JOURNAL OF CLINICAL MICROBIOLOGY, May 1995, p. 1344–1347 Vol. 33, No. 5
0095-1137/95/$04.00+0

Diagnostic Assay for *Helicobacter hepaticus* Based on Nucleotide Sequence of Its 16S rRNA Gene

JANE K. BATTLES,[1] JAMES C. WILLIAMSON,[1] KRISTEN M. PIKE,[1] PETER L. GORELICK,[2] JERROLD M. WARD,[3] AND MATTHEW A. GONDA[1]*

Laboratory of Cell and Molecular Structure[1] and Laboratory Animal Sciences Program,[2] Program Resources, Inc./DynCorp, and Veterinary and Tumor Pathology Section, Office of Laboratory Animal Science,[3] National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201

Received 17 November 1994/Returned for modification 3 January 1995/Accepted 7 February 1995

Conserved primers were used to PCR amplify 95% of the *Helicobacter hepaticus* 16S rRNA gene. Its sequence was determined and aligned to those of related bacteria, enabling the selection of primers to highly diverged regions of the 16S rRNA gene and an oligonucleotide probe for the development of a PCR-liquid hybridization assay. This assay was shown to be both sensitive and specific for *H. hepaticus* 16S rRNA gene sequences.

Helicobacter hepaticus is a recently identified species of gram-negative, microaerophilic, urease-positive, spiral bacterium that was originally isolated from the livers of mice with chronic active hepatitis at the National Cancer Institute-Frederick Cancer Research and Development Center. This bacterium also colonizes the cecal and colonic mucosa of mice (9, 40, 41).

The type species of the genus *Helicobacter* is *H. pylori* (12), which is a common human pathogen that has been associated with chronic active gastritis, peptic and duodenal ulcers, and carcinoma of the stomach (1, 21, 29, 42). Other members of this genus have also been isolated from gastric lesions in ferrets (10), cats and dogs (18, 27), cheetahs (4), and macaques (2). A few *Helicobacter* species have also been found in the intestinal mucosae of rodents (19) and in the feces of humans (7, 37) and cats and dogs (33, 34). In addition, several other *Helicobacter*-like organisms await classification in this genus (31, 32).

rRNAs are functionally and evolutionarily homologous in all organisms. Phylogenetic comparison of rRNA sequences has become a powerful method for the systematic classification of microbial organisms because of their conserved nature and universal distribution (8, 13, 17, 25, 26). In bacteria, there are three types of rRNAs that are readily identifiable by size: the 120-nucleotide (nt) 5S rRNA, the 1,600-nt 16S rRNA, and the 3,000-nt 23S rRNA (25). 16S rRNA genes contain three types of domains: universally conserved domains, semiconserved domains, and nonconserved domains (13). Based on the universally conserved regions, primers can be devised to either directly sequence or PCR amplify 16S rRNA genes from a wide range of bacteria (5, 17, 43). These primers are especially useful for studying extremely fastidious or highly pathogenic bacterial species because culturing is not required (43).

On the basis of rRNA analyses, members of the genus *Helicobacter* are placed into rRNA cluster III of superfamily VI (38). Comparison of the 16S rRNA sequences of *Helicobacter* species and related bacteria reveals conserved and divergent sequences that can be used as probes for the specific identification of *H. pylori* (3, 22, 28), *Helicobacter mustelae* (11), and *Helicobacter canis* (34). Many PCR-based techniques have been developed to amplify 16S rRNA sequences of *H. pylori* and related organisms (3, 6, 15, 16, 20, 24, 39, 44).

In the present report, the objective was to develop a species-specific PCR-based assay to detect and distinguish *H. hepaticus* 16S rRNA sequences from closely related species in mouse tissues. Of the known *Helicobacter* and *Helicobacter*-like species, two species, *Helicobacter muridarum* (19) and "*Flexispira rappini*" (31), were expected to be the most closely related to *H. hepaticus* because both species have been found in mice.

The strain of *H. hepaticus* used in this study, FRED1, was isolated initially from the liver of a retired breeder male SCID/NCr mouse (National Cancer Institute-Frederick Cancer Research and Development Center) with a natural infection. The liver was removed aseptically from the mouse; homogenized in a sterile tissue grinder; spread over the surface of *Brucella* blood agar containing trimethoprim, vancomycin, and polymyxin (Remel Laboratories); and incubated at 37°C under microaerophilic conditions for 5 to 7 days. The surfaces of the plates were swabbed and suspended in sterile phosphate-buffered saline (Remel Laboratories) for subsequent passages. Additional isolates were obtained from livers of male A/J mice (Jackson Laboratory, Bar Harbor, Maine) that had been experimentally infected with the initial isolate by intraperitoneal injection. Animal care was provided in accordance with federal guidelines (23).

Prior to assay development, it was necessary to amplify, clone, and sequence the 16S rRNA gene. To obtain template DNA for PCR, chromosomal DNA was obtained from cultures of *H. hepaticus*, as previously described (15). PCR was then performed with highly conserved *Escherichia coli* 16S rRNA primers (Table 1) to amplify the majority of this gene. A 1.5-kb fragment representing 95% of this gene was amplified with the conserved primers HB1 and HB2 (Table 1). Two additional conserved primers, HB8 and HB5 (Table 1), were used in conjunction with HB1 and HB2 to generate two smaller overlapping fragments (1.1 and 1 kb) within the HB1-HB2-amplified fragment. All three PCR-generated fragments were cloned into the pCRII plasmid (Invitrogen Corp.) and transformed into the DH5α strain of *E. coli* (Life Technologies, Inc.).

Plasmid DNAs were prepared according to the double-stranded DNA template preparation method (36). DNA sequences of the plasmid clones HB1-HB2/pCRII, HB1-HB8/pCRII, and HB5-HB2/pCRII were determined with a model 370A automated DNA sequencer (Applied Biosystems Divi-

* Corresponding author. Mailing address: Laboratory of Cell and Molecular Structure, Program Resources, Inc./DynCorp, National Cancer Institute-Frederick Cancer Research and Development Center, P.O. Box B, Frederick, MD 21702-1201. Phone: (301) 846-1290. Fax: (301) 846-5427. Electronic mail address: gonda@lcms-1.ncifcrf.gov.

TABLE 1. Oligonucleotide primers used in PCR amplification of the *H. hepaticus* 16S rRNA gene[a]

Primer	*E. coli* nt	Sequence
HB1	8–27	5′-aatttgaattcccatgg**AGAGTTTGATCCTGGCTCAG**-3′
HB2	1510–1492	5′-aatttaagcttctgcag**GGTTACCTTGTTACGACTT**-3′
HB5	515–533	5′-**GTGCCAGCMGCCGCGGTAA**-3′
HB8	1115–1100	5′-**AGGGTTGCGCTCGTTG**-3′

[a] All numbers are in RNA sense. *E. coli* DNA sequences are in boldface capital letters and correspond to the nucleotide positions cited. Underlined letters are restriction enzyme sites that were incorporated to facilitate cloning (in HB1, *Eco*RI-*Nco*I; in HB2, *Hin*dIII-*Pst*I). The remaining lowercase letters represent spacers incorporated into the 5′ ends of primers HB1 and HB2 to facilitate restriction enzyme digestion. Primers HB5 and HB8 are identical to primers 515F and 1100R, and the *E. coli* portions of primers HB1 and HB2 were obtained from primers 8FPL and 1492RPL, respectively (32).

sion, Perkin-Elmer) using DyeDeoxy terminator cycle sequencing (Applied Biosystems Division, Perkin-Elmer) and universal M13 primers. *H. hepaticus*-specific primers were synthesized to fill in the sequence as needed.

The *H. hepaticus* 16S rRNA gene sequence was compared with the 16S rRNA gene sequences of several related organisms. It shares 97.8, 97.2, 93.1, and 77.7% similarity at the nucleotide level with *H. muridarum* (GenBank accession number M80205), "*F. rappini*" (M88137), *H. pylori* (M88157), and *E. coli* (J01695), respectively. Two regions on the *H. hepaticus* 16S rRNA gene were identified as having the least homology with *H. muridarum*, its closest relative. Oligonucleotide primers based on these areas were designed for use in a two-part, species-specific PCR-liquid hybridization diagnostic assay (Table 2). Oligonucleotide HB15, the forward PCR primer, shares 80, 67, 73, and 60% similarity with the corresponding regions in *H. muridarum*, "*F. rappini*," *H. pylori*, and *E. coli*, respectively. Oligonucleotide HB17, the reverse PCR primer, shares 71, 65, 59, and 41% similarity with the corresponding regions in *H. muridarum*, "*F. rappini*," *H. pylori*, and *E. coli*, respectively. In the first part of the assay, oligonucleotide primers HB15 and HB17 were used to generate a 405-bp PCR product. With these PCR primers, it was predicted that the limited sequence similarity (only 80% for HB15 and 71% for HB17) with the corresponding regions in the 16S rRNA gene of its closest relative, *H. muridarum*, would ensure the specific PCR amplification of *H. hepaticus* DNA under the conditions described below. Occasionally, unwanted nonspecific PCR products, often in the size range of the expected product, are obtained during the amplification process; this can lead to ambiguous interpretation of results in ethidium bromide-stained gel analyses. In addition, the PCR product can be in too low abundance to be visualized in ethidium bromide-stained gels. Therefore, to increase both the sensitivity and specificity of this assay, the PCR fragment amplified in the first part was hybridized in solution to a ^{32}P-labeled oligonucleotide probe, HB16. This internal probe shares 93, 93, 47, and 33% similarity with the corresponding regions in *H. muridarum*, "*F. rappini*," *H. pylori*, and *E. coli*, respectively. Only PCR products that hybridize to the probe are visible on the autoradiograph. This two-part approach incorporates the initial high sensitivity and specificity of PCR and an added enhancement in sensitivity and specificity through liquid hybridization.

DNAs from a variety of tissues from *H. hepaticus*-infected and uninfected mice were tested as templates in this assay. Upon solubilization in lysis buffer (Applied Biosystems Division, Perkin-Elmer) and proteinase K digestion, DNA from liver, stomach, cecum, and colon was extracted once each with phenol and chloroform and then ethanol precipitated as described elsewhere (30). After homogenization in Dulbecco's phosphate-buffered saline without calcium and magnesium chloride, DNA from feces was obtained by processing through a QiaAMP column (Qiagen Inc.). DNA from paraffin-embedded tissue sections was processed with the EX-WAX kit (ONCOR). Negative and positive controls were included in each run of the assay. Negative controls were no DNA and pUC12N plasmid DNA. Positive controls were 10 and 1,000 copies of target sequences in HB1-HB2/pCRII plasmid DNA. PCR amplification was performed in a reaction volume of 40 μl consisting of 4 μl of sample DNA (100 ng/μl), 2 μl of oligonucleotide HB15 (5 μM), 2 μl of oligonucleotide HB17 (5 μM), 4 μl of deoxynucleoside triphosphates (0.5 mM mixture), 4 μl of 10× PCR buffer II (Perkin-Elmer), 4 μl of 25 mM $MgCl_2$, 0.2

TABLE 2. Sequence comparison of the oligonucleotide primers used in the *H. hepaticus* PCR-liquid hybridization diagnostic assay

Primer and organism	nt[a]	Sequence[b]	% Similarity to *H. hepaticus*
HB15 (forward PCR primer)			
H. hepaticus	591–605	5′-GAAACTGTTACTCTG-3′	100
H. muridarum	594–608	5′-GAAACT**A**TTA**G**TCT**A**-3′	80
"*F. rappini*"	597–611	5′-GAAACTG**ACCA**TCT**A**-3′	67
H. pylori	599–613	5′-GAAACT**AC**TA**T**TCT**A**-3′	73
E. coli	638–652	5′-GA**T**ACTG**GC**A**AG**CTT-3′	60
HB16 (internal probe)			
H. hepaticus	788–802	5′-CCTTGCTT-GTCAGGG-3′	100
H. muridarum	791–805	5′-CCTTGCTT-G**A**CAGGG-3′	93
"*F. rappini*"	794–808	5′-CC**C**TGCTT-GTCAGGG-3′	93
H. pylori	797–811	5′-**GAGG**GCTTA**G**TC**TCT**-3′	47
E. coli	835–849	5′-**GTGCC**CTT-G**AGGC**G**T**-3′	33
HB17 (reverse PCR primer)			
H. hepaticus	995–979	5′-TCAAGCTCCCCGAAGGG-3′	100
H. muridarum	1000–984	5′-TCAAGCTCC**AC****AG**A**AG**T-3′	71
"*F. rappini*"	1003–987	5′-TCAAGCTC**TGGC**AAG**CC**-3′	65
H. pylori	1008–992	5′-TCAA**G**TC**TAGC**AAG**CT**-3′	59
E. coli	1040–1024	5′-**CACG**G**TT**CCC**GA**A**GG**CA**-3′	41

[a] Nucleotide positions for *H. hepaticus*, *H. muridarum*, "*F. rappini*," *H. pylori*, and *E. coli* were obtained from GenBank, accession numbers L39122, M80205, M88137, M88157, and J01695, respectively.

[b] Boldface letters indicate nucleotides that are different from the *H. hepaticus* sequence. Dashes were inserted to facilitate alignment.

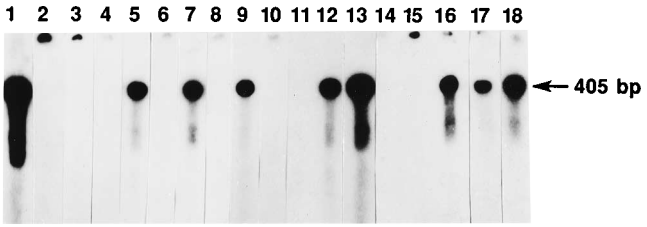

FIG. 1. PCR-liquid hybridization of DNAs from various mouse tissues with *H. hepaticus*-specific 16S rRNA oligonucleotide *H. hepaticus* primers. Tissues in lanes 1, 3, 5, 7, and 9 were obtained from an infected SCID/NCr mouse. Tissues in lanes 2, 4, 6, 8, and 10 were obtained from a noninfected SCID/NCr mouse. Tissue in lane 16 was obtained from an *H. hepaticus*-infected A/JCr mouse. DNA templates used for PCR amplification were obtained from the following tissues or sources: lanes 1 and 2, liver; lanes 3 and 4, stomach; lanes 5 and 6, cecum; lanes 7 and 8, colon; lanes 9 and 10, feces; lane 11, dH_2O; lanes 12 and 13, 10^1 and 10^3 molecules of HB1-HB2/pCRII, respectively; lane 14, dH_2O; lane 15, pUC12N; lane 16, paraffin-embedded liver (94-47055); and lanes 17 and 18, 10^1 and 10^3 molecules of HB1-HB2/pCRII, respectively. Thirty and 50 cycles of PCR amplification were used in lanes 1 to 13 and 14 to 18, respectively.

μl of AmpliTaq DNA polymerase (5 U/μl), and 19.8 μl of distilled H_2O (dH_2O). Initial denaturation was carried out for 2 min at 94°C. Thirty-five cycles of amplification were performed in a GeneAmp PCR System 9600 thermal cycler (Perkin-Elmer). Each cycle consisted of three steps: denaturation for 45 s at 94°C, annealing for 1 min at 55°C, and extension for 90 s at 72°C. An additional extension step for 5 min at 72°C was performed at the end of amplification. For DNA from paraffin-embedded sections, the number of cycles of amplification was increased to 50. The probe for liquid hybridization was radiolabeled for 1 h at 37°C in a 20-μl reaction mixture containing 4 μl of oligonucleotide HB16 (3 μM), 2 μl of 10× kinase buffer, 3 μl of [γ-^{32}P]ATP (3,000 Ci/mmol; Amersham), 2 μl of T4 polynucleotide kinase (New England Biolabs), and 9 μl of dH_2O. dH_2O (18 μl), 2 μl of 0.5 M EDTA, and 50 μl of 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) were added to stop the reaction. dH_2O (160 μl) was added to dilute the probe. For the liquid hybridization, 5 μl of the ^{32}P-labeled probe was added to a 15-μl aliquot of the PCR-amplified product and incubated for 5 min at 94°C and 15 min at 55°C. Four microliters of 6× loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, and 40% [wt/vol] sucrose) was added, and 4 μl of this mixture was electrophoresed on a 5% polyacrylamide gel cast in a 1-mm minigel cassette (NOVEX) at 150 V in 0.5× Tris-borate-EDTA buffer in a Mini-Cell (NOVEX). The gel was dried at 80°C for 2 h in a vacuum gel dryer and exposed to XAR film.

Examples of PCR-liquid hybridization results using DNA obtained from various tissues from *H. hepaticus*-infected and noninfected SCID/NCr mice are shown in Fig. 1 (lanes 1 to 10). The SCID/NCr mice were obtained from different isolators at two buildings at the National Cancer Institute-Frederick Cancer Research and Development Center and were shown to be infected or noninfected by histopathology (hepatitis and Steiner stain positive for helical bacteria). DNA was also obtained from the paraffin-embedded liver (Fig. 1, lane 16) from an infected A/JCr mouse with hepatitis.

To verify the sequence of the PCR-amplified fragments and to examine the sequence diversity within this 405-bp region, HB15-HB17-amplified PCR products were generated from DNA obtained from different tissues (liver, cecum, colon, and feces) and bacterial DNA obtained from an *H. hepaticus* culture and cloned into the pCRII plasmid. Plasmid DNAs were purified with Qiagen columns and sequenced as described above. The sequence data (not shown) demonstrated that the sequences were identical, the assay was specific for *H. hepaticus*, and the amplified target area is quite homogeneous.

The HB1-HB2/pCRII clone served as a readily available source of positive target DNA of known concentration. To assess the sensitivity of this assay, the number of molecules of the target DNA sequence (HB15-HB17 fragment) was calculated for a known concentration of HB1-HB2/pCRII plasmid DNA. Dilutions containing 10^1 and 10^3 molecules per μl were routinely included in every assay (Fig. 1, lanes 12, 13, 17, and 18). Our results show that this assay can detect as few as 10 molecules of target DNA in a standard 20-μl PCR assay mixture. Although it is not known how many copies of the 16S rRNA gene are contained in each *H. hepaticus* cell, in both *H. pylori* and *E. coli*, it is estimated that there are about three copies per cell (14, 35). Therefore, the sensitivity of our assay is about three bacterial cells.

The specificity of the assay for *H. hepaticus* DNA sequences was established further by testing two closely related species, *H. muridarum* and *H. pylori*, and *E. coli*. DNAs were extracted from *H. muridarum* (ATCC 49282) and *E. coli* (DH5α strain) cultures. *H. pylori* DNA was extracted from paraffin-embedded stomach tissue from a human with chronic gastritis and *H. pylori* infection. All three DNAs were negative in the assay (data not shown). To ascertain that there was PCR-amplifiable DNA in the *H. muridarum* preparation, PCRs were performed with the conserved primers from the *E. coli* 16S rRNA gene (Table 1). The data (not shown) indicated that *H. muridarum* DNA is PCR amplifiable with the conserved primers from *E. coli* but not with the *H. hepaticus* diagnostic primers. Therefore, this assay appears to be highly specific for *H. hepaticus.*

After the development of our assay, partial sequences from three strains of *H. hepaticus*, Fox Hh-1, Fox Hh-2, and Fox Hh-3 (GenBank accession numbers U07573, U07574, and U07575, respectively), became available (9). All three of these 16S rRNA gene entries contain many gaps and unidentified nucleotides. For the remaining sequences that could be compared (1,237, 1,142, and 1,140 nt in files U07573, U07574, U07575, respectively), the alignments showed that these entries were identical to our 16S rRNA gene sequences determined for *H. hepaticus.*

In conclusion, we have PCR amplified, cloned, and sequenced 95% of the 16S rRNA gene of *H. hepaticus*. This 1,457-nt sequence corresponds to nt 8 to 1464 of the *E. coli* 16S rRNA and lacks only the very proximal 5′ and terminal 3′ ends. Thus, our data represent the most complete DNA sequence obtained for any strain of *H. hepaticus* 16S rRNA to date. On the basis of sequence comparisons with 16S rRNA gene sequences of related bacteria, we have devised primers and a probe for use in an *H. hepaticus*-specific PCR-liquid hybridization assay. We have demonstrated that this sensitive and species-specific assay is a useful research tool to detect *H. hepaticus* infections in a variety of mouse tissues. In addition, there was strong correlation between PCR diagnostic and histopathologic results. The present method is applicable to DNA obtained from fresh, frozen, and formalin-fixed tissues and DNA obtained from fecal material. This assay could be made more sensitive if rRNAs, instead of rRNA genes, are used as templates in reverse transcription PCR because there are up to 10,000 copies of each rRNA per cell, as compared to 2 to 4 copies of each rRNA gene per cell (28). Studies to increase the sensitivity, specificity, and speed of PCR-based diagnostics for *H. hepaticus* are in progress.

Nucleotide sequence accession number. The nucleotide sequence for the FRED1 strain of *H. hepaticus* has been assigned GenBank accession number L39122.

We thank J. Tully for *H. muridarum* cultures; T. Enomoto for paraffin-embedded *H. pylori* gastritis; D. Hutchison for sequencing reactions; J. Elser and G. Tobin for sequence analyses; K. Nagashima, V. Marshall, and S. Rivard for photographic assistance; and G. Serig for manuscript preparation.

REFERENCES

1. **Blaser, M. J.** 1987. Gastric *Campylobacter*-like organisms, gastritis, and peptic ulcer disease. Gastroenterology **93:**371–383.
2. **Bronsdon, M. A., C. S. Goodwin, L. I. Sly, T. Chilvers, and F. D. Schoenknecht.** 1991. *Helicobacter nemestrinae* sp. nov., a spiral bacterium found in the stomach of a pigtailed macaque (*Macaca nemestrina*). Int. J. Syst. Bacteriol. **41:**148–153.
3. **Desai, M., D. Linton, R. J. Owen, H. Cameron, and J. Stanley.** 1993. Genetic diversity of *Helicobacter pylori* indexed with respect to clinical symptomatology, using a 16S rRNA and a species-specific DNA probe. J. Appl. Bacteriol. **75:**574–582.
4. **Eaton, K. A., M. J. Radin, J. G. Fox, B. Paster, F. Dewhirst, S. Krakowka, and D. R. Morgan.** 1991. *Helicobacter acinonyx*, a new species of *Helicobacter* isolated from cheetahs with gastritis. Microb. Ecol. Health Dis. **4:**S104.
5. **Edwards, U., T. Rogall, H. Blöcker, M. Emde, and E. C. Böttger.** 1989. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res. **17:**7843–7853.
6. **Engstrand, L., A.-M. H. Nguyen, D. Y. Graham, and F. A. K. El-Zaatari.** 1992. Reverse transcription and polymerase chain reaction amplification of rRNA for detection of *Helicobacter* species. J. Clin. Microbiol. **30:**2295–2301.
7. **Fennell, C. L., P. A. Totten, T. C. Quinn, D. L. Patton, K. K. Holmes, and W. E. Stamm.** 1984. Characterization of *Campylobacter*-like organisms isolated from homosexual men. J. Infect. Dis. **149:**58–66.
8. **Fox, G. E., E. Stackebrandt, R. B. Hespell, J. Gibson, J. Maniloff, T. A. Dyer, R. S. Wolfe, W. E. Balch, R. S. Tanner, L. J. Magrum, L. B. Zablen, R. Blakemore, R. Gupta, L. Bonen, B. J. Lewis, D. A. Stahl, K. R. Luehrsen, K. N. Chen, and C. R. Woese.** 1980. The phylogeny of prokaryotes. Science **209:**457–463.
9. **Fox, J. G., F. E. Dewhirst, J. G. Tully, B. J. Paster, L. Yan, N. S. Taylor, M. J. Collins, Jr., 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.
10. **Fox, J. G., B. M. Edrise, E. B. Cabot, C. Beaucage, J. C. Murphy, and K. S. Prostak.** 1986. *Campylobacter*-like organisms isolated from gastric mucosa of ferrets. Am. J. Vet. Res. **47:**236–239.
11. **Fox, J. G., B. J. Paster, F. E. Dewhirst, N. S. Taylor, L.-L. Yan, P. J. Macuch, and L. M. Chmura.** 1992. *Helicobacter mustelae* isolation from feces of ferrets: evidence to support fecal-oral transmission of a gastric helicobacter. Infect. Immun. **60:**606–611.
12. **Goodwin, C. S., J. A. Armstrong, T. Chilvers, M. Peters, M. D. Collins, L. Sly, W. McConnell, and W. E. S. Harper.** 1989. Transfer of *Campylobacter pylori* and *Campylobacter mustelae* to *Helicobacter* gen. nov. as *Helicobacter pylori* comb. nov. and *Helicobacter mustelae* comb. nov., respectively. Int. J. Syst. Bacteriol. **39:**397–405.
13. **Gray, M. W., D. Sankoff, and R. J. Cedergren.** 1984. On the evolutionary descent of organisms and organelles: a global phylogeny based on a highly conserved structural core in small subunit ribosomal RNA. Nucleic Acids Res. **12:**5837–5852.
14. **Greisen, K., M. Loeffelholz, A. Purohit, and D. Leong.** 1994. PCR primers and probes for the 16S rRNA gene of most species of pathogenic bacteria, including bacteria found in cerebrospinal fluid. J. Clin. Microbiol. **32:**335–351.
15. **Ho, S.-A., J. A. Hoyle, F. A. Lewis, A. D. Secker, D. Cross, N. P. Mapstone, M. F. Dixon, J. I. Wyatt, D. S. Tompkins, G. R. Taylor, and P. Quirke.** 1991. Direct polymerase chain reaction test for detection of *Helicobacter pylori* in humans and animals. J. Clin. Microbiol. **29:**2543–2549.
16. **Hoshina, S., S. M. Kahn, W. Jiang, P. H. R. Green, H. C. Neu, N. Chin, M. Morotomi, P. LoGerfo, and I. B. Weinstein.** 1990. Direct detection and amplification of *Helicobacter pylori* ribosomal 16S gene segments from gastric endoscopic biopsies. Diagn. Microbiol. Infect. Dis. **13:**473–479.
17. **Lane, D. J., B. Pace, G. J. Olsen, D. A. Stahl, M. L. Sogin, and N. R. Pace.** 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci. USA **82:**6955–6959.
18. **Lee, A., S. L. Hazell, J. O'Rourke, and S. Kouprach.** 1988. Isolation of a spiral-shaped bacterium from the cat stomach. Infect. Immun. **56:**2843–2850.
19. **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.
20. **Mapstone, N. P., D. A. F. Lynch, F. A. Lewis, A. T. R. Axon, D. S. Tompkins, M. F. Dixon, and P. Quirke.** 1993. Identification of *Helicobacter pylori* DNA in the mouths and stomachs of patients with gastritis using PCR. J. Clin. Pathol. **46:**540–543.
21. **Marshall, B. J., and J. R. Warren.** 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet **i:**1311–1315.
22. **Morotomi, M., S. Hoshina, P. Green, H. C. Neu, P. LoGerfo, I. Watanabe, M. Mutai, and I. B. Weinstein.** 1989. Oligonucleotide probe for detection and identification of *Campylobacter pylori*. J. Clin. Microbiol. **27:**2652–2655.
23. **National Institutes of Health.** 1985. Guide for the care and use of laboratory animals. NIH publication no. 86-23. National Institutes of Health, Bethesda, Md.
24. **Nguyen, A.-M. H., L. Engstrand, R. M. Genta, D. Y. Graham, and F. A. K. El-Zaatari.** 1993. Detection of *Helicobacter pylori* in dental plaque by reverse transcription-polymerase chain reaction. J. Clin. Microbiol. **31:**783–787.
25. **Olsen, G. J., D. J. Lane, S. J. Giovannoni, and N. R. Pace.** 1986. Microbial ecology and evolution: a ribosomal RNA approach. Annu. Rev. Microbiol. **40:**337–365.
26. **Pace, N. R., G. J. Olsen, and C. R. Woese.** 1986. Ribosomal RNA phylogeny and the primary lines of evolutionary descent. Cell **45:**325–326.
27. **Paster, B. J., A. Lee, J. G. Fox, F. E. Dewhirst, L. A. Tordoff, G. J. Fraser, J. L. O'Rourke, N. S. Taylor, and R. Ferrero.** 1991. Phylogeny of *Helicobacter felis* sp. nov., *Helicobacter mustelae*, and related bacteria. Int. J. Syst. Bacteriol. **41:**31–38.
28. **Romaniuk, P. J., and T. J. Trust.** 1989. Rapid identification of *Campylobacter* species using oligonucleotide probes to 16S ribosomal RNA. Mol. Cell. Probes **3:**133–142.
29. **Romaniuk, P. J., B. Zoltowska, T. J. Trust, D. J. Lane, G. J. Olsen, N. R. Pace, and D. A. Stahl.** 1987. *Campylobacter pylori*, the spiral bacterium associated with human gastritis, is not a true *Campylobacter* sp. J. Bacteriol. **169:**2137–2141.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Schauer, D. B., N. Ghori, and S. Falkow.** 1993. Isolation and characterization of "*Flexispira rappini*" from laboratory mice. J. Clin. Microbiol. **31:**2709–2714.
32. **Solnick, J. V., J. O'Rourke, A. Lee, B. J. Paster, F. E. Dewhirst, and L. S. Thompkins.** 1993. An uncultured gastric spiral organism is a newly identified *Helicobacter* in humans. J. Infect. Dis. **168:**379–385.
33. **Stanley, J., A. P. Burnens, D. Linton, S. L. W. On, M. Costas, and R. J. Owen.** 1992. *Campylobacter helveticus* sp. nov., a new thermophilic species from domestic animals: characterization, and cloning of a species-specific DNA probe. J. Gen. Microbiol. **138:**2293–2303.
34. **Stanley, J., D. Linton, A. P. Burnens, F. E. Dewhirst, R. J. Owen, A. Porter, S. L. W. On, and M. Costas.** 1993. *Helicobacter canis* sp. nov., a new species from dogs: an integrated study of phenotype and genotype. J. Gen. Microbiol. **139:**2495–2504.
35. **Taylor, D. E., M. Eaton, N. Chang, and S. M. Salama.** 1992. Construction of a *Helicobacter pylori* genome map and demonstration of diversity at the genome level. J. Bacteriol. **174:**6800–6806.
36. **Titus, D. E.** 1991. Promega protocols and applications guide, 2nd ed. Promega Corp., Madison, Wis.
37. **Totten, P. A., C. L. Fennell, F. C. Tenover, J. M. Wezenberg, P. L. Perine, W. E. Stamm, and K. K. Holmes.** 1985. *Campylobacter cinaedi* (sp. nov.) and *Campylobacter fennalliae* (sp. nov.): two new *Campylobacter* species associated with enteric disease in homosexual men. J. Infect. Dis. **151:**131–139.
38. **Vandamme, P., E. Falsen, R. Rossau, B. Hoste, P. Segers, R. Tytgat, and J. De Ley.** 1991. Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov. Int. J. Syst. Bacteriol. **41:**88–103.
39. **van Zwet, A. A., J. C. Thijs, A. M. D. Kooistra-Smid, J. Schirm, and J. A. M. Snijder.** 1994. Use of PCR with feces for detection of *Helicobacter pylori* infections in patients. J. Clin. Microbiol. **32:**1346–1348.
40. **Ward, J. M., M. R. Anver, D. C. Haines, and R. E. Benveniste.** 1994. Chronic active hepatitis in mice caused by *Helicobacter hepaticus*. Am. J. Pathol. **145:**959–968.
41. **Ward, J. M., J. G. Fox, M. R. Anver, D. C. Haines, C. V. George, M. J. Collins, Jr., P. L. Gorelick, K. Nagashima, M. A. Gonda, R. V. Gilden, J. G. Tully, R. J. Russell, R. E. Benveniste, B. J. Paster, F. E. Dewhirst, J. C. Donovan, L. M. Anderson, and J. M. Rice.** 1994. Chronic active hepatitis and associated liver tumors in mice caused by a persistent bacterial infection with a novel *Helicobacter* species. J. Natl. Cancer Inst. **86:**1222–1227.
42. **Warren, J. R., and B. Marshall.** 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet **i:**1273–1275.
43. **Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane.** 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173:**697–703.
44. **Weiss, J., J. Mecca, E. da Silva, and D. Gassner.** 1994. Comparison of PCR and other diagnostic techniques for detection of *Helicobacter pylori* infection in dyspeptic patients. J. Clin. Microbiol. **32:**1663–1668.