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Cloning and Characterization of a Gene (*msdA*) Encoding Methylmalonic Acid Semialdehyde Dehydrogenase from *Streptomyces coelicolor*

YING-XIN ZHANG,[1] LI TANG,[1] AND C. RICHARD HUTCHINSON[1,2]*

School of Pharmacy[1] and Department of Bacteriology,[2]
University of Wisconsin, Madison, Wisconsin 53706

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A homolog of the *mmsA* gene of *Pseudomonas aeruginosa*, which encodes methylmalonic acid semialdehyde dehydrogenase (MSDH) and is involved in valine catabolism in pseudomonads and mammals, was cloned and sequenced from *Streptomyces coelicolor*. Of the two open reading frames (ORFs) found, which are convergently transcribed and separated by a 62-nucleotide noncoding region, the deduced amino acid sequence of the *msdA* ORF (homologous to *mmsA*) is similar to a variety of prokaryotic and eukaryotic aldehyde dehydrogenases that utilize NAD^+, particularly to the MmsA protein from *P. aeruginosa*. No significant similarity was found between the deduced product of *ORF1* and known proteins in the databases. An *S. coelicolor msdA* mutant, constructed by insertion of a hygromycin resistance gene (*hyg*) into the *msdA* coding region, lost the MSDH activity and the ability to grow in a minimal medium with valine or isobutyrate as the sole carbon source but grew on propionate. The *msdA*::*hyg* mutation was complemented by introduction of the *msdA* gene on a plasmid. When the *S. coelicolor msdA* gene was overexpressed in *Escherichia coli* under the control of the T7 promoter, a protein of 51-kDa, corresponding to the approximate mass of the predicted *S. coelicolor msdA* product (52.6 kDa), and specific MSDH activity were detected. These results strongly suggest that *msdA* indeed encodes the MSDH that is involved in valine catabolism in *S. coelicolor*.

Streptomycetes produce a phenomenal variety of secondary metabolites, including antibiotics. Numerous gene clusters involved in the formation of antibiotics have been intensively studied, leading to considerable understanding of the genetic elements governing the biosynthesis of these compounds. However, comparatively little attention has been devoted to the mechanisms that operate in the transition from primary to secondary metabolism and control the carbon flow for the required building blocks. Several studies have shown that valine catabolism can play an important role in supplying fatty acid precursors for macrolide and polyether antibiotic formation (4, 12, 14, 15, 17, 18, 23).

Valine catabolism has been investigated in microbes and higher animals (4, 6, 13, 17, 19, 20, 22, 25). There is general agreement on the initial catabolic events, which include oxidative deamination and decarboxylation to yield isobutyryl coenzyme A (isobutyryl-CoA). It is known that valine is degraded to propionate by some microorganisms and mammals and that 3-hydroxyisobutyryl-CoA and 2-methylmalonic acid semialdehyde are intermediates in this pathway. The aldehyde carbon of 2-methylmalonic acid semialdehyde, which comes from C-3′ of 3-hydroxyisobutyrate, becomes the carboxyl of propionate in this system as shown in Fig. 1, pathway A. However, the results of isotope labeling experiments suggest that streptomycetes metabolize isobutyrate through a different route (Fig. 1, pathway B), because C-1 of the propionate that is incorporated into macrolide or polyether antibiotics comes from the carboxyl carbon of isobutyrate (C-2 of valine) (14, 15, 17, 18).

Genes encoding the valine (branched-chain amino acid) dehydrogenase and branched-chain 2-keto acid dehydrogenase, two of three enzymes that comprise a common pathway catalyzing the conversion of valine, isoleucine, and leucine to their respective acyl-CoA derivatives, have been cloned and characterized in *Streptomyces* spp. (4, 22, 23). The acyl-CoA metabolites formed subsequent to the common pathway in pseudomonads are catabolized by three separate series of enzymes, one specific for each amino acid (13). We are interested in elucidating the pathway of valine catabolism in streptomycetes and characterizing the genes involved in the route leading to methylmalonyl-CoA and propionyl-CoA. As an extension of our work on the valine dehydrogenase genes (22, 23), the *mms* operon DNA from *Pseudomonas aeruginosa* (19) was used as a probe to look for the homologous sequences in streptomycetes. The *mms* operon contains *mmsA*, which encodes methylmalonic acid semialdehyde dehydrogenase (MSDH), and *mmsB*, which encodes 3-hydroxyisobutyrate dehydrogenase (19). MSDH, an enzyme involved in the distal pathway of valine catabolism in pseudomonads and mammals, catalyzes the direct conversion of methylmalonic acid semialdehyde to propionyl-CoA, with the concurrent reduction of NAD^+. The 3-hydroxyisobutyrate dehydrogenase catalyzes the NAD^+-dependent oxidation of 3-hydroxyisobutyrate to methylmalonic acid semialdehyde. In this paper, we report the cloning and nucleotide sequencing of a homolog of the *mmsA* gene from *Streptomyces coelicolor*. In addition, an insertionally inactivated mutant of this *S. coelicolor* gene was generated and analyzed for growth characteristics and enzymatic activity. We have also expressed the *S. coelicolor* MSDH (*msdA*) in *Escherichia coli* and an *S. coelicolor msdA* mutant.

MATERIALS AND METHODS

Strains, plasmids, and culture conditions. *E. coli* DH-5α (16), GM2929 (*dam dcm*), obtained from Doug MacNeil, Merck Co. Research Laboratories, and JM105 (16), used as hosts for plasmids or for M13 DNA sequencing, were grown at 37°C on LB and 2xYT media (16), respectively. *S. coelicolor* J802 (*dagA1 agaA7*) (9), obtained from David Hodgson, was grown on R2YE plates (10) at 30°C for general use or minimal medium containing 0.05% K_2HPO_4, 0.02%

* Corresponding author. Phone: (608) 262-7582. Fax: (608) 262-3134. Electronic mail address: CRHUTCHI@FACSTAFF.WISC.EDU.

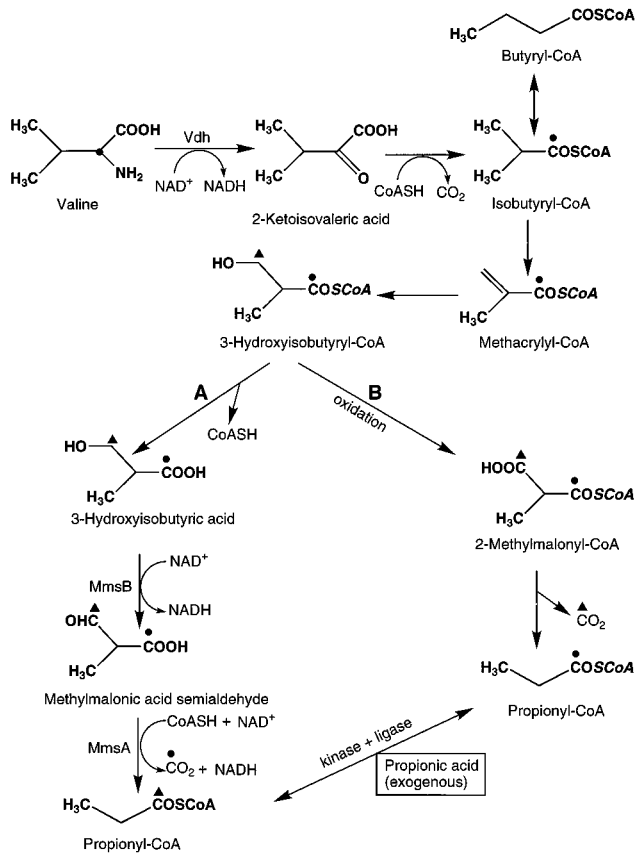

FIG. 1. A hypothesis for the catabolism of valine in bacteria. The pathway may diverge after the formation of 3-hydroxyisobutyryl-CoA along route A in pseudomonads and route B in some streptomycetes. The symbols indicate the predicted relationships between carbons that are retained or lost during the catabolism in each pathway. CoA esters that have not been confirmed by experiment are shown in italics.

$MgSO_4 \cdot H_2O$, 0.001% $FeSO_4 \cdot H_2O$, and 1% agarose (SeaKem ME agarose; FMC BioProducts) with 50 mM $NaNO_3$ and valine or other compounds as sole nitrogen and carbon sources as described in the text for analysis of growth. *S. coelicolor* transformation was performed by standard procedures (10) except with plasmid DNA isolated from *E. coli* GM2929, and transformants were selected on R2YE plates supplemented with 25 μg of thiostrepton or 200 U of hygromycin (Sigma) per ml. Plasmid pJRS78, kindly provided by John R. Sokatch, University of Oklahoma, was used as a source of the *mmsA* and *mmsB* genes.

DNA preparation and amplification. General DNA manipulation and single-stranded M13 DNA isolation were performed as described previously (22). Streptomycete genomic DNA was isolated by the lysozyme-sodium dodecyl sulfate (SDS) method of Hopwood et al. (10). Oligodeoxynucleotides for PCR primers were synthesized by an Applied Biosystems model 391 DNA synthesizer and purified according to the manufacturer's protocols.

The primers at the 5′ end of the *mmsA* gene and 3′ end of *mmsB* gene used in the PCR with *mmsAB* DNA were 5′-CCCAGTCCGCCACCTCATCG CGG-3′ and 5′-GGATGCTGGAGAAGTCCAGC-3′, respectively. Each primer (0.5 μg) was incubated with 30 ng of the 2.0-kb *Hin*dIII-*Eco*RI fragment of pJRS78 as a template, which contains the *mmsAB* coding region, in 20 mM Tris-HCl (pH 8.3)–1.2 mM $MgCl_2$–20 mM KCl–0.1% Triton X-100–100 mg of bovine serum albumin per ml–5% (vol/vol) formamide–120 mM dCTP and dGTP–80 mM dATP and dTTP in a total volume of 99 μl. The reaction was carried out in a model 480 DNA thermal cycler (Perkin-Elmer Cetus). After being overlaid with mineral oil, the reaction mixture was boiled for 5 min and then cooled to 70°C, and 4.5 U of *Taq* polymerase (Sigma) was added. Amplification was achieved with 25 cycles of denaturation at 96°C for 50 s followed by annealing and extension at 70°C for 3.5 min, with the addition of an extra 4.5 U of *Taq* polymerase at cycles 6, 12, and 18. The resulting PCR product was purified by an Ultrafree-MC unit (Millipore Co.) and analyzed by restriction enzyme digestion.

DNA hybridization, cloning, and sequencing. Southern blot hybridization and colony hybridization were performed with Hybond-N membranes (Amersham, Arlington Heights, Ill.) by standard techniques (16). The digoxigenin-alkaline phosphatase (dig-AP) labeling, hybridization, and detection were done with a Genius kit as instructed by the manufacturer (Boehringer Mannheim, Indianapolis, Ind.). The blots were washed two times with 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS for 5 min at room temperature and then two times in 0.5× SSC–0.1% SDS for 15 min at room temperature (low stringency). When plasmid pWHM1070 was used as a probe, the blot was washed two times with 1× SSC–0.1% SDS for 5 min at room temperature and two times in 0.1× SSC–0.1% SDS for 15 min at 68°C (high stringency).

A minilibrary of *S. coelicolor* J802 genomic DNA was constructed with genomic DNA that was double digested with *Sph*I and *Sst*I and size fractionated by electrophoresis on a 1% agarose gel, and 5.0- to 6.0-kb *Sph*I-*Sst*I DNA fragments excised from the gel were cloned in pUC18 (26). The genomic DNA minilibrary was screened by colony hybridization with the PCR product of the *mmsA* and *mmsB* genes as a probe to obtain clone pWHM1070.

DNA segments for sequencing were purified and subcloned into M13mp18 or M13mp19 (26). The nucleotide sequence of the resulting single-stranded DNA from both strands was determined by the dideoxy-chain termination method as described previously (22). Sequence data were read from dried gels, using the DNASTAR (Madison, Wis.) software and digitizer. The Genetics Computer Group software (5) version 8.0 was used for sequence analysis. Nucleotide sequence-deduced amino acid sequence data were compared with available databases by using the FASTA and TFASTA programs (5).

Gene disruption. To construct the plasmid used for genomic replacement in *S. coelicolor*, an 1.34-kb *Pvu*II fragment from pWHM1070 was subcloned into the *Hin*cII site of pUC18. The resulting plasmid was digested with *Bbs*I, and the ends were filled in with Klenow polymerase and ligated to the 1.7-kb *Sma*I-*Eco*RV fragment of pXH106 (7), carrying the hygromycin resistance gene (*hyg*). A 3.04-kb *Hin*dIII-*Xba*I fragment was isolated from this plasmid and cloned into pWHM1065 (22), a temperature-sensitive vector, to give plasmid pWHM1090 (Fig. 2). pWHM1090 was introduced into *S. coelicolor* J802, and then thiostrepton-sensitive, hygromycin-resistant (Th^s Hg^r) transformants were selected on R2YE plates as described previously (22, 23).

Construction and use of expression plasmids. For the *E. coli* expression plasmid, an *Nde*I site was introduced into the *msdA* translational start codon. The primers used for PCR were 5′-CACATATGAAGGAGTTCTCGGTCAT GACGAACATCGTC-3′ (nucleotides [nt] 1319 to 1357 in Fig. 3) and 5′-GGTG GTGACCTCGCCGGTCG-3′ (nt 1424 to 1443 in Fig. 3). The *msdA* gene appears to start at position nt 1342 (ATG) and is preceded by a plausible ribosomal binding site. However, we initially thought that the GTG 18 nt upstream of this ATG could serve as a start codon and thus that the oligodeoxynucleotide corresponding to the 5′ end of *msdA* starts from nt 1319, which may have resulted in the addition of six amino acids (Fig. 3) to the N terminus of the MsdA protein. The PCR was carried out with 2.5 U of *Taq* polymerase, 0.5 μg of each primer, and 50 ng of pWHM1070 DNA as the template for 25 cycles as described above except that the annealing-extension step was shortened to 0.5 min. The 0.12-kb PCR product was recovered by 2% agarose gel electrophoresis and ligated into the *Hin*cII site of pUC18 to give pWHM1091, whose insert was verified by DNA sequence analysis. The entire *msdA* gene was assembled by three-piece ligation of the 0.12-kb *Nde*I-*Bst*EII fragment of pWHM1091, the 2.05-kb *Bst*EII-*Hin*dIII fragment of pWHM1092, and the *Nde*I-*Hin*dIII fragment of pT7-7 (21) to give pWHM1093. pWHM1092 was prepared by subcloning the 2.3-kb *Asc*I-*Nco*I fragment containing the entire *msdA* gene from pWHM1070 into the *Hin*cII site of pUC18 (Fig. 2).

pWHM1093 was introduced by transformation into *E. coli* BL21(DE3) (Novagen, Madison, Wis.). Transformants were grown at 37°C in LB plus 100 μg of ampicillin per ml until the optical density at 600 nm reached 0.6 and then induced by addition of isopropylthiogalactopyranoside (IPTG) to a final concentration of 0.4 mM, and the incubation continued for another 3 h. Proteins were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE).

For the *Streptomyces* expression plasmid, the 2.3-kb *Bam*HI-*Hin*dIII fragment of pWHM1092 was subcloned into pWHM3 (24), a high-copy-number vector, to give pWHM1094. pWHM1094 was introduced into the *S. coelicolor msdA* mutant, and transformants were grown on R2YE plus 200 U of hygromycin and 25 μg of thiostrepton per ml.

MSDH assay. *S. coelicolor* cultures were grown at 30°C in 5 ml of R2YE for 48 h with shaking at 300 rpm and then transferred to 50 ml of fresh R2YE and incubated for another 24 h. Mycelial cells were collected and washed two times with PGEM buffer (50 mM potassium phosphate [pH 7.5], 10% [vol/vol] glycerol, 1 mM EDTA, 5 mM β-mercaptoethanol). Cell extracts were prepared by sonication in PGEM buffer followed by centrifugation. MSDH activity was measured by using methylmalonic acid semialdehyde to initiate the reaction as described by Hatter and Sokatch (8), and the activity determination was based on the rate of NADH production at 340 nm. The methylmalonic acid semialdehyde was prepared by the method of Kupiecki and Coon (11). Ethyl methylmalonate-semialdehyde diethyl acetal was kindly provided by J. R. Sokatch. Protein concentration was determined by the method of Bradford (3).

Nucleotide sequence accession number. The nucleotide sequence reported here has been deposited at EMBL and GenBank under accession number L48550.

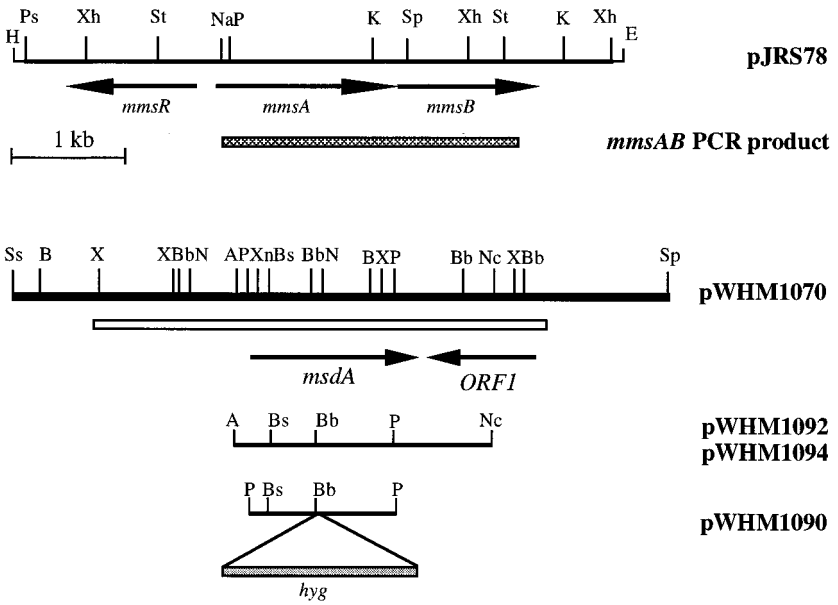

FIG. 2. Restriction maps of pJRS78, pWHM1070, and related plasmids. The arrows indicate the direction of the transcription for the ORFs. The box beneath pWHM1070 indicates the region that was sequenced. Abbreviations: A, *Asc*I; B, *Bgl*II; Bb, *Bbs*I; Bs, *Bst*EII; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; N, *Nsp*I; Na, *Nar*I; Nc, *Nco*I; P, *Pvu*II; Ps, *Pst*I; Sp, *Sph*I; Ss, *Sst*I; St, *Stu*I; X, *Xma*I; Xh, *Xho*I; Xn, *Xmn*I.

RESULTS

Cloning of the homolog of the *P. aeruginosa mmsA* gene from *S. coelicolor* J802. The 1.3-kb *Nar*I-*Kpn*I and 0.6-kb *Sph*I-*Stu*I fragments of pJRS78 (19) containing the *P. aeruginosa mmsA* and *mmsB* genes, respectively, were dig-AP labeled and used to identify cross-hybridizing bands in a Southern blotting experiment with *S. coelicolor* J802 genomic DNA digested with several restriction enzymes. The high G+C content of *Pseudomonas* spp. (about 67%) and *Streptomyces* spp. (about 70%) chromosomal DNA was expected to make it possible to probe *S. coelicolor* DNA successfully with the *mmsA* and *mmsB* genes. Hybridization bands were detected at low stringency with the *mmsA* probe as described in Materials and Methods, indicating the possible presence of a closely related sequence in the *S. coelicolor* genome, but not with the *mmsB* probe (data not shown). The 5.0- to 6.0-kb *Sst*I-*Sph*I genomic DNA fragments corresponding in size to the hybridization band of interest were cloned in pUC18 as described in Materials and Methods. Since the *mmsA* and *mmsB* probes derived from pJRS78, when excised from an agarose gel, gave strong hybridization bands with the vector pUC18 DNA at low stringency, a dig-AP-labeled PCR product (Materials and Methods) of the *mmsA* and *mmsB* genes (Fig. 2) was used as the probe to screen the *S. coelicolor* J802 minilibrary. A positive clone, designated pWHM1070, was isolated by colony hybridization experiments. Restriction mapping (Fig. 2) demonstrated that it represented approximately 5.5 kb of genomic DNA.

Southern analysis using a dig-AP-labeled probe derived from pWHM1070 DNA indicated that a corresponding *msdA* gene (homologous to *mmsA*) is also present in the chromosome of the spiramycin-producing *Streptomyces ambofaciens* ATCC 15154 and the tylosin-producing *Streptomyces fradiae* C373.1, although the intensity of the signal was much weaker in *S. fradiae* (data not shown).

Nucleotide sequencing of the *msdA* gene and the flanking region. The DNA sequence of a 4.0-kb fragment from pWHM1070 containing the *msdA* locus and flanking sequence was determined. CODON PREFERENCE analysis (5) showed that in this region are two major open reading frames (ORFs) having the characteristic codon usage pattern for *Streptomyces* DNA (2). The convergently transcribed *msdA* and *ORF1* genes are separated by only a 62-nt noncoding region (Fig. 3). The 1,500-nt *msdA* ORF appears to begin with an ATG at position 1342 and terminates in a TGA at position 2842 and should encode a 500-amino-acid protein. A region centered about 10 nt 5′ to the first codon of the *msdA* gene (Fig. 3) has a significant degree of complementarity to the 3′ end of 16S rRNA of *Streptomyces lividans* (5′-GAUCACCUCCUUUCU-3′) (1) and should serve as the ribosome binding site. This ORF is followed by a potential transcription termination site that may be shared with *ORF1*. CODON PREFERENCE analysis (5) of the sequence data failed to demonstrate an ORF upstream of the *msdA* gene (up to a distance of 1.34 kb).

Another ORF was found downstream of the *msdA* gene on the opposite strand and is temporarily designated *ORF1*. This 855-nt ORF is likely to begin with ATG at position 3759 and terminate in TGA at position 2904 and to encode a 285-amino-acid protein. A putative ribosome binding site was found at a suitable distance from the ATG start codon (Fig. 3).

A search of the GenBank and EMBL databases (versions 88 and 42) by using FASTA and TFASTA (5) revealed significant sequence similarities between the deduced MsdA protein and a variety of prokaryotic and eukaryotic aldehyde dehydrogenases (NAD^+ dependent), particularly with the MmsA protein from *P. aeruginosa*, but no significant homology was found between the deduced product of *ORF1* and known proteins by database searching. The nucleotide sequences and their deduced products of the *P. aeruginosa mmsA* and *S. coelicolor msdA* genes were compared; this comparison revealed approximately 63 and 47% identity between the nucleotide and deduced amino acid sequences, respectively, of the two genes but no significant homology between *ORF1* and *mmsB* or *mmsR* at the DNA or protein levels (data not shown).

Functional analysis of the *msdA* gene. To test the idea that *msdA* indeed encodes the MSDH and to confirm the role of MSDH in the valine catabolic pathway in *S. coelicolor*, the *S.*

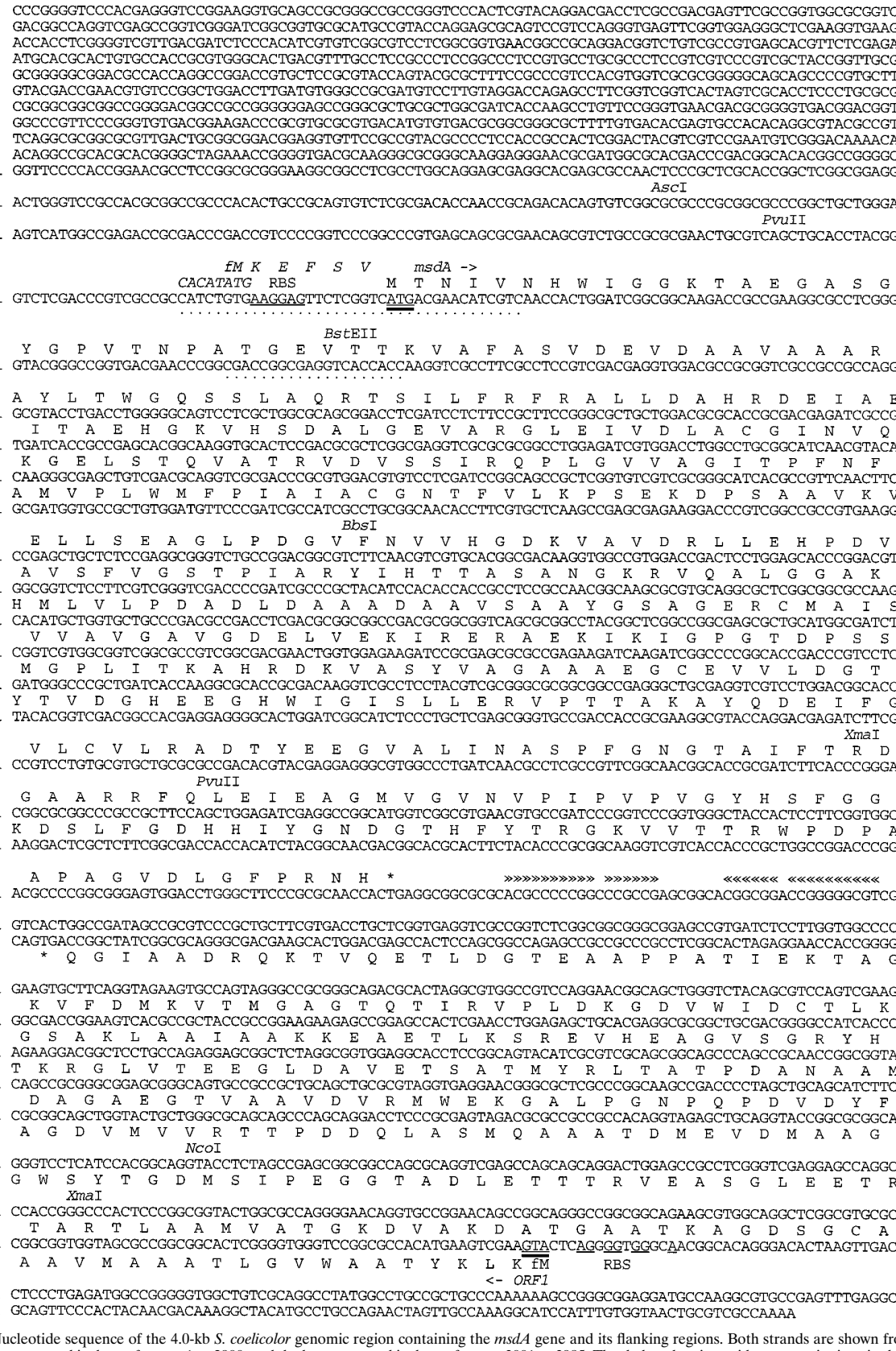

FIG. 3. Nucleotide sequence of the 4.0-kb *S. coelicolor* genomic region containing the *msdA* gene and its flanking regions. Both strands are shown from nt 2901 to 3000, only the top strand is shown from nt 1 to 2900, and the bottom strand is shown from nt 3001 to 3985. The deduced amino acid sequence is given in the single-letter code above (for the *msdA* gene) or below (for the *ORF1*) the nucleotide sequence. Selected restriction enzyme sites are listed above their recognition sequences. The ATG translational start sites of the *msdA* and *ORF1* genes are double underlined. RBS indicates the presumed ribosome binding sites (underlined). Dyad repeats in the sequence as a potential transcriptional terminator are indicated by arrowheads showing the direction of repeated sequence. The positions of the PCR primers used to construct pWHM1093 are indicated by dotted lines below the sequence. The italicized nucleotide and deduced amino acid sequences above the nucleotide sequence at position 1319 indicate the introduced *Nde*I site and the possible N-terminal amino acid sequence fused with MsdA when it was produced in *E. coli.*

TABLE 1. Growth and MSDH specific activity of wild-type and mutant strains and transformants

Strain	MSDH sp act[a]	Growth on[b]:			
		G	V	IB	P
S. coelicolor					
J802	27.6	+	+	+	+
WMH1640	0[c]	+	−	−	+
J802(pWHM1094)	29.2	+	+	+	+
WMH1640(pWHM1094)	34.8	+	+	+	+
E. coli(pT7-7)	0[c]				
E. coli(pWHM1093)	356.4				

[a] Expressed as the formation of nmol of NADH per min per mg of protein.
[b] + or −, ability or inability to grow, respectively, for 6 to 7 days at 30°C when fresh spore suspension was used to inoculate minimal medium with 50 mM glucose (G), valine (V), isobutyrate (IB), or propionate (P) as the sole carbon source.
[c] Below the limit of detection (specific activity, ≤1.0 nmol/min/mg).

coelicolor J802 gene was inactivated by insertion of the *hyg* gene into the *msdA* coding region at the *Bbs*I site (Fig. 2). The *msdA*::*hyg* construct was cloned in a temperature-sensitive *Streptomyces* plasmid pWHM1065 (22) to give pWHM1090 (Materials and Methods). After *S. coelicolor*(pWHM1090) transformants were grown at 39°C to eliminate the plasmid, two Th^s Hg^r colonies were isolated for further study. Chromosomal DNA isolated from the Th^s Hg^r mutant strains (one of them was named WHM1640) and the wild-type parental strain (J802) were used to analyze the region around the *msdA* gene by Southern blot analysis. The dig-AP-labeled 1.34-kb *Pvu*II fragment from pWHM1070 hybridized to a 3.6-kb *Xma*I fragment for strain WMH1640 in place of the 1.9 kb fragment for the wild-type strain. Digestion of WMH1640 genomic DNA with *Nsp*I-*Xmn*I gave a 2.6-kb fragment in place of the 0.9-kb fragment for the wild-type strain (data not shown). Consequently, the results of the Southern blot analysis confirmed that the 1.7-kb *hyg* gene had inserted into the *msdA* coding region in strain WMH1640 through the expected double crossover.

The *msdA*::*hyg* mutant lost the ability to grow on a minimal medium containing valine or isobutyrate as the sole carbon source but still grew as well as strain J802 on glucose or propionate as the sole carbon source (Table 1). It also lacked detectable MSDH activity as determined by the method of Kupiecki and Coon (11). The *msdA*::*hyg* mutation could be complemented by introduction of the *msdA* gene cloned as pWHM1094 (Fig. 2) to give the wild-type level of MSDH activity in the WMH1640 transformant and enable this transformant to grow on minimal medium with valine or isobutyrate as the sole carbon source (Table 1). These results indicate that the *S. coelicolor msdA* gene encodes the MSDH protein that is involved in valine catabolism, most likely by the route shown in Fig. 1, pathway A.

Overexpression of the *S. coelicolor msdA* gene in *E. coli*. To test the enzymatic activity of the *msdA* gene product, a pT7-7 (21) derivative, pWHM1093, containing the complete *msdA* coding region expressed from the T7 promoter under control of an IPTG-inducible gene for T7 RNA polymerase was constructed as described in Materials and Methods. *E. coli* BL21 (DE3) containing pWHM1093 was analyzed for *msdA* expression by SDS-PAGE (Fig. 4) and enzymatic assay. Upon IPTG induction, a protein of 51 kDa, which is the approximate mass of the predicted *S. coelicolor msdA* product (52.6 kDa), was detected in the protein extract from *E. coli*(pWHM1093) but not from the pT7-7 control strain (Fig. 4). Since the pWHM1093 construct has two translational start codons and corresponding ribosomal binding sites (one from the T7 expression vector and *Nde*I site and another corresponding to the original *msdA* ORF), the MsdA protein from *E. coli* (pWHM1093) could be an N-terminal KEFSVM-MsdA fusion protein or a mixture of fusion protein and native protein. Nevertheless, as shown in Table 1, MSDH assays of the crude cell extract of *E. coli*(pWHM1093) exhibited strong MSDH activity.

DISCUSSION

Although *S. coelicolor* is not known to produce antibiotics made from the valine catabolites, propionate, methylmalonate, and isobutyrate, these compounds are used for macrolide production in other streptomycetes, in which a defect in valine utilization by inactivation of the *vdh* gene (Fig. 1) greatly decreased antibiotic production (23). *S. coelicolor* was chosen for study of the *msdA* gene because information about the genetics of valine catabolism can be integrated into knowledge about this genetically well-characterized organism and serve as a model for other *Streptomyces* spp. The MSDH activity, expressed by the *msdA* gene cloned from *S. coelicolor* J802, catalyzes the oxidative decarboxylation of a 3-keto acid with the formation of an acyl-CoA thioester (Fig. 1). MSDH belongs to the aldehyde dehydrogenase (NAD^+) superfamily (6). It is involved in the pathway of valine catabolism, where it catalyzes the direct conversion of methylmalonic acid semialdehyde to propionate in some microorganisms and mammals, and the catabolism of thymine and compounds that are catabolized by the way of β-alanine, including uracil and cytidine in mammals (6). Since disruption of the *S. coelicolor msdA* gene caused complete loss of the MSDH activity and ability to grow on minimal medium with valine or isobutyrate as the sole carbon source, with restoration of growth by propionate, it appears that the MSDH enzyme acts late in the valine catabolic pathway, similar to the route in *Pseudomonas* spp. (19,

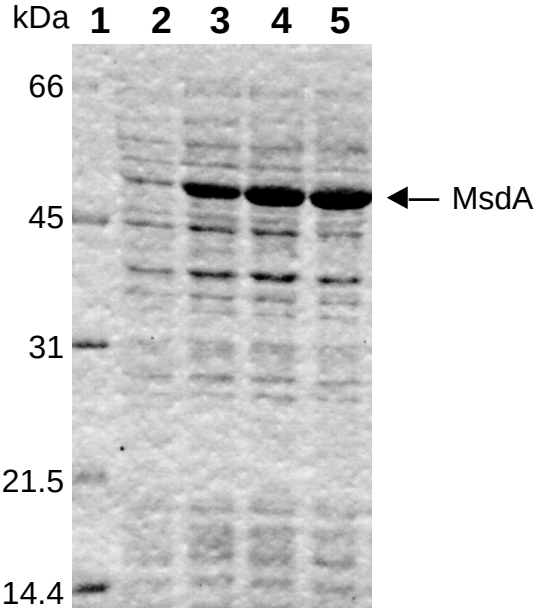

FIG. 4. Overexpression of the *msdA* gene in *E. coli*. SDS-PAGE (12.5% polyacrylamide gel) analysis of cell extracts from *E. coli* BL21(DE3) containing pT7-7 (lane 2) and pWHM1093 (lane 3, 4, and 5, for which IPTG induction was carried out for 1, 2, and 3 h). Lane 1 contains molecular weight standards.

25), as shown in Fig. 1, pathway A. In both organisms, it is assumed that externally added propionate is converted to propionyl-CoA before it enters the mainstream of metabolism.

There is a dichotomy in this hypothesis, however, because in several cases, the pattern of isotopic labeling of secondary metabolites that are formed from valine catabolites like 2-methylmalonate and propionate suggests that two routes to propionate exist. Pathways A and B in Fig. 1 illustrate this idea and show that the carbon atom at position 2 of valine is lost as CO_2 in pathway A but retained in the propionate formed in pathway B. Although pathway A is supported by the activity of the MSDH from *S. coelicolor* and the corresponding enzyme produced by the *P. aeruginosa mmsA* gene, the labeling pattern of macrolide and polyether antibiotics from [2-^{13}C]valine or [1-^{13}C]isobutyrate (14, 15, 17, 18) can be explained only by operation of pathway B. If both pathways are functional, which could be determined by the amount of 3-hydroxyisobutyryl-CoA that escapes into pathway B without hydrolysis of the CoA ester, then the *S. coelicolor msdA* mutant would have been expected to utilize valine or isobutyrate as the carbon source. Since strain WMH1640 cannot grow on these carbon sources but requires exogenous propionate in the absence of glucose or another carbon source that bypasses branched-chain amino acid metabolism, there appears to be a single catabolic route to propionate from valine. Therefore, either pathway B operates only in a select group of *Streptomyces* spp. producing polyketides that are assembled in part from propionate and 2-methylmalonate, such as tylosin (14) and lasalocid (17), or there is an explanation for the labeling results reported above that we cannot imagine from the current knowledge of bacterial metabolism. For instance, pathway B may operate only at the time of antibiotic production. The lack of rearrangement of methylmalonyl-CoA to succinyl-CoA or decarboxylation to propionyl-CoA are unlikely alternative explanations. Thus, it will be worthwhile to determine whether the *S. coelicolor* and *S. fradiae msdA* mutants have identical phenotypes with respect to valine catabolism and how tylosin is labeled by [2-^{13}C]valine in the latter mutant.

The *S. coelicolor msdA* genotype reveals significant differences between the genetics of valine catabolism in *Pseudomonas* and *Streptomyces* spp. A homolog of *mmsB* gene encoding 3-hydroxyisobutyrate dehydrogenase is not found adjacent to the *msdA* gene, which suggests that expression of the genes governing the final two steps in valine catabolism is regulated differently in the two microorganism. In *P. aeruginosa*, the upstream regulatory gene (*mmsR*) (Fig. 2) plays a key role in this process through the *mmsAB* operon (19).

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