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

HIROYUKI INOUE,1 KENJI INAGAKI,1 SHIN-ICHI ERIGUCHI,1 TAKASHI TAMURA,1 NOBUYOSHI ESAKI,2 KENJI SODA,2 AND HIIDEHIKO TANAKA1*  

Department of Bioresearch Resources Chemistry, Faculty of Agriculture, Okayama University, Okayama 700,1 Institute for Chemical Research, Kyoto University, Kyoto 611,2 and Faculty of Engineering, Kansai University, Osaka 564,3 Japan

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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-ketoisobutyrate rather than pyruvate. These results suggest that MdeB plays an important role in the metabolism of α-ketoisobutyrate produced by MdeA from L-methionine. Accordingly, mdeB encodes a novel E1 component, α-ketoisobutyrate 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 mdeI. 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 t-methionine through α-ketoisobutyrate by three main pathways (36): (i) conversion of methionine to cystathionine through S-adenosylmethionine and homocysteine and then to cysteine, α-ketoisobutyrate, and ammonia; (ii) deamination to α-keto-γ-methylthiobutyrate and the subsequent dethiomethylation to α-ketoisobutyrate; and (iii) simultaneous deamination and dethiomethylation to α-ketoisobutyrate by t-methionine γ-lyase.

1. Methionine γ-lyase (EC 4.4.1.11), a pyridoxal 5’-phosphate-dependent enzyme, catalyzes the direct conversion of t-methionine into α-ketoisobutyrate, 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). t-Methionine γ-lyase is induced by the addition of t-methionine to the medium and is regarded as a key enzyme in methionine catabolism. α-Ketoisobutyrate, a main product of t-methionine catabolism, is converted to propionyl-coenzyme A by pyruvate dehydrogenase complex (2, 19) or to α-aceto-α-hydroxyisobutyrate by pyruvate by acetolactate synthase, which is the isoleucine biosynthetic enzyme (6, 41). It has been proposed that high α-ketoisobutyrate levels interfere with a number of metabolic pathways by several mechanisms (5, 41). Therefore, a study of the t-methionine catabolism pathway (iii above) should be considered along with a study of the metabolism of α-ketoisobutyrate.

Recently, we cloned the t-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 t-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-ketoisobutyrate 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 (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 Hybrid-N+ nylon membranes (Amersham) by the standard techniques (32). A PstI-SacI 272-bp fragment containing a part of the mdeB gene (Fig. 1A) and a HindIII-PstI 453-bp fragment containing a part of the mdeA gene were used as probes. These probes were labeled with [32P]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 BamHI and separated by electrophoresis on a 0.7% agarose gel. The 15-kb BamHI DNA fragments excised from the gel were cloned into Charomid 928. 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 PstI-SacI 272-bp fragment as a probe to obtain the clone pYH1001 (Fig. 1). Several restriction fragments from pYH1001 were subcloned into pUC118 or

* Corresponding author. Mailing address: Department of Bioresearch Resources Chemistry, Faculty of Agriculture, Okayama University, Tsushima-naka 1-1-1, Okayama-shi, Okayama 700, Japan. Phone: 81-86-251-8298, Fax: 81-86-254-0714, E-mail: hitanaka@cc.okayama-u.ac.jp.
TABLE 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Description</th>
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<tr>
<td><strong>Bacterial strains</strong></td>
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<tr>
<td><em>P. putida</em> ICR3460</td>
<td>Wild type</td>
<td>27</td>
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<tr>
<td>E. coli DH1</td>
<td>supE44 lacI217 recA1 endA1 gspA96 thi-1 relA1</td>
<td>32</td>
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<tr>
<td>MV1184</td>
<td>araD(lac-proAB) rpsL thi-1 (q80 lacZDelta15)Delta(orl-recA306; Tn10(TcR) F’(traD36 proAB+ lacI+ lacZDelta15)</td>
<td>32</td>
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<td><strong>Plasmids</strong></td>
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<tr>
<td>Charomid 9-28</td>
<td>Cosmid Ap∗</td>
<td>31</td>
</tr>
<tr>
<td>pUC118</td>
<td>ColE1 ori Ap∗</td>
<td>42</td>
</tr>
<tr>
<td>pUC119</td>
<td>ColE1 ori Ap∗</td>
<td>42</td>
</tr>
<tr>
<td>pMW218</td>
<td>pSC101 ori Km∗</td>
<td>Nippon Gene</td>
</tr>
<tr>
<td>pET-11a</td>
<td>Ap∗; contains a T7 promoter with start codon and designed ribosome binding site</td>
<td>Novagen</td>
</tr>
<tr>
<td>pYH1001</td>
<td>15-kb insert (genomic BamHI fragment) from <em>P. putida</em> ICR3460 DNA in Charomid 9-28</td>
<td>This study</td>
</tr>
<tr>
<td>pYH2</td>
<td>2.4-kb insert (genomic SacI fragment) from <em>P. putida</em> ICR3460 DNA in pUC118</td>
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<td>4.6-kb insert (SalI-SacI fragment of pYH6) in pUC118</td>
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<td>pYH6</td>
<td>6.9-kb insert (BamHI-SacI fragment of pYH1001) in pUC118</td>
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<td>pYH7</td>
<td>2.8-kb insert (SstI-SacI fragment of pYH6) in pUC118</td>
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<td>pYH1010</td>
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<td>This study</td>
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<tr>
<td>pMUS5</td>
<td>0.7-kb insert (StuI-HindIII fragment of pYH7) in pMW218</td>
<td>This study</td>
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</table>

∗ Ap, ampicillin; Km, kanamycin.

*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 MgCl2) 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-dichlorophenolphindophenol (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 MgCl2, 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 hydroxozone 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 electrophoretically separated from an SDS-polyacrylamide gel as described previously (13).

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**FIG. 1.** (A) Restriction map of the *BamHI* 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 of the lac promoter are indicated by short arrows. MdeA activators of transformants are presented in Table 3. B, *BamHI*; Sa, *SalI*; St, *StuI*; P, *PstI*; Sc, *Sacl*; H, *HindIII*; Sm, *SmaI*; E, *EcoRI*.
amide gel in 25 mM CAPS [3-(cyclohexylamino)-1-propanesulfonic acid] 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/cm². 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 PstI-SacI region containing mdeA and the 5' part of the mdeB gene from 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.
P. putida ICR3460 as reported previously (13). To clone the entire mde operon, we used the 272-bp PstI-SacI fragment containing mdeB as a probe (Fig. 1A). A 15-kb BamHI hybridization band was identified by Southern blot analysis of P. putida genomic DNA digested with several restriction enzymes. We constructed the BamHI 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-

![Diagram](image)

**TABLE 2. Expression of mdeB in E. coli MV1184**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>pYH1010 grown at:</th>
<th>pUC119 grown at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28°C</td>
<td>37°C</td>
</tr>
<tr>
<td>α-Ketobutyrate</td>
<td>4.80</td>
<td>0.44</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>3.70</td>
<td>3.08</td>
</tr>
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</table>

*E. coli MV1184 transformants were grown at 28 or 37°C in LB.

One unit of the enzyme is defined as the amount that reduces 1 μmol of DCPIP per min at 30°C.
agreement with the approximate mass of the predicted an inclusion body. A protein of 94 kDa, which is in reasonable MV1184/pYH1010 was grown at 37°C, MdeB was expressed as E. coli prepared from materials and Methods (Fig. 3A). Soluble and insoluble fractions signed ribosome binding site of pET-11a as described in Ma-constructed an expression plasmid (pYH1010) containing structural gene corresponding to a protein showing E1-like

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-keto- 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-keto 4-ketoisovalerate 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.** 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 hetero- 

<table>
<thead>
<tr>
<th>TABLE 3. Expression of mdeA in E. coli MV1184</th>
<th>Specific activity (mU/mg) in:</th>
</tr>
</thead>
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<tr>
<td>Plasmid</td>
<td>LB</td>
</tr>
<tr>
<td>--------</td>
<td>----</td>
</tr>
<tr>
<td>pYH2</td>
<td>ND</td>
</tr>
<tr>
<td>pYH2pMUS5</td>
<td>34</td>
</tr>
<tr>
<td>pYH2pMW219</td>
<td>ND</td>
</tr>
<tr>
<td>pYH4</td>
<td>ND</td>
</tr>
<tr>
<td>pYH7</td>
<td>17</td>
</tr>
<tr>
<td>pYH5</td>
<td>18</td>
</tr>
<tr>
<td>pYH6</td>
<td>17</td>
</tr>
</tbody>
</table>

* 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.
* Medium containing 0.25% L-methionine.
* 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 StuI-HindIII 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 form an α-ketobutyrate dehydrogenase complex with E2 (unclustered) 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.

REFERENCES


VOL. 179, 1997 mde OPERON OF P. PUTIDA 3961
58:466–490.
inhibitors of two enzymes of the branched-chain amino acid pathway in
efficient cloning and mapping of large or small restriction fragments. Proc. Natl.
Acad. Sci. USA 82:8664–8668.
ization of the Alcaligenes eutrophus pyruvate dehydrogenase complex and
176:4394–4408.
164:310–315.
237:674–684.
ation of the pyruvate dehydrogenase complex of Pseudomonas putida branched-
BkdR-DNA binding in the expression of the bkd operon of Pseudomonas putida.
Pseudomonas putida is required for expression of the bkd operon and en-
3940.
1984. Purification and properties of L-methionine γ-lyase from Aeronomous sp.
421–424.
19. Platt, T. 1986. Transcription termination and the regulation of gene expres-
cloning and mapping of large or small restriction fragments. Proc. Natl.
Acad. Sci. USA 83:8664–8668.
laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold
Spring Harbor, N.Y.
1995. Cloning and sequencing of cDNAs encoding branched-chain keto acid dehy-
oxidase activity with 3-methyl-2-benzothiazolone hydrazo hydrachloride.
pyruvate dehydrogenase complex of Escherichia coli K12. Nucleotide se-
133:155–162.
Molecular cloning of genes encoding branched-chain keto acid dehydro-
mapping of pyruvate, 2-ketogluatrate, and branched-chain keto acid dehy-
products in α-ketobutyrate metabolism by Salmonella typhimurium. Mol.
zation of Lrp, an Escherichia coli regulatory protein that mediates a global