Genetic and Biochemical Diversity of Ureases of *Proteus*, *Providencia*, and *Morganella* Species Isolated from Urinary Tract Infection

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Bacterial urease, particularly from *Proteus mirabilis*, has been implicated as a contributing factor in the formation of urinary and kidney stones, obstruction of urinary catheters, and pyelonephritis. Weekly urine specimens ($n = 1,135$) from 32 patients, residing at two chronic-care facilities, with urinary catheters in place for $\geq$30 days yielded 5,088 phenotypically and serotypically diverse bacterial isolates at $\geq$10^5 CFU/ml. A total of 86% of specimens contained at least one urease-positive species, and 46% of 3,939 gram-negative bacilli were urease positive. For investigation of genetic relatedness of urease determinants, whole-cell DNA from 50 urease-positive isolates each of *Providencia stuartii*, *Providencia rettgeri*, *P. mirabilis*, *Proteus vulgaris*, and *Morganella morganii* were hybridized with a urease gene probe derived from within the urease operon of *Providencia stuartii* BE2467. The percentage of strains hybridizing with the gene probe was 98 for *Providencia stuartii*, 100 for *Providencia rettgeri*, 70 for *P. mirabilis*, 2 for *M. morganii*, and 0 for *P. vulgaris*. Electrophoretic mobilities of ureases from representative isolates revealed nine different patterns among the five species. The urease gene probe hybridized with fragments of *Hind*III-digested chromosomal DNA from all isolates except *M. morganii*. Fragment sizes differed between species. Molecular sizes of the enzymes, determined by Sephacryl S-300 chromatography, were found to be 280 kilodaltons (kDa) (*P. mirabilis*), 323 to 337 kDa (*Providencia stuartii*, *Providencia rettgeri*, *P. mirabilis*, *P. vulgaris*), 620 kDa (*Providencia rettgeri*), and >700 kDa (*M. morganii*, *Providencia rettgeri*). K_ms ranged from 0.7 mM urea for *M. morganii* to 60 mM urea for a *P. mirabilis* isolate. In general, *P. mirabilis* ureases demonstrated lower affinities for substrate but hydrolyzed urea at rates 6- to 25-fold faster than did enzymes from other species, which may explain the frequent association of this species with stone formation.

Bacterial urease, an enzyme that catalyzes the hydrolysis of urea, yielding ammonia and carbon dioxide, has been implicated as a factor contributing to pyelonephritis (2, 10, 12, 20), hepatic coma (23), hyperammonemia (21), inactivation of complement (1), and urolithiasis (5, 6, 18; H. L. T. Mobley and J. W. Warren, manuscript submitted for publication). The aged, chronically catheterized patient has been found to be almost universally bacteriuric, usually with more than one species (16, 24, 25). In a recent study by our group, 86% of weekly urine specimens from 32 such patients contained urease-producing organisms (Mobley and Warren, submitted; H. L. T. Mobley, H. L. Muncie, Jr., and J. W. Warren, Abstr. IVth Int. Symp. Pyelonephritis, p. 42, 1986). Overall, 46% of the gram-negative bacilli produced urease. In the long-term catheterized patient, bacterial urease represents a potential virulence factor that is produced by nearly half of the gram-negative bacteria present at $\geq$10^5 CFU/ml of urine and may therefore represent a common pathogenic factor.

Although urease production is a common phenotype among isolates from urinary tract infection, certain species, such as *Proteus mirabilis*, are more often associated with struvite and carbonate-apatite stone formation (6) and pyelonephritis (20) than are other urease-positive isolates representing *Providencia* and *Morganella* species. These differences in pathogenicity may be caused by additional virulence factors expressed by *P. mirabilis* or simply caused by different biochemical characteristics (e.g., affinity for substrate and rate of hydrolysis) of the ureases produced by this species.

Only a few reports have appeared comparing the ureases of *Proteus*, *Providencia*, and *Morganella* species. Senior et al. (22) demonstrated that representatives of the genera of the *Proteeae* tribe produce ureases of different electrophoretic mobilities, but no molecular weights were calculated. Rosenstein et al. (19) also found that the molecular weights of single representatives of the *Proteeae* tribe differ and that *Proteus morganii* (now *Morganella morganii*) is more resistant to two urease inhibitors. The high molecular weights (all greater than 560,000) reported here, however, were not consistent with data from experiments described by Senior et al., who observed significant migration of enzymes through 6% polyacrylamide gels. Finally, Guo and Liu (7) found that *M. morganii* urease is serologically distinct from that of *P. mirabilis*, *Proteus vulgaris*, and *Providencia rettgeri*.

Because the enzymes from different bacterial species seem to possess different properties and because the few existing studies appear to provide conflicting information, we undertook a systematic comparison of the ureases of urinary tract isolates with respect to molecular weight, kinetics of urea hydrolysis, electrophoretic mobilities, isoelectric points, and genetic relatedness of these enzymes. This report describes diversity among ureases from species of *Proteus*, *Providencia*, and *Morganella* and attempts to explain why the ureases of *P. mirabilis* may be important virulence factors.

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MATERIALS AND METHODS

Bacteria. Urine specimens were collected aseptically from 32 elderly patients with urinary catheters in place for 30 days. Bacteria at concentrations of $\geq 10^5$ CFU/ml of urine were identified to the species level by using the Minitek Enterobacteriaceae II System (BBL Microbiology Systems, Cockeysville, Md.) as previously described (16, 25). Isolates were stored at $-70°C$ in Trypticase soy broth (BBL) supplemented with 15% (vol/vol) glycerol.

Urease gene probe. A 2.8-kilobase DNA sequence that fell between the sites of two transposon insertions (pMID401 and pMID403) which inactivated the cloned *Providencia stuartii* urease gene was used as a gene probe (17). Plasmid pMID204 (17) was digested with *Eco*RV and *Hin*dIII, and two adjacent fragments (1.3 and 1.5 kilobases) were electroeluted from an excised gel slice from a preparative 1% agarose gel and extracted with phenol, chloroform, and ether. DNA (0.25 μg) was labeled with [^{32}P]ATP (New England Nuclear Corp., Boston, Mass.; 800 Ci/mmol) by nick translation as described by Maniatis et al. (13).

Dot blot hybridization. Bacteria were grown overnight in 5 ml of nutrient broth (Difco Laboratories, Detroit, Mich.) at 37°C with aeration (200 rpm). Culture was mixed 1:1 with 0.6 M NaCl, 0.2 M NaOH, 0.08% sodium dodecyl sulfate. Cell lysate (15 μl) was spotted onto a gridded nitrocellulose filter (Schleicher & Schuell, Inc., Keene, N.H.) as described by Maniatis et al. (13). Filters were baked under vacuum for 2 h at 80°C and hybridized with the ^{32}P-labeled urease gene probe under stringent conditions (50% formamide, 65°C wash) as described by Maniatis et al. (13). Dried blots were placed between acetate sheets and autoradiographed for 18 h at $-70°C$ with an intensifying screen in place. *Providencia stuartii* BE2467, the source of the gene probe, was used as the positive control for each filter.

Chromosomal DNA extraction. Chromosomal DNA was isolated from bacterial cells lysed with sodium dodecyl sulfate and extracted with phenol, chloroform, and ether by the method of Marmur (14).

Urease preparations. Bacteria were grown at 37°C with aeration in 100 ml of Luria broth (15) supplemented with 0.1% (wt/vol) urea after being autoclaved. Cells were harvested by centrifugation at 4°C, washed two times with 20 mM sodium phosphate, pH 6.8, suspended in 5 ml of 20 mM sodium phosphate, pH 6.8–5 mM dithiothreitol–1 mM EDTA, and ruptured in a precooled French pressure cell at 20,000 lb/in^2. The lysate was centrifuged at 27,000 × g for 30 min, and the supernatant was removed with a Pasteur pipette and either used directly or stored in 1-ml portions at $-70°C$. Protein was determined by the method of Lowry et al. (11) by using bovine serum albumin as a standard.

Column chromatography. A urease preparation (1 ml; 10 to 15 mg of protein per ml) was loaded onto a Sephacryl S-300 column (100 by 2.5 cm) equilibrated with 20 mM sodium phosphate–0.02% sodium azide, pH 6.8. Fractions (3 ml) were collected at a flow rate of 30 ml/h. The column was calibrated with standard proteins of known molecular weight (Pharmacia Biotechnology Products, Piscataway, N.J.). Fractions with peak activity were determined by adding samples (100 μl) of each fraction to wells of microtiter dishes containing 100 μl of 0.04% cresol red–100 mM urea. Determinations were made in triplicate.

Nondenaturing polyacrylamide gel electrophoresis. Urease preparations (20 μl) were mixed with 20 μl of 50% sucrose–0.1% bromphenol blue and loaded onto a 5.5% polyacrylamide gel (16 by 16 by 0.15 cm) (1:32 bisacryl-

amide-acrylamide; U.S. Biochemical Co., Cleveland, Ohio) with a 4% stacking gel and electrophoresed for 4 h at 250 V). Gels were equilibrated with 0.02% cresol red–0.1% EDTA (22) and then immersed in 1.5% (wt/vol) urea. The points of migration of ureases were recorded on Kodachrome 64 film (Eastman Kodak Co., Rochester, N.Y.).

Spectrophotometric urease assay. Rates of urea hydrolysis were measured by the spectrophotometric assay of Hamilton-Miller and Gargan (8) and as described previously (17). The assay was calibrated by comparing changes in optical density at 560 nm with ammonia liberation as measured by an ammonia electrode (Corning Glass Works, Corning, N.Y.) connected to a pH meter (model 071; Beckman Instruments, Inc., Fullerton, Calif.), which was calibrated with 10^{-6} to 10^{-1} molar ammonium chloride as described by the manufacturer.

Isoelectrofocusing in polyacrylamide gels. Gels were prepared as described for the LKB Ultromould system (LKB Instruments, Inc., Rockville, Md.). Urease preparations (30 μl) were loaded approximately 2 cm from the cathode onto a 5% polyacrylamide gel (100 by 245 by 0.1 mm) (1:32 bisacrylamide-acrylamide; U.S. Biochemical Co.) with 7.7% ampholytes (pH range, 3.5 to 9.5; LKB). Gels were electrophoresed at a constant power setting of 3 W for 6 h and cooled with circulating ice water. The pH gradient was measured by using a surface electrode (Beckman), and the gel was equilibrated in 0.02% cresol red–0.1% EDTA. The point of migration of ureases (isoelectric point) was determined by immersion of the gel in 1.5% urea as described above for nondenaturing polyacrylamide gels.

RESULTS

To study genetic and biochemical diversity of ureases, two variables were examined for a large number of strains to select unique enzymes for more detailed analyses. Strains were examined for the ability of whole-cell DNA to hybridize to a urease gene probe derived from a *Providencia stuartii* strain. On the basis of the degree of hybridization, a series of isolates was used to determine the relative electrophoretic mobilities of the ureases on nondenaturing polyacrylamide activity gels. Isolates producing ureases with unique electrophoretic mobilities were then selected for characterization.

Hybridization with a *Providencia stuartii* gene probe. Whole-cell DNA from the species listed in Table 1 were used to prepare dot blots. Filters were hybridized with a ^{32}P-labeled *Providencia stuartii* urease gene probe under stringent conditions. The gene probe recognized homologous sequences with all but one urease-positive strain of *Providencia stuartii* (Fig. 1). Hybridizations were as strong as with *Providencia stuartii* BE2467, the strain from which the original recombinant clone was derived. The probe appeared to be specific, showing absolutely no hybridization with urease-negative *Providencia stuartii* strains (Fig. 1; Table 1) or plasmid vector pBR322. Excellent hybridization (100% positive) also was seen with strains of the closely related species *Providencia rettgeri*. In addition, 70% of *P. mirabilis* isolates showed positive hybridization but generally gave a much weaker signal, probably indicating significant homology but to a lesser degree than for isolates of *Providencia stuartii* or *Providencia rettgeri*. Little or no hybridization was seen with isolates of *P. vulgaris*, *M. morganii*, or *Klebsiella pneumoniae* by the dot blot procedure.

Electrophoretic mobilities of ureases. Ten isolates from each of the species representing each hybridization class

TABLE 1. Dot blot hybridization of bacterial isolates from urinary tract infection with a ^{32}P-labeled *Providencia stuartii* urease gene probe

Bacterial species[a]	No. of isolates tested	No. of positive hybridizations[b]		Negative hybridi-zation[e]	% Positive
		Strong[c]	Weak[d]		
Providencia stuartii					
Urease positive	47	44	2	1	98
Urease negative	11	0	0	11	0
Providencia rettgeri	48	21	27	0	100
Proteus mirabilis	50	4	31	15	70
Proteus vulgaris	15	0	0	15	0
Morganella morganii	51	0	1	50	2
Klebsiella pneumoniae	51	0	3	48	6
Escherichia coli	5	0	2	3	40
Citrobacter diversus	4	1	1	2	50
Pseudomonas aeruginosa	4	0	0	4	0
Klebsiella oxytoca	3	0	0	3	0
Pseudomonas sp./ alcaligenes group	1	0	0	1	0
Acinetobacter anitratus	1	0	0	1	0
Other gram-negative spp.	8	3	4	1	88

[a] All urease positive except where indicated.
[b] 50% formamide, 65°C wash.
[c] X-ray emulsion as dark as control DNA (*Providencia stuartii* BE2467).
[d] X-ray emulsion exposed, but not as dark as control DNA.
[e] X-ray emulsion not exposed.

(strong, weak, or negative) within a species were examined for urease electrophoretic mobilities on nondenaturing activity gels. Mobilities of the enzymes were compared with that of the urease of *Providencia stuartii* BE2467. Nine isolates among the five species revealed unique urease patterns and were selected for further study (Fig. 2). In all cases, a rapidly
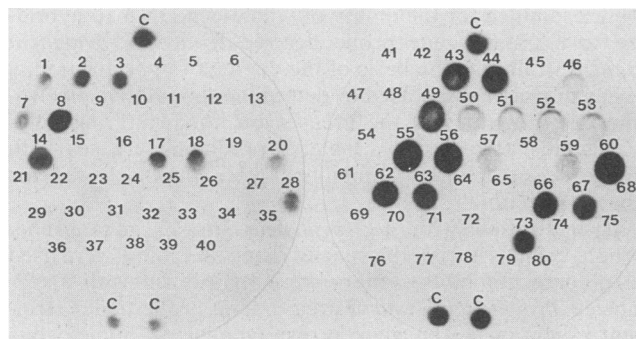

FIG. 1. Dot blot hybridization of isolates from urinary tract infection with a ^{32}P-labeled urease gene probe derived from *Providencia stuartii* BE2467. Cells from overnight cultures were lysed with alkaline sodium dodecyl sulfate and spotted on nitrocellulose filters. Filters were hybridized under stringent conditions (13) with a ^{32}P-labeled gene probe derived from *Providencia stuartii* BE2467, washed, dried, and autoradiographed. Samples are directly below the identifying number. *Providencia stuartii* BE2467, the source of the gene probe, was used as a positive control (C). Dots: Urease-negative *Providencia stuartii*: 21, 22, 35, 37, 38; urease-positive isolates of *Providencia stuartii*: 1, 2, 7, 8, 17, 18, 20, 28, 43, 44, 49, 55, 56, 60, 66, 73; *Providencia rettgeri*: 23, 24, 46, 50–53, 57, 59, 62, 63, 67; *P. mirabilis*: 3, 4, 13, 14, 19, 25, 36, 40, 47, 48, 68, 74, 76, 80; *M. morganii*: 9–12, 32, 41, 42, 45, 54, 61, 65, 71, 78, 79; *P. vulgaris*: 30, 31, 58, 69, 75; *K. pneumoniae*: 5, 6, 15, 16, 34, 39, 64, 70, 72, 77; *Pseudomonas aeruginosa*: 26; *Escherichia coli*: 27; and *Citrobacter diversus*: 29, 33. Weak but significant hybridization with *P. mirabilis* isolates on the original X-ray film often did not reproduce well in the figure.

developing band appeared on the gels after incubation with urea followed by the appearance of more slowly developing bands.

Phenotypes of isolates expressing unique ureases. To aid in interpretation of homology data, biochemical phenotypes were determined for the isolates with unique urease patterns (Table 2). All isolates conformed to current taxonomic definitions (3, 9). The four *P. mirabilis* phenotypes differed by phenotypic single reactions; one isolate utilized citrate, one was sucrose positive, and one did not exhibit swarming motility. The two *Providencia rettgeri* isolates differed in rhamnose fermentation.

Kinetics of urea hydrolysis. One index of diversity of enzymes is the relative affinity (K_m) for the substrate urea. K_ms for the isolates with unique ureases were determined along with rates of hydrolysis at saturating urea concentrations (Table 3). The enzymes of *Providencia stuartii* and *Providencia rettgeri* strains that gave positive hybridization had similar K_m values, ranging from 9 to 12 mM urea, and were not significantly different. All four *P. mirabilis* strains produced enzymes with lower substrate affinities (two- to sixfold higher K_ms) and significantly higher rates of hydrolysis than any other species ($P < 10^{-5}$). *M. morganii* TA43 produced an enzyme with significantly higher affinity for urea than that of enzymes produced by other isolates.

Molecular sizes of ureases. Estimation of molecular sizes by Sephacryl S-300 chromatography revealed four general groups of enzyme size: approximately 280 kilodaltons (kDa),
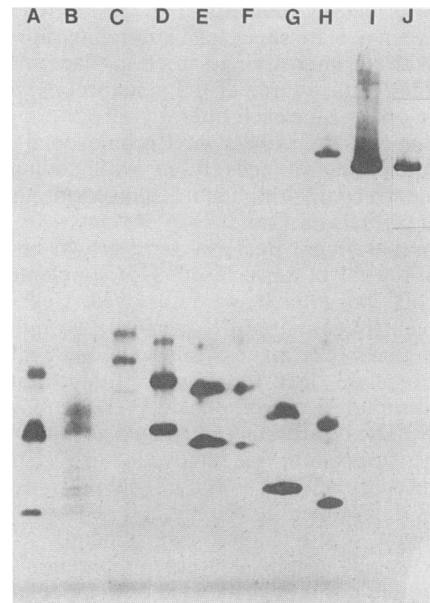

FIG. 2. Urease activity in nondenaturing polyacrylamide gels of cell lysates from *Proteus*, *Providencia*, and *Morganella* species. Cells grown in Luria broth supplemented with 0.1% urea were harvested, washed, and ruptured in a French pressure cell at 20,000 lb/in[2]. Insoluble material was removed by centrifugation. Soluble protein was loaded onto a 5.5% polyacrylamide gel and electrophoresed for 4 h at 4°C. The gel was equilibrated with 0.02% cresol red–0.1% EDTA and then transferred to a 1.5% urea solution. Bands denote an intense color development at the points of migration of ureases. Lanes: A, *Providencia stuartii* BE2467; B, *P. mirabilis* BR2528; C, *P. mirabilis* HI4320; D, *P. mirabilis* BU517; E, *P. mirabilis* BU7354; F, *P. vulgaris* GO1232; G, *Providencia rettgeri* TA1738; H, *Providencia rettgeri* SI5453; I, *M. morganii* TA43; J, (inset) immediate color development of *M. morganii* TA43.

TABLE 2. Biochemical phenotypes of isolates of *Providencia*, *Proteus*, and *Morganella* species which produce ureases of unique electrophoretic mobility

Organism	Phenotype[a]												
	NR	H₂S	IND	VP	CIT	UR	OR	AD	I	RA	R	SU	SWARM
Providencia stuartii BE2467	+	−	+	−	+	+	−	−	−	+	−	+	−
Providencia rettgeri TA1738	+	−	+	−	+	+	−	+	+	−	−	−	−
Providencia rettgeri SI5453	+	−	+	−	+	+	−	+	+	−	+	−	−
Proteus mirabilis HI4320	+	+	−	+	+	+	+	−	−	−	−	−	+
Proteus mirabilis BR2528	+	+	−	+	−	+	+	−	−	−	−	+	+
Proteus mirabilis BU517	+	+	−	+	−	+	+	−	−	−	−	−	+
Proteus mirabilis BU7354	+	+	−	+	−	+	+	−	−	−	−	−	−
Proteus vulgaris GO1232	+	+	+	−	−	+	−	−	−	−	−	+	−
Morganella morganii TA43	−	+	+	−	−	+	+	−	−	−	−	−	−

[a] NR, Nitrate reductase; H₂S, hydrogen sulfide; IND, indole; VP, Voges-Proskauer; CIT, citrate; UR, urease; OR, ornithine decarboxylase; AD, adonital; I, inositol; RA, raffinose; R, rhamnose; SU, sucrose; SWARM, swarming motility on agar. All isolates were positive for phenylalanine deamination and glucose. All isolates were negative for *o*-nitrophenyl galactosidase, lysine decarboxylase, arginine decarboxylase, malonate, arabinose, sorbitol, and lactose.

represented by two strains of *P. mirabilis*; 323 to 339 kDa, represented by *P. vulgaris*, two strains of *P. mirabilis*, a strain of *Providencia rettgeri*, and *Providencia stuartii*; 850 kDa, represented by an isolate of *Providencia rettgeri*; and 712 kDa from the *M. morganii* isolate. *Providencia rettgeri* SI5453 expressed two distinct enzymes with molecular sizes estimated to be 315 and 850 kDa.

Isoelectric points of ureases. Each of the nine ureases with unique electrophoretic mobility was characterized according to its isoelectric point. The enzymes were electrophoresed in polyacrylamide gels containing ampholytes which formed a pH gradient from 3.5 to 9.5. The urease from each strain revealed a distinct and unique isoelectric point (Table 3). In all cases, a predominant band developed in the gel when the gel was soaked in urea, and the band was followed by the appearance of more slowly developing secondary bands. With the exception of the high-molecular-weight urease of *Providencia rettgeri* SI5453, which had a pI of 6.8, all species produced enzymes with pI's ranging from 5.1 to 5.9.

Hybridization with chromosomal DNA digests. Chromosomal DNA isolated from each of the nine strains was digested with *Hind*III, electrophoresed, and transferred to nitrocellulose. The DNA was then hybridized with the ³²P-labeled *Providencia stuartii* urease gene probe under stringent conditions. Hybridization was very strong with the positive control *Providencia stuartii* BE2467 as well as with

P. mirabilis BR2528, *Providencia rettgeri* TA1738, and *Providencia rettgeri* SI5453. Weaker hybridization was also seen with *P. mirabilis* HI4320 and *P. mirabilis* BU517. It was surprising that very weak hybridization was also observed for *P. mirabilis* BU7354 and *P. vulgaris* GO1232, because they had not demonstrated homology to the probe by dot blot hybridization. No hybridization was seen with *M. morganii* DNA, even at reduced stringency. The urease gene probe hybridized strongly with *Hind*III chromosomal DNA restriction fragments of *P. mirabilis* BR2528 and *Providencia rettgeri* SI5453 with the same electrophoretic mobility of the fragment derived from *Providencia stuartii* BE2467. All four *P. mirabilis* strains, including strain BR2528, possessed a smaller *Hind*III fragment of equal size that shared some homology with the gene probe. In addition, *Providencia rettgeri* TA1738 and *P. vulgaris* GO1232 possessed fragments different from those of all other isolates that hybridized with the gene probe.

Hybridization with plasmid DNA. Plasmid DNA was isolated from each of the nine strains, electrophoresed, transferred to nitrocellulose, and hybridized with the ³²P-labeled *Providencia stuartii* urease gene probe under stringent conditions. Although six of nine isolates carried plasmids of various mobilities, the probe hybridized only with the high-molecular-weight plasmid of *Providencia stuartii* BE2467 (16, 17) from which the probe was derived.

TABLE 3. Characteristics of ureases of unique electrophoretic mobility from representative isolates of *Providencia*, *Proteus*, and *Morganella* species

Organism	Strain	Hybridization with urease gene probe[a]	K_m (mM urea)[b]	Maximum hydrolysis by induced lysate (μmol of NH₃/min per mg of protein)[b]	Molecular size (kDa)[c]	Isoelectric point (pH)[d]
Providencia stuartii	BE2467	+ (Control)	12[e]	4	337 ± 18	5.4
Providencia rettgeri	TA1738	+W	11	6	335 ± 13	5.2
	SI5453	+	ND	9	315 ± 11, 850 ± 18	5.1, 6.8
Proteus mirabilis	HI4320	+W	39	51	281 ± 8	5.9
	BR2528	+	22	40	338 ± 15	5.3
	BU517	+W	24	35	282 ± 12	5.4
	BU7354	−	60	55	322 ± 11	5.2
Proteus vulgaris	GO1232	−	10	3	323 ± 12	5.7
Morganella morganii	TA43	−	0.7	0.4	712 ± 32	5.4
Jack bean		ND	10		574	5.1

[a] +, Strong hybridization; +W, weak hybridization; −, no hybridization by dot blot hybridization (Table 1); ND, not determined.
[b] Values represent means of three determinations.
[c] Values represent means ± standard deviations for three determinations.
[d] Values represent averages of two determinations.
[e] Two distinct ureases were produced by this isolate.

DISCUSSION

Proteus, *Providencia*, and *Morganella* species are distinguished from other genera of the *Enterobacteriaceae* by the production of urease. This positive phenotype characterized by the hydrolysis of urea is indeed represented by a highly diverse group of proteins which differ within and between species with respect to size, charge, affinity for substrate, and DNA homology of the genes which encode these enzymes.

In previous studies comparing ureases of *Proteus*, *Providencia*, and *Morganella* species, certain aspects of diversity were noted. In a serological study, cross-precipitation of ureases by antisera directed against urease preparations of *P. vulgaris*, (four strains), *P. mirabilis*, (three strains), *M. morganii* (three strains), and *Providencia rettgeri* (three strains) was reported. Guo and Liu (7) observed that the ureases of *P. vulgaris*, *P. mirabilis*, and *Providencia rettgeri* were immunologically cross-reactive but were distinct from the enzyme produced by *M. morganii*. That the *Morganella* urease is distinct from that of *Proteus* and *Providencia* species is also supported by our data. No significant hybridization of the *Providencia* gene probe was observed with 51 *Morganella* isolates. In addition, the electrophoretic mobility and molecular size of 712 kDa, which makes it one of the largest enzymes observed, were distinct from those of the other enzymes studied, and this urease demonstrated the highest affinity (lowest K_m) for substrate. Although we saw genetic cross-reactivity of *P. mirabilis*, *Providencia rettgeri*, and *Providencia stuartii*, the 15 isolates of *P. vulgaris* did not hybridize on dot blots, indicating a significant lack of homology with the gene probe. However, a *Hind*III restriction fragment of *P. vulgaris* chromosomal DNA did hybridize weakly with the gene probe, whereas no such hybridization was seen for *M. morganii* even at reduced stringency.

In a study of electrophoretic mobilities of ureases for *Proteus*, *Providencia*, and *Morganella* species, Senior et al. (22) previously noted differences in rates of migration through nondenaturing polyacrylamide gels. However, no molecular weights were reported. As we report, the greatest variability in mobilities within species occur with *P. mirabilis* and *Providencia rettgeri* as ureases showed similar patterns within species for *Providencia stuartii*, *P. vulgaris*, and *M. morganii*.

Rosenstein et al. (19) examined single isolates of *P. mirabilis*, *P. vulgaris*, *Providencia rettgeri*, and *M. morganii* for a number of biochemical parameters. Molecular sizes (from a Sephadex G-200 column) were reported to be from 560 to 800 kDa and, with the exception of *M. morganii*, are not in agreement with values reported here. For K_ms, although our values are somewhat different from those reported by Rosenstein et al. (19), the general trend that *M. morganii* exhibits a higher affinity for substrate than do other species is in agreement with our data.

Indeed, the most significant observation in this report may relate to the relative affinities of the ureases for substrate and the maximum rates of urea hydrolysis by lysates of induced strains. The habitat of these isolates is urine, which has been reported to contain 400 to 500 mM urea (6). At this concentration, undoubtably, ureases from any species would be saturated with substrate and would be working at the V_{max}. Species producing high-affinity ureases, such as *M. morganii* TA43, would have no selective advantage over other strains listed in Table 2 with lower affinity enzymes because of the plentiful substrate in urine. All four *P. mirabilis* strains had ureases with significantly higher K_m values (and thus lower
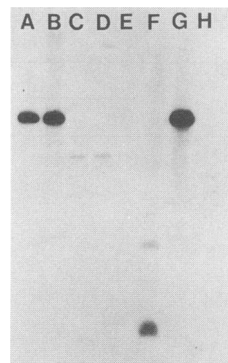

FIG. 3. Southern blot hybridization of chromosomal digests with the urease gene probe. Chromosomal DNA was isolated from the strains found to produce ureases with unique biochemical characteristics (Table 3). DNA was digested with *Hind*III, electrophoresed on a 0.7% agarose gel, transferred to nitrocellulose, and hybridized under stringent conditions (50% formamide, 65°C wash) with the ^{32}P-labeled urease gene probe derived from *Providencia stuartii* BE2467. Blots were washed, dried, and autoradiographed. Lanes: A, *Providencia stuartii* BE2467; B, *P. mirabilis* BR2528; C, *P. mirabilis* HI4320; D, *P. mirabilis* BU517; E, *P. mirabilis* BU7354; F, *Providencia rettgeri* TA1738; G, *Providencia rettgeri* SI5453; H, *P. vulgaris* GO1232; I, *M. morganii* TA43. The high-molecular-weight fragment in lane D was caused by incomplete digestion of DNA.

affinity) than the enzymes from all other species ($P < 0.023$) but, importantly, demonstrated rates of urea hydrolysis that were six- to thirty-fold higher than for any other isolate. This observation alone may explain why *P. mirabilis* is so often linked with urinary and kidney stone formation (6), obstruction of urinary catheters (Mobley and Warren, submitted), and pyelonephritis (2, 12, 20), whereas such reports for other *Proteus*, *Providencia*, and *Morganella* species are seen much less often.

Genetic relatedness of urease genes as determined by hybridization of whole-cell DNA and chromosomal DNA restriction fragments with a *Providencia stuartii* urease gene probe demonstrated that the genes encoding the enzyme of *Providencia rettgeri* are very homologous. Strong and weak hybridization was seen for 44 and 56% of the *Providencia rettgeri* isolates, respectively. One isolate that initially did not hybridize produced a high-molecular-weight, high affinity ($K_m = 2$ mM urea) enzyme and was later reidentified as *M. morganii*. Isolates of *P. mirabilis* also showed significant hybridization with the gene probe, but strong hybridization occurred with only 8% of isolates; 62% hybridized weakly. Three of four strongly hybridizing strains produced a urease of molecular weight not significantly different from that of *Providencia stuartii* BE2467. The other strongly hybridizing isolate produced a smaller enzyme of 280 kDa. Surprisingly, none of the 15 isolates of *P. vulgaris* tested hybridized on dot blots of whole-cell DNA, indicating that, although the molecular size of the enzyme may be similar to that of both *P. mirabilis* and *Providencia stuartii* isolates, the genes are not as closely related. In addition, *M. morganii* urease was also genetically unrelated and was different from all other ureases characterized in every category tested.

Certain observations can be explained on the basis of hybridizations with restriction enzyme-digested chromosomal DNA. *P. mirabilis* BR2528 (Fig. 3B) and *Providencia rettgeri* SI5453 (Fig. 3G) demonstrated strongly hybridizing *Hind*III fragments of the same size as the parent, *Providencia stuartii* BE2467. Both of these strains show evidence for two distinct ureases (stepladder formation in Fig. 2B and

high and low patterns in Fig. 2H). Molecular weights of one of the *P. mirabilis* BR2528 ureases may overlap with a *Providencia stuartii* enzyme-like enzyme produced by that strain, and the smaller *Providencia rettgeri* enzyme has a molecular weight similar to that of *Providencia stuartii*. We can postulate that these secondary ureases may have been acquired at some point by genetic exchange with a *Providencia stuartii* plasmid-encoded urease (4, 16). This theory is supported by the fact that identical copies of the *Providencia stuartii* urease gene appear to reside both on a conjugative plasmid and on chromosomal sequences in *Providencia stuartii* BE2467.

The data presented here summarize a systematic analysis of urease diversity in *Proteus*, *Providencia*, and *Morganella* isolates cultured from the urine of patients with urinary catheters in place for ≥30 days. The characterization of these enzymes gives clues to why the ureases of *P. mirabilis* may be more important virulence factors in urinary tract infection than the ureases of other common urease-producing species. Furthermore, the diversity observed among these three closely related genera suggests that the substrate specificity for urea may be one of the few similarities for an otherwise dissimilar group of proteins.

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