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Characterization of the *mac-1* Gene Encoding a Putative ABC Transporter from *Myxococcus xanthus*[1]

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The *mac-1* gene of *Myxococcus xanthus* TA, an antibiotic TA producer, encoded a protein with strong sequence similarity to the antibiotic ATP-binding cassette (ABC) transporter for macrolide antibiotics. The *mac-1* gene encoding protein (Mac-1) had two ATP-binding domains containing Walker A and B motifs, and no hydrophobic transmembrane regions. Insertional inactivation of *mac-1* caused enhanced sensitivity to oleandomycin, a macrolide antibiotic, while the *mac-1* mutant showed normal export of antibiotic TA into the extracellular fluid. The *mac-1* mutant could form mounds, but was unable to form fruiting bodies or sporulate under nutrient starvation. A primary role for Mac-1 in *M. xanthus* may be as a transporter which exports or imports a molecule required for the sporulation process.

Key words: ABC transporter, antibiotic, development, *Myxococcus xanthus*, sporulation.

Myxobacteria are gram-negative, gliding bacteria, which exhibit complex multicellular life cycles (*1–4*). Their multicellular behaviors are coordinated through transmission of intercellular signals. They live in soil and mainly feed cooperatively on other microbes. For the lysis of other microbes, they produce a variety of antibiotics and bacteriolytic enzymes (*5*, *6*). In the absence of nutrients, swarms of them aggregate to form fruiting bodies in which the rod-shaped cells differentiate into spherical, environmentally resistant myxospores.

The *Myxococcus xanthus* TA and Mx1 strains excrete antibiotic TA and saframycin Mx1 into the media under nutritionally limited conditions, respectively (*7–9*). Antibiotic TA is a macrocyclic compound that inhibits the polymerization step in cell wall formation (*10*), and its mode of action is similar to that of vancomycin. Saframycin Mx1 belongs to the group of heterocyclic quinone antibiotics that bind covalently to DNA, and inhibits cellular DNA and RNA synthesis (*11*, *12*). ATP-binding cassette (ABC) transporters participate in the incorporation into and secretion from cells of many different molecules. Many antibiotic-producing actinomycetes possess at least one antibiotic ABC transporter that is responsible for secretion of the antibiotic outside the cells (*13*).

Recently, we identified a DD-carboxypeptidase (*pdcA*) gene from a type strain of *M. xanthus* (*14*). The characteristics of the *pdcA* gene product were similar to those of penicillin-resistant DD-carboxypeptidases of vancomycin-resistant enterococci. The region downstream of the *pdcA* gene, an incomplete open reading frame (ORF), was present, and its deduced gene product (Mac-1) showed a high degree of similarity with antibiotic ABC transporters. This paper describes the primary structure determination and deletion analysis of the antibiotic ABC transporter, Mac-1, from the antibiotic TA producer, *M. xanthus* TA. Also, the role of Mac-1 in development is discussed.

METHODS AND MATERIALS

Bacterial Strains and Growth Conditions—M. xanthus TA (ATCC 31046) was used as the wild-type strain in this study. The *M. xanthus* wild-type or mutant strains were grown at 28°C in Casitone-yeast extract (CYE) medium with vigorous shaking (*15*). CF medium, used for testing fruiting body formation, was prepared as previously described (*16*). Cells were grown to the mid-exponential growth phase, harvested, and then concentrated in TM buffer (10 mM Tris-HCl, pH 7.5, and 8 mM $MgSO_4$). Aliquots (10 μl) of the cell suspension (2×10^7 cells) were spotted onto CF agar plates. The plates were incubated for 7 days at 30°C, and then examined for the formation of fruiting bodies and spores under a light microscope.

DNA Manipulation and Sequencing—In order to obtain a complete *mac-1* gene, a positive clone containing the *pdcA* and *mac-1* genes (*14*) was used in this experiment. Phage DNA was prepared from the clone and digested with several restriction enzymes. The DNA fragments were separated in a 0.7% agarose gel and then transferred to a nylon membrane for Southern blot analysis. The 1.3 kb *Apa*I–*Pst*I fragment containing a partial *mac-1* gene was used to prepare a DIG-labeled probe with a random primer labeling kit (Roche Diagnostics). The 4 kb *Sma*I and 1.6 kb *Apa*I fragments were hybridized with the probe and used for sequence analysis. Nucleotide sequencing was performed by the dideoxynucleotide chain termination method of Sanger *et al.* (*17*), using an ALF DNA sequencer (Amersham Pharmacia Biotech) with an AutoRead sequencing kit (Amersham Pharmacia Biotech).

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Construction of a mac-1 Insertion Mutant—To investigate the biological function of Mac-1, we constructed a *mac-1* insertion mutant. First, a 2.0 kb fragment of *mac-1* in the 4 kb *Sma*I fragment was amplified by PCR using the primers, 5′-CCGGCGCGTTCAAGAGGGATTG-3′, which anneals at positions 70 to 91, and 5′-AGCGGCTCAGCTCTTCGTA-3′, which anneals at positions 2018 to 2036. The PCR product was ligated into a vector pT7 Blue. This plasmid was designated as pTMAC. A 1.2-kb fragment containing the kanamycin-resistance (Km^r) gene from TnV (*18*) was inserted into pTMAC digested with *Bal*I (Fig. 1). The 3.2-kb DNA fragment, which contains the *mac-1* gene::Km^r, was amplified by the PCR procedure using the above two primers. The insertion mutation was moved into the chromosome of *M. xanthus* TA by the electroporation method of Plamann *et al.* (*19*). *M. xanthus* kanamycin-resistant colonies were grown in CYE medium containing 70 μg/ml kanamycin, and chromosomal DNAs were prepared from the mutants. Replacement of the wild-type *mac-1* gene by the defective gene was confirmed by Southern blot hybridization and PCR analyses.

Biological Assay for Antibiotic TA Production—0.5% Casitone (0.5 CT) medium was used for the production of antibiotic TA (*20*). The *M. xanthus* TA wild-type and mutant strains were grown in 0.5 CT medium at 28°C with gyratory shaking for 1 to 4 days. Antibiotic TA was extracted from the culture supernatants and cells with 0.8 volume of chloroform. The chloroform layer was washed once with distilled water and then concentrated *in vacuo*. The residue was suspended in a small volume of chloroform. Antibiotic TA activity was determined by the paper disc assay method, with *Escherichia coli* NovaBlue as the test organism. *E. coli* NovaBlue was grown overnight in LB medium, and 0.1 ml aliquots of the culture were mixed with 3 ml of soft agar and spread over LB plates. Disks (6-mm diameter) containing different concentrations of the antibiotic were then placed on the indicator bacteria, and then the plates were incubated at 37°C for 16 h.

RT-PCR—Total RNA was isolated from *M. xanthus* at the exponential growth phase and stationary phase, and during development as described (*21*). Development was induced by plating cells that had been vegetatively grown in CYE medium with aeration until the mid-log phase on CF agar. Contaminating DNA was removed by digestion with DNase. Reverse transcription RNA (1 μg) was reverse transcribed and amplified using a *Bca*BEST RNA PCR kit (Takara Shuzo). The following primer pair was used for the amplification: forward 5′-ATGGCCCAGAATTTCATCTTCACG-3′, which anneals at positions 351 to 374, and reverse 5′-GGCCATCTCGATGGTGCGG-3′, which anneals at positions 779 to 797. The amplification products were separated by electrophoresis on 1.0% agarose gels, stained with ethidium bromide, and detected with a LAS-1000 system (Fuji Film).

Nucleotide Sequence Accession Number—The nucleotide sequence data reported here will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases under accession no. AB041227.

RESULTS

Cloning and Sequencing of the mac-1 Gene—In a previous paper, we reported that an incomplete open reading frame (ORF1) was present downstream of the penicillin-resistant DD-carboxypeptidase (*pdcA*) gene in the 3.5-kb *Pst*I fragment of *M. xanthus* IFO 13542 chromosome, and the *orf1* gene has the characteristics of ABC transporters (*14*). By subcloning the cloned DNA, the complete *orf1* gene was obtained from the 4.0-kb *Sma*I fragment DNA of the clone (Fig. 1). We determined the nucleotide sequences of both strands of the 2.5-kb *Apa*I (1)–*Sma*I (2) fragment (Fig. 2). The *orf1* gene, designated as *M. xanthus mac-1*, had two potential start codons, one at positions 351–353, and the other at positions 375–377. However, the first one is most likely the initiation codon, since it had an upstream purine-rich sequence (AGGAAGG) that could function as a ribosome-binding site. The *mac-1* gene had a high percentage of G+C nucleotides (96%) at the third position of the codons. The predicted translation product, Mac-1, had a deduced amino acid sequence of 559 amino acids corresponding to an estimated molecular weight of 62.1 kDa. A typical inverted repeat sequence composed of a 14-bp stem, which is a common feature of a transcription terminator, was located immediately downstream of the terminal codon.

Based on the sequence of the *mac-1* gene, two primers were synthesized and used to amplify the *mac-1* gene from *M. xanthus* TA (ATCC 31046) chromosomal DNA. The sequences of the two primers were 5′-ATGGCCCAGAATTTCATCTTC-3′ and 5′-TCAGCTCTTCGTAATCGGGCG-3′.

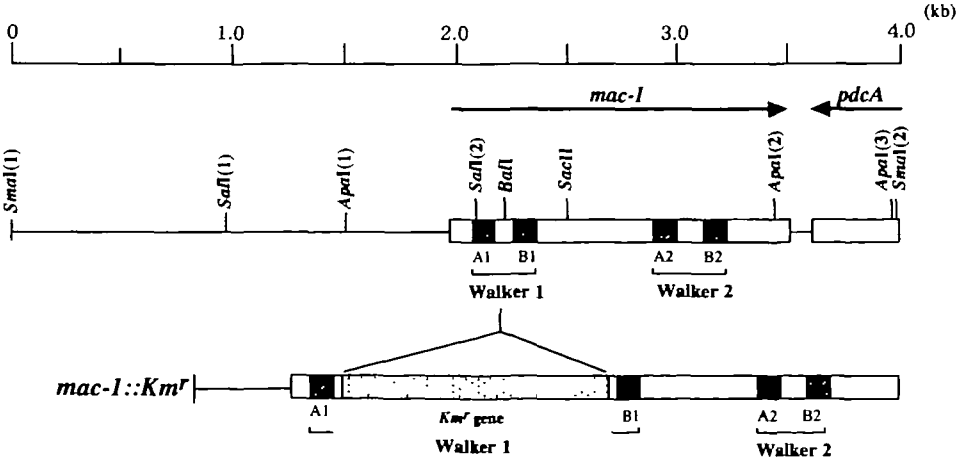

Fig. 1. **Restriction map of the cloned *Sma*I fragment harboring the *mac-1* gene of *M. xanthus*.** The upper part of the figure shows a restriction map of the 4.0-kb *Sma*I chromosomal fragment, the *mac-1* gene being denoted by a open bar. The dotted bars indicate the positions of two ATP-binding domains (Walker 1 and 2). The lower part of the figure shows the position of insertion of the kanamycin-resistance (Km^r) gene into the *Bal*I site of the *mac-1* gene.

The resulting 1.7-kb *mac-1* gene from *M. xanthus* TA was also sequenced. The sequence was consistent with that of the *mac-1* gene from the type-strain except for five nucleotides; at positions 502 (C for T), 1324 (C for T), 1795 (C for A), 1796 (G for C), and 1797 (C for G), some of which resulted in four amino acid substitutions, at positions 51 (Thr for Ile), 325 (Ala for Val), 482 (Thr for Asn), and 483 (Leu for Val).

Deduced Properties of the Mac-1 Polypeptide—A computer search with the BLAST program in the GenBank database indicated that the deduced amino acid sequence for Mac-1 exhibited significant similarity to proteins belonging to the ABC transporter superfamily. The polypeptide sequence deduced from Mac-1 exhibited 61% identity along the entire length to the product of hypothetical gene HI1252 in *Haemophilus influenzae* (*22*). They also indicated clear similarity to some genes known to confer antibiotic resistance in different organisms, with the following

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GGGCCCTCACCCTGGGGGCCGGGCGGTTTCGGCGGGCCTGCCGGCTGTTCCTGTACTTCGCGCTCGATGCCG    72
GCGCGTTCAAGAGGGATTGAAAGAAGATGGACGCCGTCATGGAGCAGCCGGAGCTGGCCGCCACGACGGAAT   144
AGGCCCAGGGGGGGCCTGGCGGCACCGTTCGCTTTTCCACGCCTGCGGCGCGGGCCAAGGGGGCGACGGCTT   216
GTTCCGGGCTGCCCCCACCTGCCGGGGCCGGTCGTTCGAGCCCCACAGGGTTGGGGGACGCTCTCGCCTTGA   288
GTGCGCTGTTGCACCCTGGTATCCGCAGAGCCCCTCACTCCACCTTAAGCAGGAAGGCCCAGATGGCCCAGA   360
                                                          M  A  Q      3
ATTTCATCTTCACGATGCAGGACCTGCGCAAGGTCAAGAACGGCAAGGAGATCCTCAAGGGCATCTACCTCT   432
N  F  I  F  T  M  Q  D  L  R  K  V  K  N  G  K  E  I  L  K  G  I  Y  L     27
CGTTCTTCCCCGGCGCGAAGATTGGCGTCATTGGCCCCAACGGCTCCGGTAAGTCGACGCTGCTGCGCATCA   504
S  F  F  P  G  A  K  I  G  V  I  G  P  N  G  S  G  K  S  T  L  L  R  I     51
TGGCGGGCGTGGACACGGAGTTCTTCGGTGTCGCCAAGCCGGACCCCAGCGCCAAGGTTGGCTACCTGGCGC   576
M  A  G  V  D  T  E  F  F  G  V  A  K  P  D  P  S  A  K  V  G  Y  L  A     75
AGGAGCCACAGCTCGATGCCTCGCTCGACGTGAAGGGCAACGTGGAGCTGGGCCTGAAGGAGATTCGCGCCA   648
Q  E  P  Q  L  D  A  S  L  D  V  K  G  N  V  E  L  G  L  K  E  I  R  A     99
CGCTGGACCGCTTCAACGAGGTCAGCGCGAAGTTCGCCGAGCCCATGAGCGACGCGGAGATGGAGAAGCTGC   720
T  L  D  R  F  N  E  V  S  A  K  F  A  E  P  M  S  D  A  E  M  E  K  L    123
TGGCCGAGCAGGGCCGGCTGCAGGACGCCATCGACGCGGTGAATGGTTGGGAGCTGGACCGCACCATCGAGA   792
L  A  E  Q  G  R  L  Q  D  A  I  D  A  V  N  G  W  E  L  D  R  T  I  E    147
TGGCCATGGACGCGCTGCGCCTGCCGCCGGGCGACGCGGACGTGACGAAGCTGTCCGGTGGTGAGAAGCGCC   864
M  A  M  D  A  L  R  L  P  P  G  D  A  D  V  T  K  L  S  G  G  E  K  R    171
GCGTGGCGCTGTGCCGCATCCTGCTGGAGAAGCCGGACCTGCTCCTCCTGGACGAGCCCCACCAACCACCTGG   936
R  V  A  L  C  R  I  L  L  E  K  P  D  L  L  L  L  D  E  P  T  N  H  L    195
ACGCGGAGAGCGTCGCGTGGCTGGAGCAGGCGCTCAAGGAGTACAAGGGCACCATCGTGTGCATCACCCACG  1008
D  A  E  S  V  A  W  L  E  Q  A  L  K  E  Y  K  G  T  I  V  C  I  T  H    219
ACCGGTACTTCCTGGACAACGCGGCCGAGTGGATTCTGGAGCTGGACCGCGGCGAGGGTGTGCCCTGGAAGG  1080
D  R  Y  F  L  D  N  A  A  E  W  I  L  E  L  D  R  G  E  G  V  P  W  K    243
GCAACTACTCCAGCTGGCTGGAGCAGAAGCAGAAGCGCCTGGAGCTGGAGGAGAAGTCGGAGAGCCACCGCC  1152
G  N  Y  S  S  W  L  E  Q  K  Q  K  R  L  E  L  E  E  K  S  E  S  H  R    267
AGAAGACGCTCAAGCGCGAGCTGGAGTGGGTGCGTGCCTCCCCGAAGGCCCGTCAGGCCAAGAGCAAGGCGC  1224
Q  K  T  L  K  R  E  L  E  W  V  R  A  S  P  K  A  R  Q  A  K  S  K  A    291
GCATCGCGGCCTACGAGGAGCTGCTCAACCAGACGCAGGACAAGCGCGACGCGACGGGCGAGGTCATCATCC  1296
R  I  A  A  Y  E  E  L  L  N  Q  T  Q  D  K  R  D  A  T  G  E  V  I  I    315
CGCCCGGCCCGCAGCTCGGGGGGCTGGTGGTCGAGGCCAAGGGGCTGCGCAAGGCGTACGGCGACCGGCTGC  1368
P  P  G  P  Q  L  G  G  L  V  V  E  A  K  G  L  R  K  A  Y  G  D  R  L    339
TCATCGAGGACCTGAACTTCAAGCTCCCGCGCGGTGGCATCGTGGGCGTCATCGGTCCCAACGGCGCGGGCA  1440
L  I  E  D  L  N  F  K  L  P  R  G  G  I  V  G  V  I  G  P  N  G  A  G    363
AGACGACGCTGTTCCGGATGATGACGGGCGTGGAGAAGCCGGACGAGGGCGAGCTGAACATCGGCGAGACGG  1512
K  T  T  L  F  R  M  M  T  G  V  E  K  P  D  E  G  E  L  N  I  G  E  T    387
TGAAGCTGGCCTACGTGGACCAGAGCCGCGACGCGCTGGACGGCGACAACTCGGTGTTCCAGGAGGTGAGCG  1584
V  K  L  A  Y  V  D  Q  S  R  D  A  L  D  G  D  N  S  V  F  Q  E  V  S    411
GCGGGCTGGACCACCTGGACCTGGGCAAGGCGGGGCAGGTGCCCAGCCGCGCGTACCTGGCGGCTTTCGCCT  1656
G  G  L  D  H  L  D  L  G  K  A  G  Q  V  P  S  R  A  Y  L  A  A  F  A    435
TCAAGGGGCAGGACCAGCAGAAGCGGGTGAAGGACCTGTCCGGTGGTGAGCGCAACCGCGTGCACCTGGCGA  1728
F  K  G  Q  D  Q  Q  K  R  V  K  D  L  S  G  G  E  R  N  R  V  H  L  A    459
AGATGCTCAAGAGCGGCGGCAACCTCTTGCTGCTCGACGAGCCCACCAACGACCTGGACGTGGAGAACGTGC  1800
K  M  L  K  S  G  G  N  L  L  L  L  D  E  P  T  N  D  L  D  V  E  N  V    483
GCAGCCTGGAGGACGCGCTGCTCGGCTTCGCGGGCTGCGCCGTGGTCATCAGCCACGACCGCTGGTTCCTCG  1872
R  S  L  E  D  A  L  L  G  F  A  G  C  A  V  V  I  S  H  D  R  W  F  L    507
ACCGCATCGCCACGCACATCCTGGCGTTCGAGGGCGACAGCAAGGCGTTCTTCTTCGAGGGCAACTTCGAGG  1944
D  R  I  A  T  H  I  L  A  F  E  G  D  S  K  A  F  F  F  E  G  N  F  E    531
ACTACGAGGCGGACAAGAAGAAGCGCCTGGGCCCCGAGGCCCTGGAGCCGCACCGCATCCGCTACCGCCCGA  2016
D  Y  E  A  D  K  K  K  R  L  G  P  E  A  L  E  P  H  R  I  R  Y  R  P    555
TTACGAAGAGCTGAGCCGCTGGAAGTCCGGAGCCACCGTACCTGCAAGGTGCGGTGGCTCGACGGCGCTTCA  2088
I  T  K  S  *                                                              559
CCTCAGCACCGCGAACGCCATGAGCAGCTTCTTCATGTCGCTCTCCTGCGTCAGGGCCGGTACTCCCAGTGC  2160
CAGGGCTCGGACGGGACGGTGCGCACGAAGCCGAAGTTCTTCGCGTTGTTGGCCATCCACCGGTACGTCGAG  2232
GTGCCCGTGCCGCCCGTGTTGACGTCCACCGCGATGCCGCCCTGGTGGTTGGAGTAGCCCGGAGGCGCGGCC  2304
AGGTTGCCGGTGCCGTTCTTGTACGCGCGGTACAGCGCTTCCTGCTCCGCCATGCTGCGGAAGCCGCTGTTC  2376
ACCTTCAGGGTGATGCCCTGCGCGCGCGCCGCCGCGTACATGCGGTTGTACGCCGCCGCCGCGTCCGAGCGC  2448
ATCTCCTTGCCGTTCGGGACCGACGAGAGCGAAATCTGCCGCGGCTTCCCGTTCACATAGCCCGTGACGACC  2520
CGACCGCCACCGCCACCGCCCGGG                                                   2545
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Fig. 2. **Nucleotide and deduced amino acid sequences of the *mac-1* gene of *M. xanthus* IFO 13542 (ATCC 25232).** A putative ribosome-binding site is double underlined. Arrows indicate the position of the palindrome sequence.

percentages of identical/similar amino acids: *carA*, a carbomycin-resistance gene from *Streptomyces thermotolerans*, 34%/47% (*23*), *tlrC*, a tylosin-resistance gene from *S. fradiae*, 29%/46% (*24*), *oleB*, an oleandomycin-resistance gene from *S. antibioticus*, 31%/46% (*25*), and *srmB*, a spiramycin-resistance gene from *S. ambofaciens*, 32%/44% (*26*). These products and Mac-1 contained a duplicated ATP-binding domain with two well-conserved amino acid regions around the so-called Walker A and B motifs characteristic of ABC transporters (*27*) (Fig. 3). Mac-1 also contained a motif that resembles the characteristic loop 3 of ABC sites. It is located between the Walker A and B motifs, and carries the conserved sequence SxG. This loop is thought to interact with the cell membrane (*28*). A hydropathy plot of the Mac-1 sequence according to the algorithm of Kyte and Doolittle (*29*) indicated the protein is hydrophilic (data not shown).

Sensitivity Test against Antibiotics—The *M. xanthus* TA wild-type and *mac-1* deletion mutant strains were tested for their sensitivity to eleven antibiotics (oleandomycin, erythromycin, tylosin, spiramycin, lincomycin, penicillin G, streptomycin, kanamycin, chroramphenicol, tetracycline, and polymixin B). The wild-type and mutant strains were cultured in CYE medium containing various concentrations of the antibiotics. The mutant exhibited greater sensitivity to oleandomycin, a macrolide antibiotic, than the wild-type (Fig. 4). In the presence of 2.5 μg/ml of oleandomycin in CYE medium, mutant cells showed a nearly 10-fold decrease in viability compared with that of wild-type cells. The mutant also exhibited slightly enhanced sensitivity (approximately 1.5-fold) to erythromycin when the strains were cultured in CYE medium containing 0.1 to 0.2 μg/ml of erythromycin (data not shown). The mutant did not differ significantly in the ability to grow in the presence of the other antibiotics.

Bioassaying of Antibiotic TA—Since macrolide antibiotics and antibiotic TA consist of a macrocyclic lactone ring (*30*, *31*), we investigated whether or not antibiotic TA can be secreted by the Mac-1 protein. The *M. xanthus* TA wild-type and mutant strains were grown in 0.5 CT medium at 27°C with gyratory shaking for 1 to 4 days. Antibiotic TA was extracted from the culture fluid and cells, and antibiotic TA activity was determined by the paper disc assay method with *E. coli* NovaBlue as the test organism. Since there were no apparent differences in the secreted or accumulated amounts of antibiotic TA between the *M. xanthus* TA wild-type and mutant strains, the Mac-1 transporter was not involved in the secretion of antibiotic TA (data not shown).

Developmental Assay—To investigate the function of Mac-1 during development, the *M. xanthus mac-1* mutant was assayed for developmental defects by plating on starvation agar (CF agar). Wild-type cells clearly aggregated, forming mounds after 32 h, and formed distinct fruiting bodies at 48–64 h (Fig. 5a). Mutant cells formed mounds about 24 to 36 h later than the wild-type strain, whereas fruiting bodies and spores had not been formed after 7 days of development (Fig. 5b). The mutant cells within mounds were slender rods. In the growth medium, CYE, the *M. xanthus mac-1* mutant grew as well as the wild-type. However, when subcultured in CYE medium and stocked at 15°C, the mutant cells had lost their viability by 4 weeks, *i.e.* 3 to 4 weeks earlier than in the case of the wild type (data not shown).

Expression of the mac-1 Gene—The expression of *mac-1* during development was examined by RT-PCR. The expected 447-bp RT-PCR product was amplified from RNA

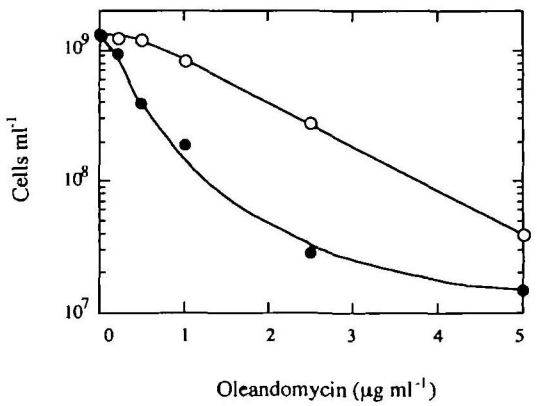

Fig. 4. **Growth of the *M. xanthus* wild-type (open circle) and *mac-1* mutant (closed circle) strains in CYE medium containing various concentrations of oleandomycin.** Cultures were inoculated at 2×10^7 cells/ml. The growth of each strain was determined by counting in a hemacytometer.

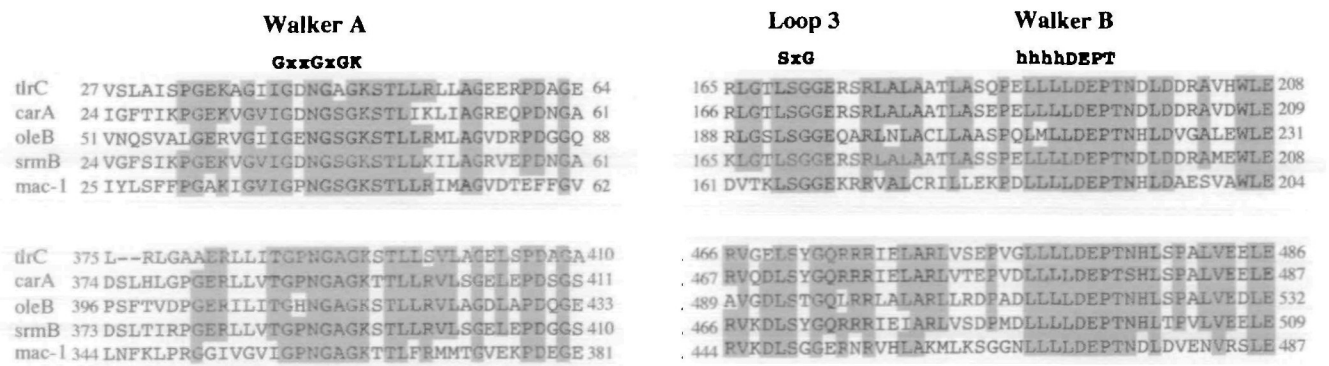

Fig. 3. **Alignment of the regions encoding potential ATP-binding domains of *mac-1* with those of homologous ABC transporters.** The amino acid regions corresponding to the Walker A and B motifs, and to loop 3 are aligned, and the amino acids are indicated. x refers to any amino acid and h to any hydrophobic residue. The following antibiotic-resistance genes are represented: *tlrC*, tylosin-resistance gene from *S. fradiae*; *carA*, carbomycin-resistance gene from *S. thermotolerans*; *oleB*, oleandomycin-resistance gene from *S. antibioticus*; and *srmB*, spiramycin-resistance gene from *S. ambofaciens*.

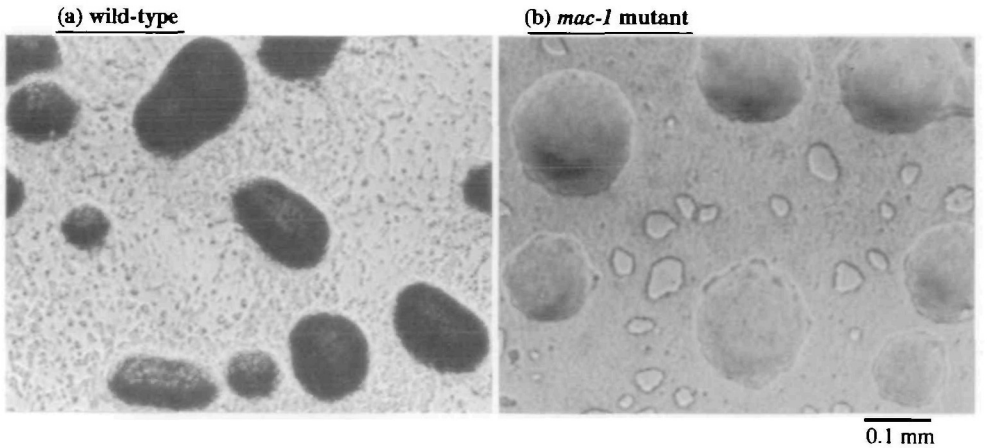

Fig. 5. **Development of the *M. xanthus* wild-type (a) and *mac-1* mutant (b) strains on CF agar.** The photographs in panels (a) and (b) were taken at 3 and 7 days after inoculation, respectively.

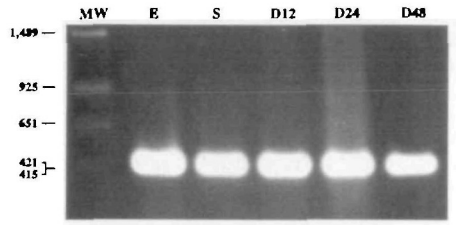

Fig. 6. **Agarose gel electrophoresis of the *mac-1*–specific RT-PCR products from *M. xanthus* mRNA.** Total RNA was prepared from cultures at the exponential growth phase (E) and the stationary phase (S), and during development at 12 h (D12), 24 h (D24), and 48 h (D48), and was used for RT-PCR analysis.

extracted from growing and developing cells (Fig. 6). The amount of the RT-PCR product was similar for all RNA preparations, indicating that there is little relative change in the *mac-1* mRNA level during growth and development. We checked that the expected product was not amplified without a reverse transcriptase (*Bca*BEST polymerase).

DISCUSSION

The ABC transporters comprise a large family of membrane-associated export and import systems present in prokaryotes and eukaryotes. Antibiotic resistance in producer organisms can also be mediated by ABC-transporters. Mendez and Salas classified the antibiotic ABC transporters into three different groups, types I to III, according to the number and organization of the nucleotide-binding domains, and the composition of the transporter system (*13*). The *mac-1* encoded protein is characteristic of type II, which includes antibiotic transporters only consisting of a gene encoding a hydrophilic polypeptide containing two nucleotide-binding domains. This type of transporter has been mainly reported for macrolide producers: carbomycin (*23*), tylosin (*24*), oleandomycin (*25*), and spiramycin (*26*). A membrane counterpart has been identified in none of them so far. Spiramycin and tylosin each consist of 16-membered macrocyclic lactone bearing three sugar substituents (*32*, *33*), and oleandomycin consists of a 14-membered macrocyclic lactone bearing two sugar substituents (*34*). Among the different macrolides, the *M. xanthus mac-1* mutant only exhibited high sensitivity to oleandomycin, and no sensitivity to spiramycin or tylosin. The results indicate that the Mac-1 protein may mainly excrete oleandomycin into the extracellular fluid. In *S. antibioticus*, oleandomycin transporter (OleB) confers specific resistance to oleandomycin but not to other macrolide antibiotics (*25*). The *M. xanthus* TA strain produces and excretes antibiotic TA, which consists of a macrocyclic ring, however, the Mac-1 protein is not involved in the export of antibiotic TA.

M. xanthus has been shown to utilize extracellular signaling during development (*3*). There appear to be at least five extracellular signals (A to E) involved in cell–cell interactions during development. Two ABC transporters (AbcA and RfbAB) required for development have been found in *M. xanthus* (*35*, *36*). The AbcA protein, which shows strong homology to a family of repeat-in-toxin transporters, plays a developmental aggregation role and is thought to function as a transporter of a molecule required for the autochemotactic process. The *rfbA* and *rfbB* gene products contain an integral membrane domain and an ATPase domain, respectively, of the ABC transporter that functions as an exporter of *M. xanthus* O-antigen. The *rfbABC* mutant was slow to develop and the spore yield of the mutant strain was approximately 14% that of the *rfbABC*$^+$ strain (*37*).

The *mac-1* mutant of *M. xanthus* was also defective in normal development. The mutant cells could aggregate and form normal mounds, but were unable to form fruiting bodies or sporulate under nutrient starvation. The phenotype of the *mac-1* insertion mutant was not due to a polar effect of the mutation, because *mac-1* was the last gene of the operon. Also, expression of the *mac-1* gene was detected during starvation-induced development of *M. xanthus*. Since *M. xanthus* does not usually encounter oleandomycin in natural environments, it is likely that the ABC transporter, Mac-1, in *M. xanthus* mainly contributes to the export or import of a developing signal or a structural component for sporulation.

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