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A Highly Specific and Sensitive DNA Probe Derived from Chromosomal DNA of *Helicobacter pylori* Is Useful for Typing *H. pylori* Isolates

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_Hin_dIII-digested DNA fragments derived from an _Eco_RI-digested 6.5-kb fragment of chromosomal DNA prepared from _Helicobacter pylori_ ATCC 43629 (type strain) were cloned into the pUC19 vector. A 0.86-kb insert was identified as a potential chromosomal DNA probe. The specificity of the probe was evaluated by testing 166 non-_H. pylori_ bacterial strains representing 38 genera and 91 species which included aerobic, anaerobic, and microaerophilic flora of the upper and lower gastrointestinal tracts. None of the 166 non-_H. pylori_ strains hybridized with this probe (100% specificity), and the sensitivity of this probe was also 100% when _H. pylori_ isolates from 72 patients with gastritis and with the homologous ATCC type strain were tested by dot blot hybridization. The capability of this probe for differentiating between strains of _H. pylori_ was evaluated by Southern blot hybridization of _Hae_III-digested chromosomal DNA from 68 clinical isolates and the homologous ATCC type strain of _H. pylori_. Fifty-one unique hybridization patterns were seen among the 69 strains tested, demonstrating considerable genotypic variation among _H. pylori_ clinical isolates. We propose that this probe would be of significant value for conducting epidemiologic studies.

Helicobacter pylori is now recognized as the most common cause of active chronic gastritis in humans. It probably plays a significant pathogenic role in the development and recurrence of gastric and duodenal ulcers (6, 12, 17, 34). Recently, several lines of evidence have suggested that *H. pylori* infection may be associated with gastric cancer (7, 8, 26). However, little is known about the route of transmission of *H. pylori* and other aspects of its epidemiology. Evidence that humans are the probable reservoir of these infections include the recovery of viable *H. pylori* from the dental plaque of one patient (30), the detection of *H. pylori* DNA in human saliva by polymerase chain reaction (13), and the isolation of the same strain from several members of a family (4, 15). Strains similar but not identical to *H. pylori* have been recovered from the pig, baboon, and various species of monkeys. Recently, *Helicobacter mustelae* has been isolated from the feces of ferrets (9). Documentation of the route of transmission of *H. pylori* has been hindered by the lack of a reliable typing method. Because many patients have a relapse soon after apparently successful antibacterial treatment, it is important to differentiate between reinfection by the same organism and infection by a new organism. Unlike most long-established bacterial pathogens, typing schemes such as biotyping, serotyping, or phage typing have been of little use for the precise identification of individual strains of *H. pylori* (5, 23). Fingerprinting based on DNA patterns and ribotypes has shown that there is considerable genomic diversity among *H. pylori* isolates (19, 22, 24). The genomic heterogeneity of *H. pylori* isolates from different patients has been reported from the United Kingdom, The Netherlands, Australia, and Canada (16, 18, 21, 31). A reliable method of typing *H. pylori* is required to investigate the source of the infection. This is the first report of the use of a cloned *H. pylori* chromosomal DNA fragment as a probe to identify strain variations among *H. pylori* isolates.

MATERIALS AND METHODS

Source of bacterial strains and growth conditions. The *H. pylori* strains used in the present study included the American Type Culture Collection (ATCC) type strain (ATCC 43629) and 72 *H. pylori* isolates from human gastric biopsy specimens. The biopsy specimens for culture were obtained from the antrum of the stomach. The specimens were initially inoculated onto an *H. pylori* selective medium (Belo-Horizonte medium) (27) and a nonselective sheep blood agar plate. After incubation under microaerophilic conditions at 37°C for 5 days and an additional 3 days if necessary, suspect colonies that exhibited typical colonial morphology were subcultured and identified as *H. pylori* on the basis of Gram stain morphology and by the oxidase, catalase, and rapid urease tests (1). The growth derived from several colonies was stored at −80°C in aqueous methylcellulose (32).

Fourteen bacterial species closely related to *H. pylori* were used in specificity studies. They included *Helicobacter mustelae* ATCC 43772, *Helicobacter fennelliae* ATCC 35684, *Campylobacter concisus* ATCC 33237, *Campylobacter sputorum* subsp. *sputorum* ATCC 35980, *Campylobacter sputorum* subsp. *bubulus* ATCC 33491, *Campylobacter jejuni*, *Campylobacter coli* (clinical isolates), *Campylobacter laridis* (Skirrow, E152283), *Campylobacter upsaliensis*, *Campylobacter cryaerophila*, *Campylobacter fetus* subsp. *fetus*, *Campylobacter cinaedi*, *Campylobacter* sp. clinical isolates, and *Wolinella succinogenes* ATCC 29543. Another 150 bacterial strains representing 77 different species were used to assess the specificity of the probe for *H. pylori*

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TABLE 1. Reference bacteria tested by dot blot hybridization with the ^{32}P-labeled cloned probe

Organism	No. of isolates tested[a]	Source information[b]
Helicobacter mustelae	1	Type strain, ATCC 43772
Helicobacter fennelliae	1	Type strain, ATCC 35684
Acinetobacter anitratus	1	Clinical isolate, ETSU
Acinetobacter lwoffii	1	Clinical isolate, ETSU
Aerococcus viridans	1	Clinical isolate, ETSU
Alcaligenes faecalis	2	Clinical isolate, ETSU
Alcaligenes oderans	1	Clinical isolate, ETSU
Alcaligenes xylosoxidans subsp. *xylosoxydans*	2	Clinical isolate, ETSU
Bacillus brevis	1	Clinical isolate, ETSU
Bacillus cereus	3	Clinical isolate, ETSU
Bacteroides asaccharolyticus	1	Type strain, ATCC 25260
Bacteroides bivius	1	Type strain, ATCC 29303
Bacteroides disiens	1	Type strain, ATCC 29426
Bacteroides distasonis	1	Type strain, ATCC 8503
Bacteroides fragilis	1	Clinical isolate, ETSU
Bacteroides gingivalis	1	Type strain, ATCC 33277
Bacteroides intermedius	1	Type strain, ATCC 25611
Bacteroides melaninogenicus	1	Type strain, ATCC 24845
Bacteroides splanchnicus	1	Type strain, ATCC 29572
Bacteroides thetaiotaomicron	1	Type strain, ATCC 29148
Bacteroides sp. (new species)	1	Clinical isolate, ETSU
Bordetella bronchiseptica	1	Clinical isolate, ETSU
Bordetella parapertussis	1	Clinical isolate, ETSU
Campylobacter sp.	1	Clinical isolate, ETSU
Campylobacter cinaedi	1	Clinical isolate, ETSU
Campylobacter coli	1	Clinical isolate, ETSU
Campylobacter concisus	1	Type strain, ATCC 33237
Campylobacter cryaerophila	1	Clinical isolate, ETSU
Campylobacter fetus subsp. *fetus*	1	Clinical isolate, ETSU
Campylobacter jejuni	3	Clinical isolate, ETSU
Campylobacter laridis	1	Skirrow, E152283
Campylobacter sputorum subsp. *bubulus*	1	Type strain, ATCC 33491
Campylobacter sputorum subsp. *sputorum*	1	Type strain, ATCC 35980
Campylobacter upsaliensis	1	Clinical isolate, ETSU
Capnocytophaga sp.	1	Clinical isolate, ETSU
Capnocytophaga sputigena	1	Clinical isolate, ETSU
Cardiobacterium hominis	1	Clinical isolate, ETSU
Citrobacter diversus	1	Clinical isolate, ETSU
Citrobacter freundii	2	Clinical isolate, ETSU
Clostridium perfringens	1	Clinical isolate, ETSU
Corynebacterium diphtheriae	1	Clinical isolate, ETSU
Enterobacter aerogenes	4	Clinical isolate, ETSU
Enterobacter agglomerans	2	Clinical isolate, ETSU
Enterobacter cloacae	6	Clinical isolate, ETSU
Enterococcus faecalis	3	Clinical isolate, ETSU
Enterococcus casseliflavus	2	Clinical isolate, ETSU
Escherichia adecarboxylata	1	Clinical isolate, ETSU
Escherichia coli	1	FDA strain ATCC 25922
Escherichia coli	7	Clinical isolate, ETSU
Fusobacterium necrophorum	1	Type strain, ATCC 25286
Fusobacterium nucleatum	1	Type strain, ATCC 25586
Gardnerella vaginalis	1	Clinical isolate, ETSU
Hafnia alvei	1	Clinical isolate, ETSU
Haemophilus aphrophilus	1	Clinical isolate, ETSU
Haemophilus influenzae	3	Clinical isolate, ETSU
Haemophilus paraphrophilus	1	Clinical isolate, ETSU
Haemophilus parainfluenzae	1	Clinical isolate, ETSU
Klebsiella pneumoniae	7	Clinical isolate, ETSU
Listeria monocytogenes	1	Clinical isolate, ETSU
Micrococcus luteus	2	Clinical isolate, ETSU
Moraxella catarrhalis	2	Clinical isolate, ETSU
Morganella morganii	4	Clinical isolate, ETSU
Neisseria lactamica	1	Clinical isolate, ETSU
Neisseria sicca	1	Clinical isolate, ETSU
Neisseria subflava	1	Clinical isolate, ETSU
Oligella ureolytica	1	Clinical isolate, ETSU
Pasteurella multocida	2	Clinical isolate, ETSU
Pasteurella pneumotropica	1	Clinical isolate, ETSU

Continued on following page

TABLE 1—*Continued*

Organism	No. of isolates tested[a]	Source information[b]
Plesiomonas shigelloides	1	Clinical isolate, ETSU
Proteus mirabilis	4	Clinical isolate, ETSU
Providencia rettgeri	3	Clinical isolate, ETSU
Pseudomonas acidovorans	1	Clinical isolate, ETSU
Pseudomonas aeruginosa	1	MIC strain, ATCC 27853
Pseudomonas aeruginosa	11	Clinical isolate, ETSU
Pseudomonas fluorescens	4	Clinical isolate, ETSU
Pseudomonas putida	2	Clinical isolate, ETSU
Rhodococcus sp.	1	Clinical isolate, ETSU
Serratia liquefaciens	2	Clinical isolate, ETSU
Serratia marcescens	1	Clinical isolate, ETSU
Staphylococcus aureus	5	Clinical isolate, ETSU
Staphylococcus epidermidis	4	Clinical isolate, ETSU
Staphylococcus intermedius	1	Clinical isolate, ETSU
Staphylococcus simulans	1	Clinical isolate, ETSU
Staphylococcus warneri	1	Clinical isolate, ETSU
Streptococcus agalactiae	2	Clinical isolate, ETSU
Streptococcus sp. group G	1	Clinical isolate, ETSU
Streptococcus milleri I	1	Clinical isolate, ETSU
Streptococcus mitis	1	Clinical isolate, ETSU
Streptococcus pneumoniae	2	Clinical isolate, ETSU
Streptococcus pyogenes	1	Clinical isolate, ETSU
Streptococcus salivarius	1	Clinical isolate, ETSU
Wolinella succinogenes	1	ATCC 29543
Xanthomonas maltophilia	6	Clinical isolate, ETSU

[a] A total of 166 strains representing 38 genera and 91 species were tested.
[b] ETSU, East Tennessee State University; FDA, U.S. Food and Drug Administration.

(Table 1). The strains 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 (1).

Preparation of genomic DNA. Genomic DNAs 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. The cells were scraped and washed twice by centrifugation in phosphate-buffered saline (pH 7.2) and twice in TE buffer (10 mM Tris hydrochloride, 1 mM sodium EDTA [pH 8.0]). The pellet was suspended in 0.5 ml of 50 mM Tris · HCl (pH 8.0)–0.25 M EDTA (pH 8.0)–1% sodium lauryl sarcosine–50 μg of RNase A (bovine pancreas type XII-A; Sigma), and the mixture was incubated for 30 min at 37°C. Proteinase K (final concentration, 100 μg/ml) was then added, and the lysate was incubated either for 3 h at 56°C or overnight at 37°C. DNA was extracted with an equal volume of phenol-chloroform (1:1; vol/vol), precipitated with 0.3 M sodium acetate and 2.5 volumes of absolute ethanol, and harvested by centrifugation at 16,000 × *g* for 15 min at 4°C. The pellet was dissolved in TE buffer. Human genomic DNA was prepared from leukocytes as described previously (29). The concentration and quality of DNA preparations were determined spectrophotometrically by measuring absorbance at A_{260} and A_{280} and by agarose gel electrophoresis. The DNA preparations were stored at −20°C.

Development of the *H. pylori* DNA probe. The *Hin*dIII-digested DNA fragments derived from an *Eco*RI-digested 6.5-kb DNA from *H. pylori* ATCC 43629 (type strain) were cloned into the pUC19 plasmid vector which was digested with *Hin*dIII. One (0.86-kb insert) clone was selected as the potential chromosomal DNA probe. The DNA insert was separated from the plasmid vector by digestion with *Hin*dIII, electrophoresed on a 2.0% agarose gel, and recovered on a DEAE-cellulose membrane (Nytran, NA-45; Schleicher & Schuell, Inc., Keene, N.H.) (29).

Testing the sensitivity and specificity of the probe. The sensitivity and specificity of the 0.86-kb DNA fragment used as a probe for *H. pylori* were tested by dot blot hybridization. Seventy-three *H. pylori* isolates and 166 non-*H. pylori* strains (representing 38 genera and 91 species and including aerobic, anaerobic, and microaerophilic flora of the upper and lower gastrointestinal tracts) were tested. DNAs prepared from *H. pylori* and non-*H. pylori* bacteria and human leukocytes were spotted onto nylon filters (Nytran; Schleicher & Schuell, Inc.) by using the Bio-Dot apparatus (GIBCO Bethesda Research Laboratories, Gaithersburg, Md.). One hundred nanograms of DNA from each *H. pylori* isolate, 500 ng from each non-*H. pylori* strain, and human genomic DNA were blotted. The DNA was fixed and denatured by the method of Sambrook et al. (29). The probe was labeled with [^{32}P]dCTP (Amersham, Arlington Heights, Ill.) by using the Prime-a-Gene labeling system (Promega, Madison, Wis.) to a specific activity of 1×10^9 to 2×10^9 cpm/μg of DNA. The filters were prehybridized at 68°C for 0.5 to 1 h in 0.5 M $NaPO_4$–7% sodium dodecyl sulfate (SDS)–1 mM EDTA and were hybridized overnight at 68°C in prehybridization solution plus the ^{32}P-labeled DNA probe. After hybridization, the filters were washed twice for 15 min each time in 1 mM EDTA–40 mM $NaPO_4$ (pH 7.2)–5% SDS at room temperature and then three times in 1 mM EDTA–40 mM $NaPO_4$ (pH 7.2)–1% SDS at 65°C. The filters were exposed to Kodak X-Omat XAR-5 film with intensifying screens for up to 7 days at −70°C.

Evaluation of the probe for typing *H. pylori*. The genomic DNAs from *H. pylori* strains were digested with *Hae*III overnight at 37°C (in accordance with the instructions of

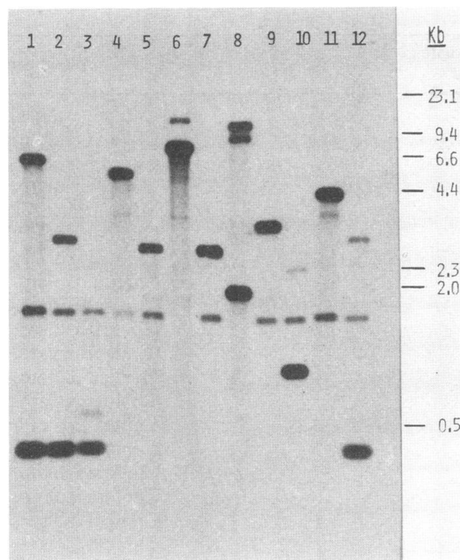

FIG. 1. Hybridization patterns for *Hae*III digests of genomic DNAs from *H. pylori* clinical isolates probed with a [^{32}P]dCTP–0.86-kb DNA probe. Lane 1, *H. pylori* type strain ATCC 43629; Lanes 2 to 12, *H. pylori* clinical isolates.

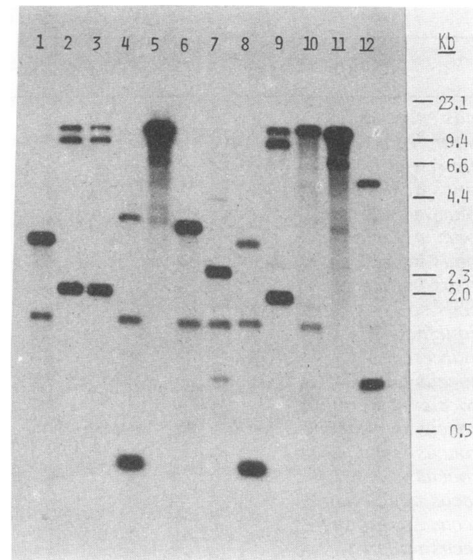

FIG. 2. Hybridization patterns for *Hae*III digests of genomic DNA from *H. pylori* clinical isolates probed with a [^{32}P]dCTP–0.86-kb DNA probe; lanes 2 and 3, *H. pylori* strains isolated from gastric biopsy and saliva of the same patient, respectively; lanes 1 and 4 to 12, *H. pylori* clinical isolates.

Promega). The DNA fragments were electrophoresed on 0.8% agarose gels at 30 V for 18 h in TAE buffer (40 mM Tris-acetate, 1 mM EDTA) and were transferred to nylon filters for Southern blot analysis (29). The hybridization was performed under the conditions mentioned above. Following this, the probe was removed from the complementary sequences on the filters by washing the filters for 60 min at 65°C in 50% formamide–2× SSEP (29). The same filters were rehybridized with the pUC19 plasmid DNA which was labeled with [^{32}P]dCTP by the Prime-a-Gene labeling system (Promega).

RESULTS

Sensitivity and specificity. By dot blot hybridization, the 0.86-kb probe detected all 73 *H. pylori* isolates, which indicates a sensitivity of 100%. The probe did not hybridize with any of the 166 non-*H. pylori* clinical isolates representing 38 genera and 91 species or with human genomic DNA and therefore demonstrated 100% specificity.

Typing. The ability of the probe to type *H. pylori* isolates was evaluated by Southern blot hybridization. Restriction endonuclease *Hae*III digestion of the genomic DNAs from the *H. pylori* isolates yielded clear and distinct bands in most cases. However, DNA preparations from four *H. pylori* isolates were not cleaved into detectable fragments by the *Hae*III restriction enzyme. In the remaining 69 preparations, Southern blot hybridization demonstrated heterogeneity among the clinical isolates (Fig. 1 and 2). The majority of the *H. pylori* isolates had distinct DNA hybridization patterns, with three to five strong bands at sizes of between 0.46 and 15.0 kb. Some strains also showed several intermediate or weak bands. The most homologous sequence corresponded to a single fragment of approximately 1.6 kb which appeared in 75% (52 of 69) of *H. pylori* isolates (Fig. 1 and 2). Hybridization to a 0.46-kb fragment was observed in DNA from 36% (25 of 69) of *H. pylori* DNA preparations (Fig. 1 and 2). Fifty-one distinct hybridization patterns were produced from the 69 *H. pylori* isolates that hybridized with this probe after digestion with restriction enzyme *Hae*III. Twenty-seven isolates shared identical hybridization patterns with at least one other strain and were grouped into nine typing patterns as shown in Table 2. The remaining 42 *H. pylori* isolates (62%) had hybridization patterns which were different from those of all other isolates tested. No hybridization was observed when the same filters were rehybridized with the [^{32}P]dCTP-pUC19 plasmid DNA, which confirmed that the signals that were seen with the cloned *H. pylori* probe were due to hybridization of the homologous sequences of *H. pylori* and not to homology with vector sequences.

DISCUSSION

To increase the sensitivity of detection of *H. pylori* isolates, a number of ^{32}P-labeled and nonradioactive genomic DNA probes from *H. pylori* have been applied and evaluated in both dot blot (39) and in situ (38) hybridizations. A specific oligoprobe homologous to 16S rRNA sequences has been developed for the detection of *H. pylori* isolates (20). Alternatively, a 17-kb DNA fragment was selected as a

TABLE 2. DNA hybridization patterns shared by more than one isolate

Hybridization pattern[a]	No. of isolates with the same pattern	Hybridization band sizes (kb)
R3	8	13.6, 10.1, 2.1
R30	2	4.2, 2.1
R32	2	4.2, 1.6
R37	3	3.5, 1.6
R45	2	3.2, 1.6, 0.46
R49	3	2.6, 1.6, 1.0
R50	3	2.6, 1.6
R51	2	2.1, 1.6, 0.46
R52	2	1.6, 0.64, 0.46

[a] Laboratory-designated patterns.

DNA probe from cloned *H. pylori* genomic DNA in *Escherichia coli* K-12 (3). The sensitivity of detection was further improved by several protocols that use the polymerase chain reaction (PCR); these included the use of a DNA sequence analysis of a cryptic fragment cloned from the *H. pylori* genome (37), the sequences of the 16S rRNA gene (14) and the urease gene (2), and a species-specific antigen of *H. pylori* (13). However, large clinical epidemiologic studies with these protocols have not been published, and a full evaluation of the sensitivity and specificity of these DNA probes for *H. pylori* has yet to be performed.

The probe described in this report was developed from the chromosomal DNA of the *H. pylori* type strain ATCC 43629. Initially, a 6.5-kb DNA fragment derived from *Eco*RI-digested chromosomal DNA from *H. pylori* ATCC 43629 detected eight of eight *H. pylori* strains (sensitivity, 100%) and yielded a specificity of 95.7% when tested against 23 other bacterial species. The 6.5-kb DNA fragment hybridized very weakly with *C. concisus* (data not shown). In order to increase the specificity, the 6.5-kb DNA fragment was digested with *Hin*dIII and cloned into the pUC19 plasmid vector, which was digested with *Hin*dIII, and a 0.86-kb insert was selected as a probe. This 0.86-kb probe hybridized with genomic DNAs from 73 *H. pylori* isolates by dot blot hybridization. None of the 166 DNA preparations from non-*H. pylori* bacterial isolates (frequently found in mouth and stool, representing 38 genera and 91 species including *H. mustelae*, *H. fennelliae*, *H. cinaedi*, *C. jejuni*, *C. cinaedi*, *C. sputorum* subsp. *sputorum*, and *W. succinogenes*) hybridized with this probe. It has been shown (28, 36) previously that the most closely related species are *W. succinogenes*, *H. fennelliae*, and *C. cinaedi* and that the true campylobacters are genetically more distantly related. *H. mustelae* was included in the same genus as *H. pylori* on the basis of the similarity of five major taxonomic features (11), but the DNA-DNA hybridization method revealed that it is a separate species. The 0.86-kb DNA fragment that we cloned is derived from sequences which appear to be unique to *H. pylori*; this property allows its application as a specific probe.

Typing of *H. pylori* isolates is of importance in studying the epidemiology of infection by this organism and in investigating the route of transmission and relapse. DNA fingerprints based on restriction endonuclease digestion patterns provide a sensitive and reproducible method of identifying strains of *H. pylori*. The genomic variation of *H. pylori* isolates was initially demonstrated by Langenberg et al. (16) by using restriction endonuclease analysis with *Hin*dIII digests. Majewski and Goodwin (18) found similar strain variation in *Hin*dIII digests of DNA from *H. pylori* isolates, but observed different digestion patterns with time in consecutive isolates from the same patient following treatment and suggested that a typing scheme by means of restriction endonuclease analysis may not be a suitable method for epidemiologic studies. In addition, the DNA digestion patterns produced by restriction endonucleases are too complex and *H. pylori* strains are too diverse to allow this method to be used for typing large numbers of *H. pylori* isolates, although it has been shown to be useful for typing a small number of strains in comparative studies (16, 18, 21, 31).

Ribotyping based on rRNA gene restriction patterns comprises fewer, more discrete bands and offers greater potential as a means of typing isolates of *H. pylori*. Morgan and Owen (19) studied a small sample of *H. pylori* isolates by using a biotinylated *E. coli* 16S plus 23 rRNA probe. Tee et al. (35) reported that 77 ribotypes were obtained from *Hin*dIII digestion of 126 strains of *H. pylori* from 100 unrelated symptomatic patients by using the plasmid pKK 3535 probe, which encodes the 5S, 16S, and 23S rRNAs and the tRNA^{2GLU} genes of *E. coli*. More recently, however, Owen et al. (25) used the computer-assisted numerical analysis of *Hae*III ribopatterns of 122 strains from nine countries on four continents and suggested that the *Hae*III ribopatterns were too discriminatory for large-scale epidemiologic typing purposes because no rational basis for grouping the strains was evident. More recently, Foxall et al. (10) used the PCR technique to amplify the urease structural subunit genes *ureA* and *ureB*. *Hae*III digestion of PCR-amplified 2.4-kb products from 22 clinical isolates produced 10 distinct restriction patterns and 2 patterns shared between five and six strains. They proposed that *Hae*III digestion of PCR-amplified urease genes can be used as the basis for the typing of *H. pylori* isolates. Since urease genes are well conserved among bacterial species, these PCR-amplified urease genes cannot be used for the primary identification of *H. pylori*.

To evaluate our cloned probe for typing *H. pylori*, genomic DNAs from *H. pylori* isolates were digested with *Hae*III and transferred to the nylon filters, and Southern blot hybridization was performed. The probe hybridized with *Hae*III-digested chromosomal DNA from 69 *H. pylori* isolates and yielded at least three to five bands of 15.0 to 0.46 kb. It is interesting that 52 of 69 DNA preparations from *H. pylori* isolates shared the 1.6-kb band and 25 of 69 shared a 0.46-kb fragment, respectively. Fifty-two different *Hae*III restriction patterns were seen from the 69 clinical isolates (Fig. 1 and 2). Twenty-seven isolates shared identical hybridization patterns with at least one other isolate and were grouped into nine typing patterns, as shown in Table 2. Forty-two other *H. pylori* isolates had their own unique *Hae*III DNA hybridization patterns. The 51 hybridization patterns obtained by our probe from 69 unrelated patients suggest that the *H. pylori* isolates are heterogeneous and represent a wide diversity of genetic patterns.

Most DNA hybridization patterns reported here have been determined on repeat testing at least three or four times, with identical results. Furthermore, we also have DNA samples prepared from six *H. pylori* isolates that have gone through multiple replications. The stabilities of these six isolates have been noted to be unchanged even after storage at −80°C for 6 to 18 months. In addition, we recently isolated *H. pylori* from the saliva of a patient. Its DNA profile was identical to that of an isolate from a gastric biopsy specimen from the same patient (Fig. 2, lanes 2 and 3). This proves that the probe is useful in identifying and tracking strains.

The genomic variations of *H. pylori* isolates observed with our probe are consistent with the observations of others who used DNA restriction digest analyses, ribotyping, and pulsed-field gel electrophoresis (16, 18, 19, 21, 22, 25, 31, 33, 35). In addition, our probe revealed this diversity while only producing three to five major bands per strain. We conclude that the hybridization patterns generated by our probe are convenient and practical for comparative studies of *H. pylori* isolates and have great potential for use in epidemiologic studies of infections caused by this organism.

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