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Transformation of Isopropylamine to L-Alaninol by *Pseudomonas* sp. Strain KIE171 Involves N-Glutamyalted Intermediates

Susana I. de Azevedo Wäsch, Jan R. van der Ploeg, Tere Maire, Alice Lebreton, Andreas Kiener, and Thomas Leisinger

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*Pseudomonas* sp. strain KIE171 was able to grow with isopropylamine or L-alaninol [5-(+)-2-amino-1-propanol] as the sole carbon source, but not with D-alaninol. To investigate the hypothesis that L-alaninol is an intermediate in the degradation of isopropylamine, two mini-Tn5 mutants unable to utilize both isopropylamine and L-alaninol were isolated. Whereas mutant KIE171-BI transformed isopropylamine to L-alaninol, mutant KIE171-BII failed to do so. The two genes containing a transposon insertion were cloned, and the DNA regions flanking the insertions were sequenced. Two clusters, one comprising eight *ipu* (isopropylamine utilization) genes (*ipuABCDEFGH*) and the other encompassing two genes (*ipuI* and *orf259*), were identified. Comparisons of sequences of the deduced Ipu proteins and those in the database suggested that isopropylamine is transported into the cytoplasm by a putative permease, IpuG. The next step, the formation of γ-glutamyl-isopropylamide from isopropylamine, ATP, and L-glutamate, was shown to be catalyzed by IpuC, a γ-glutamyl synthetase. γ-Glutamyl-isopropylamide is then subjected to stereospecific monoxygenation by the hypothetical four-component system *IpaABDE*, thereby yielding γ-glutamyl-L-lalaninol [γ-(L-glutamyl)-L-hydroxy-isopropylamid]. Enzymatic hydrolysis by a hydrolyase, IpuE, was shown to finally liberate L-alaninol and to regenerate L-glutamate. No gene(s) encoding an enzyme for the next step in the degradation of isopropylamine was found in the *ipu* clusters. Presumably, L-alaninol is oxidized by an alcohol dehydrogenase to yield L-2-aminopropanaldehyde or it is deaminated by an ammonia lyase to propionaldehyde. Genetic evidence indicated that the aldehyde formed is then further oxidized by the hypothetical aldehyde dehydrogenases IpuI and IpuII to either L-alanine or propionic acid, compounds which can be processed by reactions of the intermediary metabolism.

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Isopropylamine is used as a solvent and as a raw material in manufacturing various chemicals. The compound occurs as a constituent of the herbicides atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine] and propachlor (2-chloro-N-isopropylacetanilide), from which it is liberated during microbial degradation (16, 21, 27). Little information is available on the microbial metabolism of isopropylamine. Studies with *Pseudomonas* sp. strain KIE171, which grows with isopropylamine and L-alaninol but not with D-alaninol, have provided genetic and biochemical evidence that isopropylamine degradation in this strain proceeds via γ-glutamyl-isopropylamide and γ-glutamyl-L-lalaninol to L-alaninol.

**MATERIALS AND METHODS**

**Materials.** Reagents for molecular biology were obtained from Fermentas (Vilnius, Lithuania) and New England Biolabs. γ-(L-Glutamyl)-isopropylamide and γ-(L-glutamyl)-L-1-hydroxy-isopropylamide were obtained from Bachem (Bubendorf, Switzerland). All other chemicals were reagent grade or better and were obtained from Aldrich, Fluka, or Sigma.

**Growth media and bacterial strains.** *Pseudomonas* sp. strain KIE171 and its derivatives were cultivated aerobically at 30°C in the mineral salts medium described by Kulla et al. (15) with a 10 or 20 mM carbon source. Cells were grown in 500-ml Erlenmeyer flasks containing 100 ml of liquid medium and shaken at 140 rpm. *Escherichia coli* was grown aerobically and shaking at 140 rpm. *Escherichia coli* was grown aerobically in Luria-Bertani medium (22). Growth was monitored as turbidity at 650 nm.

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*Pseudomonas* sp. strain KIE171 has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as DSM 12360. The isopropylamine and L-alaninol-utilizing mutants KIE171-B, KIE171-BL, and KIE171-BII carry the Deutsche Sammlung von Mikroorganismen und Zellkulturen numbers DSM 11521, DSM 11629, and DSM 13380, respectively. *E. coli* strains DH5α (GIBCO/BRL Life Technologies) and XLI-Blue (Strategene) were used for cloning, and *E. coli* BL21(DE3) (Novagen) was used as the host for the overex-
expression of proteins. E. coli strain S17-1 Ap(a) (17) was the donor in biparental mating.

Enrichment and isolation of an isopropylamine utilization. Ten milliliters of sewage sludge from the Lonza AG wastewater treatment plant was mixed with 90 ml of mineral salts medium containing 20 mM isopropylamine, and the mixture was incubated at 30°C under nonsterile conditions without shaking. After growth had occurred, 1 ml aliquots of the enrichment were transferred into 99 ml of the same medium and incubated under the above-mentioned conditions. After four subcultures in sterile medium with shaking, the final enrichment was serially diluted and spread onto plates containing mineral salts medium with 20 mM isopropylamine. The majority of the colonies were of one type. Purification of a typical colony yielded a pure culture of the isopropylamine utilizing Pseudomonas sp. strain KIE171.

Mutagenesis. Chemical mutagenesis of strain KIE171 was performed with N-methyl-N-nitro-N-nitrosoguanidine (MNGO) according to the protocol of Foster (10). A suspension of mutagenized cells containing approximately 50% survivors was plated on minimal medium containing 20 mM L-glutamate. A total of 2,000 colonies were replica plated on minimal plates containing 20 mM isopropylamine or 20 mM L-alanine as the sole carbon source. One mutant, strain KIE171-B, unable to grow with these compounds, was chosen for further study.

Transposon mutagenesis was carried out by using the mini-Tn5 system (7). The transposon was introduced into strain KIE171 by plate conjugation on Luria-Bertani medium at 30°C for 8 h with a donor/recipient ratio of 1:1. E. coli S17-1 Ap(a) (pUT-miniTn5Kn) was used as the donor strain. Exconjugants were selected on mineral salts agar containing 10 mM L-lactate and 10 mM L-alanine plus 50 μg of kanamycin per ml. A total of 3,000 kanamycin-resistant strain KIE171 exconjugants were then replicated onto mineral salts plates containing 50 μg of kanamycin per ml and 20 mM concentration of either isopropylamine, L-alanine, or L-lactate as the carbon source. Mutants impaired in growth with one or several of these carbon sources were chosen for further study.

DNA manipulations. Isolation of plasmids and of genomic DNA, restriction enzyme digestion, agarose gel electrophoresis, Southern analysis, and transformation of E. coli were carried out using standard methods (2).

RNA isolation and primer extension analysis. RNA isolation and primer extension were performed as described before (3). Oligonucleotide ipupe (5'-CACTATCGTCTTTGGAATGTCGT-3') was used to prime the reverse transcription reaction. The plasmid used for generation of a sequencing ladder was pME4771, which was constructed by insertion of a 0.7-kb ApaI/PstI fragment containing the ipuf promoter region in pBluescript II KS.

Construction of an ipuf::lacZ fusion strain. Plasmid pME4268 contains a 2.7-kb Smal/SstI fragment harboring the ipuf gene (S. L. de Azevedo Wasm, unpublished data). For the construction of an ipuf::lacZ fusion in strain KIE171, the 2.4-kb Smal fragment from pXM1918GT (24) was inserted in the blunt BamHI site of pME4268 to give pME4762. The Smal/HindIII fragment from pME4762 was then cloned in the vector pEX18Tc (24), resulting in pME4763. Plasmid pME4763 was introduced in strain KIE171 by conjugation, and integrants were selected on Luria-Bertani plates containing tetracycline (15 μg/ml). To select for second crossover events, integrants were subsequently plated on Luria-Bertani medium containing 5% sucrose. Sucrose-resistant colonies were obtained, and one such colony was designated KIE171-BV. PCR was used to verify whether correct replacement had occurred.

Construction of ipufC and ipuf expression plasmids. For the production of IpuC as an N-terminal histidine-tagged fusion protein, the ipufC gene was amplified by PCR from genomic DNA of strain KIE171 with the oligonucleotide primers ipufC-NT (5'-AACAGGTGATATATATAGGGCAAG-3') and ipufC-CT (5'-TTTGAAAGCTTGAAGCTTGCGGCG-3'), with the changes to introduce Ndel and HindIII restriction sites, respectively, underlined. The 1.4-kb PCR product was digested with Ndel and HindIII, and the resulting fragment encompassing ipufC was ligated into Ndel-HindIII-digested pET-28at (+) (Nova-gen), resulting in plasmid pME4275, in which ipufC is under the control of the T7 polymerase promoter. The ipufC sequence of plasmid pME4275 was sequenced to confirm that no changes had been introduced during PCR amplification. For the production of the wild-type IpuC, the Ndel-HindIII ipufC insert of pME4275 was placed under the control of the T7 promoter of vector pET24at (+) (Nova-gen), generating plasmid pME4277.

For the production of IpuF as an N-terminal histidine-tagged fusion protein, the ipuf gene was amplified by PCR from plasmid pME4275 by using the primers 5'-GCGCGCATATGGCATGGTAT-3' and 5'-TCTAAGCTTGAAGCTTGCGGCG-3', with the changes to introduce Ndel and SacI restriction sites, respectively, underlined. The 909-bp PCR product was digested with Ndel and SacI, and the resulting 891-bp fragment containing the ipuf gene was cloned into the vector pET28at (+), resulting in plasmid pME4275. The plasmid that no changes had been introduced during PCR amplification, the ipuf gene of plasmid pME4275 was sequenced. Plasmid pME4276, which expresses wild-type IpuF, was obtained by cloning the Ndel-Sacl insert of pME4275 into the expression vector pET24b (+).

Enzyme assays. γ-Glutamylaminde synthetase (IpuF) activity was assayed by measuring the substrate-dependent formation of inorganic phosphate from ATP and glutamate derivatives. The reaction mixture (0.4 ml) contained 10 mM ATP, 10 mM substrate, 10 mM L-glutamate, 50 mM MgCl2, 50 mM imidazole-HCl (pH 7.0), 3.5 mM NaCl, and 25 μg of enzyme preparation. The reaction was started by the addition of enzyme, and it was run at 37°C. To stop the reaction, 0.9 ml of ferrous sulfate reagent (0.8% FeSO4·7H2O in 15 mM HSO4) and 0.075 ml of ammonium molybdate reagent [6.6% (NH4)6MoO4·4H2O in 7.5 mM H2SO4] were added to 0.1 ml of incubation mixture. The sample was mixed vigorously and color was allowed to develop to read the expression plasmid pME4751 was performed at 18°C for 5 h to a final A540 of 1. The same procedure was used for the production of wild-type IpuC from the expression plasmid pME4756.

Induced E. coli BL21(DE3) cells (0.5 g) expressing His6-IpuC or His6-IpuF were suspended in 4 ml of the appropriate lysis buffer containing DNase I (10 μg/ml). Cell extract was obtained by two passages through a French pressure cell at 5.5 MPA and subsequent centrifugation at 40,000 x g for 30 min. His6-IpuC was purified by metal chelate affinity chromatography on a 2.5-ml HisBind resin column (Novagen) according to the manufacturer's instructions, and the pure protein was stored at −20°C in 200 mM imidazole, 500 mM NaCl, 20 mM Tris-HCl, 20% (vol/vol) glycerol (pH 8.0).

Crude extract containing His6-IpuF was supplemented with glycerol to a final concentration of 15% (vol/vol). His6-IpuF was purified on a 1-ml Ni-nitrilotriacetic acid agarose column (Qiagen) as described by the manufacturer and stored at −20°C in 250 mM imidazole, 300 mM NaCl, 50 mM Na2HPO4, 15% (vol/vol) glycerol (pH 8.0).

SDS-PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a Mini-PROTEAN II system (Bio-Rad) with 12% polyacrylamide gels under denaturing conditions (22). The broad range molecular weight markers used were from Bio-Rad or from New England Biolabs. Protein concentrations were measured using the method of Bradford (4) with Bio-Rad dye concentrate. Bovine serum albumin was used as a standard.

HPLC. HPLC was carried out with an alliance HPLC system (Waters), using MILLIENNUM software. Phenylisothiocyanate (PITC)-derivatized amines, including isopropylamine, t-alaninol, γ-glutamylaminde, t-glutamate, ATP, and ADP, were separated on a Nucleosil-C18 reversed-phase column (250 by 4.6 mm; particle size, 7 μm) by applying a step gradient from 5 mM potassium phosphate buffer, pH 6.5, to the start to 80% (vol/vol) methanol as the solvent. The flow rate was 1 ml/min. Compounds eluting from the column were detected by measuring the A254 and identified by cochromatography. For derivatization with PITC, 15 μl of sample was mixed with 15 μl of ethanol and subsequently with 140 μl of derivatization mixture (70% [vol/vol] ethanol, 20% [vol/vol] triethylamine, 10% [vol/vol] PITC). After 10 min at room temperature, the sample was hyophilized, resuspended in

Vol. 68, 2002 ISOPROPYLAMINE TO L-ALANINOL 2369
DNA sequencing and analysis. DNA was sequenced on both strands by using PCR methods with fluorescent deoxynucleotide terminators and an ABI Prism automatic sequencer (Perkin-Elmer). DNA sequences and derived amino acid sequences were analyzed using the Genetics Computer Group Wisconsin package, version 10. Similarity searches were performed using the gapped BLAST program (1) against public protein and gene databases.

Nucleotide sequence accession numbers. The sequence of the ipuABC [259] gene cluster of Pseudomonas sp. strain KIE171 has been deposited in the GenBank database under accession number AJ311161. The sequence of the ipuABC-DEFGH gene cluster carries accession number AJ311159 and that of the strain KIE171 16S rRNA gene has the accession number AJ11160.

RESULTS

Properties of Pseudomonas sp. strain KIE171. Pseudomonas sp. strain KIE171 was isolated from sludge of the Lonza AG wastewater treatment plant by enrichment on minimal medium containing 20 mM isopropylamine as the sole carbon source. Isopropylamine, L-alaninol, t-alaninol, D-alanine, t-lactate, proionic acid, aminoethanol, propane-1,2-diol, and L-glutamate supported growth, whereas d-alaninol, D,L-alaninol, and acetone did not. Based on its 16S ribosomal DNA (rDNA) sequence and its profile of fatty acids, strain KIE171 was classified within the γ-subdivision of the proteobacteria into rRNA similarity group 1 of Pseudomonas (18). The 16S rDNA sequence of strain KIE171 was 99% identical to that of Pseudomonas aeruginosa, whereas its physiological characteristics matched most closely those of Pseudomonas aeruginosa (95.8% 16S rDNA identity). A clear attribution of strain KIE171 to a particular species of RNA group 1 thus was not obtained.

Accumulation of L-alaninol by an isopropylamine-nonutilizing mutant. To establish that t-alaninol is an intermediate in the degradation of isopropylamine, a mutant defective in the utilization of these compounds was generated by mutagenesis with MNNG and isolated as described in Materials and Methods. This mutant, strain KIE171-B, exhibited no growth after 5 days of incubation in minimal medium containing either 20 mM isopropylamine or 20 mM t-alaninol. Whole cells of strain KIE171-B suspended in minimal medium with 20 mM isopropylamine formed 8 mM t-alaninol within 40 h. Based on HPLC analysis, the supernatant of the medium after 40 h of incubation did not contain d-alaninol. Incubation of the cell suspension for more than 40 h resulted in the disappearance of t-alaninol (not shown). This effect is believed to be due to the activity of a putative aldehyde dehydrogenase encoded by ipuH (see below).

Identification of genes involved in isopropylamine utilization. For the identification of the genes encoding the enzymes of the isopropylamine degradation pathway, we isolated mini-Tn5 transposon insertion mutants of Pseudomonas sp. strain KIE171 that are unable to grow with isopropylamine. Two Ip− (isopropylamine utilization-negative) mutants were obtained that displayed no growth with isopropylamine or t-alaninol as the sole carbon source but displayed normal growth with all other carbon sources tested. Of these mutants, strain KIE171-BI transformed isopropylamine to L-alaninol, whereas strain KIE171-BII failed to do so. The genes whose insertional inactivation caused loss of the ability to grow with isopropylamine were isolated by selection of the kanamycin resistance gene present on the mini-transposon (7). The DNA fragments carrying a transposon insertion were sequenced and found to represent two apparently unlinked loci. These were termed cluster A (mutant KIE171-BI) and cluster B (mutant KIE171-BII) and appear to constitute two transcriptional units comprising two and eight genes, respectively. A schematic representation of the 10 open reading frames (ORFs) identified in the DNA sequences of clusters A and B is shown in Fig. 1.

With the exception of that encoded by orf259, the polypeptides encoded in clusters A and B displayed significant sequence identity to proteins of known function (Table 1). Based on the function of their homologs, these proteins can be arranged in a hypothetical pathway for the degradation of isopropylamine that is presented in Fig. 2. In this pathway isopropylamine is thought to be transported into the cytoplasm by IpuG, which shows weak sequence similarity to amino acid permeases of the amino acid-polyamine-organocation (APC) superfamily (13). The degradation of isopropylamine is then initiated by IpuC. This protein exhibits about 30% sequence identity to glutamine synthetases. Preliminary characterization revealed that IpuC catalyzes the ATP-dependent conversion of isopropylamine and L-glutamate to γ-glutamyl-isopropylamide and that ammonium is not a substrate (see below). IpuC thus is a γ-glutamyl-isopropylamide synthetase.

The next step in the hypothetical pathway is catalyzed by a four-component monooxygenase system that is proposed to stereospecifically hydroxylate γ-glutamyl-isopropylamide to γ-glutamyl-L-alaninol. It is thought to be composed of IpuD, which in its sequence is 30% identical to P-450CAM of Pseudomonas putida (23), and of IpuA, IpuB, and IpuE. IpuA exhibits 28% sequence identity to various thioredoxin reductases. Sequence alignments revealed that the two active-site cysteines typical for thioredoxin reductases (29) are absent in IpuA. Since the two flavin adenine dinucleotide binding domains and the NAD(P) binding site are present, it seems to be a NAD(P)H-dependent ferredoxin reductase, but not a thioredoxin reductase. IpuB and IpuE show strong sequence identity (48 and 40%, respectively) to ferredoxins. A [4Fe-4S] and a [3Fe-4S]...
cluster are present in IpuB, whereas IpuE contains a single [3Fe-4S] cluster. This suggests that IpuB and IpuE function as electron transport components and, together with IpuA and IpuD, constitute a four-component cytochrome P-450-based monooxygenase system responsible for the stereospecific hydroxylation of γ-glutamyl-isopropylamide.

In a further step of the proposed pathway, γ-glutamyl-l-alaninol is hydrolyzed to l-alaninol and l-glutamate. This reaction was shown to be catalyzed by purified IpuF (see below). IpuF is related to the N-terminal part of guanosine 5’-phosphate synthetase. This enzyme catalyzes the final step in guanine ribonucleotide biosynthesis, and its N-terminal domain is responsible for the hydrolysis of glutamine, thus providing ammonium for the ATP-dependent formation of guanosine 5’-phosphate from xanthosine 5’-phosphate by the C-terminal synthetase domain (31).

None of the ORFs encoded in clusters A and B is similar to an enzyme reacting with an amino-alcohol such as l-alaninol. The reactions proposed to be involved in the degradation of this compound (Fig. 2) are, however, completely speculative. L-Alaninol thus are entirely speculative. L-Alaninol is possibly oxidized by an alcohol dehydrogenase to L-2-aminoadipate as an intermediate in the degradation of L-alaninol is catalyzed by a hypothetical protein with 30% sequence identity to NAD-dependent aldehyde dehydrogenase. Evidence for the formation of an aldehyde as an intermediate in the degradation of l-alaninol is provided by the growth properties of mutant KIE171-BI. Its inability to grow with isopropylamine and l-alaninol is due to insertional inactivation of ipuI in cluster A, a gene encoding a protein with 30% sequence identity to NAD-dependent aldehyde dehydrogenases. It appears unlikely that the phenotype of strain KIE171-BI is caused by a polar effect on the expression of orf259, which encodes a polypeptide similar to a hypothetical protein of unknown function from P. aeruginosa (Table 1). Another protein with sequence similarity to NAD-dependent aldehyde dehydrogenases is encoded by ipuH, the most-downstream gene in cluster B. Degradation of l-alaninol thus appears to involve two aldehyde dehydrogenases. This view is supported by our observation that, in order to obtain a strain stably accumulating l-alaninol from isopropylamine, it is necessary to inactivate both ipuH and ipuI (de Azevedo Wäsch et al., unpublished).

Evidence for the formation of γ-glutamyl-isopropylamide by IpuC. In the pathway suggested by analysis of the ipu genes (Fig. 2), IpuC is postulated to catalyze the ATP-dependent formation of γ-glutamyl-isopropylamide from isopropylamine and l-glutamate. To verify this reaction, IpuC was expressed in E. coli as an N-terminally histidine-tagged fusion protein (His6-IpuC) and purified in one step by metal chelate-affinity chromatography (Fig. 3). A crude E. coli extract containing His6-IpuC had a specific activity of 0.67 U/mg of protein, while a crude extract from E. coli expressing wild-type IpuC exhibited an activity of 0.75 U/mg of protein (Fig. 4). When purified His6-IpuC was incubated for 5 h in 1 ml of standard incubation mixture, 6.5 μmol of isopropylamine, 7.1 μmol of l-glutamate, and 4.7 μmol of ATP were consumed, and the products detected were 5.6 μmol of γ-glutamyl-isopropylamide, 4.9 μmol of ADP, and 5.6 μmol of inorganic phosphate. Except for inorganic phosphate, which was determined by a colorimetric assay, substrates and products were measured by HPLC analysis and identified by cochromatography with pure compounds (see Materials and Methods). 4-Aminobutyrate, glutarate, d-glutamate, l-aspartate, and l-2-amino adipate were not accepted as substrates by the enzyme. However, IpuC showed a broad substrate range with respect to primary amines. Amino-alkanes; amino-alcohols, including l-alaninol and d-alaninol; and amino-esters were substrates (Table 2). Ammonium and primary amines with a positive or a negative charge on the side chain were not.

IpuF is a γ-glutamyl-l-alaninol hydrolase. IpuF catalyzes the third step in the proposed pathway, the hydrolysis of γ-glutamyl-l-alaninol to l-alaninol and l-glutamate (Fig. 2). An N-terminally histidine-tagged fusion protein of IpuF (His6-IpuF) was expressed in E. coli and purified by metal chelate-affinity chromatography (not shown). Purified His6-IpuF catalyzed the hydrolysis of γ-glutamyl-l-alaninol, γ-glutamyl-isopropylamide, γ-glutamyl-ethylamidine, l-glutamine, and γ-glutamyl-p-nitroanilide but did not react with reduced glutathione or with γ-glutamyl-l-alanine. Since γ-glutamyl-isopropylamide was the best substrate, enzyme activity was routinely determined with this compound. Crude extract containing wild-type IpuF exhibited about the same specific activity for

<table>
<thead>
<tr>
<th>Gene or ORF</th>
<th>Length (amino acids)</th>
<th>Gene</th>
<th>Inferred function</th>
<th>Sequence comparison of of representative hit</th>
<th>% Identity</th>
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<tr>
<td>Cluster A</td>
<td></td>
<td></td>
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<tr>
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<td></td>
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<td>Aldehyde dehydrogenase</td>
<td>ALDH2 (Homo sapiens) (P05091)</td>
<td>43.9 (481)</td>
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a Accession numbers from PIR, SwissProt, and Trembl databases are shown in parentheses.
b The numbers of amino acids considered for comparison are given in parentheses.
FIG. 2. Postulated pathway for the degradation of isopropylamine by Pseudomonas sp. strain KIE171. Arrows with broken lines indicate hypothetical reactions, for which no experimental evidence is available. Abbreviations: GIPA, \( \gamma \)-glutamyl-isopropylamide; GALO, \( \gamma \)-glutamyl-L-alaninol.

2372
isopropylamine as crude extract containing His_{6}-IpuF, that is, 1.44 and 1.35 U/mg of protein, respectively. Purified His_{6}-IpuF was stable for several weeks upon storage at −20°C in buffer containing 20% (vol/vol) glycerol. However, during incubation at 30°C, the activity of the enzyme dropped with a half-life of 19 min. The enzyme showed a Michaelis-

\[ \text{Substrate Relative activity (%)} \]

<table>
<thead>
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<th>Substrate</th>
<th>Relative activity (%)</th>
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<td>Isobutylamine</td>
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<td>186</td>
</tr>
<tr>
<td>3-Amino-2-propanol</td>
<td>140</td>
</tr>
<tr>
<td>R-2-Amino-1-propanol</td>
<td>142</td>
</tr>
<tr>
<td>S-2-Amino-1-propanol</td>
<td>140</td>
</tr>
<tr>
<td>S-2-Amino-1-butanol</td>
<td>133</td>
</tr>
<tr>
<td>R-2-Amino-1-butanol</td>
<td>132</td>
</tr>
<tr>
<td>Glycine methyl ester</td>
<td>50</td>
</tr>
<tr>
<td>4-Aminobutyric acid methyl ester</td>
<td>42</td>
</tr>
<tr>
<td>2-Amino-1,3-propanediol</td>
<td>79</td>
</tr>
</tbody>
</table>

* A relative specific activity of 100% corresponds to 1.16 U/mg of protein.

DISCUSSION

Analysis of the genes responsible for the utilization of isopropylamine as a carbon source by Pseudomonas sp. strain KIE171 leads us to propose a pathway that converts the substrate in three enzyme-catalyzed steps to l-alaninol. This compound is then further degraded by as yet unknown reactions, of which at least one appears to be catalyzed by two aldehyde dehydrogenases, whose genes have been identified (Fig. 2). The pathway from isopropylamine to l-alaninol involves two N-glutamylated intermediates, and its occurrence was supported by the following lines of physiological and biochemical evidence. Resting cells of a mutant of strain KIE171, unable to grow with l-alaninol, quantitatively accumulated l-alaninol from isopropylamine. The purified enzymes γ-glutamyl-l-isopropylamide synthetase (IpuC) and γ-glutamyl-l-alaninol hydro-
anism of glutamine synthetases, are changed in (9). These residues, which are central to the enzymatic mechanism and accepts a proton from the adduct to form glutamine stabilize the tetrahedral glutamine adduct in the transition state. The sequencing ladder obtained with plasmid pME4771 as a template is shown. The major primer extension product is indicated by an arrow, and the position of the transcriptional start is marked by an asterisk.

The pathway from isopropylamine to L-alaninol consumes 1 mol of ATP per mol of substrate degraded. A more energy-efficient version of the pathway would involve transfer of the glutamyl residue of γ-glutamyl-L-alaninol onto isopropylamine, rather than its hydrolytic removal. Based on amino acid sequence analysis it is unlikely that IpuF is able to catalyze such a glutamyl-transfer reaction. However, it cannot be excluded that a host enzyme encoded outside of the ipu gene clusters acts as glutamyl-transferase and thereby participates in isopropylamine degradation. The IpuF enzyme is about half the size of guanosine 5′-phosphate synthetase of E. coli, and its sequence aligns with the N-terminal part, the amidotransferase domain (31), of the latter. The catalytically important residues are present in both enzymes, thus suggesting that the mechanism of hydrolysis of γ-glutamyl-L-alaninol is similar to that for the hydrolysis of glutamine of amidotransferase enzymes (20).

The ipuABCDEFGH gene cluster was found to be flanked by two putative IS elements (Fig. 1), features reminiscent of a catabolic transposon for isopropylamine degradation. ISL, the left-hand element, exhibited sequence similarity to IS 2 (8), but inverted repeats and the start codon of a putative transposase gene could not be identified. The sequence of ISR was similar to that of IS 3 (8). It consisted of a 1,334-bp DNA sequence with similarity to genes encoding transposition proteins. This sequence was interrupted by several frameshifts, and it contained flanking 30-bp imperfect inverted repeats. These features and the absence of target site duplications resulting from transposon insertion make it appear unlikely that the apparent ipu transposon is functional. However, the possibility that it becomes transposable by site-specific recombination with an element containing a functional transposase gene cannot be excluded (28). Support for the view that the ipu genes have been introduced into Pseudomonas sp. strain KIE171 by lateral transfer is provided by their GC content of 51%. This value differs significantly from the overall GC content of members of the genus Pseudomonas, which varies between 58 and 68% (19).

Sequencing of the genes involved in aniline degradation by P. putida (11) and Acinetobacter sp. strain YAA (26) has revealed ORFs that encode, in addition to a putative aniline oxidase, a protein with homology to glutamine synthetases (TdnQ and AtdA1, respectively) and one with homology to the amidotransferase domain of GMP synthetases (TdnQ and AtdA1, respectively). These proteins were proposed to transfer the amino group of the substrate to an unknown acceptor or to release ammonia (11). However, by analogy to the isopropylamine degradation pathway described in the present study, it seems more likely that they are involved in the N-glutamyl-
group by a glutamyl residue prior to hydroxylation of a neighboring carbon atom thus appears to be followed in at least one other degradative pathway for a primary amine.

ACKNOWLEDGMENTS

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REFERENCES