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# Molecular Characterization of the *mde* Operon Involved in L-Methionine Catabolism of *Pseudomonas putida*

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**A 15-kb region of *Pseudomonas putida* chromosomal DNA containing the *mde* operon and an upstream regulatory gene (*mdeR*) has been cloned and sequenced. The *mde* operon contains two structural genes involved in L-methionine degradative metabolism: the already-identified *mdeA*, which encodes L-methionine γ-lyase (H. Inoue, K. Inagaki, M. Sugimoto, N. Esaki, K. Soda, and H. Tanaka. J. Biochem. (Tokyo) 117:1120–1125, 1995), and *mdeB*, which encodes a homologous protein to the homodimeric-type E1 component of pyruvate dehydrogenase complex. A rho-independent terminator was present just downstream of *mdeB*, and open reading frames corresponding to other components of α-keto acid dehydrogenase complex were not found. When MdeB was overproduced in *Escherichia coli*, the cell extract showed the E1 activity with high specificity for α-ketobutyrate rather than pyruvate. These results suggest that MdeB plays an important role in the metabolism of α-ketobutyrate produced by MdeA from L-methionine. Accordingly, *mdeB* encodes a novel E1 component, α-ketobutyrate dehydrogenase E1 component, of an unknown α-keto acid dehydrogenase complex in *P. putida*. In addition, we found that the *mdeR* gene was located on the opposite strand and began at 127 bp from the translational start site of *mdeA*. The *mdeR* gene product has been identified as a member of the leucine-responsive regulatory protein (Lrp) family and revealed to act as an essential positive regulator allowing the expression of the *mdeAB* operon.**

Methionine plays a central role in the metabolism of sulfur amino acids. Many bacteria and eukaryotes catabolize L-methionine through α-ketobutyrate by three main pathways (36): (i) conversion of methionine to cystathionine through *S*-adenosylmethionine and homocysteine and then to cysteine, α-ketobutyrate, and ammonia; (ii) deamination to α-keto-γ-methylthiobutyrate and the subsequent dethiomethylation to α-ketobutyrate; and (iii) simultaneous deamination and dethiomethylation to α-ketobutyrate by L-methionine γ-lyase.

L-Methionine γ-lyase (EC 4.4.1.11), a pyridoxal 5′-phosphate-dependent enzyme, catalyzes the direct conversion of L-methionine into α-ketobutyrate, methanethiol, and ammonia. This enzyme has been demonstrated to be present in various bacteria, such as *Pseudomonas putida* (27, 40), *Aeromonas* sp. (26), and *Clostridium sporogenes* (16), and in the parasite *Trichomonas vaginalis* (20). L-Methionine γ-lyase is induced by the addition of L-methionine to the medium and is regarded as a key enzyme in methionine catabolism. α-Ketobutyrate, a main product of L-methionine catabolism, is converted to propionyl-coenzyme A by pyruvate dehydrogenase complex (2, 19) or to α-aceto-α-hydroxybutyrate with pyruvate by acetolactate synthase, which is the isoleucine biosynthetic enzyme (6, 41). It has been proposed that high α-ketobutyrate levels interfere with a number of metabolic pathways by several mechanisms (5, 41). Therefore, a study of the L-methionine catabolism pathway (iii above) should be considered along with a study of the metabolism of α-ketobutyrate.

Recently, we cloned the L-methionine γ-lyase gene (termed *mdeA*) from *P. putida* (13). We reported the presence of a part of an open reading frame (*mdeB*) in the 3′-flanking region of *mdeA* and that these genes form an operon which was termed the *mde* operon. The deduced amino acid sequence of MdeB showed a high homology with that of the N-terminal region of the E1 component of the pyruvate dehydrogenase complex from *Escherichia coli* (13). To obtain more information about the genes involved in L-methionine catabolism, we have cloned and characterized the genes containing the entire *mde* operon from *P. putida*. We have demonstrated that the *mdeB* gene product shows a high substrate specificity towards α-ketobutyrate rather than pyruvate. In addition, we have also identified a regulatory gene, termed *mdeR*, upstream of *mdeA*, which may relate to the expression of the *mde* operon.

## MATERIALS AND METHODS

**Strains, plasmids, and culture conditions.** The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* cells were cultured aerobically at 37 or 28°C in Luria broth (LB) (32) or a medium (pH 7.2) containing 0.25% L-methionine (Met medium) as described previously (27). *P. putida* cells were cultured aerobically at 28°C in LB, Met medium, or a medium (pH 7.2) containing 0.3% glucose and 0.3% diammonium hydrogen phosphate in place of L-methionine (Glc medium). Ampicillin (50 μg/ml) and/or kanamycin (50 μg/ml) was added to the media for *E. coli* as a selection marker, when necessary.

**DNA hybridization, cloning, and sequencing.** Southern blot hybridization, Northern blot hybridization, and colony hybridization were performed with Hybond-N$^+$ nylon membranes (Amersham) by the standard techniques (32). A *Pst*I-*Sac*I 272-bp fragment containing a part of the *mdeB* gene (Fig. 1A) and a *Hin*dIII-*Pst*I 453-bp fragment containing a part of the *mdeA* gene were used as probes. These probes were labeled with [α-$^{32}$P]dCTP (NEN Research Products) by using 6-bp random primers (Takara Shuzo, Kyoto, Japan) and Klenow fragment.

The chromosomal DNA of *P. putida* was isolated by the method of Saito and Miura (30). To construct a *P. putida* genomic DNA library, genomic DNA was digested with *Bam*HI and separated by electrophoresis on a 0.7% agarose gel. The 15-kb *Bam*HI DNA fragments excised from the gel were cloned into Charomid 9-28. The library was packaged in λ with LAMBDA INN (Nippon Gene, Toyama, Japan) and then plated on *E. coli* DH1. The library was screened by colony hybridization with a *Pst*I-*Sac*I 272-bp fragment as a probe to obtain the clone pYH1001 (Fig. 1).

Several restriction fragments from pYH1001 were subcloned into pUC118 or

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TABLE 1. Bacterial strains and plasmids used in this study

| Strain or plasmid | Description[a] | Reference or source |
|---|---|---|
| Bacterial strains | | |
| *P. putida* ICR3460 | Wild type | 27 |
| *E. coli* | | |
| DH1 | *supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1* | 32 |
| MV1184 | *ara* Δ(*lac-proAB*) *rpsL thi* (ϕ80 *lacZ*ΔM15) Δ(*srl-recA*)*306*::Tn*10*(Tet$^r$) F′(*traD36 proAB*$^+$ *lacI*$^q$ *lacZ*ΔM15) | 32 |
| Plasmids | | |
| Charomid 9-28 | Cosmid Ap$^r$ | 31 |
| pUC118 | ColE1 *ori* Ap$^r$ | 42 |
| pUC119 | ColE1 *ori* Ap$^r$ | 42 |
| pMW218 | pSC101 *ori* Km$^r$ | Nippon Gene |
| pET-11a | Ap$^r$; contains a T7 promoter with start codon and designed ribosome binding site | Novagen |
| pYH1001 | 15-kb insert (genomic *Bam*HI fragment) from *P. putida* ICR3460 DNA in Charomid 9-28 | This study |
| pYH2 | 2.4-kb insert (genomic *Sac*I fragment) from *P. putida* ICR3460 DNA in pUC118 | 13 |
| pYH4 | 2.7-kb insert (genomic *Pst*I-*Sac*I fragment) in pUC118 | 13 |
| pYH5 | 4.6-kb insert (*Sal*I-*Sac*I fragment of pYH6) in pUC118 | This study |
| pYH6 | 6.9-kb insert (*Bam*HI-*Sac*I fragment of pYH1001) in pUC118 | This study |
| pYH7 | 2.8-kb insert (*Stu*I-*Sac*I fragment of pYH6) in pUC118 | This study |
| pYH1010 | 2.7-kb insert (*Xba*I-*Bam*HI fragment of *mdeB* containing a start codon and designed ribosome binding site of pET-11a) in pUC118 | This study |
| pMUS5 | 0.7-kb insert (*Stu*I-*Hin*dIII fragment of pYH7) in pMW218 | This study |

[a] Ap, ampicillin; Km, kanamycin.

pUC119. The single-stranded DNA was prepared with the helper phage M13KO7 (42). The DNA sequence was determined by the dideoxy chain termination method (33). Sequencing was carried out with M13-specific M4 primer (Takara Shuzo) radiolabeled with [γ-$^{32}$P]ATP (NEN Research Products) and *Bca*BEST DNA sequencing kit (Takara Shuzo). The DNA sequence data and deduced amino acid sequences were analyzed with the GENETYX-Mac version 7.0.9 software (Software Development, Tokyo, Japan).

**Construction of expression plasmid.** To construct the expression plasmid for the *mdeB* gene product, an *Nde*I site was introduced into the proposed translational start codon of *mdeB* by PCR. The 1,152-bp fragment corresponding to the 5′-terminal half of the coding sequence was amplified by PCR with the PCR primers P1 (5′-GGGCATATGGTGGCAATGATGAACCTTGTGC-3′) and P2 (5′-GGCAAAGGCGGCATACAGCTTGC-3′), which corresponded to the sequences from nucleotides 1866 to 1890 and from 2989 to 3011 (complementary sequence) (Fig. 2), respectively. The preceding six bases (GGGCAT) of the *mdeB* initiating codon, ATG (underlined in the text), was substituted for the wild-type sequence (AATGCA). The PCR product was purified and subcloned into the *Sma*I site of pUC119. Subsequently, the *Nde*I-*Bam*HI fragment excised from the resultant plasmid was subcloned into pET-11a (termed pYH1008). To construct the entire *mdeB* structural gene, a *Sac*I-*Bam*HI fragment containing a *Sac*I-*Sma*I fragment of the 3′-part of *mdeB* was inserted into pYH1008 to give pYH1009. Finally, an *Xba*I-*Bam*HI fragment of pYH1009 was inserted into pUC119 to yield the expression plasmid pYH1010 (Fig. 3A).

**Expression of *mdeB* in *E. coli*.** The expression plasmid pYH1010 was transformed into *E. coli* MV1184. A 0.5-ml overnight culture of the cells was inoculated into 150 ml of LB and cultivated at 28°C for 30 h under aeration. The soluble fraction of the cell extract was prepared by sonication in buffer A (10 mM potassium phosphate buffer [pH 7.2] containing 0.1 mM thiamin pyrophosphate [TPP] and 3 mM $MgCl_2$) followed by centrifugation. The cell debris was washed twice with buffer A, and then the inclusion body fraction containing MdeB was obtained as a precipitate from a 10-min centrifugation at 3,000 × *g*. Each fraction was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (18).

**Enzyme assays.** E1 activity was measured by an assay involving the reduction of 2,6-dichlorophenolindophenol (DCPIP) (21). In the assay, 50 μl of 100 mM α-keto acid (pyruvate or α-ketobutyrate) was added to 1,050 μl of mixture containing 0.1 M potassium phosphate (pH 7.4), 0.1 mM DCPIP, 0.2 mM TPP, 0.1 mM $MgCl_2$, and 0.5 to 1.0 mg of cell extract at 30°C. L-Methionine γ-lyase activity was routinely followed by the determination with 3-methyl-2-benzothiazolone hydrozone hydrochloride of the amount of α-ketobutyrate formed, as described previously (35). A cell extract from the *E. coli* MV1184 transformant containing *mdeA* was prepared as described previously (13). Protein was measured by the method of Lowry et al. (22) with bovine serum albumin as a standard.

**N-terminal amino acid analysis.** For the determination of the N-terminal amino acid sequence, the enzyme was electroblotted from an SDS-polyacryl-

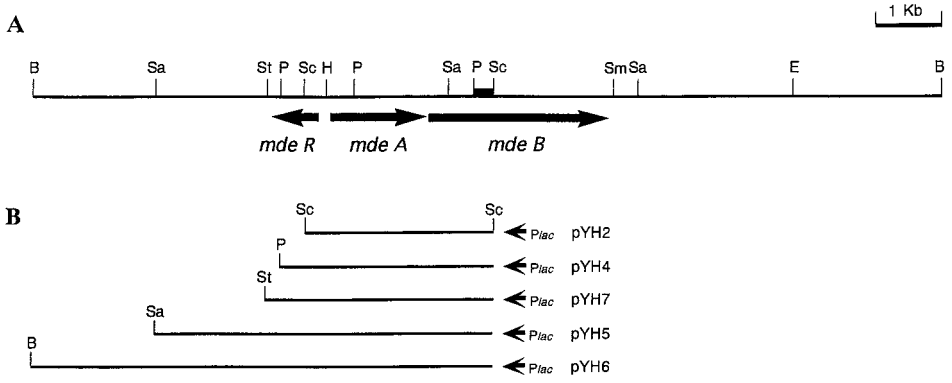


FIG. 1. (A) Restriction map of the *Bam*HI fragment of pYH1010 containing the *mde* operon. The positions and directions of transcription of the genes are indicated by arrows. The fragment used as a probe for cloning is indicated with a bold line. (B) The DNA fragments used in promoter analysis of *mdeA*. The directions of transcription by the *lac* promoter are indicated by short arrows. MdeA activities of transformants are presented in Table 3. B, *Bam*HI; Sa, *Sal*I; St, *Stu*I; P, *Pst*I; Sc, *Sac*I; H, *Hin*dIII; Sm, *Sma*I; E, *Eco*RI.

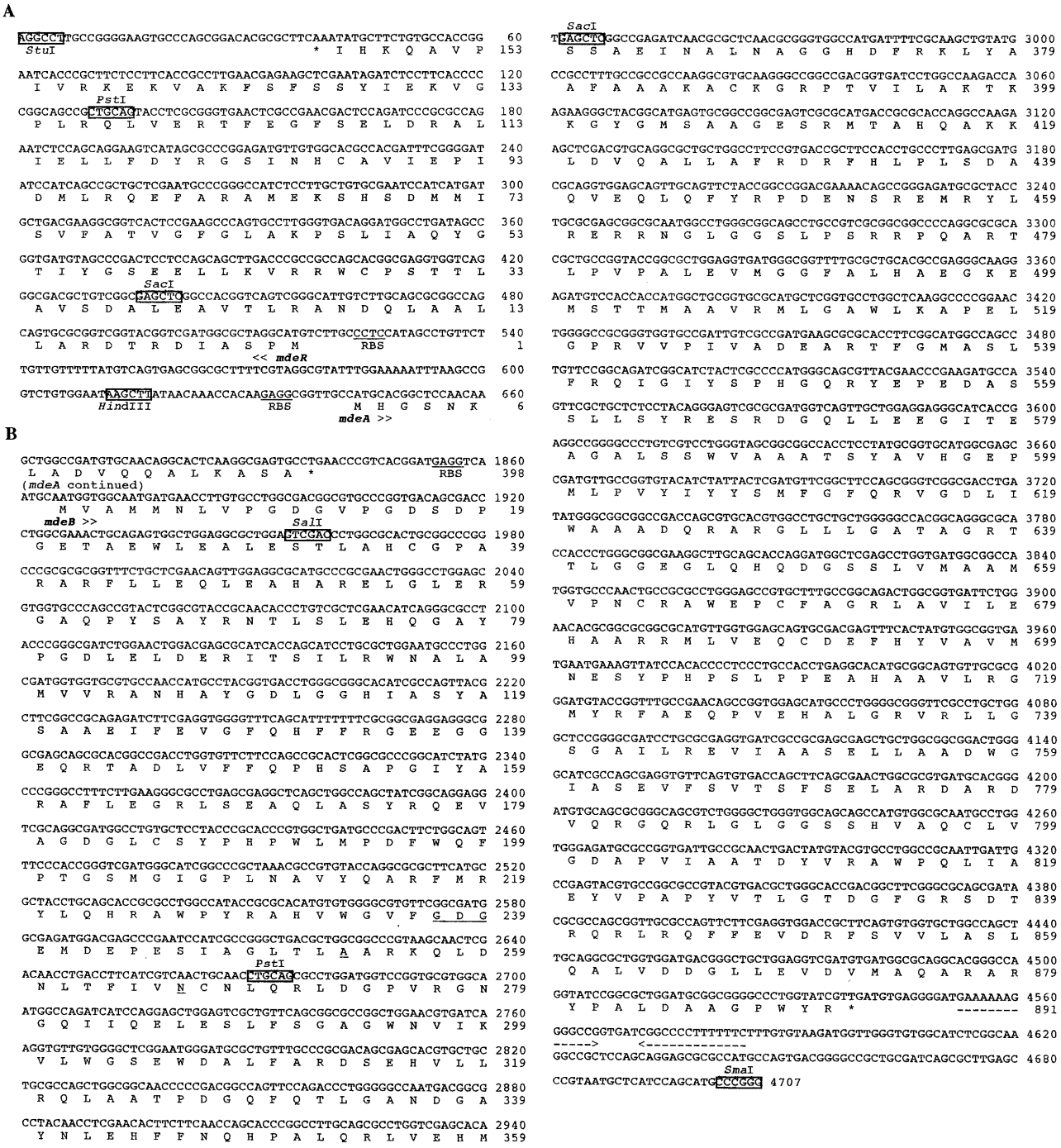


FIG. 2. Nucleotide sequences of *mdeR* (A) and *mdeB* (B) genes. The amino acid sequences of *mdeR*, *mdeB*, and part of *mdeA* are represented by single-letter code. Putative ribosome binding sites (RBS) and a putative TPP-binding site of the *mdeB* gene product are underlined. Only restriction sites used in experiments are boxed. The stop codons are indicated with asterisks. The location of a potential rho-independent terminator structure is marked by facing arrows.

amide gel in 25 mM CAPS [3-(cyclohexylamino)-1-propanesulfonicacid] buffer–10% (vol/vol) methanol (pH 11) onto a polyvinylidene fluoride membrane with a Problott (Applied Biosystems, Foster City, Calif.) at a constant current of 5 mA/cm$^2$. Proteins on the membrane were stained with Coomassie brilliant blue R250 (Bio-Rad). Areas of the membrane corresponding to required proteins were cut out and subjected to sequence analysis with a 477A pulsed liquid-phase protein-peptide sequencer and a 120A on-line phosphothio-hydantoin amino acid analyzer (both from Applied Biosystems) as instructed by the manufacturer.

**Nucleotide sequence accession number.** The nucleotide sequence reported here has been submitted to EMBL, GenBank, and DDBJ with accession no. D89015.

## RESULTS

**Cloning of the *mde* operon.** We had cloned a 2.8-kb *Pst*I-*Sac*I region containing *mdeA* and the 5′ part of the *mdeB* gene from

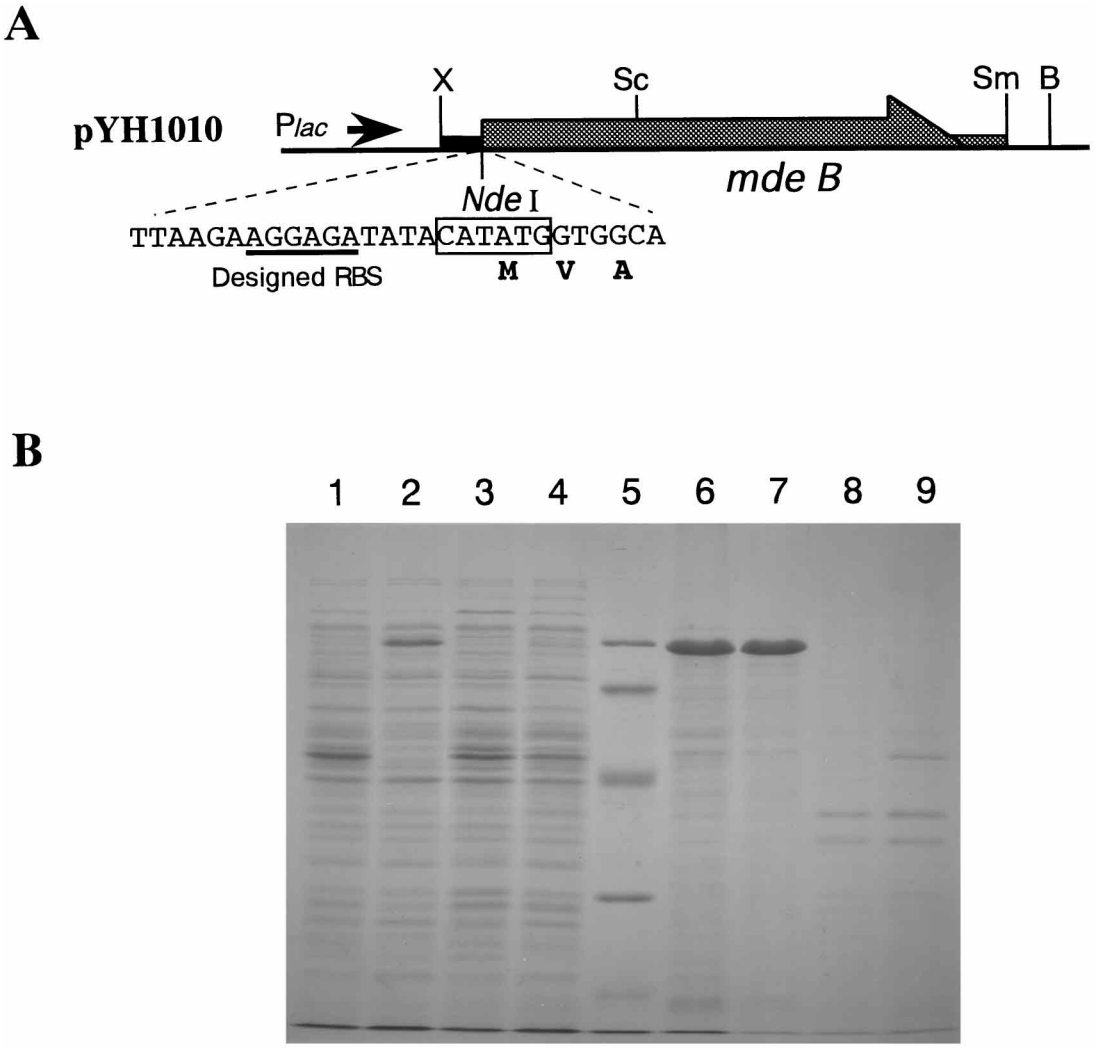


FIG. 3. (A) Schematic drawing of the expression construct, pYH1010, used to express MdeB in *E. coli* MV1184. A designed ribosome binding site (RBS) from pET-11a is underlined. The *Xba*I-*Nde*I region is from pET-11a. X, *Xba*I; Sc, *Sac*I; Sm, *Sma*I; B, *Bam*HI. (B) SDS-PAGE analysis of the cell extracts of *E. coli* transformants. *E. coli* MV1184/pYH1010 and MV1184/pUC119 (control) cells were incubated at 28 and 37°C. Each cell extract (insoluble and soluble fractions) was run on SDS-PAGE (12.5% acrylamide). Lanes 1 to 4 and 6 to 9 indicate soluble fractions and insoluble fractions, respectively. Lanes 1 and 6, *E. coli* MV1184/pYH1010 incubated at 37°C; lanes 2 and 7, *E. coli* MV1184/pYH1010 incubated at 28°C; lanes 3 and 8, *E. coli* MV1184/pUC119 incubated at 37°C; lanes 4 and 9, *E. coli* MV1184/pUC119 incubated at 28°C; and lane 5, molecular mass standards (top to bottom: 94, 67, 43, 30, and 20.1 kDa).

*P. putida* ICR3460 as reported previously (13). To clone the entire *mde* operon, we used the 272-bp *Pst*I-*Sac*I fragment containing *mdeB* as a probe (Fig. 1A). A 15-kb *Bam*HI hybridization band was identified by Southern blot analysis of *P. putida* genomic DNA digested with several restriction enzymes. We constructed the *Bam*HI genomic library in Charomid 9-28 as described in Materials and Methods. A positive clone, designated pYH1001, was isolated by colony hybridization, and it was confirmed by Southern blot analysis that the cloned fragment was the objective fragment. Restriction mapping showed that a portion of this clone overlapped the whole region of the previously cloned fragment corresponding to the insert pYH4.

**Organization and sequence analysis of the *mde* operon.** Figure 1A shows the structural organization of the *mde* operon and an upstream regulatory gene (*mdeR*). The *mde* operon contained two structural genes (*mdeAB*). The nucleotide sequences and deduced amino acid residues for *mdeR* and *mdeB* are shown in Fig. 2. The *mdeR* coding region contained 477 bp beginning 127 bp from the 5′ end of *mdeA* on the opposite strand and encoded a 159-amino-acid-residue protein with a calculated molecular weight of 17,836. The 5′ end of *mdeB* is separated from the 3′ end of *mdeA* by a 28-bp noncoding region that contains a putative ribosome binding site (Fig. 2B). The *mdeB* coding region contained 2,673 bp encoding an 891-

TABLE 2. Expression of *mdeB* in *E. coli* MV1184[a]

| Substrate | MdeB (mU/mg)[b] in: | | | |
|---|---|---|---|---|
| | pYH1010 grown at: | | pUC119 grown at: | |
| | 28°C | 37°C | 28°C | 37°C |
| α-Ketobutyrate | 4.80 | 0.44 | 0.46 | 0.96 |
| Pyruvate | 3.70 | 3.08 | 3.28 | 4.40 |

[a] *E. coli* MV1184 transformants were grown at 28 or 37°C in LB.
[b] One unit of the enzyme is defined as the amount that reduces 1 μmol of DCPIP per min at 30°C.

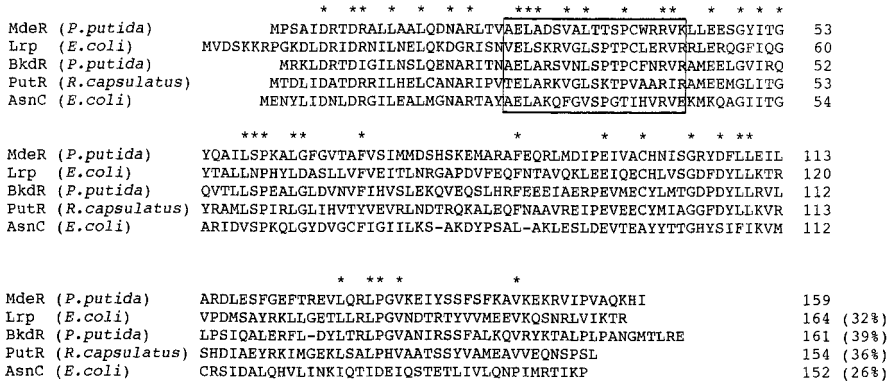


FIG. 4. Alignment of the deduced amino acid sequence of *P. putida* MdeR with homologous proteins from other organisms. These sequences were aligned by introducing gaps (hyphens) to maximize identities. A putative DNA binding helix-turn-helix motif is boxed. Percentages of identity to MdeR are given at the end of each sequence. Common residues in four or five proteins containing MdeR are shown with asterisks.

amino-acid-residue protein with a calculated molecular weight of 98,082. A stem-loop structure followed by T residues, which probably encoded a rho-independent terminator (29), was found immediately after the stop codon of *mdeB*, and additional downstream open reading frames were not found. The G+C contents of *mdeR*, *mdeA*, and *mdeB* were 62.7, 66.3, and 66.9 mol%, respectively. The G+C contents of these genes correspond to the genomic G+C content of the *P. putida* genome (62.5 mol%) (17). The intergenic region between *mdeR* and *mdeA* was only 45.7 mol% G+C. To analyze the expression of the *mde* operon in *P. putida* ICR3460, RNA was isolated from cells grown on Met medium or Glc medium. In Northern blot hybridization, two radiolabeled probes complementary to *mdeA* and *mdeB* hybridized significantly at the same single position with the RNA isolated from cells grown on Met medium (data not shown). These results indicate that *mdeB* is transcribed only from the *mdeA* promoter and thus follows the same tight induction pattern as *mdeA*.

**Identification of the *mdeB* gene product.** A computer search with the deduced amino acid sequence of *mdeB* in data libraries revealed 50.5, 53.3, and 65% amino acid homology to the E1 component of the pyruvate dehydrogenase complexes of *E. coli* (AceE) (37) and *Alcaligenes eutrophus* (PdhA and PdhE) (9, 10), respectively, which are known as homodimeric-type pyruvate dehydrogenase complexes, in contrast to the heteromeric (α and β subunits) type from eukaryotes and gram-positive bacteria (9). The conserved 30 amino acid residues of the putative TPP-binding region (beginning with the sequence GDG and ending with the sequence NN or C), which had previously been found in the sequences of various TPP-dependent enzymes, were also observed in the sequence of the protein encoded by *mdeB* (amino acid residues 237 to 267) (8) (Fig. 2B). These results indicate that *mdeB* might represent the structural gene corresponding to a protein showing E1-like activity (decarboxylase activity) of the pyruvate dehydrogenase complex.

To test the enzymatic activity of the *mdeB* gene product, we constructed an expression plasmid (pYH1010) containing *mdeB* in the same orientation in the *lac* promoter and a designed ribosome binding site of pET-11a as described in Materials and Methods (Fig. 3A). Soluble and insoluble fractions prepared from *E. coli* carrying pYH1010 and pUC119 (control) were analyzed by SDS-PAGE (Fig. 3B). When *E. coli* MV1184/pYH1010 was grown at 37°C, MdeB was expressed as an inclusion body. A protein of 94 kDa, which is in reasonable agreement with the approximate mass of the predicted *mdeB* product (98 kDa), was observed in the insoluble fraction (Fig. 3B, lane 6). MdeB could be detected in both soluble and insoluble fractions when MV1184/pYH1010 was grown at 28°C (Fig. 3B, lanes 2 and 7). No protein of 94 kDa was found in either fraction of the MV1184/pUC119 control strain. Only the pYH1010 transformant grown at 28°C exhibited strong α-ketobutyrate dehydrogenase E1-like activity, 10 times higher than that of the control strain (Table 2). However, the increase in the pyruvate dehydrogenase E1 activity in the transformant was insignificant. These results suggest that MdeB is an α-keto acid decarboxylase which is highly specific for α-ketobutyrate rather than pyruvate. The E1-like activity was not detected in the insoluble fraction of MV1184/pYH1010 (data not shown). The sequence of the first five N-terminal amino acids of the insoluble MdeB was VAMMN, which was in agreement with the predicted amino acid sequence except for the first methionine residue.

**Identification of the *mdeR* gene product as an Lrp-family regulatory protein.** The deduced amino acid sequence of the *mdeR* gene product showed a homology to regulatory proteins that belong to the leucine-responsive regulatory protein (Lrp) family (Fig. 4). The identity scores of MdeR are 32% for Lrp (43) (from *E. coli*), 26% for AsnC (15) from *E. coli*, 39% for BkdR (25) from *P. putida*, and 36% for PutR (14) from *Rhodobacter capsulatus*. Although Lrp is a global positive or negative transcriptional regulatory protein (4), the other Lrp-like proteins, especially BkdR and PutR, have been reported to function as positive transcriptional regulators of the genes of their respective catabolic pathways. They are involved in the

TABLE 3. Expression of *mdeA* in *E. coli* MV1184

| Plasmid | Specific activity (mU/mg)[a] in: | |
|---|---|---|
| | LB | Met medium[b] |
| pYH2 | ND[c] | ND |
| pYH2/pMUS5 | 34 | 24 |
| pYH2/pMW219 | ND | ND |
| pYH4 | ND | ND |
| pYH7 | 17 | 7.8 |
| pYH5 | 18 | 12 |
| pYH6 | 17 | 4.1 |

[a] One unit of the enzyme is defined as the amount that catalyzes the formation of 1 μmol of α-ketobutyrate per min at 37°C.
[b] Medium containing 0.25% L-methionine.
[c] ND, not detected.

catabolism of branched keto acid (BkdR) and proline (PutR) (14, 25). To investigate the influence of the *mdeR* gene product on the *mde* operon expression, we constructed plasmids, containing 0.2 to 4.7 kb of the 5′-flanking region of *mdeA* and the entire *mdeA* gene, which were introduced into pUC118 (Fig. 1B). The MdeA activity was assayed with the cell extract of these *E. coli* MV1184 transformants. Only transformants carrying plasmids containing the entire *mdeR* gene (pYH5, pYH6, and pYH7) showed MdeA activity (Table 3). The MdeA activity of *E. coli* carrying pYH2 was also detected when complemented in *trans* by pMUS5, which was constructed from the *Stu*I-*Hin*dIII fragment containing a complete copy of *mdeR* inserted into pMW219 (a compatible plasmid for the pUC vector) (Table 3). These results suggest that the *mdeR* gene product acts as an essential positive regulator of the expression of the *mde* operon. However, L-methionine had a repressing, rather than an activating, effect on *mde* operon promoter activity in *E. coli* MV1184 (Table 3).

## DISCUSSION

To our knowledge, this is the first report on the genes responsible for L-methionine catabolism in the form of an operon. Molecular and biochemical characterization of the structural genes of the *mde* operon revealed an effective degradation pathway of L-methionine for *P. putida*. The predicted amino acid sequences of MdeA and MdeB revealed that each of these enzymes showed sequence similarities to enzymes from other sources catalyzing similar reactions. MdeA and MdeB show extensive homology with cystathionine γ-synthase (13) and the homodimeric-type E1 component of pyruvate dehydrogenase complex, respectively. The genes encoding these proteins are commonly found in various bacteria as genes of distinct operons (1, 10, 37). These observations suggest that the L-methionine catabolism pathway with the *mde* operon from *P. putida* evolved from the reconstitution of preexisting enzymes.

Our results suggest that MdeB is a novel E1 component of α-keto acid dehydrogenase complex. A 10-fold increase in E1-like activity, in comparison to the background activity, was found for α-ketobutyrate of the cell extract from the MdeB overexpression strain (Table 2). Interestingly, E1-like activity for pyruvate of the MdeB overexpression strain did not show any significant increase. Therefore, in spite of amino acid sequence similarities, the E1 component of pyruvate dehydrogenase complex and MdeB are clearly distinct in substrate specificity and physiological role. It is generally accepted that pyruvate dehydrogenase complex can use α-ketobutyrate as a substrate (2, 19, 41). However, the pyruvate dehydrogenase complex possessing a homodimeric E1 component demonstrates a low relative activity for α-ketobutyrate. In the pyruvate dehydrogenase complex of *E. coli*, the $K_m$ value for α-ketobutyrate is 10-fold greater than that for pyruvate and the maximum velocity shows a 5-fold reduction (2). In contrast, MdeB of *P. putida* is a homodimeric-type E1 component specific for α-ketobutyrate, which is the true substrate for this enzyme. MdeB may play an important role in reducing the toxic accumulation of α-ketobutyrate formed from L-methionine in the cell (5, 41).

It is unclear whether MdeB can form a complex with E2 (dihydrolipoamide *S*-acyltransferase) and E3 (dihydrolipoamide dehydrogenase) components. *mdeB* was not clustered with structural genes for other components of the complex. This is a remarkable result, because all other known genes encoding E1 components of α-keto acid dehydrogenase complexes which have been identified so far occur together with the respective genes for the E2 components as operons or gene clusters (10, 34, 37, 38). The only exception to this is the *pdhE* gene from *A. eutrophus*, which was recently reported by Hein and Steinbüchel (9). *A. eutrophus* possesses two distinct homodimeric-type pyruvate dehydrogenase E1 components, PdhA and PdhE. The *pdhA* gene is clustered together with *pdhB* and *pdhL*, which encode the E2 and E3 components, respectively (10). PdhE is the second *A. eutrophus* pyruvate dehydrogenase E1 component which is not clustered with structural genes for components of E2 and E3 (9). It should be noted that a high amino acid sequence identity (65%) was shown between MdeB and PdhE of the second E1 component rather than PdhA. This identity suggests that PdhE may also show a higher E1 activity for α-ketobutyrate than for pyruvate. PdhE had the ability to replace PdhA and to construct an active pyruvate dehydrogenase complex together with PdhB and PdhL (9). From this report we expect that MdeB also may form an α-ketobutyrate dehydrogenase complex with E2 (unidentified) and E3 (LPD-glc) of the pyruvate dehydrogenase complex from *P. putida* (its E1 component is of homodimeric type) (11, 28, 39).

We revealed that MdeR is an Lrp family regulatory protein and acts as a positive regulator allowing the expression of the *mde* operon. A conserved helix-turn-helix motif located in the N-terminal part of Lrp family regulatory proteins was also identified in MdeR (3, 7) (Fig. 4). The 127-bp intergenic region between *mdeA* and *mdeR* is unusually low (45.7 mol%) in G+C for *P. putida* (62.5 mol%), and this low G+C content may play a role in transcriptional regulation by facilitating DNA melting and/or bending (25). We also observed that the expression of *mdeA* containing the entire regulatory region was complemented in *trans* by MdeR produced from pMUS5 (Table 3). These results suggest that MdeR acts directly on the specific region by DNA binding.

It should be noted that L-methionine could not act as an effector of MdeR to stimulate expression of the *mde* operon in *E. coli* (Table 3). The relatively low level of MdeA expression in Met medium is probably a consequence of growth inhibition. It is likely that MdeR can bind to interact with the regulatory region of the *mde* operon in spite of the presence of effector, as reported for BkdR and PutR (12, 14, 24). However, the interaction between the activated MdeR and RNA polymerase on the regulatory region may be different in *E. coli* and in *P. putida*. Thus, it remains unclear whether L-methionine can interact directly as the true effector to activate MdeR. In addition, unknown factors of *P. putida* other than MdeR may be involved in the expression of the *mde* operon.

Finally, it is interesting that both the *bkd* operon and the *mde* operon are regulated by Lrp family proteins in *P. putida*. Since the nucleotide sequences of *mdeR* and *bkdR* including each regulatory region showed high similarities (51.4% nucleotide identity) (data not shown) (23), these genes presumably evolved after gene duplication from a common ancestor. Thus, both operons may be controlled by a similar regulatory mechanism. A comparison of MdeR and BkdR should reveal not only structural and functional properties of Lrp family proteins but also more about amino acid (L-methionine and branched amino acid) catabolism in *P. putida.*

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