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Propionyl Coenzyme A Carboxylase Is Required for Development of *Myxococcus xanthus*

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A *dcm-1* mutant, obtained by transposon mutagenesis of *Myxococcus xanthus*, could aggregate and form mounds but was unable to sporulate under nutrient starvation. A sequence analysis of the site of insertion of the transposon showed that the insertion lies within the 3′ end of a 1,572-bp open reading frame (ORF) designated the *M. xanthus pccB* ORF. The wild-type form of the *M. xanthus pccB* gene, obtained from a λEMBL library of *M. xanthus*, shows extensive similarity to a β subunit of propionyl coenzyme A (CoA) carboxylase, an α subunit of methylmalonyl-CoA decarboxylase, and a 12S subunit of transcarboxylase. In enzyme assays, extracts of the *dcm-1* mutant were deficient in propionyl-CoA carboxylase activity. This enzyme catalyzes the ATP-dependent carboxylation of propionyl-CoA to yield methylmalonyl-CoA. The methylmalonyl-CoA rescued the *dcm-1* mutant fruiting body and spore development. During development, the *dcm-1* mutant cells also had reduced levels of long-chain fatty acids (C_{16} to C_{18}) compared to wild-type cells.

Myxococcus xanthus is a gram-negative gliding bacterium that feeds on other organisms and decaying organic matter (8, 20, 36). In response to nutritional stress, development proceeds if the cells are at a sufficiently high density on a solid surface. The starved cells aggregate to form multicellular fruiting bodies. During the formation of aggregates (mounds) and then fruiting bodies, the cell shape changes from rod-like to spherical. *M. xanthus* cells coordinate their multicellular behavior through cell-cell communication by transmission of intercellular signals. The transmission of intercellular signals in *M. xanthus* has been studied by isolating development-defective mutants, dividing them into five groups (A to E), and conducting pairwise mixing tests of the different groups.

A group E mutant contains a Tn*5* insertion within a branched-chain keto acid dehydrogenase (BCKAD) gene (6, 38). The BCKAD is involved in the synthesis of branched-chain fatty acids. The branched-chain fatty acids (E signal) are released from cellular phospholipase by a developmentally regulated phospholipase during the formation of fruiting bodies. Downard and Toal suggested that the branched-chain fatty acids play a critical role in development and cell-cell communication (6).

We isolated a developmental cell morphological mutant (*dcm-1* mutant) of *M. xanthus* by Tn*V* mutagenesis. The Tn*V* insertions occur in a β subunit of the *M. xanthus* propionyl coenzyme A (CoA) carboxylase (*pccB*) gene. We describe the role of propionyl-CoA carboxylase during the development of *M. xanthus*.

MATERIALS AND METHODS

Bacterial strains, phage, and plasmids. *M. xanthus* IFO 13542 (ATCC 25232) was grown vegetatively on Casitone-yeast extract (CYE) medium at 30°C (4, 7). Fruiting body formation was assayed on clone fruiting medium containing 1.5% agar (CF agar) (10). *Escherichia coli* DH5 and MRF were used as recipient strains in P1 infection and plasmid transformation, respectively. Phage P1*clr100*Cm was used to transduce transposon Tn*V* to *M. xanthus.*

Infection of *M. xanthus* with P1::Tn*V*. Transposition of Tn*V* from P1::Tn*V* into the *M. xanthus* chromosome was performed as described previously (9, 23). Infected cells were inoculated on CYE agar plates containing 20 μg of kanamycin per ml. The plates were incubated at 30°C for 6 h and then overlaid with top agar containing kanamycin to make the final concentration in the plate 85 μg/ml. After 5 days of incubation at 30°C, Km^r cells were inoculated into CYE containing 70 μg of kanamycin per ml. The cells were harvested, washed with 10 mM Tris-HCl–8 mM $MgSO_4$ (pH 7.6; TM buffer), and spotted on CF agar plates. The plates were incubated for 7 days at 30°C and observed for formation of fruiting bodies and spores under a light microscope. Mutants that formed aggregates but not fruiting bodies were selected by using a microscope.

Identification of Tn*V* insertion region. About 5 μg of mutant genomic DNA was digested completely with *Apa*I and electrophoresed in a 0.7% agarose gel. The *Apa*I DNA fragments were transferred to a nylon membrane (GeneScreen Plus; Dupont, NEN Research Products, Boston, Mass.) and hybridized with a digoxigenin (DIG)-dUTP-labeled Tn*V* probe. Hybridization was carried out with a hybridization buffer (0.5 M sodium phosphate buffer [pH 7.2], 7% sodium dodecyl sulfate, 1 mM EDTA–2Na) for 12 h at 54°C. Hybridized DNA bands were detected with a DIG DNA detection kit (Boehringer Biochemicals, Mannheim, Germany). DNA fragments which hybridized to the probe were recovered from agarose gels following electrophoresis, self-ligated, and transformed in *E. coli* MRF to Km^r. Plasmids containing *Apa*I fragment ::Tn*V* were isolated from the Km^r transformants. The genome DNA of the mutant in the plasmid was elongated by PCR initiated from oligonucleotide 5′-GGACGCTACTTGTGTA TAAGAG-3′, which is part of IS*50* (1). The PCR product (0.8 kb) was ligated in pT7 Blue, transformed in *E. coli* MRF, and sequenced by the method of Sanger et al. (35). The PCR product was also labeled with DIG-11-dUTP by using a random primer labeling kit (Boehringer Biochemicals) and used as a probe for the cloning of the *M. xanthus pccB* gene from a genomic DNA library.

Cloning of the *M. xanthus pccB* gene from a genomic DNA library. The *M. xanthus* genomic DNA was prepared according to the method described by Avery and Kaiser (2). The genomic DNA of wild-type bacteria thus obtained was partially digested with *Sau*3AI and size fractionated by 10 to 40% sucrose density gradient centrifugation. Then, 9- to 20-kb fragments were ligated with *Bam*HI-cleaved λEMBL 3 arms. In vitro packaging of the recombinant molecules was carried out with a commercial kit. A positive plaque was selected by plaque hybridization, and a 3.3-kb *Sma*I fragment, cut from the phage DNA with *Sma*I, was hybridized with the probe. The *Sma*I fragment was ligated with *Sma*I-cut pBluescript SK and transformed *E. coli* MRF.

Nucleotide sequencing. DNA sequencing of subfragments cloned into pBluescript SK was carried out by the dideoxynucleotide chain termination method of Sanger et al. (35). Nested deletions were constructed by using a double-stranded nested deletion kit from Pharmacia (Uppsala, Sweden).

Enzyme assays. Cells used for enzyme assay were grown at 30°C for 60 h on CF agar. The cells were harvested and washed with 10 mM Tris-HCl buffer (pH 7.2). The cells were suspended in the same buffer and disrupted by sonication with a Branson sonifier (four 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). The cell debris was washed twice with 10 mM Tris-HCl buffer (pH 7.2) and treated with 2% Triton X-100 (final concentration) overnight at 4°C. After centrifugation to

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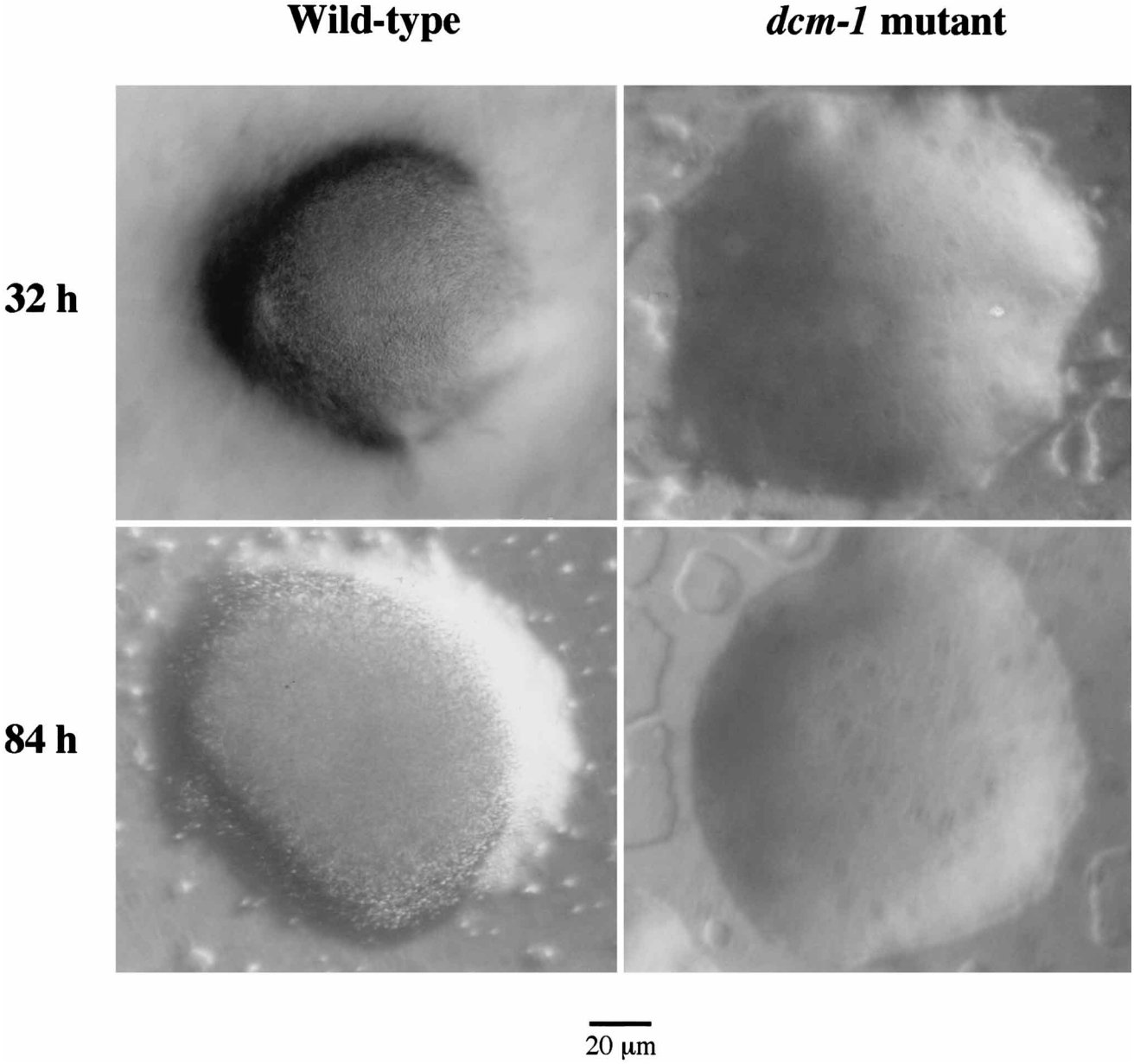

FIG. 1. Development of wild-type and *dcm-1* mutant cells. Wild-type cells formed mounds at 20 to 32 h and then constructed fruiting bodies by 84 h on a CF agar plate. The *dcm-1* mutant cells aggregated and formed mounds at 32 h on a CF agar plate but did not form fruiting bodies at 84 h. The photographs were taken at the times indicated to the left through a microscope.

remove cell debris at 12,000 × *g* for 10 min, the supernatant was used for an enzyme assay. Each enzyme activity was determined from the increase of product detected by high-performance liquid chromatography (HPLC). For propionyl- and acetyl-CoA carboxylase assays, the reaction mixture (0.2 ml) contained (in micromoles): Tris (pH 7.2), 12; ATP, 0.6; $MgCl_2$, 0.6; $KHCO_3$, 13; propionyl-CoA or acetyl-CoA, 0.02; and crude enzyme (12). Transcarboxylase activity was assayed as described previously (30). The mixtures were incubated at 30°C for 1 h and then filtered through an ultrafiltration membrane (Ultrafree C3; Millipore Co.). The filtrates were injected onto a column packed with ODS-80Ts (C18; 150 by 4.6 mm [inside diameter]; Tosoh Co., Tokyo, Japan.). Elution solvent A was 75 mM sodium acetate adjusted to pH 4.6 with acetate. Solvent B was 70% solvent A in methanol. The profile of the gradient elution was as follows: 0 min, 90% solvent A; 10 min, 60% solvent A; 30 min, 10% solvent A; 40 min, 0% solvent A (5). Absorbance measurements were made at 254 nm.

Fatty acid analysis. Wild-type and mutant cells were harvested from vegetative and developmental medium, washed twice in deionized water, and concentrated by centrifugation. The washed cells were dried under vacuum at room temperature for 60 min. The fatty acid methyl esters were liberated from the dried cells by methanolysis at 105°C for 3 h with 0.2 ml of 5% HCl-methanol and extracted three times with 3 ml of hexane (37). The supernatant extracts were evaporated to dryness and dissolved in 20 μl of acetonitrile. The fatty acid methyl esters were separated with a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector. A fused silica capillary column of cross-linked 5% phenyl methylpolysiloxane (DB-5; 0.25 mm by 30 m; 0.25-μm film thickness; J & W Scientific, Folsom, Calif.) was employed and operated at 150 to 250°C. Standard fatty acid methyl esters were obtained from Funakoshi Co. (Tokyo, Japan). Fatty acid analyses were done with duplicate cultures.

Nucleotide sequence accession number. The DNA and amino acid sequences will appear in the EMBL, GenBank, and DDBJ databases under accession no. AB007000.

RESULTS

The phenotype of *dcm-1* mutant cells. The *dcm-1* mutant obtained by Tn*V* mutagenesis grew as well as the wild type (*M.*

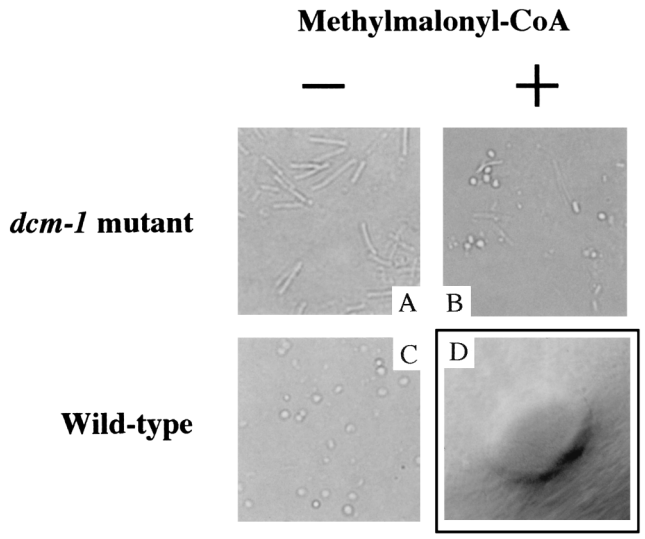

FIG. 2. Effect of methylmalonyl-CoA on the development of *dcm-1* mutant cells. The *dcm-1* mutant cells were spotted on CF agar medium with 0, 0.001, 0.05, 0.5, 5, 50, and 500 μM methylmalonyl-CoA and incubated at 30°C. The wild-type cells developed into myxospores on CF agar without methylmalonyl-CoA (C). The *dcm-1* mutant cells cultured with 5 μM methylmalonyl-CoA formed fruiting bodies (D) and spores (B). The *dcm-1* mutant fruiting body had an approximate diameter of 0.1 mm. The *dcm-1* mutant cells cultured without methylmalonyl-CoA maintained a rod-shaped morphology at 240 h within a mound (A). The photographs shown in panels B, C, and D were taken 84 h after the start of development.

xanthus IFO 13542) in the growth medium, CYE. Under these conditions, the two strains are morphologically identical. The *dcm-1* mutant cells were much less yellow than the wild-type cells. On the developmental medium, CF agar, the wild-type and mutant cells moved to aggregation centers where they assembled into multicellular structures called mounds at 24 to 32 h of development. Wild-type cells formed fruiting bodies and spores at approximately 70 to 84 h, while the *dcm-1* mutant cells maintained a rod shape within mounds and did not form fruiting bodies or spores at 10 days of development (Fig. 1 and 2). When wild-type and *dcm-1* mutant cells were mixed on CF agar, the mutant formed fruiting bodies and myxospores (data not shown).

Nucleotide sequence of the *M. xanthus pccB* gene. A 6.8-kb *Apa*I fragment containing 6 kb of Tn*V* was cloned from mutant DNA with a Tn*V* probe. The 0.8-kb insertion region around Tn*V* was sequenced and amplified by PCR. The amplified DNA was used as a probe for cloning the wild-type DNA. The insertion region of the wild-type DNA was cloned from a λ phage library. A 3.3-kb *Sma*I fragment from the λEMBL 3 clone had a sequence identical to that of the mutant DNA. The sequence contained one complete open reading frame (ORF), designated the *M. xanthus pccB* ORF, capable of encoding a 524-amino-acid protein of 57.1 kDa.

The predicted amino acid sequence was compared with those in the EMBL database by using the Blastp program. Alignments with some of the most similar proteins are shown in Fig. 3. A remarkable degree of identity between *M. xanthus* propionyl-CoA carboxylase (MxpccB) and the β chain of human propionyl-CoA carboxylase (61.6%) and between MxpccB and the carboxytransferase domain of rat liver propionyl-CoA carboxylase (61.3%) was found (22, 26). Also, the *pccB* gene shows similarity to the α subunit of methylmalonyl-CoA decarboxylase (58% identical) (17). A sequence analysis of the Tn*V* insertion regions of mutant chromosomal DNA showed that Tn*V* in the *pccB* gene had been inserted at bp 1284 at the 3′ end.

Propionyl-CoA carboxylase assay. The DNA sequence analysis suggested that the *dcm-1* mutant was deficient in propionyl-CoA carboxylase activity. To test this, the propionyl-CoA carboxylase activities of the wild-type and *dcm-1* strains were assayed in crude cell extracts (Table 1). The crude enzymes, extracellular culture fluid, cell extracts, and cell membrane extracts were used for the enzyme assay. In the wild type, about 92% of the propionyl-CoA carboxylase activity was found in cell extracts. A small amount of propionyl-CoA carboxylase (2.5%) also appeared in extracellular fluid, and that could have resulted from cell lysis rather than from a specific excretion mechanism. Relatively little activity was found in the *dcm-1* mutant developing and growing cell extracts. When acetyl-CoA was used as a substrate, the malonyl-CoA product was detected in enzyme reactions with both the wild-type and *dcm-1* mutant cell membrane extracts. The specific activities of wild-type and *dcm-1* mutant cells were 0.63 and 0.66 nmol/min/mg, respectively. In reactions with extracellular and cell extracts, the substrate and product were not detected by HPLC. Acetyl-CoA, a major metabolic intermediate, may be metabolized

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MxpccB     1 ..........  ..........  .......MDQ  TPEKDPLRAR  LEKMEKOAEL  GGGADRIAKQ
Hpccb      1  .MAAALRVA  AVGARLSVLA  SGLRAAVRSL  CSQATSVNER  IENKRRTALL  GGGQRRIDAH
Rnpccb     1 MAAVIRIRAM  AAGTRLRVLN  CGLGTTIRSL  CSQPVSVNER  IENKRHAALL  GGGQRRIDAQ
VpmmdA     1 ..........  ..........  ..........  MATVQEKIEL  LHEKLAKVKA  GGGEKRVEKQ
Pstc12     1 ..........  ..........  .MAENNNLKL  ASTMEGRVEQ  LAEQRQVIEA  GGGERLVEKQ
                                                                       ***

MxpccB    34 HESGKLTARE  RIDLLLDPGS  FCELDKFVTH  RSSEFGM-GD  K-KIPGHGVV  TGYGTVEGRK
Hpccb     59 DKRGKLTARE  RISLLLDPGS  FVESDMFVEH  RCADFGMAAD  KNKFPGDSVV  TGRGRINGRL
Rnpccb    61 HKRGKLTARE  RISLLLDPGS  FLESDMFVEH  RCADFGMAAE  KNKFPGDSVV  TGRGRINGRL
VpmmdA    31 HAQGKMTARE  RLAKLFDDNS  FVELDQFVKH  RCVNFGQ-EK  K-ELPGEGVV  TGYGTIDGRL
Pstc12    40 HSQGKQTARE  RLNNLLDPHS  FDEVGAFRKH  RTTLFGM--D  KAVVPADGVV  TGRGTILGRP
                ** ****  *   * *  *  * *  *  *  **     *   *   **  ** *   **

MxpccB    92 VFVFAQDFTV  FGGSLSGAYA  QKICKIMDLA  TRVGAPVIGL  NDSGGARIQE  GVESLAGYAD
Hpccb    119 VYVFSQDFTV  FGGSLSGAHA  QKICKIMDQA  ITVGAPVIGL  NDSGGARIQE  GVESLAGYAD
Rnpccb   121 VYVFSQDFTV  FGGSLSGAHA  QKICKIMDQA  ITVGAPVIGL  NDSGGARIQE  GVESLAGYAD
VpmmdA    89 VYAFAQDFTV  EGGSLGEMHA  AKIVKVQRLA  MKMGAPIVGI  NDSGGARIQE  AVDALAGYGK
Pstc12    98 VHAASQDFTV  MGGSAWRDAV  HEGRRDDGTA  LLTGTPFLFF  YDSGG-RIQE  GIDSLSGYGK
             *   *****  ***                 *   * *      **** ****     * **

MxpccB   152 IFVRNTGCSG  VVPQISLIMG  PCAGGAVYSP  AITDFIMMVK  DTSYMFITGP  DVIKTVTHEE
Hpccb    179 IFLRNVTASG  VIPQISLIMG  PCAGGAVYSP  ALTDFTFMVK  DTSYLFITGP  DVVKSVTNED
Rnpccb   181 IFLRNVTASG  VIPQISLIMG  PCAGGAVYSP  ALTDFTFMVK  DTSYLFITGP  EFVKSVTNED
VpmmdA   149 IFFENTNASG  VIPQISVIMG  PCAGGAVYSP  ALTDFIYMVK  NTSQMFITGP  AVIKSVTGEE
Pstc12   157 MFFANVKLSG  VVPQIAIIAG  PCAC-ASYSP  ALTDFIIMTK  KAH-MFITGP  QVIKSVTGED
              * *   **  * *** * *  ***  * ***  * ***  * *       *****     * ** *

MxpccB   212 VSKEALGGAV  THNQKSGVAH  FAAENEOAAI  VMTRELLSFL  PSNNQEEAPV  QPCEDDAFRG
Hpccb    239 VTQEELGGAK  THTTMSGVAH  RAFENDVDAL  CNLRDFFNYL  PLSSQDPAPV  RECHDPSDRL
Rnpccb   241 VTQEOLGGAK  THTTVSGVAH  RAFDNDVDAL  CNLREFLNFL  PLSNQDPASI  RECHDPSDRL
VpmmdA   209 VTAEDLGGAM  AHNSVSGVAH  FAAENEDDCI  AQIRYLLGFL  PSNNMEDAPL  VDTGDDPTRE
Pstc12   215 VTADELGGAE  PIWPSRAIYF  VAEDDDAAEL  IAK-KLLSFL  PQNNTEEASF  VNPNNDVAPN
             *    ****                          *           * *     *

MxpccB   272 RRSRSRTIVP  SNPNKPYDIK  EVIKAIVDDK  HFFEVQEHFA  KNIVIGFARM  NGRTVGVVAN
Hpccb    299 VPELD-TIVP  LESTKAYNMV  DIIHSVVDER  EFFEIMPNYA  KNIIVGFARM  NGRTVGIVGN
Rnpccb   301 VPELD-TVVP  LESSKAYNML  DIIHAVIDER  EFFEIMPNYA  KNIVIGFARM  NGRTVGIVGN
VpmmdA   269 DESLN-SLLP  DNSNMPYDMK  DVIAATVDNG  EYYEVQPFYA  TNIITCFARF  DGQSVGIIAN
Pstc12   274 -TEL-RDIVP  IDGKKGYDVR  DVIAKIVDWG  DYLEVKAGYA  TNLVTAFARV  NGRSVGIVAN
                    *                 *   *        *   *    ***   *  **   *

MxpccB   332 QPAVLAGVLD  IDASIKAARF  VRFCDCFNIP  LVTLVDVPGF  LPGTDQEWGG  IITHGAKLLY
Hpccb    358 QPKVASGCLD  INSSVKGARF  VRFCDAFNIP  LITFVDVPGF  LPGTAQEYGG  IIRHGAKLLY
Rnpccb   360 QPNVASGCLD  INSSVKGARF  VRFCDAFSIP  LITFVDVPGF  LPGTAQEYGG  IIRHGAKLLY
VpmmdA   328 QPKVMAGCLD  INASDKSSRF  IRFCDAFNIP  IVNFVDVPGF  LPGTNQEWGG  IIRHGAKMLY
Pstc12   332 QPSVMSGCLD  INASDKAAEF  VNFCDSFNIP  LVQLVDVPGF  LPVQ-QEYGG  IIRHGRKMLY
             ** *  * **  *  * *   *    *** * **     ******  **   ** **  ** **** **

MxpccB   392 AYAEATVPKV  TVITRKAYGG  AYDVMASKHI  RADMNFANFA  WPTAEIAVMG  PEGAVNIIFR
Hpccb    418 AFAEATVPKV  TVITRKAYGG  AYDVMSSKHL  CGD---TNYA  WPTAEIAVMG  AKGAVEIIFK
Rnpccb   420 AFAEATVPKI  TVITRKAYGG  AYDVMSSKHL  LGD---TNYA  WPTAEIAVMG  AKGAVEIIFK
VpmmdA   388 AYSEATVPKI  TVITRKAYGG  SYLAMCSQDL  GAD---QVYA  WPTSEIAVMG  PAGAANIIFK
Pstc12   391 AYSEATVPKI  TCLATPTAAP  TWPCATVTLV  PTPCTPV---  -PSAEIAVMG  AEGAANVIFR
             *  ******  *                                    *  ******    **  **

MxpccB   452 NELAKAPDAA  AERARLTADY  RDKFATPFKA  AELGYIDEII  RPEETRAKLI  RSLELLKDKR
Hpccb    475 GHENVEA-AQ  AE-------Y  IEKFANPFPA  AVRGFVDDII  QPSSTRARIC  CDLDVLASKK
Rnpccb   477 GHEDVEA-AQ  AE-------Y  VEKFANPFPA  AVRGFVDDII  QPSSTRARIC  CDLEVLASKK
VpmmdA   445 KDEDKDA-KT  AK-------Y  VEEFATPYKA  AERGFVDVVI  EPKQTRPAVI  NALAMLASKR
Pstc12   447 KEIKAADDPD  AMRAEKIEEY  QNGSTRRTWR  A-RGQVDDVI  DPADTRRKIA  SALEMYATKR
                       *           *  *  *  *  *  **       *    *

MxpccB   512 QENLPRKHGN  IPL
Hpccb    527 VQRPWRKHAN  IPL
Rnpccb   529 VHRPWRKHAN  VPL
VpmmdA   497 ENRAPKKHGN  IPL
Pstc12   506 QTRPAKKPWK  LPL.....
                    *     **
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FIG. 3. Amino acid sequence alignment of homologous regions in the Mxpccb protein, in the human propionyl-CoA carboxylase β subunit (Hpccb), in the rat propionyl-CoA carboxylase β subunit (Rnpccb), in the methylmalonyl-CoA decarboxylase α subunit (VpmmdA), and in the *P. shermanii* 12S subunit of transcarboxylase (Pstc12). Identical residues in all sequences compared are marked by an asterisk.

TABLE 1. Localization of propionyl-CoA carboxylase in wild-type and *dcm-1* mutant strains[a]

Fraction and strain type	Total activity (nmol/min)	Sp act (nmol/min/mg)
Extracellular		
Wild type	0.5	7.6
dcm-1 mutant	0.02	0.4
Cell extract		
Wild type	19.9	4.3
dcm-1 mutant	0.9	0.2
Cell membrane		
Wild type	1.2	0.5
dcm-1 mutant	0.1	0.02

[a] The propionyl-CoA carboxylase activity was determined with propionyl-CoA as the substrate from the rate of methylmalonyl-CoA formation by HPLC.

with other enzymes. No transcarboxylase activity was detected in either wild-type or *dcm-1* mutant extracts.

Developmental rescue of *dcm-1* by methylmalonyl-CoA. Propionyl-CoA carboxylase, which produces methylmalonyl-CoA from propionyl-CoA, lacked the *dcm-1* mutant. Some cells of the *dcm-1* mutant changed from rod shaped to spherically shaped when the *dcm-1* mutant was cultured in CF agar medium containing 5, 50, and 500 μM methylmalonyl-CoA (Fig. 2). The addition of 5 and 50 μM methylmalonyl-CoA created optimal conditions for developmental rescue. Though the methylmalonyl-CoA rescued the formation of fruiting bodies and spores, the formation frequency was lower than that of the wild-type strain without methylmalonyl-CoA. The rescued spores, which were sonicated for 1 min, were viable on CYE agar.

Fatty acids of the *dcm-1* mutant. Fatty acids were extracted from both wild-type and mutant cells after 0 and 65 h of development and analyzed by gas-liquid chromatography. The *M. xanthus* fatty acids consisted mainly of long branched-chain fatty acids (38, 40). In the vegetative cells, the total amount of the chain fatty acids with 16 to 18 carbon atoms (long-chain fatty acids) was about 7% less for the *dcm-1* mutant than for the wild-type strain (Table 2). During development, the total amount of long-chain fatty acids was reduced by 17.7% (from 35.1 to 28.9%) in the *dcm-1* mutant but increased slightly (from 41.9 to 44%) in the wild-type strain relative to vegetative cells, the difference in the total amount between the two strains being 34.3%. In contrast, the amount of short-chain fatty acids, i.e., anteiso-15:0 or -15:1, n-15:1, and n-15:0, in developing cells of the *dcm-1* mutant was increased about 4.5- to 8-fold compared to that in the wild-type strain.

DISCUSSION

Developmental mutant *dcm-1* formed aggregates (mounds) but was unable to form fruiting bodies and spores in mounds. Tn*V* was situated within a 1,572-bp ORF, denoted *pccB*, the wild-type form of which was cloned and sequenced. The predicted MxpccB protein showed significant sequence similarity to the β subunit of propionyl-CoA carboxylase (22, 26). Propionyl-CoA carboxylase is composed of nonidentical subunits (α and β). The biotin carboxylase activity and biotinylated domain were thought to reside in the α subunit, whereas the β subunit was thought to catalyze the carboxyltransferase reaction (34). The putative acyl-CoA-binding sequences conserved between residues 299 and 332 of MxpccB and between residues 89 and 142 of MxpccB might represent carboxybiotin-binding sites (28).

In the wild-type strain, the propionyl-CoA carboxylase activity increased while the rod-shaped cells differentiated into spherical forms within mounds or fruiting bodies (data not shown). Propionyl-CoA carboxylase (EC 6.4.1.3) catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA. Methylmalonyl-CoA serves as a precursor to the synthesis of branched-chain fatty acids and polyketide. Fatty acid synthetase has an inherent capacity to utilize methylmalonyl-CoA and synthesize methyl-branched fatty acids (3, 19). Also, polyketide (e.g., sorangicin) is synthesized in reactions resembling fatty acid synthesis (15). Propionyl-CoA carboxylase also functions primarily in the catabolism of fatty acids with odd-numbered chain lengths (32). *M. xanthus* releases E signal, a mixture of fatty acids, during development (6, 24, 25). Propionyl-CoA carboxylase may regulate the development of spore formation in *M. xanthus* by metabolism of the branched fatty acids and polyketides.

The deduced sequence of MxpccB protein also shows strong similarity to that of the α subunit of methylmalonyl-CoA decarboxylase (17). The decarboxylase is bound to the cell membrane and is specifically activated by Na^+ ions (13, 14). The most important role of the enzyme is as a vectorial catalyst converting part of the energy of the highly exergonic decarboxylation reaction into an Na^+ ion gradient. *M. xanthus* wild-type and *dcm-1* mutant vegetative cells grown in CYE medium containing $^{22}NaCl$ were developed on CF agar. However, no ^{22}Na-labeled ions were released from either strain during development. Nor did the developing cells of either strain take up the ^{22}Na-labeled ions in the CF agar (data not shown). These findings indicate that the phenotype of the *dcm-1* mutant does not result from a deficiency of Na^+ transport. The predicted MxpccB protein also shows significant sequence similarity to the 12S subunit of transcarboxylase (methylmalonyl-CoA–pyruvate carboxytransferase; EC 2.1.3.1) (Fig. 3). *Propionibac-*

TABLE 2. Fatty acids of wild-type and *dcm-1* mutant cells

Cell type	Amt (area %) of methyl ester:													
	n-14:0	i-15:0	ai-15:0 or -15:1	n-15:1	n-15:0	i-14:0 3OH	i-16:0	n-16:1 *cis*-11	n-16:0	i-15:0 3OH	i-17:1	i-17:0	n-18:1	i-17:0 3OH
Vegetative cell														
Wild-type	2.2	30.0	0.9	0.5	0.7	8.6	5.3	15.6	3.1	2.1	2.7	7.6	4.2	3.4
dcm-1 mutant	1.3	33.1	3.0	1.5	1.9	9.8	4.0	10.6	1.9	2.2	2.9	8.0	3.9	3.8
Developing cell														
Wild-type	1.7	32.5	0.5	0.7	1.4	10.7	6.0	13.9	1.8	2.3	3.8	10.2	4.5	3.8
dcm-1 mutant	1.6	33.2	3.3	5.7	6.3	11.5	3.3	9.6	1.4	1.8	3.6	5.6	2.2	3.2

terium shermanii transcarboxylase catalyzes a carboxyl transfer between carboxylic acid and acyl-CoA that does not require ATP or CO_2 (30, 41). The enzyme activity was not detected in *M. xanthus* wild-type extracts.

Long-chain fatty acids have a variety of effects on developing cells of *M. xanthus* and morphogenesis of *E. coli* (27). Rosenbluh and Rosenberg and Varon et al. have shown that autocide AMI is capable of accelerating aggregation and sporulation in *M. xanthus* wild-type cells (33, 39). The AMI is a mixture of fatty acids (e.g., n-16:1, i-15:0, n-16:0, and n-16:2) and appears to be an enzymatic breakdown product of phosphatidylethanolamine, a major component of the cell membranes of *M. xanthus*. They report that there is a role for autocides in the development of *M. xanthus* and that released fatty acids are intercalated into the cell membrane and alter membrane permeability. Such a membrane allows for the movement of signal compounds into and out of the cell.

The synthesis of long-chain fatty acids (C_{16} to C_{18}) is inhibited in the *dcm-1* mutant (Table 2). In *M. xanthus* cells, branched-chain fatty acids were the major fatty acids. The branched-chain fatty acids in bacteria are synthesized with branched-chain α-keto acids or branched-chain acyl-CoA esters as the primers and malonyl-CoA as the chain extender (21). The chain length of newly synthesized fatty acids appears to depend on the concentration of malonyl-CoA (16). Malonyl-CoA is normally converted from acetyl-CoA by acetyl-CoA carboxylase (EC 6.4.1.2). Yeast mutants defective in acetyl-CoA carboxylase have been isolated as auxtrophs for long-chain fatty acids (29, 31). Propionyl-CoA carboxylase catalyzes a reaction analogous to that of acetyl-CoA carboxylase (11, 12, 18). In several species, a single enzyme with dual-substrate specificity catalyzes the carboxylation of both acetyl- and propionyl-CoA. The *M. xanthus* wild-type strain had both acetyl- and propionyl-CoA carboxylases. These findings suggest the hypothesis that the long-chain fatty acid synthesis in *M. xanthus* is regulated by propionyl-CoA carboxylase through the concentration of malonyl-CoA.

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