JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1998, p. 2447–2453 Vol. 36, No. 9
0095-1137/98/$04.00+0

Development of a PCR-Restriction Fragment Length Polymorphism Assay Using the Nucleotide Sequence of the *Helicobacter hepaticus* Urease Structural Genes *ureAB*

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Received 19 February 1998/Returned for modification 5 May 1998/Accepted 10 June 1998

Infection with *Helicobacter hepaticus* causes chronic active hepatitis in certain strains of mice and is associated with hepatocellular carcinoma in A/JCr mice. Like the gastric helicobacters, *H. pylori* and *H. mustelae*, *H. hepaticus* possesses a high level of urease activity. However, the *H. hepaticus* urease structural gene sequences have not been previously determined, and the role of the urease enzyme in colonization and in pathogenesis is not known. PCR was used to amplify a portion of the urease structural genes from *H. hepaticus* genomic DNA. Amplified DNA fragments were cloned, and the nucleotide sequence was determined. The deduced amino acid sequence of the partial *H. hepaticus ureA* gene product was found to exhibit 60% identity and 75% similarity to the predicted *H. pylori* UreA. The deduced amino acid sequence of a partial *H. hepaticus ureB* gene product exhibited 75% identity and 87% similarity to the predicted *H. pylori* UreB. Diversity among *H. hepaticus* isolates was evaluated by means of a restriction fragment length polymorphism (RFLP) assay. The 1.6-kb fragments within the *ureAB* open reading frames, amplified from 11 independent isolates, were digested with the restriction endonuclease *Hha*I. Three distinct RFLP patterns were observed. Identical RFLP profiles were noted in sequential isolates of one strain of *H. hepaticus* during an 18 month in vivo colonization study, suggesting that the urease genes of *H. hepaticus* are stable. The urease genes among *H. hepaticus* strains were also well conserved, showing 98.8 to 99% nucleotide sequence identity among three isolates analyzed. These findings indicate that *H. hepaticus* has urease structural genes which are homologous to those of the gastric *Helicobacter* species and that these gene sequences can be used in a PCR and RFLP assay for diagnosis of this important murine pathogen.

The genus *Helicobacter* is a group of microaerobic gram-negative spiral to curved bacteria isolated from the stomachs and intestines of humans and animals. The type species of the genus, *Helicobacter pylori*, has been recognized in humans as the most common cause of chronic gastritis and has been unambiguously implicated in the pathogenesis of peptic ulcer disease and gastric cancer (4, 10, 38, 40). *H. mustelae*, *H. felis*, and *H. heilmannii* are closely related organisms which have been isolated from the stomachs of mammalian species. Each of these species causes various degrees of gastritis in their respective hosts (18, 19, 21, 22).

Infection with *H. hepaticus* causes chronic active hepatitis in mice and is associated with hepatocellular carcinoma in A/JCr mice (17, 20, 23). Infection with *H. hepaticus* has also been linked to inflammatory bowel disease in certain immunodeficient strains of mice as well as in A/JCr mice (6, 20, 51, 52).

Like several other murine *Helicobacter* spp. which colonize the lower intestine, *H. hepaticus* expresses an active urease (18). Whereas the role of urease expression by *Helicobacter* spp. in intestinal and hepatobiliary colonization has not been determined, the production of urease has been shown to be important in the survival of *H. pylori* in the human gastric mucosa (10, 33–35). This enzyme hydrolyzes urea, releasing ammonia, which may allow survival of the organism at a low pH. The contribution of *H. pylori* urease to pathogenesis and to the ability to colonize the stomach at acidic pHs has been assessed by utilizing urease-negative isogenic mutant strains in colonization experiments (15, 27, 48, 49). Urease-negative mutant strains of *H. pylori* are unable to colonize the gastric mucosa of gnotobiotic piglets at a normal physiological pH (14). The urease-negative mutant of *H. pylori* also lacks the ability to colonize the stomach of nude mice (49). Similar studies were conducted with urease-negative *H. mustelae* isogenic mutants. These mutants also fail to colonize the gastric mucosa of ferrets (3). However, Eaton and Krakowka showed that *H. pylori* urease-negative mutants colonize gastric mucosal explants, derived from neonatal germfree piglets, as efficiently as the wild-type strains do, suggesting that the intact host environment is a prerequisite for urease-dependent colonization (15). In addition, urease consistently elicits an immune response in the host and this immunodominant protein has been used to diagnose *H. pylori* infection (47).

It has been previously reported that the urease from *H. pylori* exhibits a peak of activity at an acidic pH, which is not observed for the urease from *H. muridarum*, another murine *Helicobacter* species that colonizes the bowel but also can colonize the mouse stomach (30). Presumably, the urease of *H. muridarum* as well as other nongastric, urease-positive helicobacters has evolved for a purpose other than buffering gastric acid, such as supplying these bacteria with nitrogen from hydrolyzed urea.

In this study, we attempted to identify differences between the ureases of *H. hepaticus* and *H. pylori* by determining the partial nucleotide sequences of the genes encoding the struc-

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TABLE 1. Primers used for amplification of *H. hepaticus* urease genes

Primer[c]	Sequence	Source of sequence (position)	Gene
HURE1	GGAATTCTAGAATAGGAGAATGAGATGA	*H. pylori* (2634–2662)[a]	*ureA*
HURE3	CCATCGATGCCCATATTATGGAAGAAGC	*H. pylori* (2777–2798)[a]	*ureA*
HURE4	GGAGATCTCCACCAATCATGGTTGTTACA	*H. pylori* (3834–3855)[a]	*ureB*
HHUF1	ATTAAATTTGGCGGAGGAAAA	*H. hepaticus* (817–837)[b]	*ureB*
HHUR1	ATATGTCCCTAAATGTTTCGA	*H. hepaticus* (922–942)[b]	*ureB*
HHUF5	TTGCATTATGCTGGGC	*H. hepaticus* (28–45)[b]	*ureA*
4324R	GCATCCGCGGCCGCTGGTGGCACACCATAAGCATGTC	*H. pylori* (4324–4346)[a]	*ureB*

[a] Sequence from the published *H. pylori* urease sequence (29) at the position indicated.
[b] Sequence from this study at the position indicated.
[c] Primers HHUF5 and 4324R were used for RFLP analysis. Primers HHUF5 and HHUR1 were used to distinguish *H. hepaticus* from other *Helicobacter* spp.

tural subunits of the enzyme synthesized by *H. hepaticus*. We have used this information to develop a PCR-based restriction fragment length polymorphism (RFLP) assay for use as an epidemiological tool for the differentiation and identification of *H. hepaticus* clinical isolates.

MATERIALS AND METHODS

Bacterial strains and growth media. The *H. hepaticus* type strain (ATCC 51449), isolated from the liver of an A/JCr mouse, was used to prepare templates for sequence determination of the urease structural subunit genes (17). All other *H. hepaticus* isolates were cultured by filtering feces or cecum homogenates from infected mice through a 0.45-μm-pore-size filter and inoculating brucella blood agar supplemented with trimethoprim, vancomycin, and polymyxin (Remel Labs, Lenexa, Kans.) with the filtrate. Plates were incubated under microaerobic conditions as previously described (43).

DNA extraction. A High Pure PCR Template Preparation Kit (Boehringer Mannheim, Indianapolis, Ind.) was used to extract DNA from bacterial pellets as outlined by the manufacturer and previously described (43).

PCR amplification. Table 1 shows the nucleotide sequences and the sources for all primers used to amplify the *H. hepaticus* urease structural genes. PCR amplification reactions were performed with a Thermal Cycler and an Expand high-fidelity PCR system (Boehringer Mannheim). The reaction mixture (100 μl) contained 1× polymerase buffer (supplied by the manufacturer but supplemented with 1 M $MgCl_2$ to a final concentration of 2.25 mM), a 0.5 μM concentration of each of the two primers, a 200 μM concentration of each deoxyribonucleotide, and bovine serum albumin (200 μg/ml). Samples were heated at 94°C for 4 min, briefly centrifuged, and cooled to 55°C. Polymerase (2.5 U) was then added, followed by an overlay of 100 μl of mineral oil. Amplification was achieved by denaturation at 94°C for 1 min, annealing at 55 to 58°C for 2 min, and elongation at 72°C for 2 min. A 15-μl portion of the sample was then electrophoresed through a 6% Visigel separation matrix (Stratagene, La Jolla, Calif.).

Southern blot analysis. For confirmation that the PCR-amplified fragment represented urease gene sequences, the PCR product (15 μl) was electrophoresed through a 1% agarose gel and transferred onto a Hybond-N nylon membrane as outlined by the manufacturer (Amersham, Arlington Heights, Ill.); this was followed by UV cross-linking (43). The filter was hybridized with a PCR product of the *H. pylori* urease gene amplified by the same primers. This 1-kb PCR product was excised from a low-melting-point agarose gel and purified with a Geneclean DNA purification kit (Bio 101, Vista, Calif.). The probe was labeled with horseradish peroxidase, hybridized overnight to the nylon membrane at 42°C, and exposed in the presence of Luminol to Hyperfilm-ECL as outlined by the manufacturer (Amersham) (43).

Cloning and sequencing of the PCR product. A TA Cloning Kit (Invitrogen Corp. San Diego, Calif.) was used for cloning PCR products. PCR products were purified from low-melting-point agarose gels with a Geneclean kit, as described above. Purified PCR product was ligated with 50 ng of PCR II vector at 15°C overnight and was then transferred into INVaF′ cells. LB plates containing ampicillin (50 μg/ml) and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (40 μg/ml) were used to select clones. Plasmid DNA was isolated from *Escherichia coli* by a High Pure Plasmid Isolation Kit (Boehringer Mannheim). The sequencing was carried out at the Biopolymers Laboratory (Center for Cancer Research, Massachusetts Institute of Technology) with a DNA sequencer (model 377; Applied Biosystems, Perkin-Elmer, Foster City, Calif.) and a *Taq* DyeDeoxy terminator cycle sequencing kit (Perkin-Elmer).

RFLP of *H. hepaticus* urease genes. Primers HHUF5 and 4324R (Table 1) were used to amplify a 1.6-kb PCR fragment from the *H. hepaticus* urease genes *ureA* and *ureB*. Amplified DNA (20 μl) was digested with 10 U of enzyme in the appropriate buffer recommended by the enzyme manufacturer at 37°C for 3 h. Restriction patterns were compared after the digested PCR products were separated on a 6% Visigel separation matrix. The restriction enzymes *Hha*I, *Alu*I, *Bfa*I, *Hin*fI, *Hae*III, and *Dpn*I were chosen on the basis of the number and placement of restriction sites predicted from the acquired nucleotide sequence.

Nucleotide sequence accession numbers. The nucleotide sequences of *H. hepaticus ureAB* from strains ATCC 51449, 96284, and 951971 have been deposited in GenBank under accession no. AF066862, AF066863, and AF066864, respectively.

RESULTS

PCR amplification and cloning of partial *ureAB* fragments. Primers HURE3 and HURE4 (Table 1) were designed to amplify a 1-kb *ureB* fragment based on conserved regions from the urease structural genes from *H. pylori* and *H. mustelae* (36, 44). However, by using this pair of primers to amplify *H. hepaticus* DNA, two bands (0.5 and 1.5 kb) were amplified. By Southern blot analysis, the PCR product amplified from *H. pylori* only hybridized to the 0.5-kb fragment (data not shown). The 0.5-kb fragment was cloned and sequenced; it has 76% similarity to the *H. pylori ureB* gene (36).

The *H. hepaticus ureB* sequence was used in conjunction with the *H. pylori ureA* sequence to design a primer to amplify upstream and downstream fragments of *ureA* and *ureB*. Primer HURE1 was chosen from the *H. pylori* urease sequence, and the primer HHUR1 was chosen from the *H. hepaticus* sequence. These primers amplified a 0.9-kb fragment containing *ureA* and part of *ureB*. Primer HHUF1, based on the *H. hepaticus* sequence, and primer 4324R, chosen from the *H. pylori* urease sequence, amplified a 0.9-kb fragment containing part of the *ureB* gene sequence. The two fragments had a 120-bp sequence overlap (Fig. 1).

Sequence analysis of *H. hepaticus* urease structural genes. Sequence analysis of the *H. hepaticus* urease fragments revealed two open reading frames, designated *ureA* and *ureB*, that were transcribed in the same direction. The nucleotide sequence showed 66% identity to the *H. pylori ureA*. The partial nucleotide sequence of the *H. hepaticus ureB* gene, approximately two-thirds of the gene according to the *H. pylori ureB*

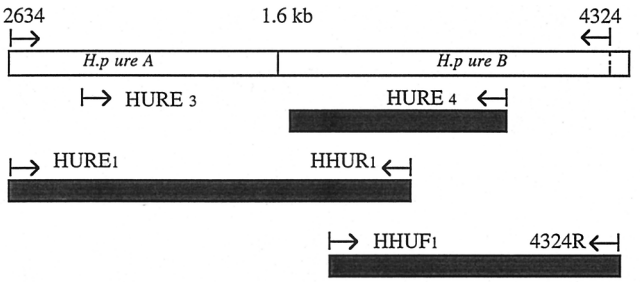

FIG. 1. PCR-amplified products of *ureAB* genes from *H. pylori* (*H.p*) (open bars) and *H. hepaticus* (shaded bars).

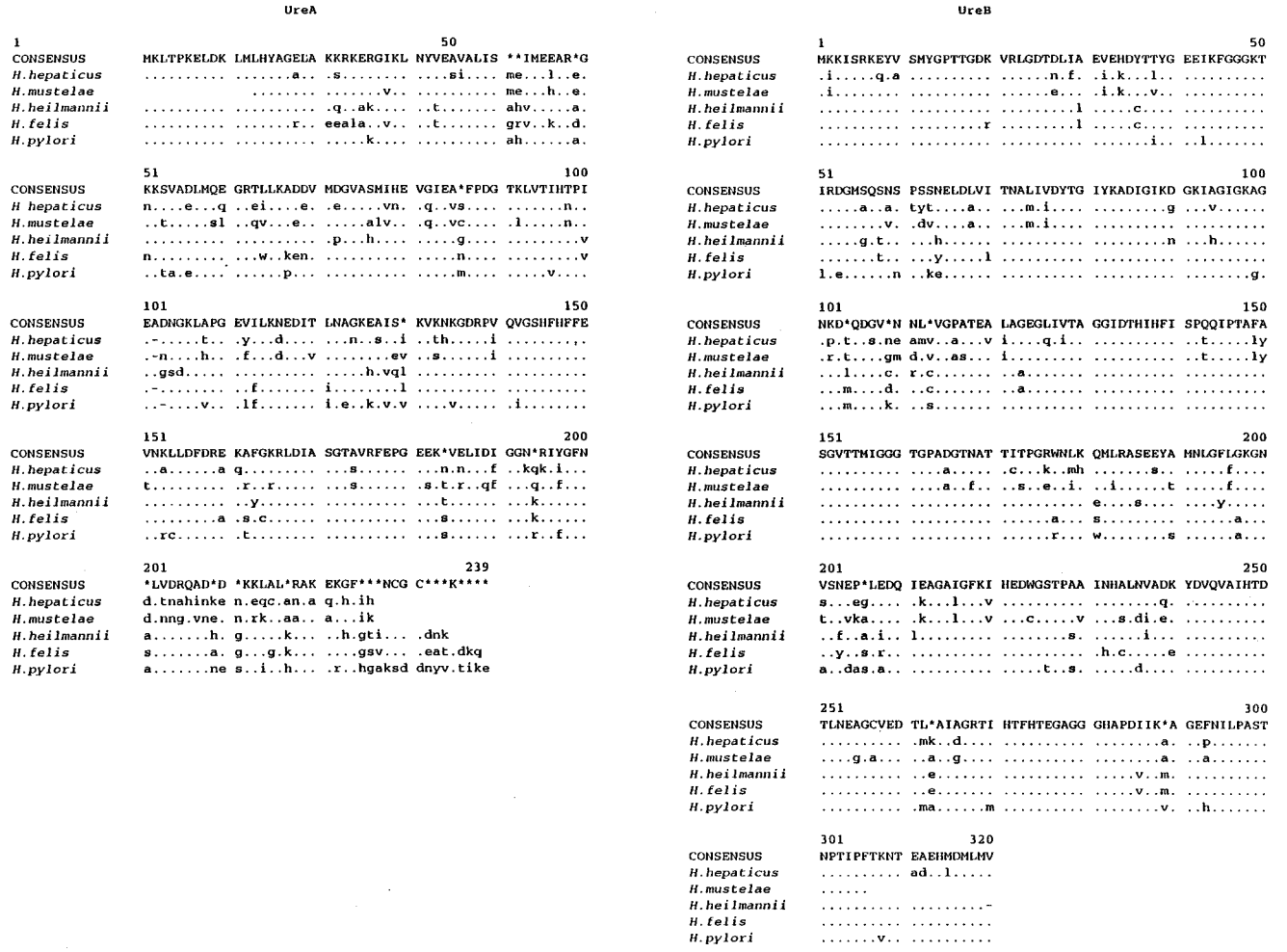

FIG. 2. Predicted amino acid sequences of UreA and UreB from *H. hepaticus* aligned with the corresponding predicted sequences from *H. felis*, *H. pylori*, *H. heilmannii*, and *H. mustelae*. ., sequence identity; *, sequence difference.

gene sequence (36), showed 71% identity to the *H. pylori ureB* gene. The predicted amino acid sequence of the *H. hepaticus* urease was compared to those of *H. felis*, *H. mustelae*, *H. heilmannii*, and *H. pylori*. The *H. hepaticus* urease was most closely related to the urease of *H. mustelae* (71% amino acid sequence identity with UreA and 83% identity with UreB) and to a slightly lesser extent was related to the urease of *H. felis* (61% amino acid sequence identity with UreA and 76% identity with UreB) (Fig. 2).

The intergenic space between *ureA* and *ureB* was found to be 21-bp for *H. hepaticus*. This intergenic space is longer than the 14 bp for *H. heilmannii*, the 9 bp for *H. felis*, and the 3 bp for *H. pylori*.

Use of PCR-amplified *H. hepaticus* urease structural genes for differentiation of *H. hepaticus* strains. Primers HHUF5 and 4324R (Table 1) successfully amplified a 1.6-kb PCR fragment (from nucleotide 2668 of *ureA* to nucleotide 4323 of *ureB* according to the *H. pylori ure* gene sequence) from 11 different *H. hepaticus* strains isolated from mice from 11 different sources. All isolates were confirmed as *H. hepaticus* by PCR using 16S rRNA primers specific for *H. hepaticus* (data not shown) (43). The PCR product digested with *Hha*I resulted in three distinct patterns after agarose gel electrophoresis (Fig. 3). Pattern A yielded fragments of about 50, 120, 150, 470, and 830 bp. Two of the strains that produced this pattern were from Europe, and the other six strains were from the United States. Two strains isolated in the United States yielded pattern B, with fragments of 50, 120, 620, and 830 bp. One strain isolated from the United States yielded Pattern C, consisting of fragments of 50, 150, 470, and 940 bp. The restriction digest fragment patterns were identical for all 11 strains when other enzymes—*Alu*I, *Bfa*I, *Hin*fI, *Hae*III, and *Dpn*I—were used for digestion (data not shown).

Using the same method, we also examined sequential *H. hepaticus* isolates cultured from mice during a long-term colonization study (20). Isolates of *H. hepaticus* from experimentally inoculated germfree mice at 3, 6, 9, 12, 15, and 18 months after inoculation were used for RFLP analysis. All of these isolates had identical pattern-A RFLP profiles (Fig. 4A). *H. hepaticus* isolates from different strains of mice originating from the same commercial animal supplier also were subjected to RFLP analysis. Among the isolates cultured from the seven different strains of mice, pattern A and pattern B were recognized (Fig. 4B).

Homology of *ure* genes among *H. hepaticus* strains. RFLP analysis with *Hha*I revealed that there was limited diversity in the *H. hepaticus* urease genes among the 18 strains analyzed. These genes appear to be highly conserved, because digestion

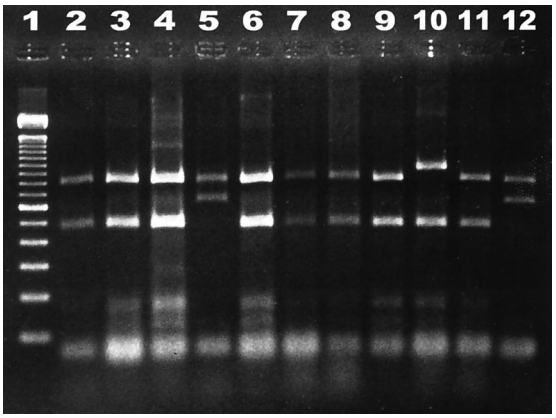

FIG. 3. Restriction digest patterns of the amplified urease gene on a 1.6-kb PCR product from 11 *H. hepaticus* isolates. Amplified DNA was digested with *Hha*I and separated by electrophoresis on a 6% Visigel. Shown are a 100-bp DNA ladder (lane 1), pattern A (lanes 2 to 4, 6 to 9, and 11), pattern B (lanes 5 and 12), and pattern C (lane 10).

with five other restriction enzymes—*Alu*I, *Bfa*I, *Hin*fI, *Hae*III, and *Dpn*I—produced identical RFLP profiles (data not shown). To investigate the similarity of *H. hepaticus* urease genes among strains, the partial urease genes of two other *H. hepaticus* strains were directly sequenced from their 1.6-kb PCR products. Strain 96284, isolated in Germany, had the same RFLP pattern as the *H. hepaticus* type strain (pattern A) and 99% nucleotide sequence identity to the urease gene of the *H. hepaticus* type strain. Strain 951971, isolated from mouse obtained from another U.S. university had a different RFLP pattern (pattern B) than the type strain. The urease genes of this strain showed 98.8% nucleotide sequence identity to that of the *H. hepaticus* type strain (Fig. 5).

Distinguishing *H. hepaticus* from other *Helicobacter* spp. Primers HHUF5 and HHUR1 (Table 1) were used to amplify a fragment from the *ure* structural genes. This set of primers amplified a 914-bp fragment from all 11 *H. hepaticus* isolates tested and did not cross-react with 8 of the other *Helicobacter* spp. analyzed (Fig. 6).

DISCUSSION

The RFLP assay we developed using amplified urease genes should provide a sensitive epidemiological tool for the typing of *H. hepaticus* clinical isolates. The use of the urease gene PCR product for restriction digest analysis readily produced identifiable differences in restriction digest patterns. Also, this assay does not require a high concentration of chromosomal DNA, special equipment for PFGE, or further extraction of the PCR product. However, because of the relatively small number of *H. hepaticus* isolates analyzed in the present study and the low degree of RFLP diversity observed, a larger number of isolates and, perhaps, additional restriction enzymes will need to be employed before the full utility of this epidemiological typing procedure can be established. Similarly, analyses of more animals, both infected and uninfected with *H. hepaticus*, will need to be tested before the true sensitivity of this assay can be determined.

We used primers to amplify a 1.6-kb urease gene fragment from 11 isolates of *H. hepaticus* obtained from mice from different sources, and three different patterns were observed. Foxall et al. (24) reported that for 22 clinical isolates of *H. pylori*, there were 10 urease gene RFLP patterns. These preliminary data suggest that the *H. hepaticus* urease genes are more conserved than that of *H. pylori*. Interestingly, *H. hepaticus* isolated from mice obtained from the same commercial source but maintained in different rooms had different RFLP patterns, indicating that different strains of mice from the same commercial source can be infected with different *H. hepaticus* strains. The different RFLP patterns detected in our study were probably not due to base substitutions during PCR, because repeated PCR amplification of individual strains produced the same restriction patterns. The urease genes also appeared to be very stable in vivo. During long-term colonization in mice, the *H. hepaticus* strain used for experimental inoculation maintained the same RFLP profile (20).

PCR amplification and analysis of restriction digest patterns of the urease structural genes also have been used to distinguish different *H. pylori* strains (1, 2, 5, 11, 13, 24, 31, 37, 46), to identify the presence of multiple strains in a single biopsy specimen (26), to ascertain whether reinfection with the same strain occurred following eradication therapy (39), and to identify similar or identical strains among family members (50). Although the epidemiology of *H. hepaticus* infection is unknown, widespread *H. hepaticus* infection in commercially maintained mice, as well as in mice being used in biomedical research, has been previously reported (43). *H. hepaticus*, like *H. pylori*, also demonstrates genetic diversity at the genomic DNA level. Saunders et al. (42) reported that *H. hepaticus* strains isolated from mice in the United States and European countries had different restriction enzyme digestion patterns of chromosomal DNA when analyzed by pulsed-field gel electrophoresis (PFGE). In their study, the strains from European countries had restriction enzyme digestion patterns of chromosomal DNA different from the pattern noted in the *H. hepati-*

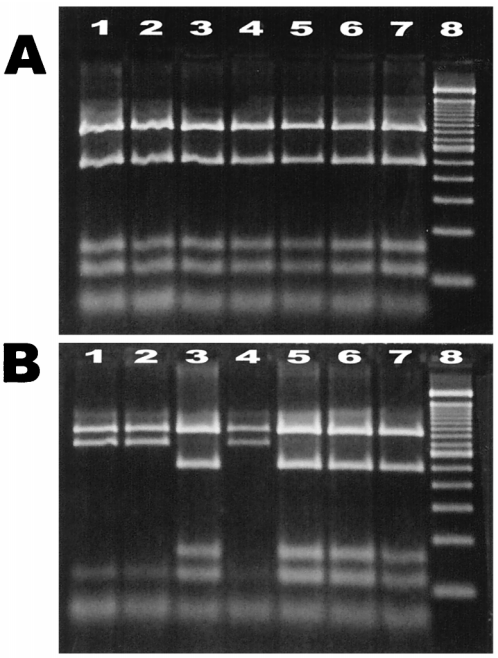

FIG. 4. (A) Restriction digest patterns of amplified urease genes of *H. hepaticus* isolates cultured from mice during a long-term colonization study. *H. hepaticus* was isolated from the mouse cecum 3 (lane 1), 6 (lane 2), 9 (lane 3), 12 (lane 4), 15 (lane 5), and 18 months (lane 6) postinoculation. Lane 7, *H. hepaticus* strain used for dosing; lane 8, 100-bp DNA ladder. (B) Restriction digest patterns of amplified urease genes of *H. hepaticus* isolated from different strains of mice housed within the same facility. Lane 1, DBA/2J; lane 2, $B6D2F_1$/D; lane 3, BALB/J; lane 4, B_6SJLF_1; lane 5, BALB/cByj-nu; lane 6, BALB/cBj; lane 7, C57BL/6c-nu; lane 8, 100-bp DNA ladder.

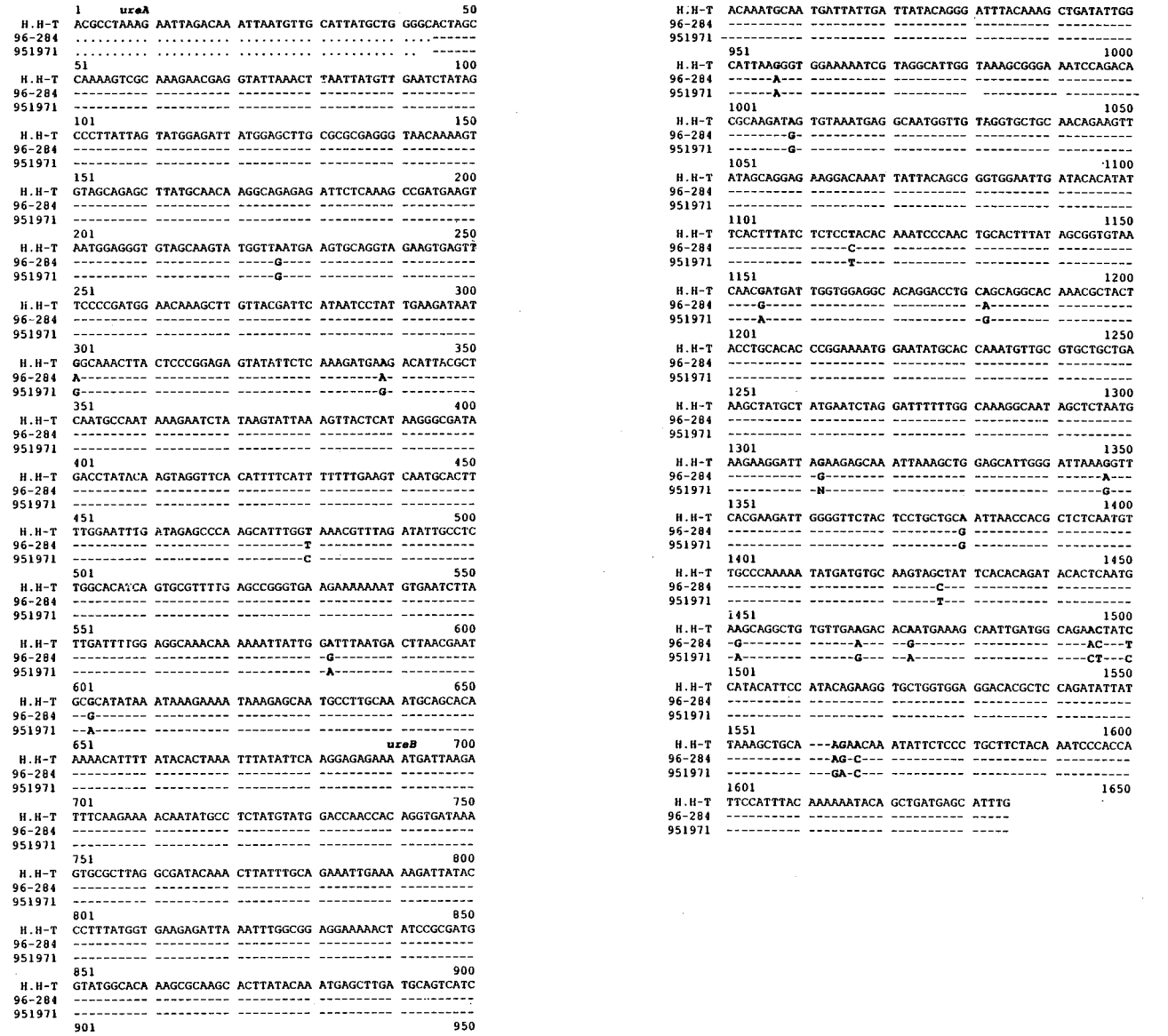

FIG. 5. Sequence comparison of the *H. hepaticus* urease genes of different strains. A dash indicates sequence identity; sequence differences are illustrated in boldface type. H.H-T, *H. hepaticus* type strain (ATCC 51449).

cus type strain. In our study, however, one of the European strains had the same RFLP pattern of the *ure* gene as the type strain. Restriction enzyme digestion of genomic DNA coupled with PFGE may be a better method with which to discriminate between individual strains of *H. hepaticus* than RFLP analysis. But the relative ease and rapidity of the RFLP method may make this a preferred method for initial analysis. Strains which are not differentiated by *ure* gene RFLP can then be analyzed for genetic polymorphism at other loci by PFGE.

It can be argued that the nucleotide sequence of such an important virulence factor as urease should be conserved among strains and that the nucleotide sequence variations detected in the *ureA* and *ureB* genes of *H. hepaticus* strains are base substitutions that nevertheless conserve amino acids or allow amino acid substitutions that do not affect enzyme activity. The different *H. hepaticus* isolates tested showed high homology within the urease gene, about 99% identity at the DNA level and 99% similarity at the amino acid level, among the three strains analyzed from different locations. Single base changes apparently are responsible for restriction site differences. Because the urease structural genes have high homology among *H. hepaticus* strains, primers selected specifically for *H. hepaticus* from the region exhibiting diversity among *Helicobacter* spp. can be used for diagnostic purposes. For example, the primers from the urease gene of *H. pylori* have been commonly used for the detection of *H. pylori* (5, 7, 32). Primers HHUF5 and HHUR1 based on *H. hepaticus* urease gene sequence amplified a 914-bp product from all of the 11 *H. hepaticus* strains but did not amplify the same size product from DNAs of 8 other *Helicobacter* spp. which either have urease activity or alternatively are urease-negative helicobacters that colonize the intestine of rodents. It is noted, however, that *H. muridarum* and *H. bilis* had nonspecific PCR products of a different size.

The presence of two structural urease subunits in *H. hepaticus* is characteristic of the genus *Helicobacter*. Urease struc-

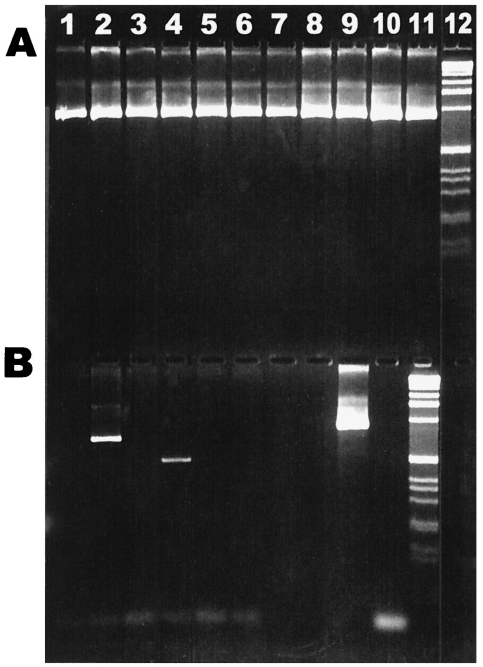

FIG. 6. Results of PCR with urease gene primers specific for *H. hepaticus*. (A) Lanes 1 to 11, 11 strains of *H. hepaticus* from 11 sources; lane 12, 1-kb DNA ladder. (B) Lane 1, *H. pylori*; lane 2, *H. bilis*; lane 3, *H. felis*; lane 4, *H. muridarum*; lane 5, *Flexispira rappini*; lane 6, *H. trogontum*; lane 7, *H. rodentium*; lane 8, *H. mustelae*; lane 9, *H. hepaticus*; lane 10, reagent control; lane 11, 1-kb DNA ladder.

tural genes from *H. hepaticus* are highly homologous to *ureA* and *ureB* from *H. mustelae* (44), *H. felis* (16, 25), *H. heilmannii* (45), and *H. pylori* (36). The homology is greatest for *ureB*, which is consistent with the fact that this subunit contains the urease catalytic site, which is highly conserved among the known *Helicobacter* spp. The conservation of *ureA* and *ureB* polypeptide sequences is consistent with an essential role for urease in the colonization and presumably the pathogenesis of some *Helicobacter* spp. (36).

The availability of urease gene DNA sequences from different species of *Helicobacter*, which differ in host range specificity, tissue specificity, and ability to produce disease, will allow further delineation of the role of urease. We have recently constructed and are currently characterizing an isogenic urease-negative mutant of *H. hepaticus*, to allow a more precise evaluation of the role of *H. hepaticus* urease in the colonization and pathogenesis of hepatobiliary and inflammatory bowel disease (6, 20). Recent evidence also suggests that immunization of mice with urease from *Helicobacter* spp. confers protection against challenge with *H. felis* and *H. pylori* (8, 9, 12, 28, 41). Studies to ascertain whether *H. hepaticus* urease can induce protective immunity and prevent *H. hepaticus* colonization in vivo may also be an important strategy in the development of an *H. hepaticus* vaccine.

In summary, we used PCR to amplify a portion of the urease structural genes from *H. hepaticus* genomic DNA. The urease genes from this nongastric *Helicobacter* sp. show a high degree of homology with other *Helicobacter* spp., and the urease genes of *H. hepaticus* were less diverse than those of *H. pylori*. A PCR-based RFLP assay using the nucleotide sequence of the *H. hepaticus* urease structural genes *ureAB* was developed to identify and differentiate clinical isolates. This assay, with further development, should prove to be useful for studying the epidemiology and pathogenesis of this murine pathogen.

ACKNOWLEDGMENTS

This work was supported by grants R01CA67529, P01CA26731, and R01AI23328 from the National Institutes of Health.

We thank Paul Foxall for sharing preliminary nucleotide sequence data.

REFERENCES

1. **Akashi, H., T. Hayashi, H. Koizuka, T. Shimoyama, and T. Tamura.** 1996. Strain differentiation and phylogenic relationships, in terms of base sequence of the ure B gene, of *Helicobacter pylori*. J. Gastroenterol. **31:**16–23.
2. **Akopyanz, N., N. O. Bukanov, T. U. Westblom, and D. E. Berg.** 1992. PCR-based RFLP analysis of DNA sequence diversity in the gastric pathogen *Helicobacter pylori*. Nucleic Acids Res. **20:**6221–6225.
3. **Andrutis, K. A., J. G. Fox, D. B. Schauer, R. P. Marini, J. C. Murphy, L. Yan, and J. V. Solnick.** 1995. Inability of an isogenic urease-negative mutant strain of *Helicobacter mustelae* to colonize the ferret stomach. Infect. Immun. **63:** 3722–3725.
4. **Bayerdorffer, E., A. Neubauer, B. Rudolph, C. Thiede, N. Lehn, S. Eidt, and M. Stolte.** 1995. Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of *Helicobacter pylori* infection. Lancet **345:**1591–1594.
5. **Bickley, J., R. J. Owen, A. G. Fraser, and R. E. Pounder.** 1993. Evaluation of the polymerase chain reaction for detecting the urease C gene of *Helicobacter pylori* in gastric biopsy samples and dental plaque. J. Med. Microbiol. **39:** 338–344.
6. **Cahill, R. J., C. J. Foltz, J. G. Fox, C. A. Dangler, F. Powrie, and D. B. Schauer.** 1997. Inflammatory bowel disease: an immune-mediated condition triggered by bacterial infection with *Helicobacter hepaticus*. Infect. Immun. **65:**3126–3131.
7. **Clayton, C., H. Kleanthous, P. Coates, D. Morgan, and S. Tabaqchali.** 1992. Sensitive detection of *Helicobacter pylori* by using polymerase chain reaction. J. Clin. Microbiol. **30:**192–200.
8. **Corthesy-Theulaz, I., N. Porta, M. Glauser, E. Saraga, A. Vaney, R. Haas, J. Kraehenbuhl, A. Blum, and P. Michetti.** 1993. *Helicobacter pylori* urease elicits protection against *Helicobacter felis* infection in mice. Acta Gastro-Enterol. Belg. **56:**64.
9. **Corthesy-Theulaz, I., N. Porta, M. Glauser, E. Saraga, A. C. Vaney, R. Haas, J. P. Kraehenbuhl, A. L. Blum, and P. Michetti.** 1995. Oral immunization with *Helicobacter pylori* urease B subunit as a treatment against *Helicobacter* infection in mice. Gastroenterology **109:**115–121.
10. **Dekigal, H., M. Murakami, and T. Kita.** 1995. Mechanism of *Helicobacter pylori* associated gastric mucosa injury. Dig. Dis. Sci. **40:**1332–1339.
11. **Desai, M., D. Linton, R. Owen, and J. Stanley.** 1994. Molecular typing of *Helicobacter pylori* isolates from asymptomatic, ulcer and gastritis patients by urease gene polymorphism. Epidemiol. Infect. **112:**151–160.
12. **Dore-Davin, C., P. Michetti, E. Saraga, A. Blum, and I. Corthesy-Theulaz.** 1996. A 37 kDa fragment of ureB is sufficient to confer protection against *Helicobacter felis* infection in mice. Gastroenterology **110:**A97.
13. **Dzierzanowska, D., A. Gzyl, E. Rozynek, E. Augustynowicz, U. Wojda, D. Celink-Cedro, M. Sankwoska, and T. Wadstrom.** 1996. PCR for identification and typing of *Helicobacter pylori* isolated from children. J. Physiol. Pharmacol. **47:**101–104.
14. **Eaton, K., and S. Krakowka.** 1995. A virulent, urease-deficient *Helicobacter pylori* colonises gastric epithelial explants ex vivo. Scand. J. Gastroenterol. **30:**434–437.
15. **Eaton, K. A., and S. Krakowka.** 1994. Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by *Helicobacter pylori*. Infect. Immun. **62:**3604–3607.
16. **Ferrero, R. L., and A. Labigne.** 1993. Cloning expression and sequencing of *Helicobacter felis* urease genes. Mol. Microbiol. **9:**323–333.
17. **Fox, J. G., F. E. Dewhirst, J. G. Tully, B. J. Paster, L. Yan, N. S. Taylor, M. J. Collins, P. L. Gorelick, and J. M. Ward.** 1994. *Helicobacter hepaticus* sp. nov, a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. J. Clin. Microbiol. **32:**1238–1245.
18. **Fox, J. G., and A. Lee.** 1997. The role of Helicobacter species in newly recognized gastrointestinal tract diseases of animals. Lab. Anim. Sci. **47:**222–255.
19. **Fox, J. G., A. Lee, G. Otto, N. S. Taylor, and J. C. Murphy.** 1991. *Helicobacter felis* gastritis in gnotobiotic rats: an animal model of *Helicobacter pylori* gastritis. Infect. Immun. **59:**785–791.
20. **Fox, J. G., X. Li, L. Yan, R. J. Cahill, R. Hurley, R. Lewis, and J. C. Murphy.** 1996. Chronic proliferative hepatitis in A/JCr mice associated with persistent *Helicobacter hepaticus* infection: a model of helicobacter-induced carcinogenesis. Infect. Immun. **64:**1548–1558.
21. **Fox, J. G., G. Otto, N. S. Taylor, W. Rosenblad, and J. C. Murphy.** 1991. *Helicobacter mustelae*-induced gastritis and elevated gastric pH in the ferret (*Mustela putorius furo*). Infect. Immun. **59:**1875–1880.
22. **Fox, J. G., J. S. Wishnok, J. C. Murphy, S. R. Tannenbaum, and P. Correa.** 1993. MNNG-induced gastric carcinoma in ferrets infected with *Helicobacter mustelae*. Carcinogenesis **14:**1957–1961.
23. **Fox, J. G., L. Yan, B. Shames, J. Campbell, J. C. Murphy, and X. Li.** 1996.

Persistent hepatitis and enterocolitis in germfree mice infected with *Helicobacter hepaticus*. Infect. Immun. **64:**3673–3681.
24. **Foxall, P., L. Hu, and H. Mobley.** 1992. Use of polymerase chain reaction-amplified *Helicobacter pylori* urease structural genes for differentiation of isolates. J. Clin. Microbiol. **30:**739–741.
25. **Gootz, T., G. Perez-Perez, J. Clancy, B. Martin, A. Tait-Kamradt, and M. Blaser.** 1994. Immunological and molecular characterization of *Helicobacter felis* urease. Infect. Immun. **62:**793–798.
26. **Hurtado, A., and R. Owen.** 1994. Identification of mixed genotypes in *Helicobacter pylori* from gastric biopsy tissue by analysis of urease gene polymorphisms. FEMS. Immunol. Med. Microbiol. **8:**307–313.
27. **Karita, M., M. Tsuda, and T. Nakazawa.** 1995. Essential role of urease in vitro and in vivo *Helicobacter pylori* colonization study using a wild-type and isogenic urease mutant strain. J. Clin. Gastroenterol. **21:**S160–S163.
28. **Kreiss, C., T. Kuclin, M. Cosma, I. Corthesy-Theulaz, and P. Michetti.** 1996. Safety of oral immunization with recombinant urease in patients with *Helicobacter pylori* infection. Lancet **347:**1630–1631.
29. **Labigne, A., V. Cussac, and P. Courcoux.** 1992. Shuttle cloning and nucleotide sequences of *Helicobacter pylori* genes responsible for urease activity. J. Bacteriol. **173:**1920–1931.
30. **Lee, A., M. W. Phillips, J. L. O'Rourke, B. J. Paster, F. E. Dewhirst, G. J. Fraser, J. G. Fox, L. I. Sly, P. J. Romaniuk, T. J. Trust, and S. Kouprach.** 1992. *Helicobacter muridarum* sp. nov., a microaerophilic helical bacterium with a novel ultrastructure isolated from the intestinal mucosa of rodents. Int. J. Syst. Bacteriol. **42:**27–36.
31. **Lopez, C., R. Owen, and M. Desai.** 1993. Differentiation between isolates of *Helicobacter pylori* by PCR-RFLP analysis of urease A and B genes and comparison with ribosomal RNA gene patterns. FEMS Microbiol. Lett. **110:** 37–43.
32. **Lopez, C. R., R. Owen, N. Banatvala, Y. Abdi, J. Hardie, G. Davies, and R. Feldman.** 1993. Comparison of urease gene primer sequences for PCR-based amplification assays in identifying the gastric pathogen *Helicobacter pylori*. Mol. Cell Probes **7:**439–446.
33. **Marshall, B., L. Barree, C. Prakash, R. McCallum, and R. Guervant.** 1990. Urea protects *Helicobacter pylori* from the bactericidal effects of acid. Gastroenterology **99:**697–702.
34. **Matsui, T., Y. Matsukawa, T. Sakai, K. Nakamura, A. Aoike, and K. Kawai.** 1995. Effects of ammonia on cell cycle progression of human gastric cancer cells. Eur. J. Gastroenterol. Hepatol. **7**(Suppl. 1)**:**S79–S81.
35. **Mobley, H.** 1996. The role of *Helicobacter pylori* urease in the pathogenesis of gastritis and peptic ulceration. Aliment. Pharmacol. Ther. **10:**57–64.
36. **Mobley, H., M. Island, and R. Hausinger.** 1995. Molecular biology of microbial ureases. Microbiol. Rev. **59:**451–480.
37. **Moore, R. A., A. Kureishi, S. Wong, and L. E. Bryan.** 1993. Categorization of clinical isolates of *Helicobacter pylori* on the basis of restriction digest analyses of polymerase chain reaction-amplified *ureC* genes. J. Clin. Microbiol. **31:**1334–1335.
38. **Nomura, A., G. N. Stemmerman, P. H. Chyou, I. Kato, G. I. Perez-Perez, and M. J. Blaser.** 1991. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. N. Engl. J. Med. **325:**1132–1136.
39. **Owen, R. J., J. Bickley, A. Hurtado, A. Fraser, and R. E. Pounder.** 1994. Comparison of PCR-based restriction length polymorphism analysis of urease genes with rRNA gene profiling for monitoring *Helicobacter pylori* infections in patients on triple therapy. J. Clin. Microbiol. **32:**1203–1210.
40. **Parsonnet, J., G. D. Friedman, D. P. Vandersteen, Y. Chang, J. H. Vogelman, N. Orentreich, and R. K. Sibley.** 1991. *Helicobacter pylori* infection and the risk of gastric carcinoma. N. Engl. J. Med. **325:**1127–1131.
41. **Rappuoli, R., A. Covacci, P. Ghiara, and J. Telford.** 1994. Pathogenesis of *Helicobacter pylori* and perspectives of vaccine development against an emerging pathogen. Behring Inst. Mitt. **95:**42–48.
42. **Saunders, K. E., K. J. McGovern, and J. G. Fox.** 1997. The use of pulsed-field gel electrophoresis to determine genomic diversity in strains of *Helicobacter hepaticus* from geographically distant locations. J. Clin. Microbiol. **35:** 2859–2863.
43. **Shames, B., J. G. Fox, F. E. Dewhirst, L. Yan, Z. Shen, and N. S. Taylor.** 1995. Identification of widespread *Helicobacter hepaticus* infection in feces in commercial mouse colonies by culture and PCR assay. J. Clin. Microbiol. **33:** 2968–2972.
44. **Solnick, J. V., C. Josenhans, S. Suerbaum, L. S. Tomkins, and A. Labigne.** 1995. Construction and characterization of an isogenic urease-negative mutant of *Helicobacter mustelae*. Infect. Immun. **63:**3718–3723.
45. **Solnick, J. V., J. O'Rourke, A. Lee, and L. S. Tompkins.** 1994. Molecular analysis of urease genes from a newly identified uncultured species of *Helicobacter*. Infect. Immun. **62:**1631–1638.
46. **Tonokatsu, Y., T. Hayashi, T. Mizuta, I. Yamamoto, Y. Fukuda, K. Tamura, and T. Tamura.** 1991. Restriction fragment length polymorphism of *Helicobacter pylori* using the urease gene probe. Gastroenterol. Jpn. **26:**788.
47. **Traumann, M., M. Moldrzyk, K. Vogt, J. Korber, T. Held, and R. Marre.** 1994. Use of a receiver operating characteristic in the evaluation of two commercial enzyme immunoassays for detection of *Helicobacter pylori* infection. Eur. J. Clin. Microbiol. Infect. Dis. **13:**812–819.
48. **Tsuda, M., T. Karata, T. Mizota, M. Morshed, K. Okita, and T. Nakazawa.** 1994. Essential role of *Helicobacter pylori* urease in gastric colonization: definite proof using a urease-negative mutant constructed by gene replacement. Eur. J. Gastroenterol. Hepatol. **6:**S49–S52.
49. **Tsuda, M., M. Karita, M. Morshed, K. Okira, and T. Nakazawa.** 1994. A urease-negative mutant of *Helicobacter pylori* constructed by allelic exchange mutagenesis lacks the ability to colonize the nude mouse stomach. Infect. Immun. **62:**3586–3589.
50. **Wang, J., J. Shew, J. Lin, T. Wang, and M. Wu.** 1993. Direct DNA amplification and restriction pattern analysis of *Helicobacter pylori* in patients with duodenal ulcer and their families. J. Infect. Dis. **168:**1544–1548.
51. **Ward, J. M., M. R. Anver, D. C. Haines, J. M. Melhorn, P. Gorelick, L. Yan, and J. G. Fox.** 1996. Inflammatory large bowel disease in immunodeficient mice naturally infected with *Helicobacter hepaticus*. Lab. Anim. Sci. **46:**15–20.
52. **Whary, M. T., T. Morgan, C. Dangler, K. Gaudes, N. Taylor, and J. G. Fox.** 1998. Chronic active hepatitis induced by *Helicobacter hepaticus* in the A/JCr mouse is associated with a Th1 cell-mediated immune response. Infect. Immun. **66:**3142–3148.