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Genetic Analysis of *Escherichia coli* Urease Genes: Evidence for Two Distinct Loci

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Studies with two uropathogenic urease-producing *Escherichia coli* strains, 1021 and 1440, indicated that the urease genes of each are distinct. Recombinant plasmids encoding urease activity from *E. coli* 1021 and 1440 differed in their restriction endonuclease cleavage sites and showed minimal DNA hybridization under stringent conditions. The polypeptides encoded by the DNA fragments containing the 1021 and 1440 urease loci differed in electrophoretic mobility under reducing conditions. Regulation of urease gene expression differed in the two ureolytic *E. coli*. The *E. coli* 1021 locus is probably chromosomally encoded and has DNA homology to *Klebsiella*, *Citrobacter*, *Enterobacter*, and *Serratia* species and to about one-half of the urease-producing *E. coli* tested. The *E. coli* 1440 locus is plasmid encoded; plasmids with DNA homology to the 1440 locus probe were found in urease-producing *Salmonella* spp., *Providencia stuartii*, and two *E. coli* isolates. In addition, the 1440 urease probe was homologous to *Proteus mirabilis* DNA.

Urease (urea amidohydrolase, EC 3.5.1.5) is produced by many species of bacteria, fungi, and plants, in which it is involved in the utilization of urea as a source of nitrogen (10). Urease catalyzes the hydrolysis of urea to ammonia and carbonic acid. Due to the number and diversity of the species that produce the enzyme, urease is involved in nitrogen assimilation in the soil, rumen, and other environments (10). Ureolytic microorganisms also play a role in specific clinical situations, for example, in human urinary tract infections. Urease increases the ammonium ion concentration in the urine, resulting in a rise in urine pH. Consequently, struvite, a magnesium ammonium phosphate salt, can be formed and deposited in the urinary tract, forming calculi (1, 9, 20, 22).

The best-studied urease is that of the jack bean plant. Jack bean urease is a relatively large protein (M_r 590,000) composed of six identical 96-kDa subunits and contains two nickel atoms bound per holoenzyme (5, 6). Microbial ureases are not as well studied at the biochemical level but are also known to be large proteins (sizes range from 200 to 600 kDa) and require nickel for function (10). In contrast to the plant enzyme, the bacterial ureases have heteromeric subunit compositions, with each subunit consisting of a polypeptide in the 65- to 73-kDa size range, two in the 11-kDa size range, and two in the 8-kDa size range. This heteropentameric subunit composition is found in urease produced by different bacterial genera including *Proteus* (12, 25), *Providencia* (18, 19), *Klebsiella* (23), *Sporosarcina* (23), and *Selenomonas* (23).

Recently, bacterial ureases have begun to be analyzed at the genetic level. The molecular cloning of the urease loci of *Proteus mirabilis* and *Providencia stuartii* has been reported (12, 18, 25). The genetic organization of these two loci is similar: they encode polypeptides of approximately the same size, and the apparent genes for these polypeptides are arranged in an equivalent order.

We are investigating the urease determinant of *Escherichia coli* and the role of this enzyme in urinary tract infections. Ureolytic *E. coli* are uncommon clinical isolates but are found at various extraintestinal sites of infection, predominantly in the urinary tract (24). The urease phenotype is unstable in a large percentage of these isolates (24). We reported the molecular cloning of a locus encoding urease from uropathogenic *E. coli* 1021 (3). The *E. coli* 1021 urease genes appear to be chromosomally encoded, and the instability of the urease phenotype in this isolate is associated with a DNA rearrangement.

Here we report the molecular cloning of the urease genes from a second uropathogenic isolate, *E. coli* 1440. Our analysis shows that the *E. coli* 1440 locus is dissimilar from the *E. coli* 1021 locus. The two loci exhibit weak DNA homology, have distinct restriction endonuclease patterns, encode different polypeptides, and differ in their regulation of gene expression. The *E. coli* 1440 urease genes are plasmid encoded, and the loss of the urease-positive phenotype in this isolate correlated with the loss of this plasmid. Ureolytic *E. coli*, therefore, can contain one of at least two distinct urease loci.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used were obtained from either the State of California Department of Health, Berkeley, or the Centers for Disease Control, Atlanta, Ga. *E. coli* 1021 and 1440 were isolated from urine samples from patients with urinary tract infections. Either urease-negative *E. coli* HB101 (F$^-$ *hsdR hsdM recA13 lacY1 leuB6 ara-14 galK2 proA2 thi-1* Sm^r) or *E. coli* DH5α (F$^-$ *endA1 hsdR17* (r_K^- m_K^-) *supE44 thi-1* λ$^-$ *recA1 gyrA96 relA1* φ80 d*lacZ*ΔM15) was the host, as indicated, for all recombinant plasmids used in this study.

Media. Urease-positive and urease-negative isolates were distinguished by using Christensen urea slants or urea-glucose-eosin Y-methylene blue (UGEM) agar as described previously (8). Bacteria were grown in L broth or on L broth solidified with 1.5% agar.

Preparation of plasmid DNA. Plasmid DNA was isolated by one of two procedures. The recombinant plasmids pURE

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and pURE14 were isolated by alkaline lysis (15). Plasmids were isolated from the clinical strains by Triton X-100 lysis by using the following protocol. An overnight broth culture (40 ml) was washed in TES (30 mM Tris hydrochloride [pH 8.0], 5 mM EDTA, 50 mM NaCl) and suspended in 0.8 ml of 25% sucrose (wt/vol) in 10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA for 5 min at 4°C. A 1% lysozyme solution was added (0.1 ml), and after a 5-min incubation at room temperature, 0.2 ml of 0.5 M EDTA (pH 8.0) was added for a second 5-min room temperature incubation. Triton X-100 (1.0 ml of 0.1% solution in 10 mM Tris hydrochloride–5 mM EDTA, pH 8.0) was then added. After 20 min at room temperature, the preparation was centrifuged for 20 min at 17,000 rpm (SS-34 rotor; Sorvall Instruments). The plasmid DNA was isolated from the resultant supernatant by centrifugation to equilibrium in cesium chloride-ethidium bromide density gradients (15).

DNA cloning and analysis. Restriction endonucleases were purchased from either New England BioLabs, Inc., Beverly, Mass., or Bethesda Research Laboratories, Inc., Gaithersburg, Md. Enzymatic reaction conditions were those recommended by the manufacturer. DNA fragments were inserted into alkaline phosphatase-treated vectors (Pharmacia, Milwaukee, Wis.).

Restriction endonuclease site-mapping experiments were performed by horizontal slab gel electrophoresis containing 0.5 to 1% agarose with Tris-acetate buffer. Bacteriophage lambda DNA digested with *Hin*dIII was used as a molecular weight standard. Urease-associated plasmids were analyzed by agarose gel electrophoresis with undigested cesium-purified plasmids as size standards.

For the deletion analysis of pURE, the 9.4-kb *Hin*dIII fragment was inserted into the *Hin*dIII site of pSP72 (Promega Biotec) and unidirectional deletions were generated as described by Henikoff (11).

Mutagenesis with Tn*5*. Insertion mutations in pURE14.8 were obtained by transposition of the kanamycin resistance transposon Tn*5* from λ *b*221 *rex*::Tn*5* *c*I857 *O*am23 *P*am80 onto the plasmid as described previously (4). Resultant plasmids conferring ampicillin and kanamycin resistance were scored for the urease phenotype, and the physical location of the Tn*5* insertion was mapped by restriction analysis. Not more than one mutation from each pool was analyzed, to ensure that each insertion was the result of an independent transposition event.

Urease assay. The urease assay measures the production of ammonia from urea and is based on the chromogenic reaction between ammonia and phenol in the presence of hypochlorite. *E. coli* strains were grown to the exponential phase with aeration in the presence or absence of urea in Christensen urea broth (0.1% peptone, 0.5% NaCl, 0.1% glucose, 0.2% monopotassium phosphate containing 2% urea when indicated). Cell cultures were then halved. One half of the cell culture was washed three times in 50 mM potassium phosphate (pH 7.5) and assayed for total protein by a modified Lowry assay (catalog no. 690-A; Sigma Chemical Co.). The other half of the culture was washed three times in Christensen urea broth without urea. To initiate the reaction, 15 μl of 40% urea, made immediately before use, was added to 185 μl of washed cells, and urea hydrolysis was allowed to proceed at 37°C. The reaction was terminated by the addition of 0.5 ml of phenol-nitroprusside followed by the addition of 0.5 ml alkaline hypochlorite. The optical density at 635 nm of each sample was measured and compared with a standard curve generated from appropriate dilutions of a freshly prepared NH_4Cl solution. Samples were measured in duplicate.

Hybridization. Whole-cell DNA digested with various restriction enzymes or purified plasmid DNA was fractionated by agarose gel electrophoresis (0.5 to 1%), transferred to nitrocellulose sheets (0.45-μm pore size; Schleicher & Schuell, Inc., Keene, N.H.) by the Southern technique (21), and hybridized under stringent conditions (67°C) as described previously (15). After hybridization, the nitrocellulose sheets were washed in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) with 0.1% sodium dodecyl sulfate at 67°C. Probes were labeled with [^{32}P]dCTP (Dupont, NEN Research Products, Boston, Mass.) by nick translation (16). Hybridization was revealed by autoradiography with XAR-OMAT films (Eastman Kodak Co., Rochester, N.Y.) in the presence of an intensifying screen.

After autoradiography, blots were stripped of radioactive probe by being washed in 2.5 mM NaOH for 6 h at room temperature when indicated. Blots were then rinsed in 2× SSC and hybridized as described above.

The DNA fragments used as the probes were isolated from low-melting-point agarose gels after digestion of the recombinant plasmids with the appropriate restriction endonucleases. Fragments isolated by this technique can be contaminated at a low level with vector sequences. Therefore, all blots were washed in 0.25 M NaOH (6 h, 25°C) to remove the initial DNA probe and then reprobed with the appropriate ^{32}P-labeled vector to control for vector-specific hybridization bands.

In vitro transcription-translation analysis. Polypeptides encoded by the recombinant plasmids were determined by in vitro transcription-translation (Amersham Corp., Arlington Heights, Ill.) as described by the manufacturer. The resultant [^{35}S]methionine-labeled polypeptides were separated by electrophoresis on a 7.5 to 20% gradient sodium dodecyl sulfate-polyacrylamide gel under reducing conditions (30% acrylamide–0.8% bisacrylamide). The gel was then fixed, stained with Coomassie brilliant blue, and dried, and the radiolabeled polypeptides were visualized by autoradiography.

RESULTS

***E. coli* 1021 urease locus.** The urease locus from *E. coli* 1021 was previously isolated, and the recombinant plasmid was termed pURE (3). pURE contains a 9.4-kb DNA fragment inserted into the *Hin*dIII site of pBR322. This 9.4-kb DNA fragment codes for at least two polypeptides that are essential for urease activity (M_r 67,000 and 27,000) (3).

The boundaries of the 1021 urease locus were defined by deletion analysis. Unidirectional deletions extending from either end of the 9.4-kb DNA fragment were constructed by the method of Henikoff (11). The 1021 urease locus encompassed a 4.6-kb DNA fragment extending from approximately 100 bp to the left of the *Xho*I site to approximately 1 kb to the right of the *Pvu*I site (Fig. 1). Insertion mutations located immediately to the right of the *Xho*I site eliminate urease activity (3), indicating that Δ4-2 defines the left end of the urease locus.

Whole-cell DNA from six other urease-producing clinical *E. coli* isolates was screened for homology to the 1021 urease genes. The DNA fragment of the 1021 urease clone encoding most of the 67-kDa urease subunit was used as the probe (1021 probe, see Fig. 4). Three of the six isolates (*E. coli* 5406, 914, and 187) screened appeared to contain a urease locus similar to that found in *E. coli* 1021 (Fig. 2A). The 1021

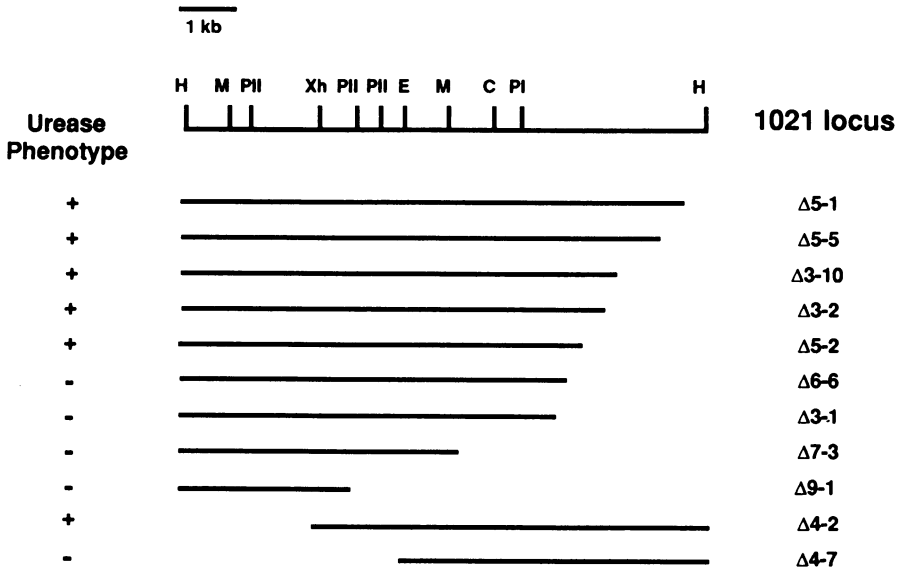

FIG. 1. Physical map and deletion analysis of the *E. coli* 1021 urease locus. Restriction map of the 9.4-kb DNA fragment insert of pURE, a recombinant plasmid encoding the urease genes of *E. coli* 1021, and deletion analysis of this fragment. Deletion mutations were generated as described in Materials and Methods. Δ, Deletion derivatives; +, deletions having no effect on urease activity; −, deletions eliminating urease activity. C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; M, *Mlu*I; PI, *Pvu*I; PII, *Pvu*II; Xh, *Xho*I.

probe hybridized to a 9.4-kb *Hin*dIII fragment in all three cases (Fig. 2A). A faint hybridization signal was detected between the 1021 urease probe and a 7.0-kb *Hin*dIII DNA fragment from the other three isolates (*E. coli* 1440 [Fig. 2A] and *E. coli* 4448-74 and 1138-77 [data not shown]). This suggested that these three isolates contain a urease locus dissimilar from the 1021 locus.

Cloning the *E. coli* 1440 urease locus. To determine whether there are distinct urease-encoding loci associated with ureolytic *E. coli*, the DNA encoding the *E. coli* 1440 urease phenotype was isolated and compared with the DNA fragment coding for the 1021 urease genes. Total cell DNA from *E. coli* 1440 was partially digested with *Sau*3AI and ligated to *Bam*HI-digested phosphatase-treated pBR322. *E. coli* DH5α was transformed with the resultant recombinant plasmids, and transformants were screened for urease activity on UGEM agar plates (8). A urease-encoding recombinant plasmid (pURE14) containing a 35-kb DNA insert was isolated from one urease-positive transformant. The urease determinant was subsequently localized to an 8-kb *Spe*I fragment. This *Spe*I fragment was inserted into the *Xba*I site of pUC19, and the resultant plasmid was named pURE14.8

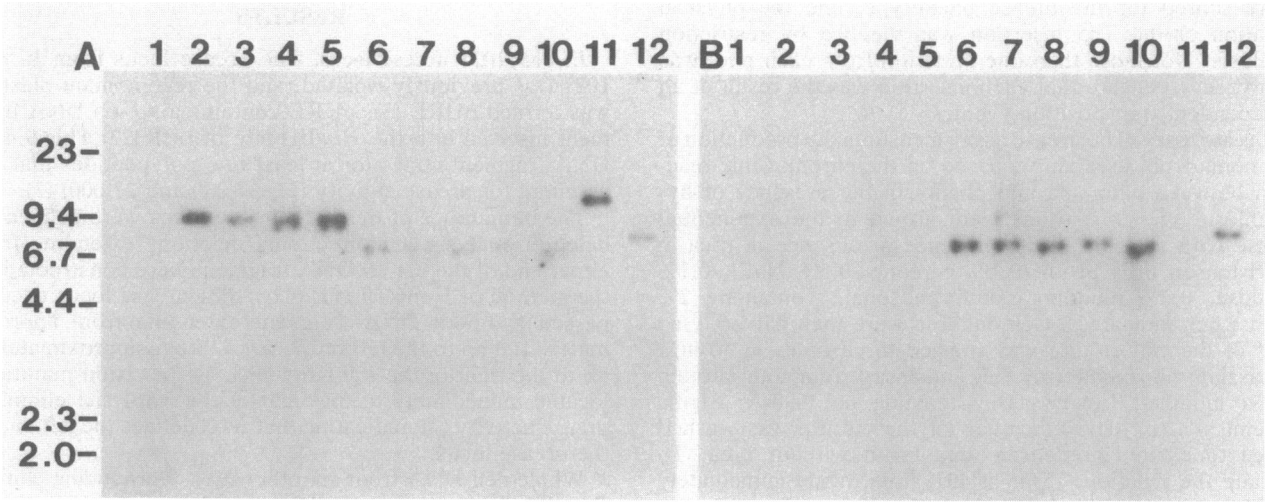

FIG. 2. Southern hybridization analysis of genomic DNA from various urease-producing members of the family *Enterobacteriaceae*. DNA samples were digested with *Hin*dIII and hybridized under stringent conditions to the *E. coli* 1021 urease gene probe (A). After autoradiography, the blot was stripped of bound probe and hybridized to the *E. coli* 1440 urease gene probe (B). DNA probes used are shown in Fig. 4. Equal amounts of digested genomic DNA were added to each lane (approximately 3 μg). Lanes: 1, *E. coli* DH5α; 2, *E. coli* 1021; 3, *E. coli* 5406; 4, *E. coli* 914; 5, *E. coli* 187; 6, *E. coli* 1440; 7, *Providencia stuartii* JH1; 8, *Providencia stuartii* JH3; 9, *S. cubana*; 10, *S. ohio*; 11, pURE; 12, pURE14.8. Molecular size markers (in kilobases) are indicated on the left side of the figure.

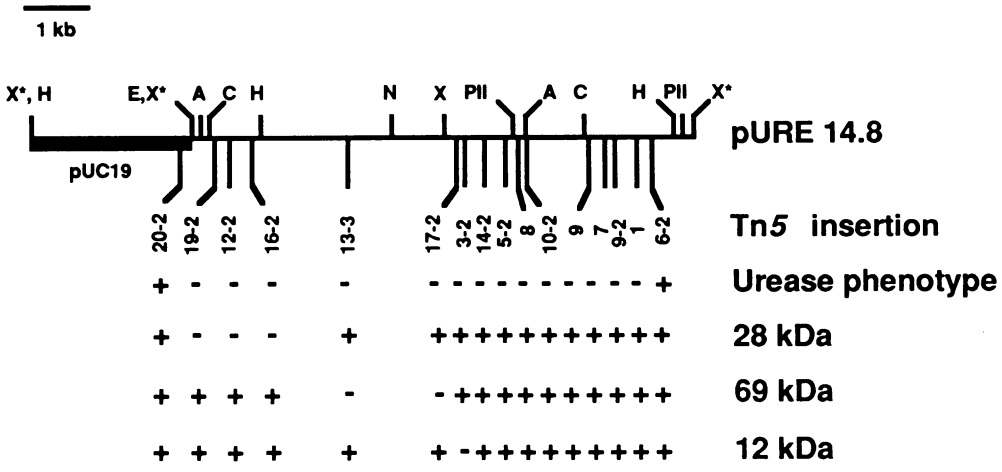

FIG. 3. Physical and genetic map of the *E. coli* 1440 urease locus. Restriction map of pURE14.8, a recombinant plasmid encoding the urease genes of *E. coli* 1440. Thick line designates pUC19 sequences. Vertical lines below the map indicate the positions of Tn*5* insertion mutations. +, Mutations having no effect on urease activity or production of designated polypeptide; −, mutations eliminating urease activity or production of designated polypeptide. X*, To construct pURE14.8, an *Spe*I DNA fragment was inserted into the *Xba*I restriction site of pUC19, eliminating both these restriction sites. A, *Ava*I; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; N, *Nru*I; PII, *Pvu*II; X, *Xba*I.

(Fig. 3). There were no similarities between the restriction endonuclease cleavage site profiles of the DNA fragments encoding the 1021 and 1440 urease loci (Fig. 4).

The region of pURE14.8 necessary for the production of urease was determined by transposon mutagenesis with Tn*5*. Nearly all of the 8-kb *Spe*I DNA fragment was needed for expression of the urease phenotype (Fig. 3).

Polypeptides encoded by the two loci differ. Both in vitro transcription-translation (Fig. 5) and maxicell analysis (data not shown) indicated that the DNA fragments containing the 1021 and 1440 loci coded for polypeptides of similar but nonidentical size. The 9.4-kb DNA fragment that contains the 1021 urease locus produces polypeptides of M_r 67,500, 29,500, 26,800, 16,500, 12,600, 11,800, and 7,700. The 8-kb DNA fragment containing the 1440 urease locus codes for polypeptides of M_r 69,000, 27,500, 19,000, 12,400, and

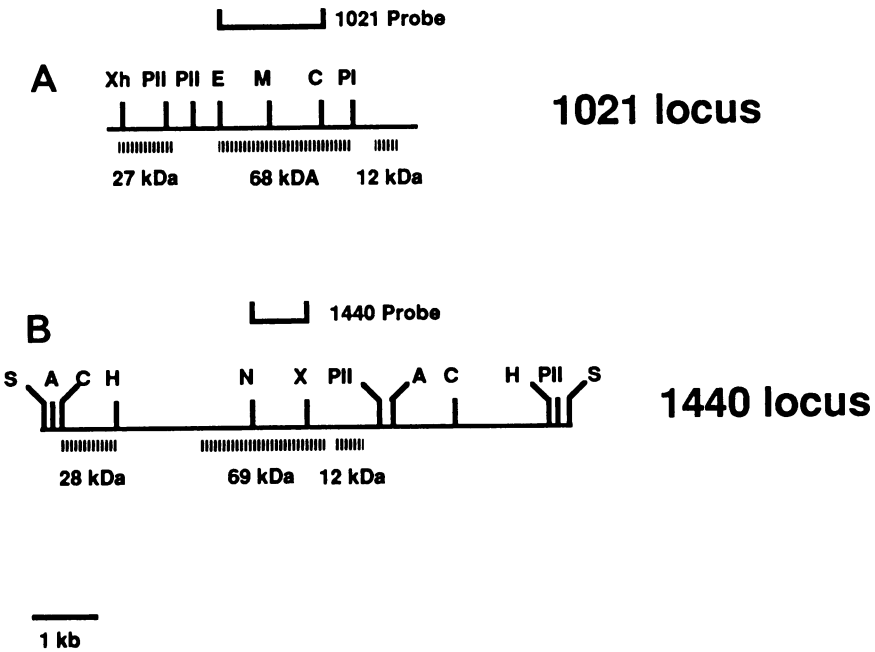

FIG. 4. Maps of the *E. coli* 1021 and 1440 urease loci as defined by deletion and transposon mutagenesis analysis. (A) Restriction map of the DNA region encoding the *E. coli* 1021 urease genes. (B) Restriction map of the DNA fragment encoding the *E. coli* 1440 urease genes. Dashed lines represent approximate positions of genes coding for urease-associated polypeptides. 1021 probe and 1440 probe identify the DNA fragments used in the hybridization analysis. Position of DNA responsible for the 68- and 27-kDa polypeptides of the 1021 locus is from reference 3. Position of gene encoding 12-kDa polypeptide of the 1021 locus is from Collins and Falkow (unpublished data). A, *Ava*I; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; M, *Mlu*I; N, *Nru*I; PI, *Pvu*I; PII, *Pvu*II; S, *Spe*I; X, *Xba*I; Xh, *Xho*I.

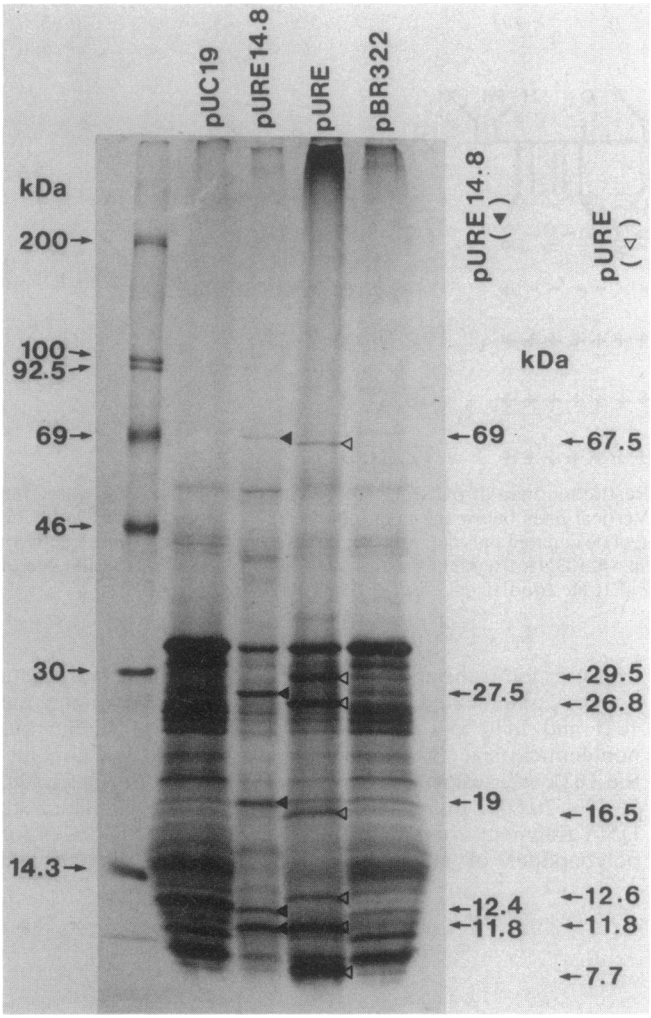

FIG. 5. In vitro transcription-translation analysis of pURE and pURE14.8. Plasmid-encoded polypeptides were labeled with [^{35}S]methionine and run under reducing conditions on a 7.5 to 20% polyacrylamide gel. ^{14}C-methylated protein mixture (Amersham Corp.) was used for molecular mass standards. Bands were visualized by autoradiography.

11,800. Each fragment encoded a polypeptide in the 67- to 69-kDa range, at least one in the 26- to 29-kDa range, and two smaller ones in the 11- to 13-kDa range.

In vitro transcription-translation analysis of the Tn*5* insertion mutations in pURE14.8 (Fig. 5) indicated that the 69-, 27.5-, and 12.4-kDa polypeptides are essential for urease activity (data not shown). Insertions 19-2 and 13-3 resulted in the production of truncated 27.5- and 69-kDa polypeptides, respectively. This suggests that the direction of transcription of the genes encoding these polypeptides proceeds from right to left. None of the insertion mutations shown affected the production of the other pURE14.8-encoded polypeptides. Previous experiments indicate that the 68- and the 27-kDa polypeptides encoded on pURE are required for expression of urease activity (3).

Regulation of urease genes differs in the two loci. The regulation of urease gene expression differed between the two loci. Urease gene expression appeared to be induced 20-fold by urea in *E. coli* 1440, whereas urease gene expression was constitutive in *E. coli* 1021 (Table 1). The recombinant plasmids encoding the respective urease gene clusters showed regulation patterns similar to those of the wild-type strains. There was less than a twofold difference in urease activity between *E. coli* DH5α containing pURE when grown with or without urea. The urease activity encoded on pURE14.8 was induced approximately threefold in the presence of urea, similar to the induction of the urease activity seen in the presence of urea with *E. coli* 1440. Why the activity associated with pURE14.8 was not induced to the same extent as the wild-type activity is not clear at this time.

TABLE 1. Urease activity of cells grown in the presence or absence of urea

Strain	Urease activity[a]	
	+Urea	−Urea
E. coli 1440	9.7	0.5
E. coli DH5α(pURE14.8)	64	21
E. coli 1021	5.5	5.0
E. coli DH5α(pURE)	37	21
E. coli DH5α	0.5	0.4

[a] Urease activity is expressed as moles of ammonia produced per minute per milligram of protein × 10^4. Assay was performed as described in Materials and Methods. Results shown are from a representative experiment; urease activities are average values obtained from duplicate samples that differed by less than 10%.

Nature of the urease-positive to urease-negative transition in *E. coli* 1440. *E. coli* 1021 and 1440 both segregate urease-negative variants at a high frequency. Although we had previously suspected that this instability was due to urease being a plasmid-encoded trait in these two isolates, we were not able to demonstrate the presence of urease-associated plasmids (3). Using a plasmid isolation technique that employs Triton X-100 to lyse the bacterial cells, we are now able to demonstrate the presence of a 160-kb plasmid in *E. coli* 1440 that encodes urease. The loss of the urease-positive phenotype in *E. coli* 1440 is associated with the loss of this plasmid.

Plasmid profiles of the urease-positive *E. coli* 1440 and an *E. coli* 1440 urease-negative segregant were compared. There are a number of plasmids in *E. coli* 1440 ranging in size from approximately 160 to 4 kb. The 160-kb plasmid is uniquely absent in the *E. coli* 1440 urease-negative segregant. Subsequent hybridization analysis with the 1440 urease probe indicated that the 160-kb plasmid encodes the 1440 urease locus (data not shown).

In contrast, we were unable to demonstrate the presence of a urease-associated plasmid in *E. coli* 1021. It has been shown that the urease-positive to urease-negative transition in *E. coli* 1021 is associated with a DNA rearrangement that retains most if not all of the urease locus (3). Thus, we conclude that the 1021 locus is probably chromosomally encoded and the 1440 locus is plasmid encoded.

Homology between the two *E. coli* loci and DNA from other urease-producing enteric bacteria. Southern hybridization analysis was performed to determine whether there was homology between the two *E. coli* loci and the DNA of other urease-producing enteric organisms. The results are summarized in Table 2. The *E. coli* 1021 urease probe produced a strong hybridization signal with DNA from urease-producing *Citrobacter*, *Klebsiella*, *Enterobacter*, and *Serratia* species and a weak signal with DNA from *Providencia stuartii* and two urease-producing *Salmonella* isolates, serotypes *S. cubana* (7) and *S. ohio*, but did not hybridize to the other species listed.

TABLE 2. Homology between *E. coli* urease loci and DNA of other urease-producing enteric bacteria[a]

Strain	*E. coli* 1021 probe	*E. coli* 1440 probe
Escherichia coli 1021	+	−
Escherichia coli 1440	±	+
Salmonella cubana	±	+
Salmonella ohio	±	+
Citrobacter diversus	+	−
Citrobacter freundii	+	−
Klebsiella ozaenae	+	−
Klebsiella pneumoniae	+	−
Enterobacter agglomerans	+	−
Enterobacter cloacae	+	−
Serratia fonticola	+	−
Serratia marcescens	+	−
Hafnia alvei	−	−
Proteus mirabilis	−	+
Providencia stuartii	±	+
Morganella morganii	−	−
Yersinia enterocolitica	−	−

[a] DNA homology was determined by Southern analysis as described in Materials and Methods. The DNA probes used are shown in Fig. 5. ±, Weak yet detectable hybridization signal.

The *E. coli* 1440 probe hybridized to *Hin*dIII-digested total DNA from urease-producing *Proteus mirabilis* and *Providencia stuartii* and two urease-producing *Salmonella* isolates but not to the other enteric bacteria tested. The 1440 probe hybridized to a 7.0-kb *Hin*dIII fragment from the *Providencia stuartii* and *Salmonella* isolates (Fig. 2B). In addition, DNA from two other urease-positive *E. coli* strains, 4448-74 and 1138-77 (24), showed the same hybridization band at 7.0 kb (data not shown).

Plasmids were isolated from the above strains that showed a 7.0-kb *Hin*dIII fragment hybridizing to the *E. coli* 1440 urease probe. Each isolate contained a plasmid that showed homology to the 1440 locus probe (data not shown). These plasmids ranged in size from approximately 80 kb to greater than 200 kb.

Providencia stuartii urease is encoded on a 82-kb plasmid in *Providencia stuartii* BE2467 (17) and on a 230-kb plasmid in *Providencia stuartii* JH1 (8). A plasmid of the expected size in each of these strains hybridized to the probe. Urease-producing *E. coli* 4448-74 and 1138-77 were suggested to contain plasmids of approximately 100 kb coding for the phenotype (24). Both these strains contained a plasmid of the appropriate size that showed DNA homology to the probe. A plasmid of approximately 200 kb from the *S. cubana* isolate hybridized to the probe. A plasmid could not be visualized from the urease-positive *S. ohio* isolate by ethidium bromide staining; however, the hybridization analysis suggested the presence of a urease-encoding plasmid greater than 100 kb but smaller than the plasmid carried by the *S. cubana* isolate (data not shown).

DISCUSSION

It was initially suggested that urease was a plasmid-encoded trait in ureolytic *E. coli* based on the observation that these are unusual clinical isolates (14, 26). In 1979, Wachsmuth et al. (24) showed that the ability to hydrolyze urea could be transferred from *E. coli* 1138-77 on a 100-kb plasmid. Here we also report evidence indicating that in some *E. coli* isolates, urease is plasmid encoded. First, the loss of a 160-kb plasmid from *E. coli* 1440 correlated with the loss of the urease phenotype. Second, an 8-kb DNA fragment that codes for urease was cloned from *E. coli* 1440 and was shown to originate from the 160-kb plasmid.

The plasmid-encoded strain 1440 urease locus is distinct from the *E. coli* 1021 urease locus. The 1440 and 1021 urease genes only show weak DNA homology under stringent hybridization conditions, do not have equivalent restriction maps, and differ in the size of the polypeptides they encode. In addition, expression of urease activity is induced by urea in *E. coli* 1440 and is constitutive in *E. coli* 1021. We reported that the 1021 urease genes are probably chromosomally encoded (3). Thus, we believe there are at least two urease loci associated with ureolytic *E. coli*, a plasmid-encoded set of genes represented by the 1440 locus and a chromosomally encoded set represented by the 1021 locus.

DNA homology studies suggested that the plasmid-encoded locus is found in two other urease-producing *E. coli*, including the strain shown by Wachsmuth et al. (24) to contain a urease-encoding plasmid (*E. coli* 1138-77), two urease-producing *Salmonella* isolates, and at least four ureolytic *Providencia stuartii* isolates. In principle, this locus could be found in many different enteric bacteria depending on the host range of the plasmid carrying the genes. The restriction cleavage patterns of the *E. coli* 1440 urease region resemble that published for the *Providencia stuartii* urease locus (19). This, in addition to the DNA homology data, suggests that these urease gene clusters are highly related.

Of the various other members of the family *Enterobacteriaceae* tested, only *Proteus mirabilis* showed DNA homology with the strain 1440 urease gene probe. The hybridization pattern observed with *Proteus mirabilis* differed from that seen with the isolates containing the urease-encoding plasmids. Therefore, the *Proteus mirabilis* urease genes differ from the plasmid-encoded genes at least in the order of their restriction sites. This observation correlates with the work published by Jones and Mobley (12) on the *Proteus mirabilis* and *Providencia stuartii* urease loci.

In contrast to the strain 1440 genes, the strain 1021 urease locus is highly homologous to DNA from urease-producing *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Serratia* species. This high degree of homology is unusual given the degree of diversity among these genera (2) and indicates that these urease genes are highly conserved.

The urease gene clusters described here each code for at least three polypeptides essential for urease activity. The 1021 and 1440 urease loci both code for a polypeptide in the 68- to 69-kDa size range, one in the 27- to 28-kDa size range, and one of 12 kDa. The urease gene clusters from *Providencia* and *Proteus* species also code for polypeptides in this size range: 73, 10, 9, and 25.5 kDa for *Providencia stuartii* (19) and 61, 12, 11, and 23 kDa for *Proteus mirabilis* (13). *Proteus mirabilis* urease is composed of 61-, 12-, and 11-kDa polypeptide subunits; *Providencia stuartii* urease is composed of 73-, 10-, and 9-kDa polypeptide subunits. Therefore, it is likely that the 68- and 12-kDa polypeptides, encoded by the *E. coli* 1021 gene cluster, and the 69- and 12-kDa polypeptides, encoded by the *E. coli* 1440 gene cluster, represent urease enzyme subunits. The function of the 27-kDa polypeptide encoded by *E. coli* 1021 and the 28-kDa polypeptide encoded by *E. coli* 1440 is unknown. A polypeptide of approximately this size has been shown to be necessary for urease activity in all urease gene clusters studied to date.

Analysis of the Tn*5* insertion mutations in pURE14.8 suggests that more than one mRNA transcript is produced from the *E. coli* 1440 urease gene cluster. Tn*5* insertions that

eliminated production of the 12-, 69-, or 27-kDa polypeptides do not eliminate the synthesis of the other polypeptides. This suggests that the 12-, 69-, and 27-kDa polypeptides are encoded on distinct mRNA transcripts. Currently, experiments are being performed to determine the number and position of promoters of the *E. coli* 1440 urease locus.

E. coli 1021, which has urease genes homologous to *Klebsiella* DNA, expresses a number of biochemical traits unusual to *E. coli* but usually associated with *Klebsiella* species. In addition to being urease positive, *E. coli* 1021 is ornithine decarboxylase negative and amylase positive. *E. coli* 1440, which contains the urease-associated plasmid, differs from the stringent definition of *E. coli* only in the production of urease. This points out that a species can include a highly heterologous group of organisms. In this instance, two *E. coli* isolates that are classified together based on their expression of an unusual biochemical trait may represent two groups of *E. coli*: one that is more like *Klebsiella* species in its biochemical characteristics and one that has acquired a unique plasmid.

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