JOURNAL OF BACTERIOLOGY, Oct. 2000, p. 5462–5469 Vol. 182, No. 19
0021-9193/00/$04.00+0
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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, CO_2 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 CO_2 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 deletion 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 *Sau*3AI, 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′-TCCGTCACGGCGTGCGCGCC-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 *Sma*I site of the pT7 Blue-T vector, the recombinant plasmid was digested with *Bam*HI and *Eco*RI. 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 *Sma*I sites (10). The resulting 1.2-kb fragment containing the Km^r gene was purified, digested with *Sma*I, and inserted into the *Sma*I 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 *Sac*I and then analyzed by Southern hybridization using a 1.4-kb *Sma*I fragment as a probe. We confirmed that the *Sac*I 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 [OD_{600}] 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 $MgCl_2$, 66 mM $KHCO_3$, 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 $MgSO_4$) 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 from the conserved sequences in the biotin carboxylase subunits of acetyl-CoA carboxylases. The 3.8-kb *Apa*I, 5.6-kb *Sac*I, and 1.4-kb *Sma*I fragments of the clone DNA hybridized with the probe. Based on the restriction map derived from hybridization data, the 4-kb *Apa*LI-*Sma*I 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 CO_2 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 CO_2 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 3.0×10^9 cells/ml for the wild type and 5.0 h and 2.5×10^9 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

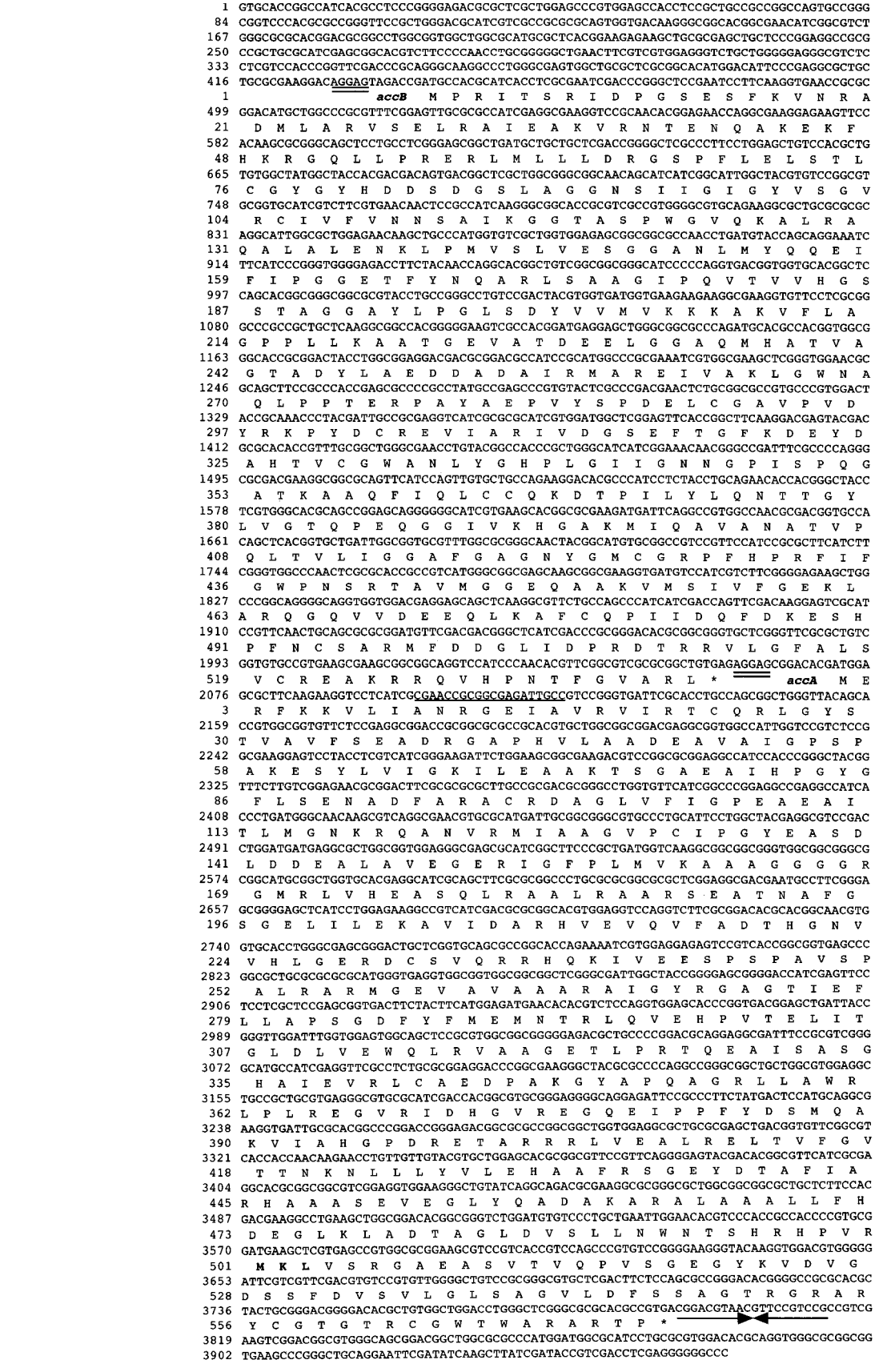

FIG. 1. Nucleotide and deduced amino acid sequences of the *accA* and *accB* genes of *M. xanthus*. Putative ribosome-binding sites are double underlined. Arrows indicate the position of the palindrome sequence. The sequence corresponding to the probe is underlined. Boldfaced amino acids represent the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

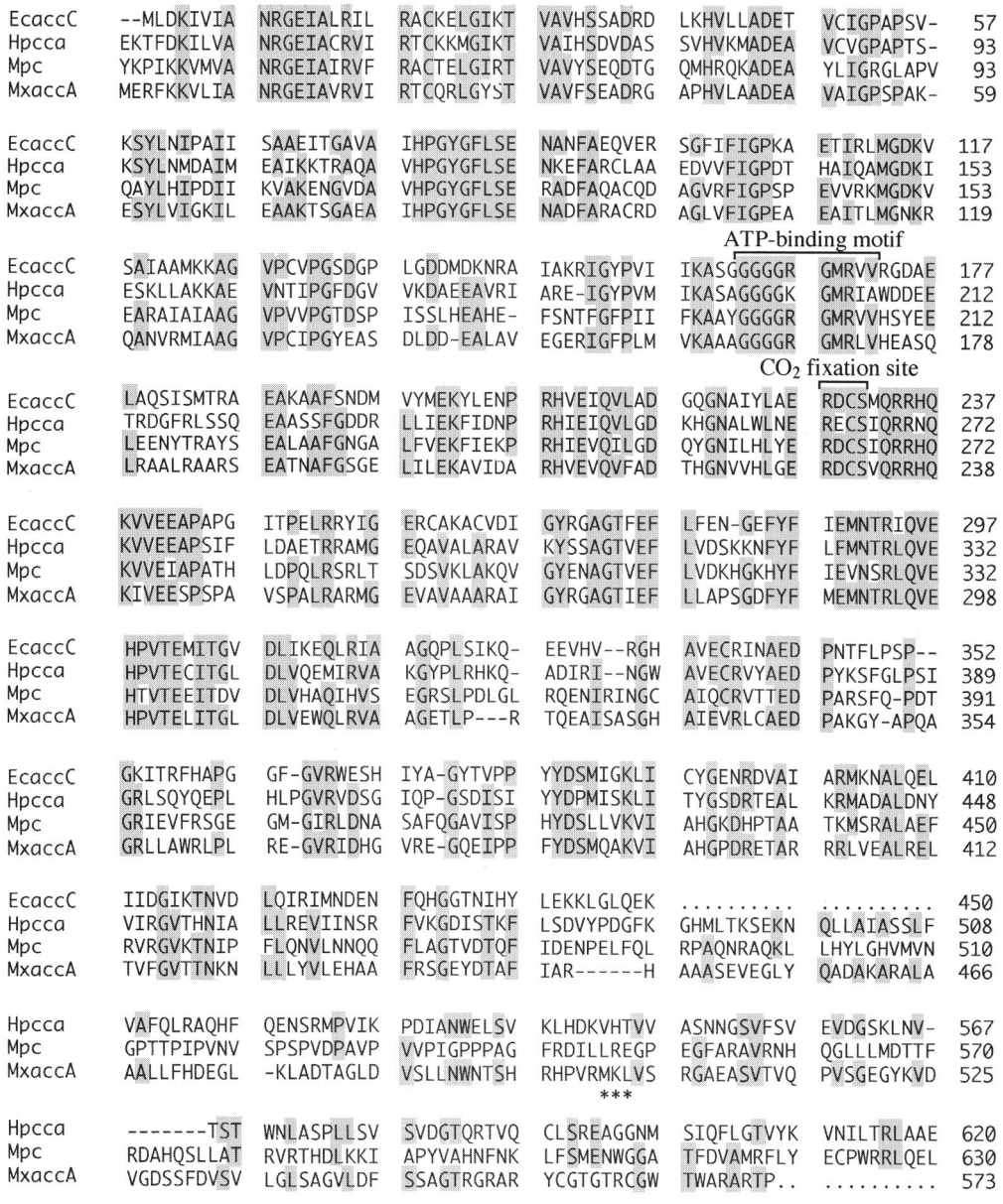

FIG. 2. Amino acid sequence alignment of homologous regions in the *E. coli* acetyl-CoA carboxylase biotin carboxylase subunit (EcaccC), the human propionyl-CoA carboxylase α subunit (HpccA), the mouse pyruvate carboxylase (Mpc), and the *M. xanthus* acetyl-CoA carboxylase biotin carboxylase subunit (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.

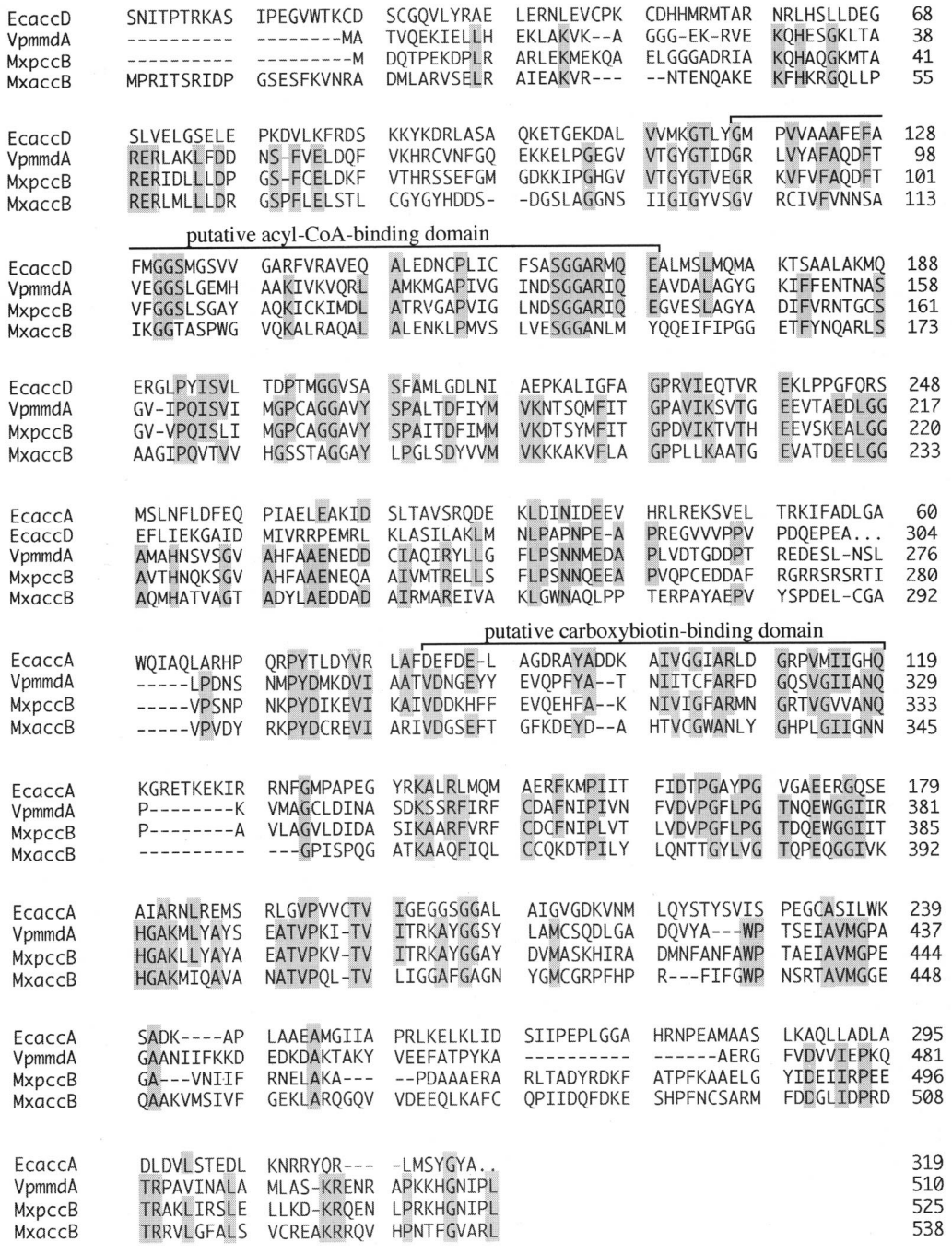

FIG. 3. Amino acid sequence alignment of homologous regions in the *E. coli* acetyl-CoA carboxylase α subunit (EcaccA) and β subunit (EcaccD) of carboxyltransferase, the *V. parvula* methylmalonyl-CoA decarboxylase α subunit (VpmmdA), the *M. xanthus* propionyl-CoA carboxylase β subunit (MxpccB), and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

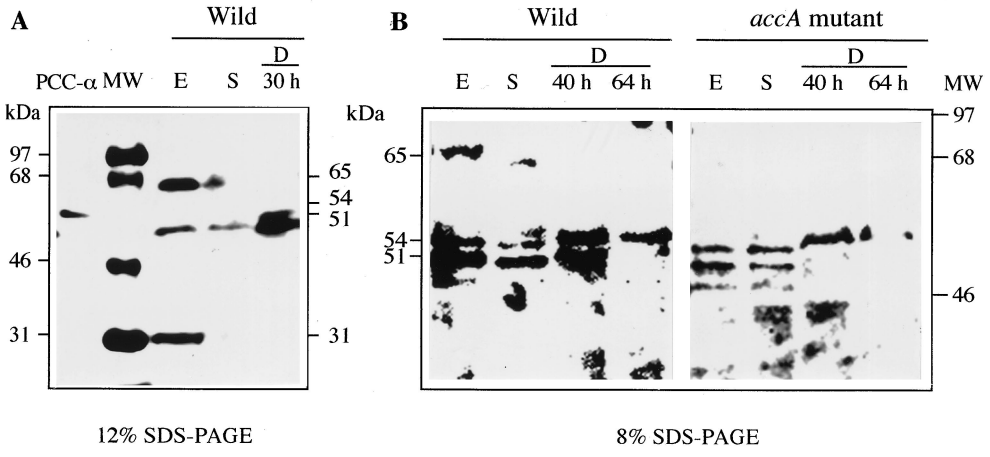

FIG. 4. Detection of biotinylated polypeptides in the protein extracts of wild-type and *accA* mutant strains. Proteins, biotinylated molecular mass standards (MW), and purified *M. xanthus* propionyl-CoA carboxylase (PCC-α) were separated by SDS–12% PAGE (A) or SDS–8% PAGE (B) and probed with streptavidin-linked 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
accA 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.

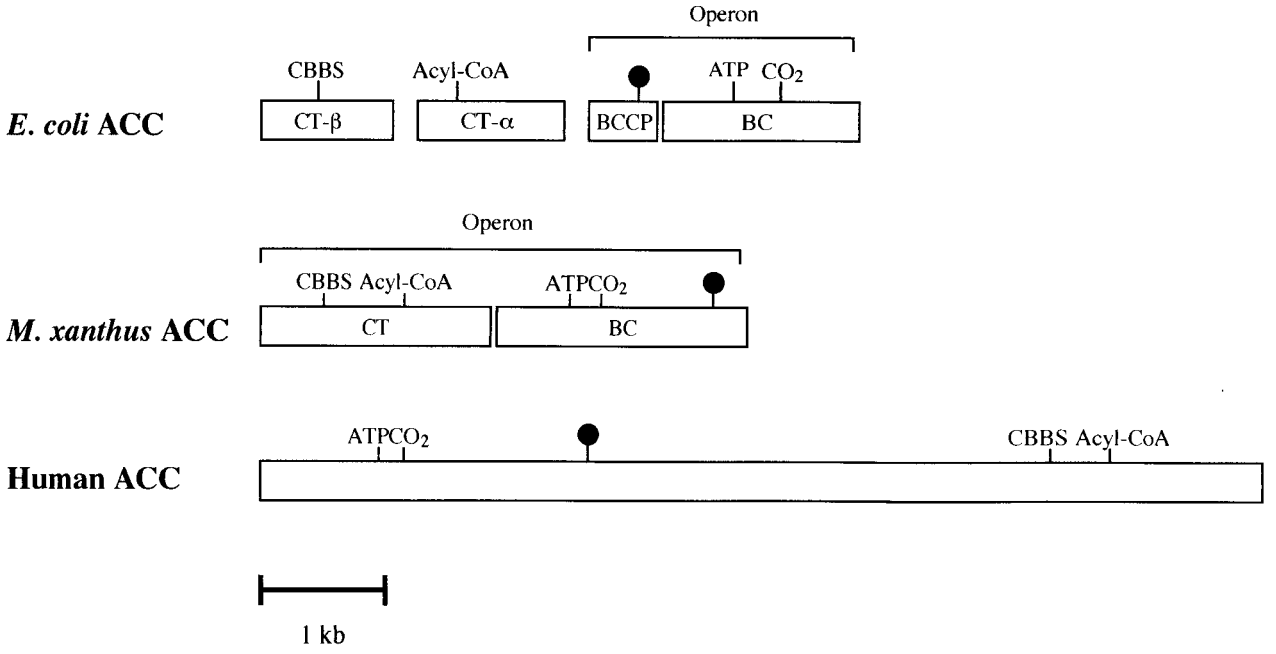

FIG. 5. Schematic diagram for the subunit structures of acetyl-CoA carboxylases. Abbreviations and symbols: CBBS, carboxybiotin-binding site; Acyl-CoA, acyl-CoA-binding site; ATP, ATP-binding site; CO_2, CO_2 fixation site; ●, biotin-binding site; ACC, acetyl-CoA carboxylase; CT, carboxyltransferase; 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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