Morganella morganii Urease: Purification, Characterization, and Isolation of Gene Sequences

LI-TAI HU, ERIC B. NICHOLSON, BRADLEY D. JONES, MARTHA J. LYNCH, and HARRY L. T. MOBLEY*

Division of Infectious Diseases, Department of Medicine, University of Maryland School of Medicine, 10 South Pine Street, Baltimore, Maryland 21201

Received 3 November 1989/Accepted 7 March 1990

Morganella morganii, a very common cause of catheter-associated bacteriuria, was previously classified with the genus Proteus on the basis of urease production. M. morganii constitutes a urease distinct from that of other uropathogens. The enzyme, purified 175-fold by passage through DEAE-Sepharose, phenyl-Sepharose, Mono-Q, and Superose 6 chromatography resins, was found to have a native molecular size of 590 kilodaltons and was composed of three distinct subunits with apparent molecular sizes of 63, 15, and 6 kilodaltons, respectively. Amino-terminal analysis of the subunit polypeptides revealed a high degree of conservation of amino acid sequence between jack bean and Proteus mirabilis ureases. \( K_u \) for urea equaled 0.8 mM. Antiserum prepared against purified enzyme inhibited activity by 43\% at a 1:2 dilution after 1 h of incubation. All urease activity was immunoprecipitated from cytosol by a 1:16 dilution. Antiserum did not precipitate ureases of other species except for one Providencia rettgeri strain but did recognize the large subunits of ureases of Providencia and Proteus species on Western blots (immunoblots). Thirteen urease-positive cosmid clones of Morganella chromosomal DNA shared a 3.5-kilobase (kb) BamHI fragment. Urease gene sequences were localized to a 7.1-kb EcoRI-Sall fragment. Tn5 mutagenesis revealed that between 3.3 and 6.6 kb of DNA were necessary for enzyme activity. A Morganella urease DNA probe did not hybridize with gene sequences of other species tested. Morganella urease antiserum recognized identical subunit polypeptides on Western blots of cytosol from the wild-type strain and Escherichia coli bearing the recombinant clone which corresponded to those seen in denatured urease. Although the wild-type strain and recombinant clone produced equal amounts of urease protein, the clone produced less than 1\% of the enzyme activity of the wild-type strain.

Morganella morganii, a gram-negative enteric bacterium, is an increasingly prevalent cause of nosocomial and catheter-associated bacteriuria (25, 35). Most (97\%) isolates are urease positive (8) and are multiply antibiotic resistant (6, 10). Senior (30) found that 34\% of 220 routine fecal specimens contained M. morganii, suggesting that the source of infecting organisms is the gastrointestinal tract. In the same study, this species was also implicated as a cause of gastroenteritis.

In the human urinary tract, the ability of M. morganii to hydrolyze urea has been linked to formation of xanthine calculi (28) as well as the more typical struvite and carbonateapatite stones (32). Management of infection stones has typically included a urease inhibitor and broad-spectrum antibiotic (1, 5, 18).

Based on urease production, M. morganii was previously classified as Proteus morganii. However, DNA-DNA hybridization studies (4) revealed that this species represented a relatively homogeneous genus that was quite distinct, exhibiting only 13 to 26\% DNA relatedness to Proteus and Providencia species.

These differences are also reflected at the level of the urease itself. Antibodies directed against crude preparations of the Proteus mirabilis enzyme did not cross-react with crude preparations of the Morganella enzyme (11). As well, we and other investigators have found that the native Morganella enzyme has a much larger apparent molecular weight than that of other species as assayed on molecular sieve columns and non-denaturing polyacrylamide enzyme activity gels (13, 29, 31). In addition, urease gene probes from Providencia stuartii (24) and Proteus mirabilis (B. Jones and H. Mobley, unpublished observation) did not hybridize with Southern blots of Morganella chromosomal DNA, even under conditions of low stringency. The Morganella enzyme also appears to have a higher affinity for urea (i.e., lower \( K_u \)) than the Proteus and Providencia enzymes (13, 29) and is produced constitutively (29).

We report here that the M. morganii urease appears distinct from the enzymes produced by Proteus mirabilis and Providencia stuartii by virtue of apparent molecular weights of native enzyme and subunit polypeptides, affinity for substrate, regulation of enzyme synthesis, and DNA homology of genes encoding the enzyme. However, some strong similarities are revealed by comparison of N-terminal amino acid sequences of subunit polypeptides. As well, some antigenic determinants of the native and denatured enzyme are apparently conserved between Morganella, Proteus, and Providencia species.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Clinical isolates of M. morganii, Proteus mirabilis, Proteus vulgaris, Providencia stuartii, Providencia rettgeri, and Klebsiella pneumoniae were isolated at concentrations of \( \geq 10^6 \) CFU/ml from the urine of long-term-catheterized patients as previously described (25, 35). Helicobacter pylori strains were isolated as previously described (22).

Escherichia coli HB101 (F" hsdR hsdM supE44 proA2 leuB6 rpsL20 recA13 lacY1 galK2 thi-1 ara-14) was the recipient for transformation with recombinant plasmids (19).

Purification. M. morganii was grown in 10 liters of Luria broth for 18 h with aeration (200 rpm) at 37°C. Cells were

* Corresponding author.
harvested by centrifugation (10,000 × g, 10 min, 4°C), washed two times in 20 mM sodium phosphate (pH 6.8), and stored as a cell pellet at −20°C. Cells (30 g [wet weight]) were suspended in 30 ml of 20 mM potassium phosphate (pH 6.8)–1 mM EDTA–1 mM β-mercaptoethanol (PEB) and ruptured by passage through a precooled French pressure cell (Amino-Bowman) at 20,000 lb/in². Unbroken cells were removed by centrifugation (10,000 × g, 10 min, 4°C), and the supernatant was centrifuged (100,000 × g, 60 min, 4°C) to remove membrane. Soluble protein was used for column application. DEAE-Sepharose chromatography was performed on a conventional column (1.5 by 7.5 cm). Phenyl-Sepharose (1.5 by 12 cm), Mono-Q (HR 5/5), and Superose 6 (1.0 by 29.5 cm) purification steps were done on a fast protein liquid chromatography system (Pharmacia Fine Chemicals, Piscataway, N.J.) at room temperature. Fractions were screened for urease activity by the phenol red spectrophotometric assay (24). Specific activity of pooled fractions was determined by the indophenol reaction (37). Protein concentration was determined by the method of Lowry et al. (17) with bovine serum albumin as a standard.

Samples were electrophoresed on sodium dodecyl sulfate (SDS)–polyacrylamide gels by the method of Hames (12). Molecular weight standards are listed in the legend to Fig. 2.

N-terminal analysis. The N-terminal amino acid sequence was determined by a modification (R. Hall and J. Collins, unpublished data) of the procedure of Moos et al. (26). Purified urease (10 μg) denatured in SDS gel sample buffer (16) for 5 min at 100°C was electrophoresed on a linear 10 to 20% polyacrylamide gradient gel. Polypeptides were transferred to polyvinyldene difluoride membrane (Immobilon-P; Millipore Corp., Bedford, Mass.) and stained with 0.1% Coomassie brilliant blue (Bio-Rad Laboratories, Richmond, Calif.) in 50% methanol. Membranes were destained with 10% acetic acid–50% methanol. Stained protein bands were excised, and N-terminal amino acid sequences were determined with a pulsed liquid-phase sequenator (model 477A; Applied Biosystems).

Urease assays. Urease hydrolysis was quantitated by two methods. In a continuous assay, enzyme was added to a cuvette containing 2.8 ml of 3 mM sodium phosphate (pH 6.8)–120 mM urea–phenol red (7 μg/ml). Reactions were performed at 23°C, and optical density was monitored at 560 nm. Rate of change of optical density was correlated with ammonia production as previously described (24).

In an endpoint assay, ammonia production was measured by indophenol formation by using the phenol-hypochlorite reaction (phenyl nitroprusside and alkali hypochlorite) (37). Optical density was measured at 625 nm with ammonium chloride as a standard.

Cell fractionation. Cells of M. morganii TA43 were harvested by centrifugation from 8-h-old Luria broth cultures (1 liter) grown at 37°C with aeration (200 rpm). Supernatant culture medium (50 ml) was saved for later assay, and cell pellets (4 g) were washed once with 10 mM Tris hydrochloride (pH 8.0) and subjected to the cold osmotic shock procedure as previously described (24). Osmotic shock fluid representing the periplasmic proteins was placed on ice for later assay. Cells were suspended in 10 ml of 20 mM sodium phosphate (pH 6.8) and ruptured by one passage through a precooled French pressure cell at 20,000 lb/in². Whole unbroken cells were removed by centrifugation (8,000 × g, 4°C, 10 min). Supernatant was centrifuged at 100,000 × g at 4°C for 60 min. Supernatant from this centrifugation represented the cytosol fraction and was placed on ice for later assay. The membrane pellet was washed two times with 20 mM sodium phosphate (pH 6.8) and then resuspended in 5 ml of 20 mM sodium phosphate (pH 6.8) and used directly for assay.

Spectrophotometric assays were used to measure enzyme activities. Urease was measured by the phenol red spectrophotometric assay. Catalase was measured by using H₂O₂ as a substrate at 240 nm. Alkaline phosphatase was measured by using p-nitrophenylphosphate as a substrate at 420 nm. NADH dehydrogenase activity was measured at 340 nm with NADH as the substrate. Protein concentration was determined by the method of Lowry et al. (17) with bovine serum albumin as a standard.

Gene bank preparation. Chromosomal DNA was isolated from M. morganii TA43 by the method of Marmur (21) and used for the preparation of a gene bank in E. coli by standard methods (20). DNA was partially digested with Sau3A, ligated into the BamHII site of pHC79, and packaged in vitro into lambda bacteriophage particles which were used to transfect E. coli HB101. Cells were plated onto Luria agar containing ampicillin at 200 μg/ml. Ampicillin-resistant colonies were screened for urease activity by replicating onto modified urea segregation agar (9) which was prepared by combining component A (4 g of yeast extract, 4 g of peptone, 0.34 g of NaH₂PO₄, 1.03 g of Na₂HPO₄, 1 g of gelatin, 5 g of NaCl, 0.90 g of KH₂PO₄, 1.10 g of K₂HPO₄, and 15 g of agar in 900 ml of distilled H₂O, autoclave sterilized) with component B (9 g of d-glucose, 6 g of urea, and 0.035 g of phenol red in 100 ml of distilled H₂O, filter sterilized). After overnight incubation, urease-positive (red) colonies were picked and used for subsequent experiments.

DNA hybridization. Dot blots of whole-cell DNA from enteric species were prepared by spotting overnight culture lysates onto nitrocellulose filters as described by Maniatis et al. (20). A 3.5-kilobase (kb) BamHII DNA fragment from within the urease operon was isolated by elution of the fragment from a preparative 0.7% agarose gel and was labeled with [α-³²P]ATP by random primer extension and used for hybridization under stringent (50% formamide, 65°C wash) and nonstringent (20% formamide, 51°C wash) hybridization conditions (20). Blots were washed, dried, and autoradiographed.

Transposon mutagenesis. Tn5 mutagenesis was performed by the method of deBruijn and Lupski (7). Briefly, E. coli HB101(pMOM203) was infected with lambda::Tn5 and selected on Luria agar containing kanamycin (50 μg/ml). Kanamycin-resistant cells were pooled and incubated overnight in Luria broth. Plasmid DNA was isolated by the method of Birnboim and Doly (3), purified by centrifugation to equilibrium on CsCl gradients, and used to transform competent cells of E. coli HB101. Kanamycin-resistant colonies were screened for urease activity, and sites of transposition insertion were determined by double digestion of plasmid DNA with EcoRI and BamHII.

In vitro transcription-translation. Purified plasmid DNA (5 μg) isolated from cells containing Tn5 insertions of plasmid pMOM203 was used for in vitro transcription-translation in the presence of [³⁵S]methionine (>600 Ci/mmol; DuPont, NEN Research Products, Boston, Mass.). Reagents were obtained from Digene (College Park, Md.), and labeling was done according to the instructions of the manufacturer. Labeled polypeptides were solubilized in gel sample buffer (16) and electrophoresed on SDS-15% polyacrylamide gels.

Preparation of antisera. Two female New Zealand White-SF rabbits (Hazelton Dutchland, Inc.) were injected subcutaneously with column-purified M. morganii urease in Freund complete adjuvant at a concentration of 100 μg/ml.
TABLE 1. Purification of urease from M. morganii

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Sp act of urease (µmol/min per mg of protein)</th>
<th>Total activity (µmol/min)</th>
<th>Total protein (mg)</th>
<th>Enzyme recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell extract</td>
<td>12</td>
<td>15,000</td>
<td>1,230</td>
<td>100</td>
</tr>
<tr>
<td>DEAE-Sepharose</td>
<td>63</td>
<td>5</td>
<td>12,000</td>
<td>190</td>
</tr>
<tr>
<td>Phenyl-Sepharose</td>
<td>284</td>
<td>23</td>
<td>5,680</td>
<td>20</td>
</tr>
<tr>
<td>Mono-Q</td>
<td>714</td>
<td>58</td>
<td>4,140</td>
<td>5.8</td>
</tr>
<tr>
<td>Superose 6</td>
<td>2,130</td>
<td>175</td>
<td>2,980</td>
<td>1.4</td>
</tr>
</tbody>
</table>

RESULTS

Purification. Urease was purified from a French press cell lysate from 30 g (wet weight) of cells by using the four-step scheme outlined in Table 1. Crude lysate was applied to a DEAE-Sepharose column, and urease was eluted at 395 mM KCl within a 0 to 500 mM KCl gradient. Peak fractions were pooled and adjusted to 1.0 M KCl and loaded onto a phenyl-Sepharose column. Urease was not retained on the column and eluted at 70 ml, prior to the elution of the majority of cell protein and before the running of the 1.0 to 0.0 M KCl gradient, which released no additional urease. Peak fractions were pooled, dialyzed against PEB buffer, and loaded onto a Mono-Q anion-exchange column. Urease eluted in a single fraction at 330 mM KCl. Protein from the active fraction was concentrated from 2.5 to 0.5 ml by a centrifugal concentrator (Centrisart I; Sartorius, Hayward, Calif.) and loaded onto a Superose 6 molecular sieve column (Fig. 1); activity eluted at 12.1 ml, which corresponded to an apparent molecular size of 590 kilodaltons (kDa). Protein from the active fractions of each purification step was denatured and electrophoresed on SDS-polyacrylamide gels

FIG. 1. Superose 6 chromatography of M. morganii urease. Pooled fractions with peak urease activity from Mono-Q anion-exchange chromatography were dialyzed against PEB buffer and chromatographed on a Superose 6 column as described in the text. Fractions (0.5 ml) were assayed for urease activity by the phenol red spectrophotometric assay. Active fractions with elution volumes between 11.5 and 13.0 ml were pooled.

FIG. 2. SDS-polyacrylamide gel electrophoresis of purified M. morganii urease. Protein samples from fractions with peak urease activity from each purification step were denatured in SDS gel sample buffer and electrophoresed on either an 8 to 15% (lanes A to H) or a 15% (lanes I and J) SDS–polyacrylamide gel. Lanes A, H, and J, High-range molecular-size markers; rabbit muscle phosphorylase b, 97.4 kDa; bovine serum albumin, 66.2 kDa; hen egg white ovalbumin, 42.7 kDa; bovine carbonic anhydrase; 31.0 kDa; soybean trypsin inhibitor, 21.5 kDa; and hen egg white lysozyme, 14.4 kDa; lane B, whole-cell lysate; lane C, DEAE-Sepharose eluate; lane D, phenyl-Sepharose eluate; lane E, Mono-Q eluate; lanes F and I, Superose 6 eluate. Lane G, Low-molecular-size markers: myoglobin, 17.0 kDa; myoglobin fragments I and II, 14.4 kDa; myoglobin fragment I, 8.2 kDa; myoglobin fragment II, 6.2 kDa.

I. 1990

(four injections of 0.25 ml each). Blood was tested at 2 weeks, and a second set of injections was administered at 3 weeks. Blood was taken again at 4 to 5 weeks. Rabbits were terminally bled at 44 days.

Western blot (immunoblot). Crude soluble protein fractions of E. coli HB101(pMOM203) and M. morganii TA43 were electrophoresed on SDS-20% polyacrylamide gel by the method of Laemmli (16) and blotted onto nitrocellulose. Western blots were developed as described by Towbin et al. (34) with the modifications of Batteiger et al. (2). Rabbit antiserum was diluted in 0.5% Tween 20 in 20 mM sodium phosphate (pH 7.4)–120 mM NaCl (phosphate-buffered saline) and incubated with nitrocellulose filters for 2 h at 37°C. Filters were washed three times for 10 min each in diluent and agitated in 1:1,000 goat anti-rabbit immunoglobulin G conjugated to alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) overnight at 4°C. Filters were thoroughly washed again and developed with Nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate.

Neutralization and immunoprecipitation. Twofold dilutions of serum were made in PEB buffer. Cytosol (100 µl containing 1 mg of protein; obtained as described above) was mixed with an equal volume of serum dilution incubated for 1 h at 4°C. A portion (75 µl) was assayed for urease activity directly. The remaining portion was centrifuged in a microcentrifuge (15,600 × g, 3 min, 4°C), and the supernatant (75 µl) was assayed for urease by the phenol red spectrophotometric assay in a reaction volume of 2.70 ml of 3 mM sodium phosphate (pH 6.8)–120 mM urea containing phenol red at 7 µg/ml.

FIG. 2. A critical step in the purification appeared to be phenyl-Sepharose (Fig. 2, lane D), eliminating greater than 80% of the contaminating protein. Some inactivation of enzyme occurred during the purification procedure.

The pure enzyme (Fig. 2, lanes F and I) was electrophoresed on both gradient (8 to 15%; left panel) and high-percentage acrylamide (15%; right panel) gels and was found to be composed of three polypeptide subunits with apparent
molecular weights of 63,000, 15,000, and 6,000 when compared with migration of standards (Fig. 2, lanes A, G, H, and J).

**Cellular localization.** Cells of *M. morganii* were fractionated into periplasmic, membrane, and cytoplasmic fractions. Unlike the ureases of *Providencia stuartii* and *Proteus mirabilis* which are clearly cytoplasmic, *Morganella* urease partitioned in both the membrane (2.5 μmol of urea hydrolyzed per min per mg of protein; 59% of total activity) and cytosol (1.76 μmol of urea per min per mg of protein; 41% of total activity). Fractionation of control enzymes indicated that membrane and cytosol were well separated; 93% of NADH dehydrogenase activity partitioned with the membrane, and 100% of catalase activity was found in the cytosol.

**N-terminal analysis.** The results of N-terminal analysis are shown in Fig. 3 with the relevant amino acid sequences of *Proteus mirabilis* (15) and jack bean (19) ureases. Comparison of *M. morganii* urease subunits with those of jack bean or *Proteus mirabilis* revealed a high percentage of sequence similarity, defined as the percentage of exactly matching and conserved amino acid residues between the compared segments. The first 15 amino acids of the 63-kDa subunit showed 80% similarity to amino acids 2 to 16 of the 61-kDa *Proteus* subunit and 53% similarity to an internal 15-amino-acid segment (amino acids 272 to 286) of jack bean urease. Likewise, the first 10 amino acids of the 6-kDa subunit showed a high degree of conservation with the first 10 amino acids of the jack bean urease (80%) and the 11-kDa subunit of *Proteus* urease (70%). The N-terminal 15 amino acids of the 15-kDa subunit shared no amino acid sequence similarity with either the *Proteus* or jack bean urease.

**Neutralization and precipitation.** Antiserum prepared against *M. morganii* urease was able to neutralize approximately 60% of urease activity when incubated with cell cytosol from *M. morganii* TA43 for 1 h. Preimmunization serum had no effect. Immune serum did not significantly inhibit the urease activities in cytosol of other *Proteus*, *Providencia*, or *Klebsiella* species.

*M. morganii* urease activity was completely precipitated by centrifugation at a titer of 1:32; that is, no activity remained in the supernatant (Fig. 4). Activity was reduced 57% at a titer of 1:64. Jack bean, *Proteus*, and *Klebsiella* ureases were apparently not precipitable by the antiserum. However, *Providencia rettgeri* showed a 40% reduction in activity at a titer of 1:8 and was the only urease other than *Morganella* urease to show a significant reduction in activity upon precipitation with antiserum.

Antiserum specifically recognized the three urease subunits on Western blots of whole-cell soluble protein from *M. morganii* electrophoresed under denaturing conditions (Fig. 5). The large subunit of the ureases from representative *Proteus* and *Providencia* species was also recognized if cells were grown in urea-containing medium, that is, if urease was induced. When culture medium did not contain urea, only the *Morganella* urease, which is constitutively produced, was recognized by the antiserum. When uninduced, the *Proteus* and *Providencia* species did not produce sufficient enzyme to give a strong signal on the Western blot. Even when induced, however, the two minor subunits of these latter species went unrecognized by specific antiserum.

**Molecular cloning of urease genes.** Of approximately 1,000 gene bank clones of *M. morganii* chromosomal DNA, 13 expressed urease activity in *E. coli* HB101 when assayed on urea segregation agar. All plasmids (approximately 45 kb in size) shared a common 3.5-kb BamHI fragment later localized within urease gene sequences. One gene bank clone, pMOM101, was selected, and genes were subcloned by HindIII deletion to produce the 20-kb pMOM202. To localize the urease genes, we mutated the plasmid with Tn5. All urease-negative insertions mapped to a 7.1-kb EcoRI-SalI fragment which contained the 3.5-kb BamHI fragment. Urease genes were further localized by digesting pMOM202 with EcoRI and SalI, religating, transforming, and screening for the smallest plasmid capable of urease activity by electrophoretic sizing. Plasmid pMOM203 (Fig. 6) contained the 7.1-kb fragment and the remaining 5.8-kb EcoRI-SalI fragment of vector pHC79. Although the clone produced urease,
the activity was extremely weak. To further localize the genes, Tn5 mutagenesis was repeated on pMOM203. Insertions resulting in inactivation were separated by 3.3 kb (Tn5-7 and Tn5-31); the maximum size of fragment necessary for activity spanned 6.6 kb (from Tn5-34 to the EcoRI sites at the vector-insert junction).

In vitro transcription-translation of pMOM202 and pMOM203 revealed the synthesis of polypeptides corresponding to 63,000, 15,000, and 6,000 molecular weight (data not shown). In addition, cell cytosol was isolated, denatured in gel sample buffer, and electrophoresed on an SDS-polyacrylamide gel, and proteins were transferred to nitrocellulose. Antisera raised against purified M. morganii urease recognized subunits with an apparent molecular weight of 63,000, 15,000, and 6,000 in cytosol from both M. morganii TA43 wild-type strain and recombinant clone E. coli HB101(pMOM203) (Fig. 7). Despite the weak urease activity of the recombinant clone, approximately equal amounts of subunit polypeptides were produced by recombinant and wild-type strains.

To determine whether DNA homology existed between Morganella urease genes and urease genes of other species, we used the 3.5-kb BamHI fragment as a DNA probe. Dot blots of whole-cell DNA from urease-positive K. pneumoniae (10 strains) and H. pylori (1 strain) were not recognized by the probe under high or low stringency. We previously determined that Morganella chromosomal DNA did not hybridize with urease gene probes from Providencia stuartii (24) or Proteus mirabilis (B. Jones and H. Mobley, unpublished observation).

**DISCUSSION**

The urease of M. morganii, a very large protein with an estimated native molecular weight of 590,000, is composed of three subunit polypeptides with apparent molecular weights of 63,000, 15,000, and 6,000. The stoichiometry of the subunit is unknown. This subunit structure reflects a general pattern seen in other genera of the family Enterobacteriaceae, including Proteus mirabilis (14), Providencia stuartii (27), and K. pneumoniae (33), which also have three unique subunits: one large subunit 61 to 72 kDa in size and two smaller subunits 8 to 12 kDa in size. This contrasts with single-subunit-type ureases reported for several other bacteria (reviewed in reference 23) as well as that produced by the jack bean. We found that the M. morganii urease shares some features with other ureases, but by other criteria, this protein is quite distinct from ureases that have been described previously.

Two of the three enzyme subunits shared amino acid similarity with subunits of the Proteus mirabilis urease as well as with portions of the jack bean sequence (Fig. 3). The first 15 amino acids of the large subunit showed 80% similarity to a 15-amino-acid sequence that begins at the second amino acid of the largest subunit of the Proteus enzyme. The first 10 amino acids of the 6-kDa subunit showed 70%
3078 HU ET AL.

FIG. 5. Western blot of soluble protein from Proteus, Providencia, and Morganella species with anti-Morganella urease antiserum. Soluble protein (50 μg) derived from bacterial cell lysates was electrophoresed on SDS-20% polyacrylamide gels and transferred to nitrocellulose by electroblocting. Blots were incubated with a 1:100 dilution of rabbit antiserum derived from animals challenged with purified enzyme, washed, incubated with goat anti-rabbit immunoglobulin G conjugated to alkaline phosphatase, washed, and developed by the addition of Nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. Top panel, Soluble protein from cells induced with 0.5% urea; bottom panel, cells uninduced. Lanes: A and B, M. morganii; B and J, Proteus mirabilis BU517; C and K, Proteus mirabilis BR2528; D and L, Proteus mirabilis H14320; E and M, Proteus mirabilis BU7354; F and N, Providencia stuartii BE2467; G and O, Proteus vulgaris GO1232; H and P, Providencia rettgeri TA1738; I and Q, Providencia rettgeri S15453. Prestained molecular size markers (Bio-Rad) are bovine serum albumin (75 kDa), ovalbumin (50 kDa), carbonic anhydrase (39 kDa), soybean trypsin inhibitor (27 kDa), and lysozyme (17 kDa).

FIG. 6. Molecular cloning of M. morganii urease gene sequences. pMOM203 was constructed by digesting pMOM202 with EcoRI and Sall and religating (see text). pMOM203 contains the 7.1-kb fragment of M. morganii chromosomal DNA able to encode an active urease and the 5.8-kb EcoRI-Sall fragment of vector pHC79. Origin of replication (ori) and the β-lactamase gene (bla) are indicated by arrows. Symbols: *, locations of Tn5 insertion mutations; −, inactivated urease; +, urease activity retained.

subunits of the ureases from Providencia and Proteus species. The antisera were unable to precipitate ureases from other species, with the single exception of partial precipitation of a Providencia rettgeri urease. That Providencia rettgeri is also reported to synthesize a high-molecular-weight enzyme (13) may account for the precipitation by the Morganella antiserum. Neutralization studies showed that antiserum directed against Morganella urease would not inactivate heterologous enzymes from all strains tested, indicating that despite other similarities seen on Western blot, the ureases differ enough in composition not to be affected by heterologous antisera.

We described the cloning of a fragment encoding the urease gene from the chromosomal DNA of M. morganii and the expression of the enzyme activity in E. coli. Between 3.3 and 6.6 kb of DNA was necessary for enzyme activity as shown by subcloning and transposon mutagenesis. The operon constitutively produces an enzyme with a native molecular weight of 590,000, making the native Morganella urease the largest bacterial urease purified to date and equalled in size only by that of jack bean (18). Most native microbial ureases range in size from 200 to 250 kDa (13, 23).

Genes encoding the subunit polypeptides appear to be well expressed in E. coli as indicated by the amount of protein recognized on Western blot by specific antisera. However, because of the very low activity of the recombinant enzyme, we must conclude that either the subunits have not been assembled into the native enzyme or the native enzyme has been inactivated in the E. coli host. To a lesser degree this inactivation was noted during the purification procedure, in which some activity was unaccountable between latter purification steps.

The Morganella urease is distinct from all other well-studied bacterial ureases. Unlike Proteus or Providencia urease, it is expressed constitutively. Antiserum directed
against the Morganella urease neither recognized the smaller subunits of the Proteus or Providencia ureases nor was able to precipitate or neutralize these enzymes. Furthermore, Proteus or Providencia urease gene probes did not hybridize with Morganella chromosomal DNA (13). Evidence that the enzymes are different is also supported by the observation that the first 15 amino acid residues of the 15-kDa subunit showed no similarity with either Proteus or jack bean urease, although the other subunits revealed significant amounts of conservation. Finally, the large size of the purified native enzyme sets Morganella urease apart from other bacterial ureases purified to date.

The urease synthesized by M. morganii displays some similarities in subunit structure, antigenic determinants of the large subunit, and amino acid sequence with ureases from other genera of the Enterobacteriaceae group. However, it is indeed a distinct enzyme with subunit sizes varying from those of other species, a higher native molecular weight, antigenic differences, and a divergent amino acid sequence of the intermediate-size (15-kDa) subunit. In addition, Morganella urease gene sequences do not hybridize with those of nearest relative Proteus species. These differences further support the division of Morganella and Proteus genera.

ACKNOWLEDGMENTS

We thank Gwynn Chippendale, Cecille Andraos Selim, and Mau- reen Fox for skilled technical assistance and Linda Horne for expert manuscript preparation. This research was supported in part by Public Health Service grant AI23328 from National Institutes of Health.

LITERATURE CITED


