

Molecular Cloning and Characterization of Two Genes for the Biotin Carboxylase and Carboxyltransferase Subunits of Acetyl Coenzyme A Carboxylase in *Myxococcus xanthus*

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We have cloned a DNA fragment from a genomic library of *Myxococcus xanthus* using an oligonucleotide probe representing conserved regions of biotin carboxylase subunits of acetyl coenzyme A (acetyl-CoA) carboxylases. The fragment contained two open reading frames (ORF1 and ORF2), designated the *accB* and *accA* genes, capable of encoding a 538-amino-acid protein of 58.1 kDa and a 573-amino-acid protein of 61.5 kDa, respectively. The protein (AccA) encoded by the *accA* gene was strikingly similar to biotin carboxylase subunits of acetyl-CoA and propionyl-CoA carboxylases and of pyruvate carboxylase. The putative motifs for ATP binding, CO₂ fixation, and biotin binding were found in AccA. The *accB* gene was located upstream of the *accA* gene, and they formed a two-gene operon. The protein (AccB) encoded by the *accB* gene showed high degrees of sequence similarity with carboxyltransferase subunits of acetyl-CoA and propionyl-CoA carboxylases and of methylmalonyl-CoA decarboxylase. Carboxybiotin-binding and acyl-CoA-binding domains, which are conserved in several carboxyltransferase subunits of acyl-CoA carboxylases, were found in AccB. An *accA* disruption mutant showed a reduced growth rate and reduced acetyl-CoA carboxylase activity compared with the wild-type strain. Western blot analysis indicated that the product of the *accA* gene was a biotinylated protein that was expressed during the exponential growth phase. Based on these results, we propose that this *M. xanthus* acetyl-CoA carboxylase consists of two subunits, which are encoded by the *accB* and *accA* genes, and occupies a position between prokaryotic and eukaryotic acetyl-CoA carboxylases in terms of evolution.

Acetyl coenzyme A (acetyl-CoA) carboxylase catalyzes the ATP-dependent carboxylation of acetyl-CoA to yield malonyl-CoA. The chain length of newly synthesized fatty acids appears to depend on the concentration of malonyl-CoA (17). In *Escherichia coli*, the rates of transcription of acetyl-CoA carboxylase genes are directly related to the rate of cell growth (27). *E. coli* and *Pseudomonas citronellolis* acetyl-CoA carboxylases consist of three functional units: carboxyltransferase, biotin carboxyl carrier protein, and biotin carboxylase (8, 13). The *E. coli* acetyl-CoA carboxylase is the only biotinylated protein in *E. coli* (9), and the enzyme does not catalyze a reaction analogous to that of propionyl-CoA carboxylase. In contrast to bacterial enzymes, eukaryotic acetyl-CoA carboxylases are unusually large enzymes and contain all components in a single protein.

Propionyl-CoA carboxylase forms methylmalonyl-CoA from propionyl-CoA by CO₂ fixation. Methylmalonyl-CoA serves as a precursor for the synthesis of branched-chain fatty acids and polyketides. All propionyl-CoA carboxylases from prokaryotes and eukaryotes consist of two nonidentical subunits, biotin carboxylase and carboxyltransferase. The bacterial acyl-CoA carboxylases isolated from *Mycobacterium pheli*, *Mycobacterium smegmatis*, and *Streptomyces erythreus* show maximal rates of carboxylation with propionyl-CoA, but the enzymes are also able to carboxylate acetyl-CoA well (7, 16, 19). In several bacteria, a single enzyme with dual-substrate specificity catalyzes the carboxylation of both acetyl- and propionyl-CoA.

Myxococcus xanthus is a gram-shaped bacterium that displays cyclic and various social behaviors (6, 34). We reported previously that an *M. xanthus* propionyl-CoA carboxylase de-

letion mutant was unable to sporulate under conditions of nutrient starvation (20). The developing cells of the mutant also showed reduced levels of long-chain fatty acids compared to wild-type cells. Since the mutant grew as well as the wild type in growth medium, *M. xanthus* appears to contain acetyl-CoA carboxylase in addition to propionyl-CoA carboxylase. We attempted to clone the acetyl-CoA carboxylase gene from *M. xanthus* using appropriate oligonucleotide probes designed from the conserved sequences in the acetyl-CoA carboxylases.

Here, we describe the cloning and sequencing of the *accA* and *accB* genes encoding acetyl-CoA carboxylase from *M. xanthus*, and we discuss the structure and function of this enzyme.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. The type strain of *M. xanthus*, IFO13542 (ATCC 25232), was grown in Casitone-yeast extract (CYE) medium at 28°C (2, 5). Fruiting body formation was assayed on clone fruiting (CF) medium containing 1.5% agar (14). Plasmids pBluescript II SK(-) (Stratagene, La Jolla, Calif.) and pT7 Blue-T (Novagen, Madison, Wis.) were used for cloning.

Cloning of the acetyl-CoA carboxylase gene. An *M. xanthus* genomic DNA library was prepared by partially digesting chromosomal DNA with *Sau3AI*, ligating the DNA with *Bam*HI-cleaved λ EMBL3, and then packaging the DNA into phage particles. For the detection of the acetyl-CoA carboxylase gene of *M. xanthus*, oligonucleotides were designed as DNA probes for hybridization experiments. The oligonucleotides were labeled with digoxigenin (DIG)-11-dUTP by using an oligonucleotide tailing kit (Boehringer GmbH, Mannheim, Germany). One positive phage was cloned by hybridization with an oligonucleotide probe (ACC1). The sequence of ACC1 is 5'-(G/C)GCGATCTC(G/C)CC(G/C)CGG TTCG-3' (oligo-1); it was designed on the basis of the consensus sequence (ANRGEIA) of acetyl-CoA carboxylases of *E. coli* and *Anabaena* sp. strain PCC 7120 (11). The 3.8-kb *Apa*I, 5.6-kb *Sac*I, and 1.4-kb *Sma*I fragments of the clone hybridized with the probe and then were subcloned into pBluescript II SK(-).

DNA sequencing. Nucleotide sequences were determined by the dideoxynucleotide chain termination method (31) using a model 4200 sequencer (Aloka, Tokyo, Japan). Both directional strands were completely analyzed by overlapping at every junction.

Insertional mutagenesis of the *accA* gene. The 2.4-kb DNA fragment, which contains the *accA* gene and part of the *accB* gene, was amplified by PCR using two primers; 5'-CTTCAAGGACGAGTACGAC-3' (oligo-2), which anneals at

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positions 1393 to 1411, and 5'-TCCGTCACGGCGTGC GCGCC-3' (oligo-3), which anneals at positions 3777 to 3796 (Fig. 1). The 2.4-kb PCR product was ligated to the pT7 Blue-T vector. For deletion of an *SmaI* site of the pT7 Blue-T vector, the recombinant plasmid was digested with *BamHI* and *EcoRI*. The ends were blunted with T4 DNA polymerase and then ligated with T4 DNA ligase. This plasmid was designated pACC1. A kanamycin resistance (*Km^r*) gene of pTF1 was amplified by PCR using suitable primers, which contain *SmaI* sites (10). The resulting 1.2-kb fragment containing the *Km^r* gene was purified, digested with *SmaI*, and inserted into the *SmaI* site of plasmid pACC1. The *accA* gene with the *Km^r* gene inserted was amplified by PCR using the same primers. The resulting 3.6-kb fragment was purified and used for electroporation, which was performed as described by Plamann et al. (29). *M. xanthus* kanamycin-resistant colonies were grown in CYE medium containing 70 µg of kanamycin per ml, and chromosomal DNAs were prepared from the mutants. The chromosomal DNAs were digested with *SacI* and then analyzed by Southern hybridization using a 1.4-kb *SmaI* fragment as a probe. We confirmed that the *SacI* digested-chromosomal DNAs from the wild type and the mutants were hybridized at 5.6-kb and 6.8-kb fragments, respectively.

Gel electrophoresis and Western blot analysis. *M. xanthus* wild-type and *accA* disruption mutant cells harvested in exponential growth phase, in stationary phase, and during development were used for Western blot analysis. Samples containing 250 µg of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (8 or 12% polyacrylamide) and electroblotted onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, Ltd.) using a Trans Blot SD semidry transfer cell (Bio-Rad Laboratories, Ltd.) according to the manufacturer's instructions. The membranes were blocked with 3% bovine serum albumin in PBS-T buffer (10 mM sodium phosphate buffer [pH 7.2], 150 mM NaCl, and 0.1% Tween 20) and then incubated with streptavidin-linked horseradish peroxidase (Amersham Pharmacia Biotech) for 1 h. The membranes were washed with PBS-T buffer, and enzyme activities were detected by ECL Western blotting detection reagents (Amersham Pharmacia Biotech).

Enzyme assays. *M. xanthus* wild-type and *accA* disruption strains were cultured in 200 ml of CYE medium at 28°C on a rotary shaker at 250 rpm. The cells were harvested at the mid-logarithmic phase of growth (optical density at 600 nm [OD₆₀₀] 0.4 to 0.6) and washed with 20 mM sodium phosphate buffer (pH 7.2). The cells were suspended in the same buffer and disrupted by sonication with a Branson Sonifier (five 30-s bursts at a power setting of 1.5). The supernatant and cell debris were separated by centrifugation (12,000 × g for 10 min). Enzyme activity was determined from the increase in the product by high-performance liquid chromatography (HPLC) (21). For acetyl- and propionyl-CoA carboxylase assays, the reaction mixture contained 60 mM Tris-HCl (pH 7.2), 1.3 mM ATP, 1.8 mM MgCl₂, 66 mM KHCO₃, 0.4 mM acetyl-CoA or propionyl-CoA, and enzyme in a total volume of 0.1 ml. The mixtures were incubated at 30°C for 10 to 45 min. One unit of activity was defined as the amount of enzyme forming 1.0 µmol of malonyl-CoA or methylmalonyl-CoA per min at 30°C.

Observation of morphology, growth, and development. Wild-type *M. xanthus* or the *accA* disruption mutant was grown in CYE medium at 28°C on a shaker. Cell numbers were estimated with a hemacytometer counting chamber. Generation times were calculated from the linear region of the growth curve by measuring the time needed for the cells of the culture to double. For spore formation, vegetative cells in TM buffer (10 mM Tris-HCl [pH 7.5]–8 mM MgSO₄) were spotted onto CF agar plates. Cell morphologies of vegetative cells and spores were observed by light microscopy.

Nucleotide sequence accession number. The sequences of the *M. xanthus accA* and *accB* genes have been deposited in the DDBJ sequence library under accession number AB039884.

RESULTS

Cloning of the structural genes for acetyl-CoA carboxylase.

One clone was isolated from an *M. xanthus* genomic DNA library by screening with an ACC1 oligonucleotide probe designed by the conserved sequences in the biotin carboxylase subunits of acetyl-CoA carboxylases. The 3.8-kb *ApaI*, 5.6-kb *SacI*, and 1.4-kb *SmaI* fragments of the clone DNA hybridized with the probe. Based on the restriction map derived from hybridization data, the 4-kb *ApaLI-SmaI* fragment was completely sequenced on both strands using synthetic oligonucleotide primers. Two open reading frames (ORFs) were identified in the 4-kb DNA fragment. The complete nucleotide sequence together with the deduced amino acid sequence is shown in Fig. 1. ORF1 and ORF2 have high percentages of G and C nucleotides (93.1 and 89.9%, respectively) in the third positions of the codons and exhibit a codon preference typical for *M. xanthus*. The putative initiation codons were preceded

by purine-rich Shine-Dalgarno-like sequences (AGGAG at nucleotides 428 to 432 and 2058 to 2762).

ORF1, designated *M. xanthus accB*, started at position 440 with an ATG and ended at position 2056 with a TGA stop codon. The *accB* gene encodes a protein of 538 amino acid residues with a calculated *M_r* of 58,100. ORF2 was located immediately downstream of the *accB* gene. ORF2, designated *M. xanthus accA*, started at position 2071 with an ATG and ended at position 3792 with a TGA stop codon. ORF2 encodes a protein of 573 amino acid residues with a calculated *M_r* of 61,500. This downstream region contained an inverted repeat, CGGACGtAACGTTcCGTCCG, that could form a stem structure.

Deduced properties of AccA and AccB polypeptides. The predicted amino acid sequences of AccA and AccB were compared with those in the GenBank database using the PSI Blast program. AccA showed considerable sequence homology to the biotin carboxylase subunits of *E. coli* acetyl-CoA carboxylase (48% identity) (26) and human propionyl-CoA carboxylase (41% identity) (23) and to the N-terminal region of mouse pyruvate carboxylase (39% identity) (35) (Fig. 2). Multiple alignment of these sequences revealed that the ATP-binding motif and CO₂ fixation site were present in AccA. The sequence Gly-Gly-Gly-Gly-Arg-Gly-Met-Arg-Leu-Val of AccA (residues 164 to 173) matched the consensus sequence of the Gly-rich motif that has been implicated in ATP binding by biotin carboxylases (30). The Cys residue of Arg-Asp-Cys-Ser (residues 229 to 232) was thought to be involved in CO₂ fixation (26). The conserved biotin-binding site Met-Lys-Met, in which the lysine residue is biotinylated, was not found, but Met-Lys-Leu was present in the C-terminal region of AccA. Replacement of the methionine residue flanking the target lysine with leucine on the biotinylation domain of the biotin carboxylase subunit of human propionyl-CoA carboxylase demonstrated that the methionine residue is not essential for correct biotinylation of the protein (24). Met-Lys-Leu as a biotinylation site is also found in the biotin carboxyl carrier protein of acetyl-CoA carboxylase from *Anabaena* sp. strain PCC 7120 (11).

AccB showed high degrees of sequence similarities with the α subunit of *Veillonella parvula* methylmalonyl-CoA decarboxylase (27% identity) (18), the β subunit of *M. xanthus* propionyl-CoA carboxylase (28% identity) (20), and the carboxyltransferase α and β subunits of *E. coli* acetyl-CoA carboxylase (19 and 13% identity, respectively) (25) (Fig. 3). The putative acyl-CoA- and carboxybiotin-binding domains, which are conserved in several carboxyltransferase subunits of acetyl-CoA and propionyl-CoA carboxylases, were found in AccB at residues 102 to 154 and 311 to 345, respectively.

Phenotypic characterization of the *M. xanthus accA* disruption mutant. Using Southern hybridization and PCR analyses, we confirmed that the kanamycin resistance gene was inserted into the *accA* gene on the mutant chromosome. Insertional inactivation of the *accA* gene of the *M. xanthus* chromosome resulted in a marked change in the growth rate. In CYE liquid medium, the wild type exhibited a lag period of about 8 h and entered the stationary phase within 48 h. In contrast to the wild-type strain, the *accA* mutant started to grow after about 18 h of lag time and reached steady-state growth at 60 h. The generation times and final yields were 3.5 h and 3.0 × 10⁹ cells/ml for the wild type and 5.0 h and 2.5 × 10⁹ cells/ml for the *accA* mutant (data not shown). In M1 defined medium (4), the wild-type and *accA* mutant strains grew at similar generation times of approximately 10 h (data not shown). No significant differences in cell morphology or sporulation were observed between the wild-type and *accA* mutant strains. When


1 GTGCACCGGCCATCACGCTCCCGGGGAGACGCGCTCGCTGGAGCCCGTGGAGCCACCTCCGCTGCCGCCGCCAGTCCGGG
84 CGGTCCACGCGCCGGGTTCGCTGGGAGCAGTCTCGCCGCGCGCAGTGGTACAAAGGGCGGCAGGCAGAACATCGCGCTC
167 GGGCGCGCAGCGAGCGGCTGGCGTGGCTGGCGCATGCGCTCAGGGAAGAAAGCTGCGCGAGTGTCCCGGAGGCGCGG
250 CGCTGGCGCATCGAGCGGACGCTTCCCAACCTCGGGGGTGAACCTCGTCTGGAGGGTCTGCTGGGAGGGGCTCTC
333 CTCGTCACCCGGTTCGACCCGAGGCAAGGCCCTGGGCGAGTGGCTCGCTCGCGGCACATGGACATCCCGGAGGCGCTGC
416 TCGCGAAGGACAGGATAGACCGATGCCACGCATCACCTCGGAATCGACCCGGGCTCCGAATCCTTCAAGGTGAACCGCGG
1 **accB** M P R I T S R I D P G S E S F K V N R A
499 GGACATGCTGGCCCGGTTTCGGAGTTGCGCGCCATCGAGGCGAAGGTCGCAACACCGGAGAACCGGCGAAGGAAAGTTC
21 D M L A R V S E L R A I E A K V R N T E N Q A K E K F
582 ACAAGCGGGGCGCTCTGCCTCGGAGCGGCTGATGCTGCTCGACCGGGGCTCGCCCTTCTGGAGCTGTCCACGCTG
48 H K R G Q L L P R E R L M L L L D R G S P F L E L S T L
665 TGTGGCTATGGCTACCACGACGACAGTACGGCTCGCTGGCGGGCGCAACAGCATCATCGGCATTGGCTACGCTGCGCGCT
76 C G Y G Y H D D S D G S L A G G N S I I G I G Y V S G V
748 GCGGTCATCGTCTTCTGAACAACCTCCGCATCAAGGGCGGCACCGCTCGCGTGGGGCGTGCAGAAGCGCTCGCGCGC
104 R C I V F V N N S A I K G G T A S P W G V Q K L A R A
831 AGGCATTCGCGTGGAGAACAAGTGCACATGGTGTGCTGGAGAGCGGCGGCCAACCTGATGTACCAGCAGGAAATC
131 Q A L A L E N K L P M V S L V E S G G A N L M Y Q Q E I
914 TTCATCCCGGGTGGGAGACCTTCTACAACAGGACCGGCTGTCGGCGGGGATCCCGCAGGTGACGGTGGACGGCTC
159 F I P G G E T F Y N Q A R L S A A G I P Q V T V H G S
997 CAGCACGGCGGGCGGCTACCTGCCGGCTGTCCGACTACGTGGTGTGATGGTGAAGAAGGCGAAGGTGTTCTCCGCGG
187 S T A G G A Y L P G L S D Y V V M V K K K A K V F L A
1080 TGTGGCTATGGCTACCACGACGACAGTACGGCTCGCTGGCGGGCGCAACAGCATCATCGGCATTGGCTACGCTGCGCGCT
214 G P P L L K A A T G E V A T D E E L G G A Q M H A T V A
1163 GGCACCGGACTACCTGGCGGAGGACGCGGACGCCATCCGCATGCGCCCGCAAACTCGTGGCAGGCTCGGTTGAACGC
242 G T A D Y L A E D D A D A I R M A R E I V A K L A R A
1246 GCAGCTTCGCGCCACCGAGCGCCCGCTATGCCGAGCCGCTGACTCGCCCGCAACCTCGCGCGCCGCTCGCGCGGAT
270 Q L P P T E R P A Y A E P V Y S P D E L C G A V P D
1329 ACCCAAACCTTACGATTGCCGCGAGTTCATCGCGCATCGTGGATGGCTCGGAGTTCACCGGCTTCAAGGACGATACGAC
297 Y R K P Y D C R E V I A R I V D G S E F T G F K D E Y D
1412 GCGCACCGTTCGCGTGGCGAACCTGTACGGCCACCCGCTGGGCATCATCGAAACACCGGCGGATTTCGCGCCAGGG
325 A H T V C G W A N L Y G H P L G I I G N N G P I S P Q G
1495 CGCGCAGGCGCGCGCAGTTCACAGTTGTGCTGCCAGAAGGACACGCCCATCTCTACCTGCAGAACCACCGGCTACCG
353 A T K A A Q F I Q L C C Q K D T P I L Y L Q N T T G Y
1578 TCGTGGCAGCAGCGGAGCAGGCGCATCGTGAAGCAGCGCGAAGATGATTACGGCCGTGGCAACCGGCGGTCGCA
380 L V G T Q P E Q G G I V K H G A K M I Q A V A N A T V P
1661 CAGCTACCGTGTGATTGGCGGTGGCTTGGCGCGGGCAACTACGGCATGTGCGCGCTCCGTTCCATCCCGGCTTACATCT
408 Q L T V L I G G A F G A G N Y G M C G R P F H P R F I F
1744 CGGTGGCCAACTCGCGCACCGGCTCATGGCGCGGAGCAAGCGCGGAGGTGATGTCATCGTCTTCGGGAGAGCTGG
436 G W P N S R T A V M G G E Q A A K V M S I V F G E K L
1827 CCCGCGGCGGCGGTTGGAGCAGGAGCAGCTCAAGCGTTCGCGCCATCATCGACAGTTCGCAAGGAGTCCGAT
463 A R Q G Q V V D E E Q L K A F C Q P I I D Q F D K E S H
1910 CCGTCAACTGCAGCGCGGATGTTGACGACGGGCTCATCGACCGCGGGACACGCGCGGCTCGCTCGGTTCCGCGCTGC
491 P F N C S A R M F D D G L I D P R D T R R V L G F A L S
1993 GGTGTCCGTAAGCGAAGCGGCGGAGTCCATCCCAACAGTTCGCGCTCGCGCGCTGTGAGAGGCGGACCGATGGA
519 V C R E A K R Q V H P N T F G V A R L * **accA** M E
2076 GCGCTCAAGAAGTCTCATCGGAACCGCGGAGATTGCGCTCCGCGGATTTCGCACTGCCAGCGGCTGGGTTCAGCA
3 R F K K V L I A N R G E I A V R V I R T C Q R L G Y S
2159 CGGTGGCGTGTTCGCGAGGCGGACCGCGCGCGCAGTGTGGCGGCGGACGAGGCGGTGGCCATTTGGTTCGCTCCG
30 T V A V F S E A D R G A P H V L A A D E A V A I G S P S
2242 GCGAAGGAGTCTACCTCGTATCGGAAGATTCTGGAAGCGGCGAAGAGCTCGCGCGGCGGAGGCGATCCACCGGGTACGG
58 A K E S Y L V I G K I L E A A K T S G A E A I H P G Y G
2325 TTTCTTGGGAGAACCGGAGTTCGCGCGGCTTCCGCGGACCGCGGCGTGGTGTTCATCGCGCGGAGCGGCGGATCA
86 F L S E N A D F A R A C R D A G L V F I G P E A E A I
2408 CCCTGATGGCAACAAGCGTCAGGCGAAGCTGCGCATGATTGGCGGGCGGCTGCCCTGCATTCCTGGTACGAGGCGTCCGAC
113 T L M G N K R Q A N V R M I A A G V P C I P G Y E A S D
2491 CTGGATGATGAGGCGCTGGCGTGGAGGGCGAGCGCATCGGCTTCCCGTGTGGTCAAGGCGGCGGCGGTTGGCGGGCGG
141 L D D E A L A V E G E R I G F P L M V K A A A G G G R
2574 CGGCATGCGGCTGTGCAGGAGCATCGAGTTCGCGCGGCGCTGCGCGGCGGCGCTCGGAGGCGGCAATGCCCTCGGGA
169 G M R L V H E A S Q L R A A L R A A R S E A T N A F G
2657 GCGGGAGCTCATCTGGAGAAGCGCTCATCGACGCGGCGCAGTGGAGGTCAGGCTTCGCGGACACCGCAGCAACGCTG
196 S G E L I L E K A V I D A R H V E V Q V F A D T H G N V
2740 GTGCACCTGGGCGAGCGGACTGCTCGGTGCGAGCGCGGCGCACGAAAATCGTGGAGGAGTCCGTCACCGCGGCGGAGCC
224 V H L G E R D C S V Q R R H Q K I V E E S P S P A S P
2823 GGCGTCCGCGCGCATGGTGAAGTGGCGGCTGGCGGCGCTCGGCGATTGGCTACCGGGGAGCGGGACCATCGATTCC
252 A L R A R M G E V A V A A A R A I G Y R G A G T I E F
2906 TCCTCGTCCGAGCGGTACTTCTACTTTCATGGAGTGAACACAGCTTCCAGGTGGAGCACCGGCTGACCGGAGTGTATCC
279 L L A P S G D F Y F M E M N T R L Q V E H P V T E L I T
2989 GGGTTGGATTGGTGGAGTGGCAGCTCCCGTGGCGGGGGGAGACGCTGCCCCGACGCGAGGAGCGATTCCCGCTCGGG
307 G L D L V E W Q L R V A A G E T L P R T Q E A I S A S G
3072 GCATGCCATCGAGTTCGCTTCTGCGGAGGAGCGGCGGAAGGCTACGCGCCCGAGGCGGGCGGCTGTCGGCTGGAGGC
335 H A I E V R L C A E D P A K G Y A P Q A G R L L A W R
3155 TGCCGCTCGTGGGCGTGGCGATCGACACGGCGTGGGAGGGGAGGAGATTCCGCCCTTCTATGACTCCATGCAGGCG
362 L P L R E G V R I D H G V R E G Q E I P P F Y D S M Q A
2328 AAGGTGATTGCGCAGCGCCGAGCGGAGACCGCGCGCGCGGCTGGTGGAGGCGCTGCCGAGCTGACGGTGTTCGCGCT
390 K V I A H G P D R E T A R R R L V E A L R E L T V F G V
3321 CACCACCAACAAGAACCTGTTGTTGACGTGCGGAGCAGCGGCGTTCGTTAGGGAGTACGACACCGGCTTCCGGA
418 T T N K N L L Y V L E H A A F R S G E Y D T A F I A
3404 GGCACGCGGCGGCTGGAGGTGAAGGCTGTATCAGGACAGCGGAAGGCGGGCGGCTGGCGGGCGGCTGCTCTCCAC
445 R H A A A S E V E G L Y Q A D A K A R A L A A A L L F H
3487 GACGAAGCGCTGAAGTGGCGGACCGGCGGCTGGATGTCCTGCTGAATGGAACACGCTCCACCGCCACCGCTCGG
473 D E G L K L A D T A G L D V S L L N W N T S H R H P V R
3570 GATGAAGCTCGTGGCGTGGCGGAAAGCGTCCGTCACCGTCCAGCCCGTTCGCGGGAAGGTTACAAGTGGACGTGGGG
501 M K L V S R G A E A S V T V Q P V S G E G Y K D V G
3653 ATTCGCTGTCGACGTCCGCTGTTGGGCTTCCGCGGCGCTGCTCGACTTCTCCAGCGCGGACCGGGCGCGCGCGC
528 D S S F D V S V L G L S A G V L D F S S A G T R G R A R
3736 TACTCGGAGCGGCGACCGCTGTGGCTGGAGTGGCTGGCGCGCGCGGCTGACCGGCTAAGCTTCCGCTCGCGCGCTG
556 Y C G T G T R C G W T W A R A R T P * 
3819 AAGTCGACGGCTGGCGAGCGGCTGGCGCGCCATGGATGGCGCATCTCGCGTGGACACGAGTGGCGGGCGGCGG
3902 TGAAGCCCGGCTCGAGAAATCGATATCAAGCTTATCGATACCGTCCAGGCGGGCGG

FIG. 1. Nucleotide and deduced amino acid sequences of the *accA* and *accB* genes of *M. xanthus*. Putative ribosome-binding sites are double underlined. Arrows indicate the position of the palindrome sequence. The sequence corresponding to the probe is underlined. Boldfaced amino acids represent the putative biotin-binding site.

EcaccC	--MLDKIVIA	NRGEIALRIL	RACKELGIKT	VAVHSSADRD	LKHVLLADET	VCIGPAPSV-	57
Hpcca	EKTFDKILVA	NRGEIACRVI	RTCKKMGIKT	VAIHSDVDAS	SVHVKMADEA	VCVGPAPTS-	93
Mpc	YKPIKKVMVA	NRGEIATRVI	RACTELGIRT	VAVYSEQDTG	QMRHQKADEA	YLIGRGLAPV	93
MxaccA	MERFKKVLIA	NRGEIAVRVI	RTCQRLGYST	VAVFSEADRG	APHVLADEA	VAIGPSPAK-	59
EcaccC	KSYLNIPAI	SAAEITGA	IHPGYGFLSE	NANFAEQVER	SGFIFIGPKA	ETIRLMGDKV	117
Hpcca	KSYLNMDAIM	EAIKKTAAQA	VHPGYGFLSE	NKEFARCLAA	EDVVFIGPDT	HAIQAMGDKI	153
Mpc	QAYLHIPPDI	KVAKENGVA	VHPGYGFLSE	RADFAQACQD	AGVRFIGPSP	EVVRKMGDKV	153
MxaccA	ESYLVIGKIL	EAAKTSGAEA	IHPGYGFLSE	NADFARACRD	AGLVFIGPEA	EAITLMGNKR	119
ATP-binding motif							
EcaccC	SAIAAMKAG	VPCVPGSDGP	LGDDMDKNRA	IAKRIGYPVI	IKASGGGGGR	GMRVVRGDAE	177
Hpcca	ESKLLAKKAE	VNTIPGFDGV	VKDAEEAVRI	ARE-I GYPVM	IKASAGGGGK	GMRIAWDDEE	212
Mpc	EARAIAIAAG	VPVPGTDSP	ISSLHEAHE-	FSNTFGFPII	FKAAYGGGGR	GMRVWHSYEE	212
MxaccA	QANVRMIAAG	VPCIPGYEAS	DLDD-EALAV	EGERIGFPLM	VKAAAGGGGR	GMLRVHEASQ	178
CO ₂ fixation site							
EcaccC	LAQISMTRA	EAKAAFSDM	VYMEKYLENP	RHVEIQVLAD	GOGNAIYLAE	RDCSMQRRHQ	237
Hpcca	TRDGFRLSSQ	EAASSFGDDR	LLIEKFIDNP	RHIEIQVLGD	KHGNALWLNE	RECSIQRRNQ	272
Mpc	LEENYTRAYS	EALAAFNGA	LFVEKFIEKP	RHIEVQILGD	QYGNILHLYE	RDCSIQRRHQ	272
MxaccA	LRAALRAARS	EATNAFGSGE	LILEKAVIDA	RHVEVQVFAD	THGNVVHLGE	RDCSVQRRHQ	238
EcaccC	KVVEEAPAPG	ITPELRRYIG	ERCAKACVDI	GYRGAGTFF	LFEN-GEFYF	IEMNTRIQVE	297
Hpcca	KVVEEAPSIF	LDAETRRAMG	EQAVALARAV	KYSSAGTVEF	LVDSKKNFYF	LFMNTRLQVE	332
Mpc	KVVEIAPATH	LDPQLRSRLT	SDSVKLAKQV	GYENAGTVEF	LVDKHGKHYF	IEVNSRLQVE	332
MxaccA	KIVEESPSPA	VSPALRARMG	EVAVAAARAI	GYRGAGTIEF	LLAPSGDFYF	MEMNTRLQVE	298
EcaccC	HPVTEMITGV	DLIKEQLRIA	AGQPLSIKQ-	EEVHV--RGH	AVECRINAED	PNTFLPSP--	352
Hpcca	HPVTECITGL	DLVQEMIRVA	KGYPLRHKQ-	ADIRI--NGW	AVECRVYAED	PYKSFGLPSI	389
Mpc	HTVTEEITDV	DLVHAQIHVS	EGRSLPDLGL	RQENIRINGC	AIQCRVTTED	PARSFQ-PDT	391
MxaccA	HPVTEELITGL	DLVEWQLRVA	AGETLP---R	TQEASISAGH	ATIEVRLCAED	PAKGY-APQA	354
EcaccC	GKITRFHAPG	GF-GVRWESH	IYA-GYTVPP	YYDSMIGKLI	CYGENRDVAI	ARMKNALQEL	410
Hpcca	GRLSQYQEPL	HLPQVRVDSG	IQP-GSDISI	YYDPMISKLI	TYGSDRTEAL	KRMADALDNY	448
Mpc	GRIEVFRSGE	GM-GIRLDNA	SAFQGAIVIS	HYDSL LVKVI	AHGKDHTPTAA	TKMSRALAEF	450
MxaccA	GRLLAWRLLP	RE-GVRIDHG	VRE-GQEIPP	FYDSMQAKVI	AHGPDRETAR	RRLVEALREL	412
EcaccC	IIDGIKTNDV	LQIRIMNDEN	FQHGGTNIHY	LEKKLGLQEK	450
Hpcca	VIRGVTHNIA	LLREVIINSR	FVKGDISTKF	LSDVYPDGFK	GHMLTKSEKN	QLLAIASSLF	508
Mpc	RVRGVKTNIP	FLQNVLNNQ	FLAGVTDTQF	IDENPELFL	RPAQNRAQKL	LHYLGHVMVN	510
MxaccA	TVFGVTTNKN	LLLYVLEHAA	FRSGEYDTAF	IAR-----H	AAASEVEGLY	QADAKARALA	466
Hpcca	VAFQLRAQHF	QENSRMPVIK	PDIANWELSV	KLHDKVHTVV	ASNNGSVFSV	EVDGSKLNV-	567
Mpc	GPTTPIPNNV	SPSPVDPAPV	VVPIGPPAG	FRDILLREGP	EGFARAVRNH	OGLLLMDTTF	570
MxaccA	AALLFHDEGL	-KLADTAGLD	VSLLNWNTSH	RHPVRMKLV	RGAEASVTVQ	PVSGEGYKVD	525

Hpcca	-----TST	WNLASPLLSV	SVDGTQRTVQ	CLSREAGNM	SIQFLGTVYK	VNILTRLAAE	620
Mpc	RDAHQSLLAT	RVRTHDLKKI	APYVAHNFNK	LFSMENWGGG	TFDVAMRFLY	ECPWRRRLQEL	630
MxaccA	VGDSSFDVSV	LGLSAGVLDL	SSAGTRGRAR	YCGTGTRCGW	TWARARTP..	573

FIG. 2. Amino acid sequence alignment of homologous regions in the *E. coli* acetyl-CoA carboxylase biotin carboxylase subunit (EcaccC), the human propionyl-CoA carboxylase α subunit (Hpcca), the mouse pyruvate carboxylase (Mpc), and the *M. xanthus* acetyl-CoA carboxylase biotin carboxylase subunit (MxaccA). The putative biotin-binding site (MKL) of *M. xanthus* AccA is marked by asterisks.

accA mutant spores were cultured in CYE medium, they were able to germinate, although more slowly, approximately 24 h later, than wild-type spores.

Biotinylated proteins in wild-type and *accA* disruption mutant cells. Biotinylated proteins are rare in bacteria, but four proteins in *M. xanthus* wild-type protein extracts reacted with streptavidin in Western blot analysis (Fig. 4A). The sizes of the four proteins were 65, 54, 51, and 31 kDa. The 65-, 51-, and 31-kDa biotin-containing proteins were mainly detected in the exponential-phase wild-type protein extract. The expression of the three proteins dropped off during the stationary phase and development. In the *accA* disruption mutant, only the 65-kDa protein was absent in the exponential-phase cells (Fig. 4B). The value of 65 kDa obtained by SDS-PAGE corresponded well with the molecular mass (61.5 kDa) of the *M. xanthus accA*

gene calculated from the predicted amino acid sequence. When the *AccA* protein was overexpressed in *E. coli*, its molecular size in SDS-PAGE was 66 kDa (data not shown). The results indicated that the product of the *accA* gene was a biotinylated protein that was expressed mainly in the exponential phase. The 54-kDa protein, which is expressed mainly during development, is thought to be the α subunit of propionyl-CoA carboxylase, because the purified propionyl-CoA carboxylase of *M. xanthus* contains a 53-kDa biotinylated protein (α subunit) (21).

Acetyl-CoA carboxylase assay. DNA sequence analysis suggested that the *accA* and *accB* genes may encode two subunits of propionyl-CoA carboxylase or acetyl-CoA carboxylase. To test this hypothesis, the enzyme activities of the wild type and the *accA* disruption mutant were assayed in crude cell extracts.

EcaccD	SNITPTRKAS	IPEGVWTKCD	SCGQVLYRAE	LERNLEVCPK	CDHHRMTAR	NRLHSLLEDEG	68
VpmmDA	-----	-----MA	TVQEKIELH	EKLAKVK--A	GGG-EK-RVE	KQHESGKLTA	38
MxpccB	-----	-----M	DQTPEKDPLR	ARLEKMEKQA	ELGGGADRIA	KQHAQGMKTA	41
MxaccB	MPRITSRIDP	GSESEFKVNRA	DMLARVSELR	AIEAKVVR---	--NTENQAKE	KFHKRGQLLP	55
EcaccD	SLVELGSELE	PKDVLKFRDS	KKYKDLASA	QKETGEKDAL	VVMKGTLYGM	PVVAFAFEFA	128
VpmmDA	RERLAKLFDD	NS-FVELDQF	VKHRCVNFQ	EKKELPGEV	VTGYGTIDGR	LVIYFAQDFT	98
MxpccB	RERIDLLLD	GS-FCLEDKF	VTHRSSEFGM	GDKKIPGHV	VTGYGTVEGR	KVFVFAQDFT	101
MxaccB	RERLMLLLDR	GSPFLELSTL	CGYGYHDS-	-DGLAGGNS	IIGIGYVSGV	RCIVFVNNSA	113
putative acyl-CoA-binding domain							
EcaccD	FMGGSMGSVV	GARFVRAVEQ	ALEDNCP LIC	FSASGGARMQ	EALMSLMQMA	KTSAALAKMQ	188
VpmmDA	VEGGSLGEMH	AAKIVKVQRL	AMKMGAPIVG	INDSGGARIQ	EAVDALAGYG	KIFFENTNAS	158
MxpccB	VFGGSLSGAY	AQKICKIMDL	ATRVGAPVIG	LNDSGGARIQ	EGVESLAGYA	DIFVRNTGCS	161
MxaccB	IKGGTASPWG	VQKALRAQAL	ALENKLPMSV	LVESGGANLM	YQEIFIPGG	ETFYNQARLS	173
EcaccD	ERGLPYISVL	TDPTMGVSA	SFAMLGDLNI	AEPKALIGFA	GPRVIEQTVR	EKLPPGFORS	248
VpmmDA	GV-IPQISVI	MGPCAGGAVY	SPALTDIFYM	VKNTSQMFIT	GPAVIKSVTG	EEVTAEDLGG	217
MxpccB	GV-VPQISLI	MGPCAGGAVY	SPAITDFIMM	VKDTSYMFIT	GPDVIKTVTH	EEVSKEALGG	220
MxaccB	AAGIPQVTVV	HGSSTAGGAY	LPGLSDYVVM	VKKKAKVFLA	GPPLLKAATG	EVATDEELGG	233
EcaccA	MSLNFDFEQ	PIAELEAKID	SLTAVSRQDE	KLDINIDEEV	HRLREKSVEL	TRKIFADLGA	60
EcaccD	EFLIEKG AID	MIVRRPEMRL	KLASILAKLM	NLPAPNPE-A	PREGVVVPPV	PDQEPEA...	304
VpmmDA	AMAHNSVSGV	AHFAAENEDD	CIAQIRYLLG	FLPSNNMEDA	PLVDTGDDPT	REDESL-NSL	276
MxpccB	AVTHNQKSGV	AHFAAENEQA	AIVMTRELLS	FLPSNNQEEA	PVQPCEDDAF	RGRRSRSRTI	280
MxaccB	AQM HATVAGT	ADYLAEDDAD	AIRMAREIVA	KLGWNAQLPP	TERPAYAEPV	YSPDEL-CGA	292
putative carboxybiotin-binding domain							
EcaccA	WQIAQLARHP	QRPYTLDYVR	LAFDFEDEL	AGDRAYADDK	AIVGGIARLD	GRPVMIIHQ	119
VpmmDA	----LPDNS	NMPYDMK DVI	AATVDNGEYY	EVQPFYA--T	NIITCFARFD	GQSVGIIANQ	329
MxpccB	----VPSNP	NKPYDIKEVI	KAI VDDKHFF	EVQEHFA--K	NIVIGFARMN	GRTVGVVANQ	333
MxaccB	----VPVDY	RKPYDCREVI	ARI VDGSEFT	GFKDEYD--A	HTVCGWANLY	GHPLGIIGNN	345
EcaccA	KGRETEKIR	RNFGMPAPEG	YRKALRLMQM	AERFKMPIIT	FIDTPGAYPG	VGAEEERGQSE	179
VpmmDA	P-----K	VMAGCLDINA	SDKSRFRIR	CDAFNPIPVN	FVDVPGFLPG	TNQEWGGIIR	381
MxpccB	P-----A	VLAGVLDIDA	SIIKARFVRF	CDCFNIP LVT	LVDVPGFLPG	TQEWGGIIT	385
MxaccB	-----	---GPISPQG	ATKAAQFIQL	CCQKDTPILY	LQNTTGYLVG	TQPEQGGIVK	392
EcaccA	AIARNLREMS	RLGVPVCTV	IGEGGSGGAL	AIGVGDKNM	LQYSTYSVIS	PEGCASILWK	239
VpmmDA	HGAKMLYAYS	EATVPKI-TV	ITRKAYGGSY	LAMCSQDLGA	DQVYA---WP	TSEIIVMGPA	437
MxpccB	HGAKLLYAYA	EATVPKV-TV	ITRKAYGGAY	DVMASKHIRA	DMNFANFAWP	TAEIIVMGPE	444
MxaccB	HGAKMIQAVA	NATVPQL-TV	LIGGAFGAGN	YGMCGRP FHP	R---FIFGWP	NSRTAVMGGE	448
EcaccA	SADK----AP	LAAEAMGIIA	PRLKELKLID	SIIPEPLGGA	HRNPEAMAAS	LKAQLLADLA	295
VpmmDA	GAANIIFKFD	EDKDAKTAKY	VEEFATPYKA	-----	-----AERG	FVDVVIPEKQ	481
MxpccB	GA---VNIIIF	RNELAKA---	--PDA AERA	RLTADYRDKF	ATPFKAAELG	YIDEIIRPEE	496
MxaccB	QAAK VMSIVF	GEKLARQQV	VDEEQLKAFK	QPIIDQFDKE	SHPFNCSARM	FDDGLIDPRD	508
EcaccA	DL DVLSTEDL	KNRRYOR---	-LMSYGYA..				319
VpmmDA	TRPAVINALA	MLAS-KREN R	APKKHGNIPL				510
MxpccB	TRAKLIR SLE	LLKD-KRQEN	LPRKHGNIPL				525
MxaccB	TRRVLGFALS	VCREAKRRQV	HPNTFGVARL				538

FIG. 3. Amino acid sequence alignment of homologous regions in the *E. coli* acetyl-CoA carboxylase α subunit (EcaccA) and β subunit (EcaccD) of carboxyltransferase, the *V. parvula* methylmalonyl-CoA decarboxylase α subunit (VpmmDA), the *M. xanthus* propionyl-CoA carboxylase β subunit (MxpccB), and the *M. xanthus* acetyl-CoA carboxylase carboxyltransferase subunit (MxaccB).

Western blot analysis indicated that AccA protein was expressed during the exponential growth phase. Therefore, the enzyme activities were assayed in extracts from exponential-phase cells. Acetyl-CoA carboxylase activities were found in both wild-type and *accA* mutant cell extracts (Table 1). However, the specific activity in *accA* mutant cells was decreased by approximately 40% compared to that in wild-type cells. In the propionyl-CoA carboxylase assay, the difference between the specific activities from wild-type and *accA* mutant extracts was not significant.

DISCUSSION

Acetyl-CoA carboxylase catalyzes the synthesis of malonyl-CoA, the first committed step in the biosynthesis of fatty acids (1). In *E. coli*, there is a direct correlation between the levels of transcription of the acetyl-CoA carboxylase genes and the rate of cell growth (27). A *Saccharomyces cerevisiae* acetyl-CoA carboxylase mutant showed inhibition of the synthesis of very-long-chain fatty acids, and the reduction in levels of these very-long-chain fatty acids resulted in marked alterations of

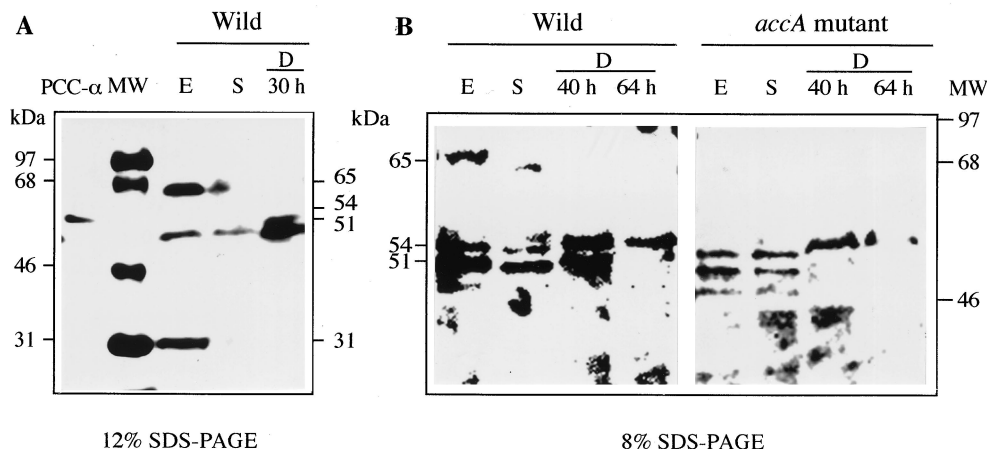


FIG. 4. Detection of biotinylated polypeptides in the protein extracts of wild-type and *accA* mutant strains. Proteins, biotinylated molecular mass standards (MW), and purified *M. xanthus* propionyl-CoA carboxylase (PCC- α) were separated by SDS-12% PAGE (A) or SDS-8% PAGE (B) and probed with streptavidin-linked horseradish peroxidase. The protein extracts were prepared from cultures at the exponential growth phase (E), at stationary phase (S), and during development (D).

the nuclear envelope (33). In the *M. xanthus accA* mutant, the total amounts of long-chain fatty acids (C_{16} to C_{18}) in vegetative and developing cells were decreased by about 4 and 6%, respectively, compared to those in wild-type cells (data not shown), but this reduction was not as marked as those observed previously in a propionyl-CoA carboxylase (*dcm-1*)-deficient mutant. *E. coli* and *S. cerevisiae* acetyl-CoA carboxylases are essential for growth (12, 28). In *M. xanthus*, disruption of the *accA* gene was not lethal, and the mutant was not completely deficient in acetyl-CoA carboxylase activity. Western blot analysis revealed the presence of 31- and 51-kDa biotinylated proteins during the exponential phase. The molecular masses on SDS-PAGE of biotin carboxyl carrier proteins of acetyl-CoA carboxylases from *E. coli*, *Anabaena* sp. strain PCC 7120, and *Pseudomonas aeruginosa* are 22 to 25 kDa (3, 11, 26). Since the 31-kDa protein was similar to these proteins in size, *M. xanthus* may have another acetyl-CoA carboxylase, like the enzyme from *E. coli*. Most higher plants have two types of acetyl-CoA carboxylases, the *E. coli*-like and eukaryotic acetyl-CoA carboxylases, which exist in plastids and the cytosol, respectively (22). The molecular mass of the biotinylated proteins of *E. coli*-like acetyl-CoA carboxylases in plants was 35 kDa on SDS-PAGE (22, 32).

The acetyl-CoA carboxylase activity was detected in growing *M. xanthus* cells. In previous studies, *M. xanthus* cells harvested in the stationary phase and during development showed very low acetyl-CoA carboxylase activity, but propionyl-CoA carboxylase activity was detected both in the stationary phase and during development and reached a maximum during the sporulation phase (21). The results of enzyme assays corresponded well to the expression of biotinylated subunits of acetyl-CoA and propionyl-CoA carboxylases as determined by

Western blot analysis in this study. The *M. xanthus accA* mutant showed a decreased growth rate, but no significant differences in cell morphology or sporulation were observed. The presence of substantial acetyl-CoA carboxylase activity in the *accA* mutant cells may account for the absence of any difference in cell morphology or sporulation in the mutant.

The acetyl-CoA carboxylases can be divided into two basic types, a bacterial type and a eukaryotic type, by their structure. The bacterial type contains four dissociated proteins, the biotin carboxylase, the biotin carboxyl carrier protein, and two carboxyltransferase subunits (α and β), organized into three functional domains (Fig. 5). The α and β subunits of the carboxyltransferase component of *E. coli* have acyl-CoA-binding and carboxybiotin-binding domains, respectively. The genes (*accA* and *accD*) encoding the two carboxyltransferase subunits are located almost directly opposite each other in the *E. coli* chromosome. The biotin carboxyl carrier protein gene (*accB*) and biotin carboxylase gene (*accC*) from *E. coli* and *P. aeruginosa* form a two-gene operon, and the two genes are cotranscribed (3, 26). In the eukaryotic type, these proteins are part of a single multifunctional polypeptide derived from the expression of a single gene. From the amino terminus, the biotin carboxylase component, biotin-binding site, carboxybiotin-binding site, and acyl-CoA-binding domain are distributed in this order along the eukaryotic acetyl-CoA carboxylase (Fig. 5). The results of this study indicated that *M. xanthus AccA* is a biotinylated biotin carboxylase subunit of acetyl-CoA carboxylase. The *accB* gene encoding the putative carboxyltransferase subunit was located upstream of the biotin carboxylase gene (*accA*). We did not confirm whether *AccB* is a carboxyltransferase subunit of acetyl-CoA carboxylase. However, since the genes encoding *AccA* and *AccB* formed a two-gene operon,

TABLE 1. Acetyl-CoA and propionyl-CoA carboxylase activities^a in wild-type and *accA* mutant strains

Fraction	Acetyl-CoA carboxylase		Propionyl-CoA carboxylase	
	Total activity (mU)	Sp act (mU/mg of protein)	Total activity (mU)	Sp act (mU/mg of protein)
Wild type	22.3	1.0	69.0	3.1
<i>accA</i> mutant	12.3	0.6	60.9	3.0

^a The acetyl-CoA and propionyl-CoA carboxylase activities were determined with acetyl-CoA and propionyl-CoA as substrates from the rate of malonyl-CoA and methylmalonyl-CoA formations, respectively. The total and specific activities were expressed as means of triplicate enzyme assays.

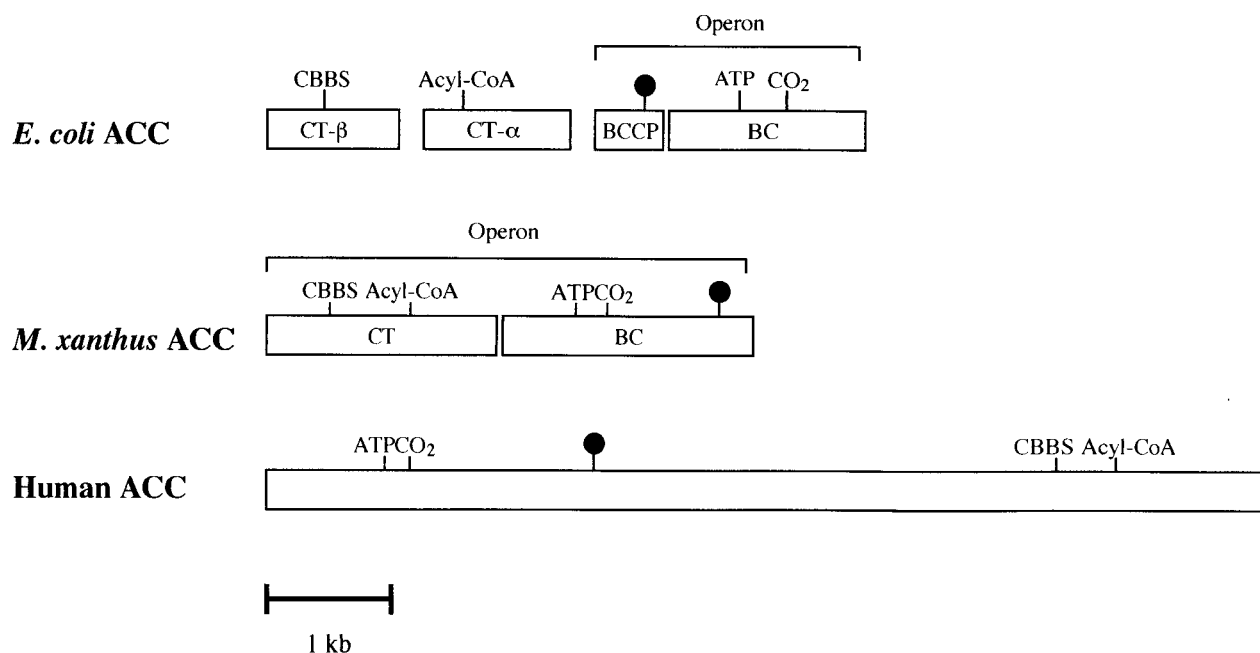


FIG. 5. Schematic diagram for the subunit structures of acetyl-CoA carboxylases. Abbreviations and symbols: CBBS, carboxybiotin-binding site; Acyl-CoA, acyl-CoA-binding site; ATP, ATP-binding site; CO₂, CO₂ fixation site; ●, biotin-binding site; ACC, acetyl-CoA carboxylase; CT, carboxyltransferase; BCCP, biotin carboxyl carrier protein; BC, biotin carboxylase.

AccB was thought to function as a carboxyltransferase subunit of the enzyme. We propose that the *M. xanthus accA* and *accB* genes may have been constructed by the fusion of *E. coli accB*- and *accC*-like genes and of *E. coli accA*- and *accD*-like genes, respectively, and may have integrated to form a single gene, such as a eukaryotic acetyl-CoA carboxylase gene.

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