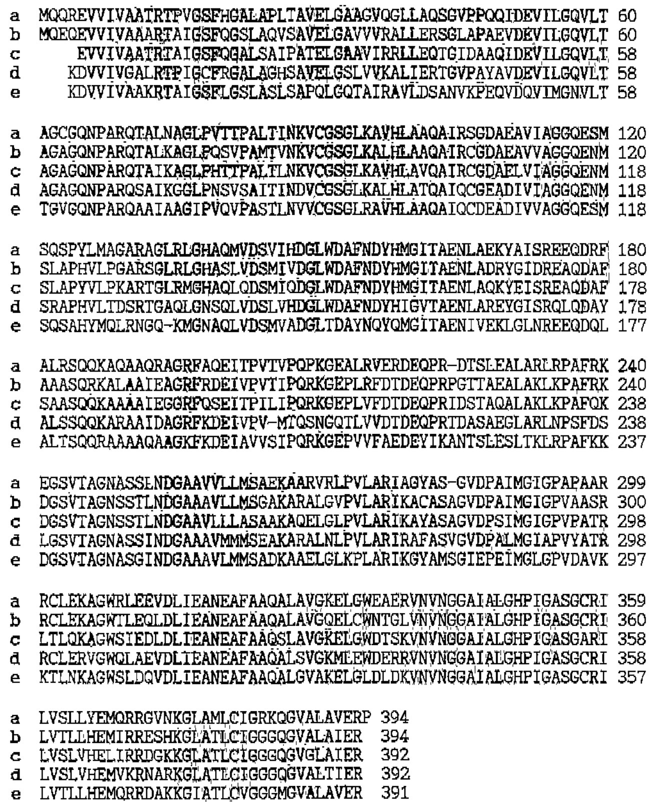

Fig. 3. Comparison of the primary structure among acetoacetyl-CoA transferase (ACOT) from different organisms.
a, ACOT of *Serratia*; b, β-ketothiolase of *Azotobacter vinelandii*; c, β-ketothiolase of *Pseudomonas* sp. 61-3; d, acetyl-CoA acetyltransferase of *Escherichia coli*; e, β-ketothiolase of *Acinetobacter* sp.

Table 1. Coenzyme A transferase activities in *E. coli* TP2139 transformed recombinant plasmids[1].

Substrate	Carboxylic acid	Plasmid			
		pKK223-4	pACO13	pACO13P	pACO14
Acetoacetyl-CoA transferase[2]	Potassium acetate	0.022(1)	0.462(21)	0.491(22.3)	0.029
	Propionic acid	0.011(1)	0.220(20)	0.212(19.3)	0.029
	β-Ketoadipate	0.004(1)	0.018(4.2)	0.0158(3.5)	0.005
Succinyl-CoA transferase	Potassium acetate	0.015(1)	0.085(5.6)	0.068(4.5)	0.009
	Propionic acid	0.016(1)	0.138(8.6)	0.120(7.5)	0.017
	β-Ketoadipate	0.044(1)	0.018(4.09)	0.015(3.4)	0.007

[1]Cultures for enzyme assay were grown in a M9 minimal medium with 100 mM carboxylic acids and 0.1% casamino acids.
[2]Transferase activity is expressed as nanomoles acetoacetyl and succinyl CoA formed per milligram of protein. Activities reported are an average of three assays of two independently prepared extracts.

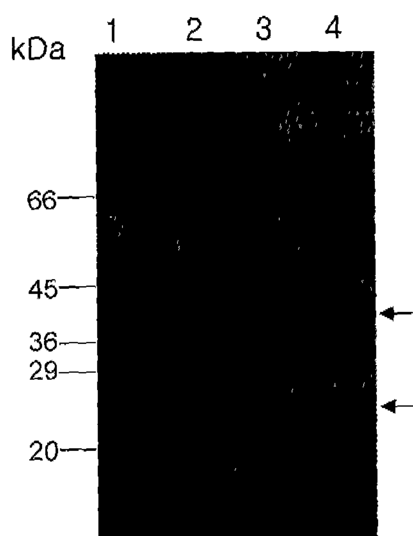

Fig. 4. SDS-polyacrylamide gel electrophoretic patterns showing the acetoacetyl-CoA transferase gene in *E. coli*.
The same amount of protein samples were separated on a 10% SDS-PAGE and stained with Coomassie Brilliant Blue. Lanes: 1, size marker; 2, pKK223-4/1mM IPTG; 3, pACO13/1mM IPTG; 4, pACO13P/1mM IPTG. The arrows indicates the approximately 42,000 Da and 23,000 Da marks.

Expression of the *S. marcescens acot* Gene in *E. coli*

Genes for the putative *S. marcescens* CoA transferase were introduced into the bacterial expression vector pKK223-4. The putative CoA-transferase gene region of pCKB13 was cut with *Eco*RI-*Bam*HI and ligated into the same sites of pKK223-4 to construct pACO13. The enzymatic activities baseline was determined using cellular lysates of *E. coli* transferase with pKK223-4 as a control. *E. coli* crude lysates expressing pACO13 showed a 21-fold increase in acetoacetate-CoA activity. In plasmid pACO14, the cloned gene is located in the opposite direction and therefore is transcribed in the anti-direction to pACO13, and it showed low level activity in the same set of *E. coli*. This result showed that the plasmid pACO13 did not contain the full promoter region. Table 1 shows the relative activity of CoA transferase with 100 mM concentration of the various carboxylic acids. The relative activity was highest with acetate, while the activity with the other carboxylic acids tested was very low.

Table 2. Purification of acetoacetyl-CoA transferase of *S. marcescens*.

Step	Protein (mg)	Total activity (units)	Specific activity (U/mg)	yield (%)
Crude extract	56	0.274	0.0049	100
DEAE-Sepharose	15	0.140	0.0093	51
CM-Sepharose	4.7	0.080	0.0170	29
Mono Q-FPLC	1.2	0.047	0.0392	17

The enzyme activity of acetoacetyl-CoA transferase is expressed as nanomoles formed per milligram of protein. Activities reported are an average of three assays of two independently prepared samples.

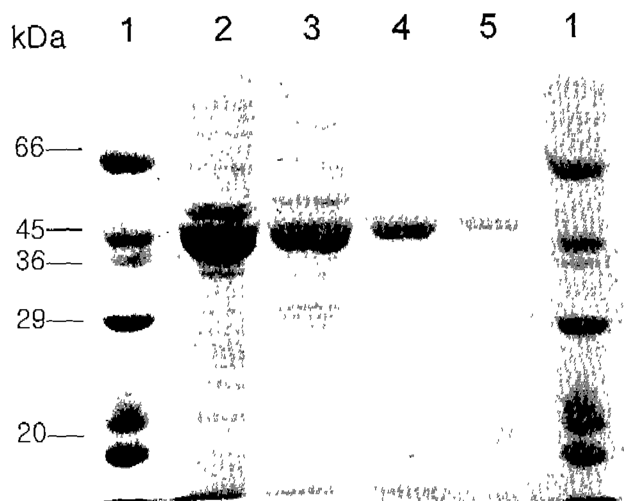

Fig. 5. SDS-polyacrylamide gel electrophoretic patterns showing purification of acetoacetyl-CoA transferase protein.
Lanes: 1, size marker; 2, pACO13P/unpurified protein; 3, DEAE Sepharose; 4, CM Sepharose; 5, FPLC.

After induction of CoA transferase with isopropyl-thio-β-D-galactopyronoside, crude extracts were prepared from cultured bacterial cells and were analyzed by SDS-PAGE. As shown in Fig. 4, distinct bands of Acot and Scot corresponding to a molecular weight of approximately 42,000 Da and 23,000 Da, respectively, are seen on lanes 3 and 4.

Purification of Acetoacetyl-CoA Transferase

Table 2 presents the purification of CoA transferase. The procedures for each step are described in Materials and Methods. The DEAE-Sepharose step of the purification procedure, although not very efficient, was necessary to obtain a pure preparation for subsequent anion-exchange chromatography on Mono-Q. Fractions from each chromatography were electrophoresed on polyacrylamide gel in the presence of sodium dodecyl sulfate (Fig. 5). All fractions corresponding to the beginning, middle, and end peak coincided with CoA-transferase activity, and showed a single peak corresponding to a molecular weight of approximately 42,000 Da.

Acknowledgment

This paper was supported by the Dong-A University Research Fund in 1999.

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