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Differentiation of *Helicobacter pylori* Strains Directly from Gastric Biopsy Specimens by PCR-Based Restriction Fragment Length Polymorphism Analysis without Culture

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Recent studies have shown the usefulness of PCR-based restriction fragment length polymorphism (RFLP) analysis for differentiating *Helicobacter pylori* strains isolated by culture. For this study, a PCR-based RFLP assay was developed for directly typing *H. pylori* strains from gastric biopsy specimens. Nineteen gastric biopsy specimens obtained from patients undergoing endoscopy for gastrointestinal complaints were cultured for isolation of *H. pylori*. Genomic DNA preparations from these gastric biopsy specimens and the corresponding *H. pylori* isolates were tested by our PCR-based RFLP assay. The 1,179-bp *H. pylori* DNA fragments amplified by the PCR assay were digested with the restriction enzymes *Hha*I, *Mbo*I, and *Alu*I and analyzed by agarose gel electrophoresis. *Hha*I, *Mbo*I, and *Alu*I digestion produced 11, 10, and 6 distinguishable digestion patterns, respectively, from the 19 *H. pylori* isolates tested and generated 13, 11, and 6 different patterns, respectively, from the 19 gastric biopsy specimens. The patterns from 13 of the 19 gastric biopsy specimens matched those of the *H. pylori* isolates from the corresponding patients. The patterns from the remaining six biopsy specimens appeared to represent infection by two strains of *H. pylori*; the pattern of one strain was identical to that of the isolate from the corresponding patient. By combining all the restriction enzyme digestion patterns obtained by using *Hha*I, *Mbo*I, and *Alu*I, we observed 19 distinct RFLP patterns from the 19 specimens. The results suggest that the PCR-based RFLP analysis method may be useful as a primary technique to identify and distinguish *H. pylori* strains directly from gastric biopsy specimens without culture of the organisms.

Helicobacter pylori is a well-recognized pathogen that causes active chronic gastritis in humans and plays a significant pathogenic role in the development and recurrence of gastric and duodenal ulcers (14). *H. pylori* infection may also be associated with gastric cancer (10) and primary gastric B-cell lymphoma (13). Epidemiologic studies have shown that *H. pylori* infection occurs worldwide, with a high prevalence rate. Recurrence of infection after apparent eradication has also been reported and is associated with the recurrence of ulcers. However, it is unclear whether the recurrence of ulcers following *H. pylori* eradication therapy is due to recrudescence of the previous infction or to exogenous reinfection by another strain. An accurate method for the detection and differentiation of *H. pylori* strains in patients, both before and after therapy, is therefore of great importance for diagnosis, monitoring of treatment, and reduction of the long-term consequences of continued but undetected disease.

Attempts to differentiate *H. pylori* isolates have been made by using a variety of conventional typing schemes, including hemagglutination (15); determination of enzymatic activity (24), cytotoxin activity (9), and plasmid profiles (29, 32); sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins (24); and immunoblotting (4). However, each of these schemes has been of little use for the precise differentiation of *H. pylori* strains. Although several nucleic acid techniques, such as the restriction endonuclease analysis of genomic DNA (6, 18, 23, 32) and Southern blot hybridization with rRNA gene probes (27), have been applied to type *H. pylori* clinical isolates from different patients, the patterns produced by these methods are complex and difficult to interpret, particularly for large-scale analyses of clinical isolates. Recently, a PCR-based restriction fragment length polymorphism (RFLP) analysis method has been developed for typing of *H. pylori* clinical isolates (1, 5, 7, 11, 12, 25). Schemes involving this method have been used to analyze *H. pylori* genes which encode urease and its accessory proteins, including the 2.4-kb *ureA-ureB* (1, 10), 1.7-kb *ureC-ureD* (1), 933-bp *ureB* (5), 1.1-kb *ureC* (25), and 820-bp *ureC* (12) genes. Randomly amplified polymorphic DNA has also been used to differentiate *H. pylori* strains (2). These data showed that PCR-based RFLP typing was a rapid and sensitive method, but these studies were limited to *H. pylori* isolates cultured from gastric biopsy specimens.

Here, we report for the first time a PCR-based RFLP analysis method developed to differentiate *H. pylori* strains directly from gastric biopsy specimens without culture of the organisms. To validate this approach, we compared the RFLP patterns of cultured *H. pylori* isolates with those of their corresponding gastric-biopsy specimens derived from 19 patients. The restriction enzymes *Hha*I, *Mbo*I, and *Alu*I generated 13, 11, and 6 different patterns, respectively, from the 19 gastric biopsy specimens. The patterns from 13 of the 19 gastric biopsy specimens matched those of *H. pylori* isolates from the corresponding patients. The patterns from the remaining six biopsy specimens appeared to represent infection by two strains of *H. pylori*; the pattern of one strain was identical to that of the isolate from the corresponding patient. Combining the three restriction enzyme digestion patterns produced 19 distinct RFLP patterns for the 19 patients tested. The results suggest

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that the PCR-based RFLP analysis protocol described here may be useful as both a primary method and a typing scheme to directly identify and distinguish *H. pylori* strains from gastric biopsy specimens without culturing the organisms.

MATERIALS AND METHODS

Bacterial strains. The type strain, ATCC 43629, was used as a positive control in this study. Nineteen clinical *H. pylori* strains were isolated from the gastric biopsy specimens of 19 patients (19, 20).

Twenty-two strains, including 5 *Helicobacter* species, 12 closely related non-*Helicobacter* species, and 4 urease-positive species not closely related to *H. pylori*, were used in specificity studies. They included *Helicobacter fennelliae* (ATCC 35684), *Helicobacter cinaedi* (clinical isolate), *Helicobacter heilmanii* (ATCC 49286), *Helicobacter felis* (ATCC 49179), *Helicobacter mustelae* (ATCC 43772), *Campylobacter concisus* (ATCC 33237), *Campylobacter sputorum* subsp. *sputorum* (ATCC 35980), *Campylobacter sputorum* subsp. *bubulus* (ATCC 33491), *Campylobacter jejuni* (two clinical isolates), *Campylobacter coli* (clinical isolate), *Campylobacter lari* (Skirrow, E152283), *Campylobacter upsaliensis* (clinical isolate), *Campylobacter fetus* subsp. *fetus* (clinical isolate), *Campylobacter rectus* (VPI 10278B), *Arcobacter cryaerophilus* (clinical isolate), *Wolinella* subsp. group 2 (VPI 10279), *Wolinella* subsp. group 3 (VPI 10296), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (clinical isolate), *Morganella morganii* (clinical isolate), and *Proteus mirabilis* (clinical isolate). All clinical isolates were obtained from the Clinical Microbiology Laboratory, Department of Microbiology, James H. Quillen College of Medicine, East Tennessee State University, and were isolated and identified by standard microbiologic procedures (8, 19, 26).

Gastric biopsy specimens. Gastric biopsy specimens were obtained from patients who underwent endoscopy for upper gastrointestinal complaints at the Veterans Affairs Medical Center, Mountain Home, Tenn. Informed consent approved by the Institutional Review Board of East Tennessee State University was obtained in all instances. Three gastric biopsy specimens were collected from similar locations in the antrum of the stomach from each patient. One piece of each specimen was histologically examined by modified Steiner (silver) staining for the detection of *H. pylori*. A second piece was used to culture for *H. pylori* as described previously (8, 19), and the remaining specimens were stored at −70°C for testing by PCR assay (20, 21).

Preparation of genomic DNA for PCR assay. Genomic DNA from *H. pylori* isolates and non-*H. pylori* strains was prepared from freshly harvested bacterial cells that were grown on 6% sheep blood agar and incubated for 1 to 4 days at 37°C under the optimal atmospheric conditions for each isolate (8, 19, 26). Genomic DNA from the frozen gastric biopsy specimens was prepared for PCR assay as described previously (20, 21). Briefly, each frozen gastric biopsy specimen was suspended in 100 μl of digestion buffer containing 10 μg of proteinase K and incubated at 55°C for 3 h. The nucleic acids were extracted with an equal volume of phenol-chloroform and precipitated with absolute ethanol. The DNA pellets were washed with 75% ethanol and finally resuspended in 50 to 100 μl of Tris-EDTA buffer. Human genomic DNA was prepared from leukocytes and used for specificity testing.

PCR amplification. The two 24-base oligonucleotides used as primers for the PCR assay were selected from published DNA sequences of the *ureC* gene in *H. pylori*, which encodes a required accessory protein for urease expression (16). The primers were designated ureC-U (5′-CCC TCA CGC CAT CAG TCC CAA AAA-3′, from −383 to −360 bp) and ureC-L (5′-AAG AAG TCA AAA ACG CCC CAA AAC-3′, from 763 to 786 bp) and synthesized at the School of Hygiene and Public Health, Johns Hopkins University, with an Eppendorf Synostat-D DNA synthesizer and purified by reverse-phase high-pressure liquid chromatography (HPLC) method. As predicted, the amplification product with these two primers for DNAs prepared from the type strain of *H. pylori*, ATCC 43629, was 1,179 nucleotide pairs in length and represented a portion of the *ureC* gene starting −386 nucleotides upstream from the AGT start codon and ending 786 nucleotides downstream from the AGT start codon.

Amplification was performed as described previously (20, 21) in 50-μl reaction mixture volumes containing 0.5 μM concentrations of each primer; 200 μM (each) dATP, dCTP, dTTP, and dGTP; 1× reaction buffer (50 mM KCl, 10 mM Tris-HCl [pH 8.3], 2.0 mM $MgCl_2$, and 0.001% [wt/vol] gelatin); 1.25 U of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, Conn.); and 2 μl of each DNA sample. Thirty cycles of amplification were performed in a DNA thermal cycler (GeneAmp 9600 system; Perkin-Elmer Cetus). Each cycle consisted of a 45-s denaturation step at 94°C, a 30-s annealing step at 59°C, and a 90-s extension step at 72°C. The final cycle included an extension step (10 min at 72°C) to ensure full extension of the product. A DNA preparation from the type strain was used as the positive control in each batch of PCR assays. For each batch of amplifications, a negative control tube containing distilled water in place of the DNA samples was also used.

Analysis of the PCR-amplified products. For identification of the amplified products, 10 μl of the PCR mixture was analyzed by electrophoresis on 1.5% agarose gels (SeaKem LE agarose; FMC Products, Rockland, Maine). The amplified fragments were then transferred from the agarose gel to a nylon membrane (Nytran; Schleicher & Schuell, Keene, N.H.) and hybridized with a [^{32}P]dCTP-labeled DNA probe which was amplified from a DNA preparation from the type strain, ATCC 43629, with the above-described primers. Southern blot hybridization was performed as described previously (19, 21).

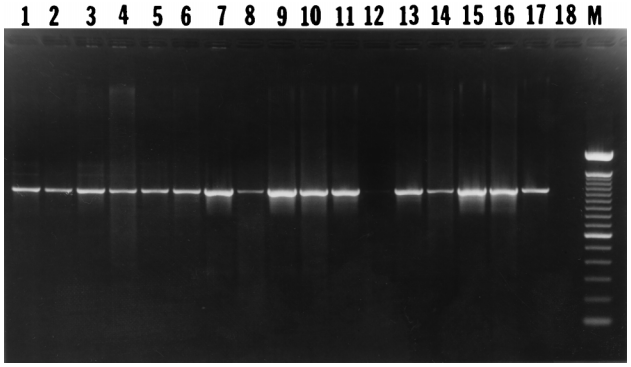

FIG. 1. Representative results for PCR-amplified 1,179-bp DNA fragments from cultured *H. pylori* isolates and their corresponding gastric biopsy samples. Lanes 1, 3, 5, 7, 9, 11, 13, and 15 are *H. pylori* isolates. Lanes 2, 4, 6, 8, 10, 12, 14, and 16 are the corresponding gastric biopsy specimens of the preceding odd-numbered lanes, respectively. Lane 17 is the *H. pylori* type strain, ATCC 43629. Lane 18 is a negative control, and lane M is a 100-bp ladder.

RFLP analysis. After amplification, the PCR products were precipitated with 2.5 volumes of ethanol (20). The pellets were washed twice with 75% ethanol and dissolved in Tris-EDTA buffer. The amplified DNA was then digested with the restriction enzymes *Hha*I, *Mbo*I, and *Alu*I in the appropriate buffered solution for 3 h at 37°C in the appropriate buffered solution recommended by the manufacturer (Promega). The digested DNA fragments were analyzed by electrophoresis on 3.5% agarose gels (MetaPhor agarose; FMC Products). The gels were examined by transillumination and photographed. The sizes of digested DNA fragments were estimated from migration distances of molecular weight standards.

RESULTS

Development of a specific PCR assay for *H. pylori*. The PCR assay successfully amplified a fragment of the expected 1,179 bp from a DNA preparation from the *H. pylori* type strain, ATCC 43629, with the ureC-U and ureC-L primers. When genomic DNAs prepared from the 19 *H. pylori* isolates and the corresponding gastric biopsy specimens, from which the *H. pylori* strains were isolated, were used as templates, the PCR assay yielded DNA fragments of the same molecular weights as those obtained with the DNA preparation from the *H. pylori* type strain, ATCC 43629 (Fig. 1). The presence of amplified *H. pylori* DNA in agarose gel electrophoresis was confirmed by Southern blot hybridization with a [^{32}P]dCTP-labeled DNA probe that was amplified from the type strain of *H. pylori.*

Human genomic DNA and 22 DNAs from 5 *Helicobacter* species (*H. mustelae*, *H. fennelliae*, *H. cinaedi*, *H. felis*, and *H. heilmanii*), 12 closely related non-*Helicobacter* species, and 4 urease-positive species not closely related to *H. pylori* were used to test the specificity of the PCR assay. All 22 of these non-*H. pylori* DNA preparations failed to amplify a product at the annealing temperature of 59°C when tested by agarose gel electrophoresis and Southern blot hybridization.

The sensitivity of the PCR assay has been tested by serial dilution of genomic DNA from *H. pylori* isolates and from gastric biopsy specimens. The 1,179-bp fragments were amplified by the PCR assay from a minimum of 0.1 pg of DNA from *H. pylori* (Fig. 2) and 2 ng of genomic DNA from gastric biopsy specimens. Since we chose the gastric biopsy specimen with the lowest density of PCR product (Fig. 1, lane 12) for the sensitivity test, the PCR assay could detect *H. pylori* DNA from a smaller amount of genomic DNA prepared from gastric biopsy specimens if the specimens contained a higher number of organisms.

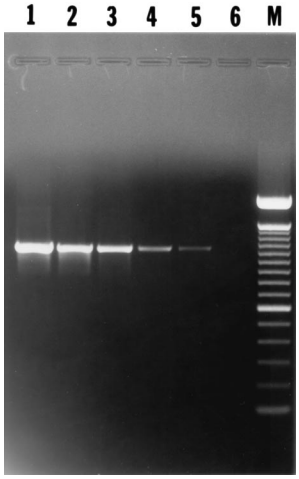

FIG. 2. The sensitivity of PCR for detection of *H. pylori* DNA was tested with serial dilutions of genomic DNA from the *H. pylori* type strain, ATCC 43629. Template DNAs in lanes 1 to 6 were in amounts of 1 ng and 100, 10, 1, 0.1, and 0.01 pg, respectively. Lane M is a 100-bp ladder.

RFLP analysis. In order to determine whether the PCR-based RFLP analysis method could differentiate *H. pylori* strains directly from gastric biopsy specimens without culture, the PCR assay was performed on genomic DNA preparations from the 19 *H. pylori* isolates and from their corresponding gastric biopsy specimens. The 1,179-bp fragments produced by PCR assay were further digested with three restriction enzymes, *Hha*I, *Mbo*I, and *Alu*I, and analyzed by agarose gel electrophoresis. When the PCR products from the 19 *H. pylori* isolates were subjected to RFLP analysis, it showed that 11, 10, and 6 distinguishable digestion patterns were generated by *Hha*I, *Mbo*I, and *Alu*I, respectively (Table 1). Combining the restriction enzyme digestion patterns obtained with *Hha*I, *Mbo*I, and *Alu*I, we observed 19 distinct RFLP patterns from the 19 *H. pylori* isolates tested (Table 2). However, when the PCR products from the 19 gastric biopsy specimens were digested by the restriction enzymes *Hha*I, *Mbo*I, and *Alu*I, we observed 13, 11, and 6 RFLP patterns, respectively. The patterns from 13 of the 19 gastric biopsy specimens matched those from the corresponding *H. pylori* isolates from each patient. The patterns from the remaining six biopsy specimens appeared to represent infections with two strains of *H. pylori*; the pattern of one of the strains was identical to that of the isolate from the corresponding patient. As shown in Table 2, gastric biopsy specimens from patients 3, 6, 7, 10, and 17 were probably colonized by two strains of *H. pylori*. With these five gastric biopsy specimens, each of the three restriction enzymes produced a combination pattern different from that of the isolate. Each combination pattern contained a pattern matching that from its corresponding isolate and a second pattern, presumably from another coinfecting strain. This suggests that the PCR-based RFLP assay can identify two, and maybe more, coinfecting *H. pylori* strains directly from gastric biopsy specimens. With the gastric biopsy specimen from patient 19, *Hha*I and *Alu*I generated combination RFLP patterns different from that of the isolate, while *Mbo*I generated a pattern identical to that of the corresponding isolate. For patient 2, *Alu*I digestion produced a combination RFLP pattern from the isolate but not from the gastric biopsy specimen. *Hha*I and *Mbo*I, however, generated identical patterns from the biopsy specimen and the corresponding isolate. Representative results of restriction enzyme digestion of PCR-amplified products from *H. pylori* isolates and their corresponding gastric biopsy specimens from each patient are shown in Fig. 3.

TABLE 1. RFLPs produced by restriction enzyme (*Hha*I, *Mbo*I, and *Alu*I) digestion of the PCR products amplified from 19 clinical isolates

Enzyme	RFLP patterns (frequencies)
*Hha*I	H1 (1), H2 (3), H3 (1), H4 (3), H5 (2), H6 (1), H7 (3), H8 (2), H9 (1), H10 (1), H11 (1)
*Mbo*I	M1 (4), M2 (4), M3 (1), M4 (1), M5 (1), M6 (2), M7 (3), M8 (1), M9 (1), M10 (1)
*Alu*I	A1 (3), A2 (7), A3 (6), A4 (1), A5 (1), A6 (1)

TABLE 2. Typing of 19 *H. pylori* clinical isolates and their corresponding biopsy specimens by PCR-based RFLP analysis

Patient	RFLP pattern(s)					
	*Hha*I		*Mbo*I		*Alu*I	
	Isolate	Biopsy specimen	Isolate	Biopsy specimen	Isolate	Biopsy specimen
1	H1	H1	M1	M1	A1	A1
2	H4	H4	M1	M1	A6, A5	A6
3	H2	H2, H13	M2	M2, M1	A2	A2, A3
4	H3	H3	M3	M3	A1	A1
5	H7	H7	M10	M10	A1	A1
6	H4	H4, H1	M5	M5, M7	A2	A2, A3
7	H5	H5, H12	M6	M6, M11	A3	A3, A2
8	H11	H11	M2	M2	A2	A2
9	H8	H8	M7	M7	A3	A3
10	H5	H5, H12	M6	M6, M11	A2	A2, A3
11	H6	H6	M2	M2	A2	A2
12	H7	H7	M7	M7	A3	A3
13	H2	H2	M1	M1	A3	A3
14	H8	H8	M8	M8	A2	A2
15	H9	H9	M7	M7	A2	A2
16	H4	H4	M2	M2	A3	A3
17	H10	H10, H5	M4	M4, M7	A4	A4, A3
18	H7	H7	M9	M9	A3	A3
19	H2	H2, H13	M1	M1	A5	A5, A6

DISCUSSION

In the present study, we successfully developed a PCR-based RFLP assay for the differentiation of *H. pylori* strains directly from gastric biopsy specimens without culture. By using the newly developed PCR-based RFLP assay, we have observed that 13, 11, and 6 distinct patterns were generated by three restriction enzymes, *Hha*I, *Mbo*I, and *Alu*I, respectively, from 19 gastric biopsy specimens. The RFLP patterns from 13 of the 19 gastric biopsy specimens were found to be identical to those of the *H. pylori* isolates from the corresponding patients. The combination RFLP patterns from the remaining six gastric biopsy specimens suggested that two strains of *H. pylori* had colonized the stomachs of these six patients, with one of the two biopsy specimen patterns always matching that from its corresponding isolate. This suggests that the PCR-based RFLP assay may be useful as a primary approach for the identification of specific *H. pylori* strains in gastric biopsy specimens without culture.

Several PCR-based RFLP analysis protocols have been proposed for the differentiation of *H. pylori* strains from clinical isolates (5, 11, 12, 22, 25). Primer pairs for detecting and typing *H. pylori*, based on amplifying the urease genes *ureA* (5), *ureA*

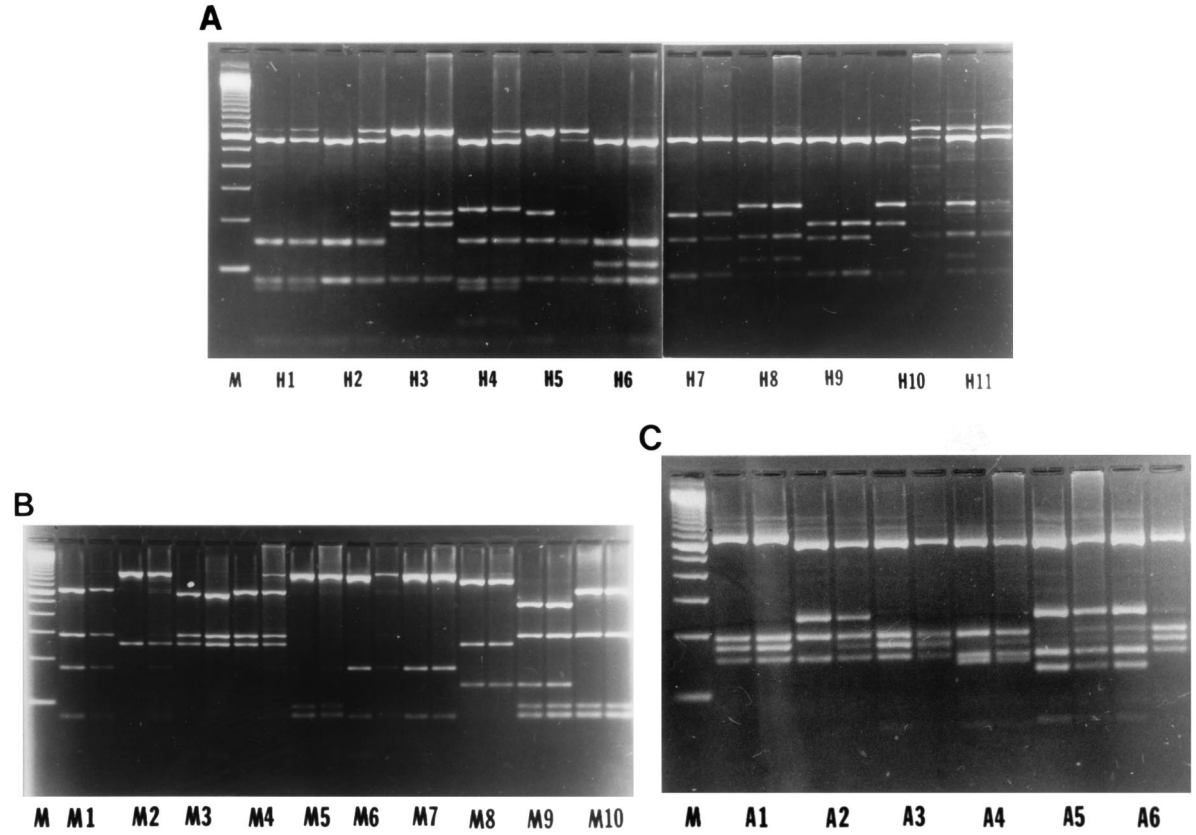

FIG. 3. PCR-amplified 1,179-bp DNA fragments from cultured *H. pylori* isolates and their corresponding gastric biopsy specimens digested with the restriction enzymes *Hha*I (A), *Mbo*I (B), and *Alu*I (C). Eleven (H1 to H11), ten (M1 to M10), and six (A1 to A6) distinguishable digestion patterns were obtained from the 19 *H. pylori* isolates and their corresponding gastric biopsy specimens. The left lane of each pair is an *H. pylori* isolate and the right lane is the corresponding gastric biopsy specimen. Lane M is a 100-bp DNA ladder.

plus *ureB* (11), and *ureC* (12, 25), have been described. These results have demonstrated a great diversity of urease genes among *H. pylori* clinical isolates. Foxall et al. (11) found that 10 distinct patterns were produced from 22 clinical isolates when the restriction enzyme *Hae*III digested the 2.4-kb PCR products amplified from the *ureA* and *ureB* genes. Lopez et al. (22) reported that the patterns generated by *Hae*III digestion of PCR products from *ureA-ureB* were almost as discriminatory as *Hae*III ribopatterns. Akopyanz et al. (1) demonstrated that eight *Mbo*I and 27 *Hae*III RFLP patterns were obtained from the 2.4-kb PCR products amplified from the *ureA-ureB* genes from 60 *H. pylori* strains and that the patterns distinguished 44 separate groups. Each isolate not distinguished from the others by RFLP analysis of *ureA-ureB* products was distinguished by *Mbo*I digestion of a 1.7-kb *ureC-ureD* segment, indicating that PCR-based RFLP analysis of the *ureC* gene may produce a greater number of RFLP patterns. Recently, Romero-Lopez et al. (31) compared urease gene primers for PCR-based RFLP assays of *H. pylori*. The data suggested that each pair of primers from urease genes was highly specific but that the sensitivity of each PCR primer pair was significantly affected by using unpurified target DNA in the assay, with detection rates varying from 0% (*ureA* plus *ureB*) to 55% (*ureA*) to 87.5% (*ureC*). Romero-Lopez et al. concluded that the smaller nucleotide product of the *ureC* primer assay was less susceptible to inhibition by a compound(s), as yet unidentified, present in heated cell lysates and that the *ureC* primer assay thus was the best of the urease gene PCR assays for the detection of *H. pylori* in clinical specimens. However, the sequence diversity of the *ureC* gene may result in some *H. pylori* organisms remaining undetected (3).

In the present study, a PCR assay employing two 24-bp primers derived from the *ureC* gene sequence (16) successfully amplified 1,179-bp fragments from 19 clinical isolates and their corresponding gastric biopsy specimens but not from human genomic DNA or from 22 DNA preparations from 5 *Helicobacter* species (*H. mustelae*, *H. fennelliae*, *H. cinaedi*, *H. felis*, and *H. heilmanii*), 12 closely related non-*Helicobacter* species, or 4 urease-positive species not closely related to *H. pylori*. The specificity of PCR with *ureC* gene primers has also been previously reported by Lage et al., who tested 25 bacterial strains from closely related genera and from various urease-positive bacterial species which occasionally occur as contaminants in gastric biopsy specimens (17). The expected PCR products were obtained only from *H. pylori* and not from other species. Bickley et al. (3) also reported that the PCR assay with *ureC* primers did not amplify the expected products from strains of *H. mustelae*, *H. cinaedi*, *H. nemestrinae*, *H. fennelliae*, or *H. felis*. Moore et al. (25) were unable to obtain PCR amplification products when genomic DNAs from several urease-positive, gram-negative species were used as templates. Together, these data suggest that the *ureC* gene is an excellent target for PCR assay of *H. pylori.*

Several studies have confirmed that PCR-based RFLP analysis of the *ureC* gene can differentiate *H. pylori* clinical isolates. Moore et al. (25) reported restriction endonuclease analyses of

a 1.1-kb PCR-amplified portion of the *ureC* gene from 21 *H. pylori* clinical isolates. The isolates were placed into four groups after *Hin*dIII digestion alone, while the strains were divided into 15 groups after being digested with *Alu*I and *Pvu*I. Fujimoto et al. (12) have shown that digestion of an 820-bp PCR-amplified portion of the *ureC* gene in *H. pylori* by the restriction enzymes *Hha*I, *Mbo*I, and *Mse*I resulted in 10, 10, and 11 different patterns, respectively. The 25 *H. pylori* clinical isolates, however, could be grouped by the 25 distinct RFLP patterns produced when the three digestion patterns were combined. In our study, we used three types of restriction enzyme analyses of the 1,179-bp PCR product amplified from a portion of the *ureC* gene of *H. pylori*. Although 11, 10, and 6 digestion patterns were generated by *Hha*I, *Mbo*I, and *Alu*I, respectively, 19 distinct RFLP patterns were observed among the 19 *H. pylori* clinical isolates when a combination of the three RFLP patterns was employed (Table 2). Our data further suggest that PCR-based RFLP analysis of a portion of the *ureC* gene of *H. pylori* is a reliable scheme for differentiating *H. pylori* isolates.

Although several PCR-based RFLP protocols have been developed for the differentiation of *H. pylori* strains, they tested only *H. pylori* isolates from gastric biopsy specimens. Culture of *H. pylori* is time-consuming and not very sensitive. Our PCR-based RFLP protocol provides a means for the direct detection and differentiation of *H. pylori* strains in gastric biopsy specimens without culture. The combination RFLP patterns generated from the gastric biopsy specimens by the three restriction enzymes suggest the presence of two infecting strains of *H. pylori* in the stomachs of six patients. It has been previously reported that two strains of *H. pylori* have been found in the stomachs of some patients (12, 28, 30). The results presented here suggest that the protocol may be useful for epidemiologic studies of *H. pylori* infections and for monitoring patients before and after eradication therapy to determine whether recurrence of ulcers following *H. pylori* eradication therapy is due to recrudescence or reinfection.

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