INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Oct. 1996, p. 916–921 Vol. 46, No. 4
0020-7713/96/$04.00+0

Helicobacter trogontum sp. nov., Isolated from the Rat Intestine

EDILBERTO N. MENDES,[1] DULCIENE M. M. QUEIROZ,[1] FLOYD E. DEWHIRST,[2] BRUCE J. PASTER,[2] SILVIA B. MOURA,[1] AND JAMES G. FOX[3]*

The Laboratory of Research in Bacteriology, Faculdade de Medicina/UFMG, Belo Horizonte, MG, 30130-100, Brazil[1]; Department of Molecular Genetics, Forsyth Dental Center, Boston, Massachusetts 02115[2]; and Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139[3]

A new *Helicobacter* species that colonizes the colonic mucosa of Wistar and Holtzman rats was isolated and characterized. This bacterium was gram negative, its cells were rod shaped with pointed ends, and its protoplasmic cylinder was entwined with periplasmic fibers. It was catalase and oxidase positive, rapidly hydrolyzed urea, and was susceptible to metronidazole and resistant to cephalothin and nalidixic acid. The new organism was microaerophilic and grew at 42°C, a feature that differentiates it from two other murine intestine colonizers, *Helicobacter hepaticus* and *Helicobacter muridarum*. On the basis of 16S rRNA sequence analysis data, the new organism was identified as a *Helicobacter* species that is most closely related to *H. hepaticus*. This bacterium is named *Helicobacter trogontum*. The type strain is strain LRB 8581 (= ATCC 700114).

Spiral microorganisms were found in the stomachs of mammals as early as 1893 (1). However, the study of these bacteria gained momentum only after the description of a spiral bacterium in the gastric mucosa of humans and the demonstration that this organism is associated with gastric disease (20, 21, 33). This bacterium, *Helicobacter pylori*, was originally placed in the genus *Campylobacter*, but subsequently became the type species of a new genus, the genus *Helicobacter*, when it and *Helicobacter mustelae* were recognized as organisms that are only distantly related to campylobacters (10). Since then, 13 formally named species and at least six other species have been added to the genus *Helicobacter*. In addition to *H. pylori*, *Helicobacter acinonyx* (4), *Helicobacter nemestrinae* (2), *Helicobacter felis* (22), *H. mustelae* (7, 22), and *Helicobacter bizzozeronii* (11) have been isolated from the stomachs of several mammalian species. Three "*Gastrospirillum*" species, gastric organisms which cannot currently be grown in pure culture, can be cultivated by passage in the stomachs of mice; these bacteria have been identified as members of the genus *Helicobacter* (16, 19, 27). The species *Helicobacter bilis* (9), *Helicobacter canis* (29), *Helicobacter cinaedi* (31), *Helicobacter fennelliae* (5), *Helicobacter hepaticus* (8), *Helicobacter muridarum* (18), *Helicobacter pametensis* (3), and *Helicobacter pullorum* (28) have been isolated from the intestinal mucosa of humans, mammals, or birds. The straight to spiral fusiform bacterium "*Flexispira rappini*" (14, 26) is also recognized as a member of the genus *Helicobacter* (32). *H. muridarum*, *H. hepaticus*, *H. bilis*, and "*F. rappini*" are natural inhabitants of the intestines of rodents. Recently, two new members of the genus, *H. hepaticus* and *H. bilis*, have been isolated from mice with active chronic hepatitis (8, 9).

Two important reasons for investigating intestinal helicobacters are to provide insights into how *H. pylori* and other gastric bacteria survive in the hostile gastric environment and to study the mechanisms of pathogenesis of members of this new genus of pathogenic bacteria.

* Corresponding author. Mailing address: Division of Comparative Medicine, Massachusetts Institute of Technology, Bldg. 45, 77 Massachusetts Avenue, Cambridge MA 02139. Phone: (617) 253-1735. Fax: (617) 252-1877.

In this paper we describe *Helicobacter trogontum*, a new *Helicobacter* species isolated from the intestinal mucosa of rats.

MATERIALS AND METHODS

Six strains of strongly urease-positive, gram-negative rods were isolated from the colonic mucosa of two Holtzman and four Wistar rats. Briefly, mucosal scrapings from the colons of these animals were streaked onto both Belo Horizonte medium (24) and brucella agar plates supplemented with trimethoprim, vancomycin, and polymyxin (Remel, Lenexa, Kans.) and incubated at 37°C in an atmosphere containing 90% nitrogen, 5% hydrogen, and 5% carbon dioxide.

Biochemical characterization. Phenotypic tests commonly used to biotype campylobacters and helicobacters were performed to identify six strains. Plates were incubated under microaerobic conditions at 37°C for up to 7 days, unless otherwise stated. Growth at 25 and 42°C and under aerobic and anaerobic conditions was determined on sheep blood agar plates. Anaerobiosis was obtained with a GasPak jar and anaerobic generator envelopes (BBL, Cockeysville, Md.). Isolates were also characterized by the Gram stain reaction and by examining motility with a phase-contrast microscope. Oxidase activity was determined by assessing the ability of the organisms to oxidize *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride (Spottest; Difco Laboratories, Detroit, Mich.). Production of catalase was assayed by inoculating bacteria into a 3% H_2O_2 solution. Production of urease was determined by observing a color change upon inoculation of urea agar (Remel). Growth in the presence of 1.0% glycine, 1.5, 2.0, and 3.0% NaCl, and 5 μg of metronidazole per ml was determined with brucella agar (Difco) containing 10% fetal calf serum and the test compound. The hippurate hydrolysis test was performed by using the protocol of Hwang and Ederer (12). H_2S production was tested on blood agar plates by using lead acetate disks (Remel). Susceptibility to nalidixic acid and cephalothin was determined on blood agar plates (Remel) by using antibiotic disks (BBL). Enzyme analysis was performed by using a RapID NH system (Innovative Diagnostics Systems, Inc., Norcross, Ga.) according to the manufacturer's protocol. Other biochemical characteristics of the six strains were determined as described previously (22).

Electron microscopy. Cells were grown on blood agar plates (Remel) at 37°C under microaerobic conditions for 48 h. Then they were gently suspended in 10 mM Tris buffer (pH 7.4) at a concentration of approximately 10^8 cells per ml. Samples were negatively stained with 1% (wt/vol) phosphotungstic acid (pH 6.5) for 20 to 30 s and examined with a JEOL model JEM-1200EX transmission electron microscope.

DNA isolation. Bacteria were cultured in 20 ml of brucella broth supplemented with 10% fetal calf serum at 37°C under microaerobic conditions. After 3 days of incubation, each culture was centrifuged at 10,000 × *g* for 20 min and crude DNA was extracted from the resulting pellet as described previously by Fox et al. (8). Briefly, the pellet was digested with lysozyme, proteinase, and other enzymes, and then the DNA was precipitated with hexadecyltrimethylammonium bromide (CTAB). Following ether-chloroform extraction, the DNA was precipitated with ethanol.

Amplification of 16S rRNA cistrons. The 16S rRNA cistrons were amplified with primers C70 and B37 (Table 1). PCR were performed with a Perkin-Elmer thermal cycler in thin-wall tubes. A 50-ng portion of each DNA preparation and each primer at a concentration of 1 μM were added to a 82-μl (final volume)

TABLE 1. PCR amplification and sequencing primers

Primer	Type	Sequence (5′-3′)[a]	Position[b]	Orientation
C70	PCR	AGAGTTTGATYMTGGC	8-23	Forward
B37	PCR	TACGGYTACCTTGTTACGA	1495-1513	Reverse
B34	Sequencing	RCTGCTGCCTCCCGT	344-358	Reverse
B35	Sequencing	GTRTTACCGCGGCTGCTG	519-536	Reverse
B36	Sequencing	GGACTACCAGGGTATCTA	789-806	Reverse
C01	Sequencing	GGTTGCGCTCGTTGCGGG	1096-1113	Reverse
C31	Sequencing	GGAATCGCTAGTAATCG	1337-1353	Forward
X91	Sequencing	CCCGGGAACGTATTCACCG	1369-1387	Reverse
B72	PCR	CATAGGTAACATGCCCCA	123-139	Forward
B39	PCR	CTGTTTTCAAGCTCCCC	1032-1047	Reverse

[a] Base designations are standard International Union of Biochemistry designations for bases and ambiguity.
[b] *E. coli* numbering.

reaction mixture. Ampliwax PCR Gem100s (Perkin-Elmer) were used in a hot-start protocol according to the manufacturer's instructions. The following conditions were employed for amplification: denaturation at 94°C for 45 s, annealing at 50°C for 45 s, and elongation at 72°C for 45 s, with 5 s added after each cycle. A total of 25 cycles were performed, and this was followed by a final elongation step at 72°C for 15 min. The purity of the amplified product was determined by electrophoresis in a 1.0% agarose gel (FMC Bioproducts). The DNA was stained with ethidium bromide and examined under UV light.

The amplified DNA was purified by precipitation with polyethylene glycol 8000 as previously described (15).

16S rRNA sequencing. The polyethylene glycol 8000-purified PCR product was directly sequenced with a TAQuence cycle sequencing kit (United States Biochemical Corp.) according to the manufacturer's instructions by using sequencing primers B34, B35, B36, C01, C31, X91, B72, and B39 (Table 1). The primers were end labeled with ^{33}P (DuPont, NEN) by using the manufacturer's protocol. Approximately 100 ng of purified DNA from the PCR was employed for sequencing. Reaction products were loaded onto 8% polyacrylamide–urea gels, electrophoresed, and detected by exposure to X-ray film for 24 to 48 h.

16S rRNA data analysis. A program set for data entry, editing, sequence alignment, secondary-structure comparison, similarity matrix generation, and dendrogram construction for 16S rRNA data was written in Microsoft QuickBASIC for use on IBM PC-AT and compatible computers. RNA sequences were entered and aligned as previously described. The sequence database used contains approximately 500 sequences determined at the Department of Molecular Genetics, Forsyth Dental Center, and another 200 sequences were obtained from GenBank or the Ribosomal Database Project. The reference strains used in the 16S rRNA analysis are shown in Table 2. Similarity matrices were constructed from the aligned sequences by using only sequence positions for which 90% of the strains had data. The similarity matrices were corrected for multiple base changes by the method of Jukes and Cantor (13). Phylogenetic trees were constructed by using the neighbor-joining method of Saitou and Nei (25, 30).

Identification of strains by PCR with specific primers. After the complete sequences of the first three isolates of *H. trogontum* were determined, PCR primers B72 and B39 (Table 1) were designed for rapid identification of additional isolates. The expected PCR product when these primers are used is 888 bases long (because *H. trogontum* 16S rRNA is shorter than *Escherichia coli* 16S rRNA, the amplicon length cannot be determined from the difference between the start of the forward primer and the end of the reverse primer [the primers are numbered relative to the *E. coli* sequence]).

The additional three strains tested were cultured on Trypticase soy blood agar plates. A loopful of cells was harvested and suspended in a PCR tube with 15 μl of GeneReleaser (Bioventures, Inc.). The GeneReleaser microwave protocol was used. PCR amplification was performed as described above for 16S rRNA, except that the annealing temperature was 63°C. The amplification products were electrophoresed on a 1% agarose gel and were visualized by ethidium bromide staining.

Nucleotide sequence accession numbers. The GenBank nucleotide sequence accession numbers and culture collection numbers for the strains examined in this study are shown in Table 2.

TABLE 2. Sources and database accession numbers of the bacterial strains studied

Strain	Other designation(s)	GenBank nucleotide sequence accession no.
Helicobacter trogontum strains		
LRB 8581^T	ATCC 700114^T	U65103
LRB 8718	ATCC 700115	
LRB 9056	ATCC 700116	
95-5368	ATCC 700117	
Reference strains		
"*Flexispira rappini*" NADC 1893^T	ATCC 43966^T	M88137
"*Gastrospirillum hominis*" 1[a]		L10079
"*Gastrospirillum hominis*" 2[a]		L10080
Helicobacter acinonyx Eaton 90-119-3^T	ATCC 51101^T, CCUG 29263^T	M88148
Helicobacter bilis Fox HB1^T	ATCC 51630^T	U18766
Helicobacter canis NCTC 12739^T		L13464
Helicobacter cinaedi CCUG 18818^T	ATCC 35683^T	M88150
Helicobacter felis Lee CS1^T	ATCC 49179^T	M57398
Helicobacter fennelliae CCUG 18820^T	ATCC 35684^T	M88154
Helicobacter hepaticus Fox Hh-2^T	ATCC 51448^T	U07574
Helicobacter mustelae Fox R-85-13-6^T	ATCC 43772^T	M35048
Helicobacter muridarum Lee ST1^T	CCUG 29262^T, ATCC 49282^T	M80205
Helicobacter nemestrinae ATCC 49396^T		X67854
Helicobacter pametensis Seymour B9^T	CCUG 29255^T	M88147
Helicobacter pullorum NCTC 12824^T		L36141
Helicobacter pylori ATCC 43504^T		M88157
Helicobacter sp. strain Bird-B^T	Seymour B-10^T, CCUG 29256^T	M88139
Helicobacter sp. strain Bird-C^T	Seymour B52^T, CCUG 29561^T	M88144
Helicobacter sp. strain CLO-3	CCUG 14564, LMG 7792	M88151

[a] This organism cannot be cultivated.

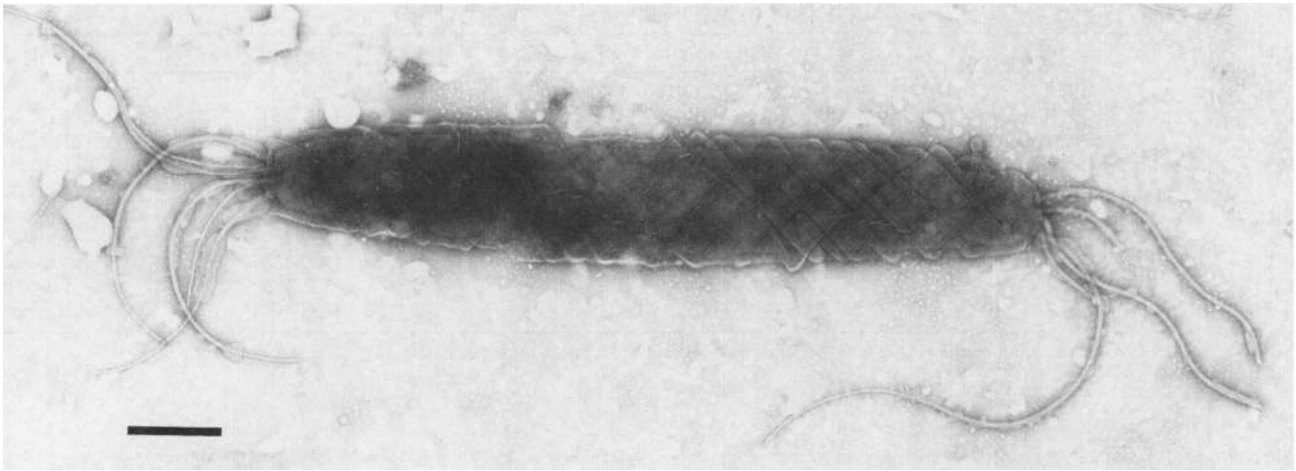

FIG. 1. Electron micrograph of a negatively stained preparation of *H. trogontum*, showing a cell with periplasmic fibers and bipolar tufts of flagella. Bar = 500 nm.

RESULTS

Isolation and growth characteristics. The bacteria initially grew as punctiform colonies and then as thin spreading films on agar media after 3 to 5 days of incubation under microaerobic conditions at 37 or 42°C, but not at 25°C. No growth was seen under aerobic or anaerobic conditions. The microorganism did not grow on brucella agar plates containing 1.5, 2.0, or 3.0% NaCl and 1.0% glycine. Many cells that tended to be ring-like, as well as coccal forms, were observed in older cultures. No hemolysis was observed.

Morphology. Cells were gram negative, rod shaped with pointed ends, 4.0 to 6.0 μm long by 0.6 to 0.7 μm wide, and motile by means of tufts of bipolar sheathed flagella. They were characterized by the presence of periplasmic fibers that were coiled around the protoplasmic cylinder, which gave a crisscross appearance to the bacterial surface (Fig. 1).

Biochemical characteristics. The bacteria were strongly urease positive and produced catalase, oxidase, and H_2S. Hippurate was not hydrolyzed. They were susceptible to metronidazole and resistant to cephalothin and nalidixic acid. Nitrate was not reduced, and tryptophan deaminase was not produced. Ornithine decarboxylase and γ-glutamyl transpeptidase were produced. β-Galactosidase and resazurin were not hydrolyzed. Acid was not produced from proline, glucose, sucrose, or fatty acid esters. Alkaline phosphatase was produced by one of the three strains examined. The biochemical and physiological characteristics of *H. trogontum* and other named and previously described *Helicobacter* species are shown in Table 3.

16S rRNA sequence. Approximately 95% of the total 16S rRNA sequence was determined for four of the six new strains (strains LRB 8581^T [T = type strain], LRB 8718, LRB 9056, and 95-5368) (Table 2). The sequences of these strains were identical. A comparison of the consensus sequence with the sequences of other bacteria in the database indicated that this sequence was most closely related to the *H. hepaticus* sequence (level of similarity, 97.0%). The levels of sequence difference identified the new strains as members of a novel species, which was named *H. trogontum*. Strain LRB 8581^T was compared

TABLE 3. Characteristics of *H. trogontum* and other *Helicobacter* species

Taxon	Catalase production	Urease activity	Nitrate reduction	Alkaline phosphatase hydrolysis	γ-Glutamyl transpeptidase activity	Indoxyl acetate hydrolysis	Growth at 42°C	Susceptibility to[a]:		Periplasmic fibers	No. of flagella per cell	Distribution of flagella
								Nalidixic acid (30-μg disc)	Cephalothin (30-μg disc)			
H. trogontum	6/6[b]	6/6	6/6	1/6	6/6	ND[c]	6/6	R[d]	R[d]	+[e]	5–7	Bipolar
H. hepaticus	+	+	+	ND	ND	+	−	R	R	−	2	Bipolar
H. muridarum	+	+	−	+	+	+	−	R	R	+	10–14	Bipolar
H. canis	−	−	−	+	ND	+	+	S	I	−	2	Bipolar
H. bilis	+	+	+	ND	ND	−	+	R	R	+	3–14	Bipolar
H. cinaedi	+	−	+	−	−	−	−	S	I	−	1–2	Bipolar
H. fennelliae	+	−	−	+	−	+	−	S	S	−	2	Bipolar
H. pullorum	+	−	+	−	ND	−	+	S	R	−	2	Bipolar
H. pylori	+	+	−	+	+	−	−	R	S	−	4–8	Bipolar
H. nemestrinae	+	+	−	+	ND	−	+	R	S	−	4–8	Bipolar
H. acinonyx	+	+	−	+	+	−	−	R	S	−	2–5	Bipolar
H. felis	+	+	+	+	+	−	+	R	S	+	14–20	Bipolar
H. pametensis	+	−	+	+	−	−	+	S	S	−	2	Bipolar
H. mustelae	+	+	+	+	+	+	+	S	R	−	4–8	Lateral

[a] R, resistant; S, susceptible; I, intermediate.
[b] Number of strains positive/number of strains tested.
[c] ND, not determined.
[d] All six strains tested are resistant.
[e] +, positive; −, negative.

TABLE 4. Similarity matrix based on 16S rRNA sequence comparisons

Taxon	% Similarity or % difference[a]																			
	Helicobacter trogontum	*Helicobacter hepaticus*	*Helicobacter muridarum*	*Helicobacter canis*	*Helicobacter bilis*	*Helicobacter cinaedi*	*"Flexispira rappini"*	*Helicobacter fennelliae*	*Helicobacter pullorum*	*Helicobacter* sp. strain CLO-3	*Helicobacter pylori*	*Helicobacter nemestrinae*	*Helicobacter acinonyx*	*"Gastrospirillum hominis" 1*	*"Gastrospirillum hominis" 2*	*Helicobacter felis*	*Helicobacter pametensis*	*Helicobacter* sp. strain Bird-C	*Helicobacter* sp. strain Bird-B	*Helicobacter mustelae*
Helicobacter trogontum		97.0	96.2	96.2	96.2	96.2	95.8	95.9	96.1	95.4	92.8	92.8	92.3	91.7	92.6	92.7	95.9	96.1	96.9	96.4
Helicobacter hepaticus	3.1		97.7	97.2	97.3	96.9	97.1	95.5	95.9	95.1	93.3	93.4	93.1	92.2	92.7	93.2	96.3	96.4	96.4	96.3
Helicobacter muridarum	3.9	2.3		96.5	96.4	95.9	96.0	95.1	95.2	94.3	93.0	92.9	92.4	91.9	92.4	92.5	95.7	96.3	96.1	95.7
Helicobacter canis	3.9	2.8	3.6		98.8	97.8	98.2	95.4	96.3	95.9	93.8	93.7	93.2	92.3	92.7	93.1	96.5	96.9	96.9	96.4
Helicobacter bilis	3.9	2.7	3.7	1.2		98.5	99.1	96.0	96.3	95.4	93.5	93.6	92.8	92.1	92.4	92.7	96.8	96.7	96.3	96.1
Helicobacter cinaedi	3.9	3.2	4.2	2.2	1.5		98.9	95.9	95.7	95.3	92.7	93.1	92.4	92.0	92.4	92.5	95.5	95.6	95.4	95.1
"Flexispira rappini"	4.3	3.0	4.1	1.9	0.9	1.1		95.5	95.7	95.3	92.9	93.1	92.4	92.0	92.2	92.4	96.2	96.1	95.9	95.7
Helicobacter fennelliae	4.2	4.7	5.1	4.7	4.1	4.2	4.7		95.9	94.8	93.0	92.6	92.3	92.6	92.8	92.9	95.5	94.7	94.6	94.4
Helicobacter pullorum	4.0	4.2	5.0	3.8	3.8	4.5	4.4	4.2		96.2	94.8	94.2	94.2	93.2	94.0	94.3	97.4	96.2	96.0	95.5
Helicobacter sp. strain CLO-3	4.7	5.0	5.9	4.2	4.7	4.9	4.8	5.4	3.9		93.8	93.3	93.1	92.8	92.9	93.2	95.1	95.4	95.1	95.3
Helicobacter pylori	7.6	7.0	7.3	6.5	6.8	7.6	7.5	7.4	5.4	6.4		98.2	97.4	94.8	95.0	95.4	94.4	94.1	93.7	93.8
Helicobacter nemestrinae	7.6	6.9	7.4	6.6	6.6	7.2	7.2	7.8	6.0	7.0	1.8		96.6	94.6	94.8	95.4	94.5	94.3	93.9	93.9
Helicobacter acinonyx	8.1	7.2	8.0	7.1	7.6	8.0	8.0	8.1	6.0	7.3	2.7	3.5		94.8	96.1	96.4	94.0	93.5	93.5	93.5
"Gastrospirillum hominis" 1	8.8	8.2	8.6	8.1	8.4	8.4	8.4	7.8	7.1	7.6	5.4	5.6	5.4		96.5	96.6	92.5	92.2	92.2	92.1
"Gastrospirillum hominis" 2	7.8	7.7	8.1	7.7	8.1	8.0	8.2	7.6	6.2	7.5	5.1	5.4	4.0	3.5		98.7	93.7	92.9	93.0	93.2
Helicobacter felis	7.6	7.2	7.9	7.2	7.6	7.9	8.0	7.4	5.9	7.1	4.7	4.7	3.7	3.5	1.3		93.8	93.1	93.3	93.4
Helicobacter pametensis	4.2	3.8	4.4	3.6	3.3	4.6	3.9	4.6	2.6	5.1	5.8	5.8	6.3	7.9	6.6	6.4		98.1	97.9	97.1
Helicobacter sp. strain Bird-C	4.0	3.7	3.7	3.1	3.4	4.5	4.0	5.5	3.9	4.7	6.2	5.9	6.9	8.3	7.5	7.2	2.0		98.3	97.9
Helicobacter sp. strain Bird-B	3.2	3.7	4.0	3.2	3.7	4.7	4.2	5.6	4.1	5.0	6.6	6.4	6.8	8.3	7.3	7.0	2.1	1.7		98.6
Helicobacter mustelae	3.7	3.8	4.4	3.7	4.0	5.0	4.4	5.8	4.7	4.9	6.4	6.3	6.8	8.4	7.1	6.9	3.0	2.1	1.4	

[a] The values on the upper right are uncorrected percentages of similarity, and the values on the lower left are percentages of difference corrected for multiple base changes by the method of Jukes and Cantor.

with *Helicobacter* reference strains (Table 2). A similarity matrix in which *H. trogontum* is compared with the species listed in Table 2 is shown in Table 4. A phylogenetic tree determined from the similarity data is presented in Fig. 2. *H. trogontum* is included in a cluster of intestinal colonizers together with *H. hepaticus*, *H. muridarum*, *H. canis*, *H. cinaedi*, and *"F. rappini."*

PCR identification of strains. PCR amplification with *H. trogontum*-specific primers B72 and B39 (Table 1) produced an 888-base fragment for *H. trogontum* strains. No amplicon was produced when DNAs from other rodent *Helicobacter* species were used. Strains Bird-B, Bird-C, and CLO-3 (Table 2) were presumptively identified by this method. The complete 16S rRNA sequence of strain 95-5368 was determined, and this sequence was identical to the sequences of previously sequenced strains.

DISCUSSION

The nucleotide sequence of the 16S rRNA gene was determined to identify the new isolates definitely. The 1,503 base pairs for which the sequence was determined showed that the new organisms are members of a new *Helicobacter* species which belongs in a cluster consisting of intestinal colonizers together with *H. bilis*, *H. hepaticus*, *H. muridarum*, *H. canis*, *H. cinaedi*, and *"F. rappini"* and is most closely related to *H. hepaticus* (level of similarity, 97.0%). Unlike *H. pullorum*, *H. canis*, *H. fennelliae*, and *H. cinaedi*, which have been isolated from humans and other mammals, the murine helicobacters produced high levels of urea. Since the gastric helicobacters have evolved properties such as high urease activity and very active motility to be able to colonize an extremely hostile

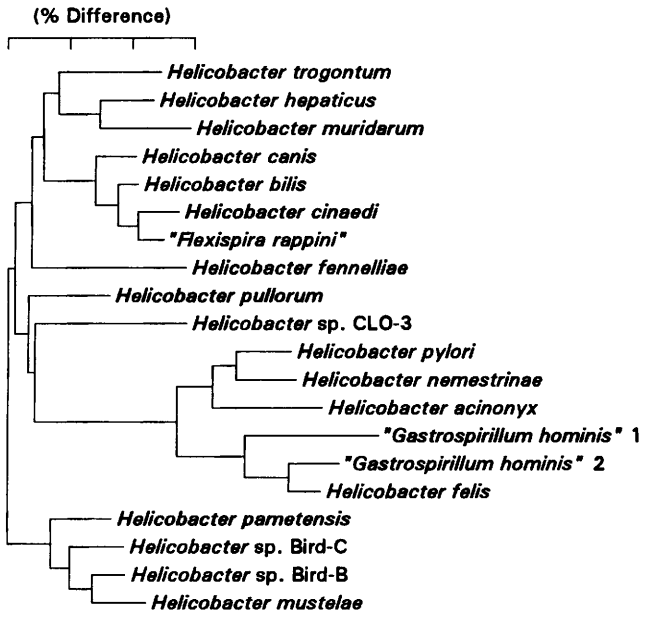

FIG. 2. Phylogenetic tree for 20 *Helicobacter* species based on levels of 16S rRNA sequence similarity. Scale bar = 3% difference in nucleotide sequence, as determined by measuring the lengths of the horizontal lines connecting any two species.

environment, such as the stomach, it would not be surprising if the urease-producing organism *H. trogontum* under certain conditions also colonizes the gastric mucosa of rats. Indeed, organisms morphologically similar to *H. trogontum* and "*F. rappini*," a urease-producing intestinal colonizer of mice, have been observed in the stomachs of dogs (19). *H. muridarum*, another urease producer, is a normal component of the intestinal flora of mice and rats which can also colonize the stomachs of mice and can apparently induce gastritis (23). The morphology of *H. trogontum* is similar to that of *H. bilis*, which also colonizes the lower intestine, but in addition *H. bilis* also colonizes livers of aged mice. *H. trogontum* inhabits the colons of rats, and organisms that have similar morphologies have been observed in bile ducts of rats experimentally infected with the liver fluke *Fasiola hepatica* (6). We are also investigating if *H. trogontum* colonizes other regions of the rat gut besides the colonic mucosa and if it colonizes the intestines of other mammals as well.

In summary, the genus *Helicobacter* now includes not only gastric bacteria, as first hypothesized (16, 18), but also intestinal colonizers (3, 5, 8, 9, 18, 27, 28, 32). Studies of the host-parasite relationship of intestinal helicobacters, of natural and experimental infections, and of the conditions under which the intestinal helicobacters can induce disease are important for understanding how *H. pylori* causes peptic ulcer disease as well as gastric cancer. Ultimately, data from such studies will help determine appropriate strategies for the management of gastric diseases.

Description of *H. trogontum* sp. nov. *H. trogontum* (tro.gon' tum. Gr. part. adj. *trogon*, gnawing; N. L. gen. pl. n. *trogontum*, of gnawing animals, since the organism was first isolated from rats). Cells are fusiform to slightly spiral and 0.6 to 0.7 μm wide by 4 to 6 μm long, have periplasmic fibers coiled around the protoplasmic cylinder, and have morphology typical of "*F. rappini*." In older cultures, coccoid forms with overlapping periplasmic fibers are common. Cells are gram negative and nonsporulating. Cells are motile by means of tufts consisting of three to seven sheathed flagella at each end. Colonies are pinpoint, but cultures often appear to be thin spreading layers on agar media. Microaerobic growth occurs at 37 and 42°C but not at 25°C. No growth occurs on brucella agar plates containing 1% glycine or 1.5, 2, or 3% NaCl. The organisms have catalase, oxidase, urease, gamma-glutamyl transpeptidase, and ornithine decarboxylase activities. Nitrate is not reduced, and H_2S is not detected on lead acetate discs. Hippurate is not hydrolyzed. Alkaline phosphatase is produced by one of the three strains examined. The bacteria are susceptible to metronidazole but resistant to cephalothin and nalidixic acid. Cells have been isolated from colonic mucosa of rats. The type strain, LRB 8581, was isolated from the colon of a rat. The type strain has been deposited in the American Type Culture Collection as strain ATCC 700114. The essentially complete 16S rRNA sequence has been deposited in the GenBank database under accession number U65103.

ACKNOWLEDGMENTS

This work was supported by CNPq-Brazil, by grants RR-01046 and RR-07036 from the National Center for Research Resources, by contract RFPS 94-69 from the National Cancer Institute, and by grants DE-10374 and DE-08303 from the National Institute of Dental Research. E.N.M. was supported in part by a CNPq-Brazil postdoctoral fellowship.

REFERENCES

1. **Bizzozzero, G.** 1893. Uber die schlauchformigen Drusen des Magendarmkanals und die Beziehungen ihres Epithels zu dem Oberflachenepithel der Schleimhaut. Arch. Mikr. Anat. **42:**82–152.
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. **Dewhirst, F. E., C. Seymour, G. J. Fraser, B. J. Paster, and J. G. Fox.** 1994. Phylogeny of *Helicobacter* isolates from bird and swine feces and description of *Helicobacter pametensis* sp. nov. Int. J. Syst. Bacteriol. **44:**553–560.
4. **Eaton, K. A., F. Dewhirst, M. J. Radin, J. G. Fox, B. J. Paster, S. Krakowka, and D. R. Morgan.** 1993. *Helicobacter acinonyx* sp. nov., isolated from cheetahs with gastritis. Int. J. Syst. Bacteriol. **43:**99–106.
5. **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.
6. **Foster, J. R.** 1984. Bacterial infection of the common bile duct in chronic fascioliasis in the rat. J. Comp. Pathol. **94:**175–181.
7. **Fox, J. G., F. E. Dewhirst, G. J. Fraser, B. J. Paster, B. Shames, and J. C. Murphy.** 1994. Intracellular *Campylobacter*-like organism from ferrets and hamsters with proliferative bowel disease is a *Desulfovibrio* sp. J. Clin. Microbiol. **32:**1229–1237.
8. **Fox, J. G., F. E. Dewhirst, J. G. Tully, B. J. Paster, L. 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.
9. **Fox, J. G., L. L. Yan, F. E. Dewhirst, B. J. Paster, B. Shames, J. C. Murphy, A. Hayward, J. C. Belcher, and E. N. Mendes.** 1995. *Helicobacter bilis* sp. nov., a novel *Helicobacter* isolated from bile, livers, and intestines of aged, inbred mouse strains. J. Clin. Microbiol. **33:**445–454.
10. **Goodwin, C. S., J. A. Armstrong, T. Chilvers, M. A. Peters, D. M. Collins, L. I. 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.
11. **Hanninen, M. L., I. Happonen, S. Saari, and K. Jalava.** 1996. Culture and characteristics of *Helicobacter bizzozeronii*, a new canine gastric *Helicobacter* sp. Int. J. Syst. Bacteriol. **46:**160–166.
12. **Hwang, M.-N., and G. M. Ederer.** 1975. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. J. Clin. Microbiol. **1:**114–115.
13. **Jukes, T. H., and C. R. Cantor.** 1969. Evolution of protein molecules, p. 21–132. *In* H. N. Munro (ed.), Mammalian protein metabolism, vol. 3. Academic Press, Inc., New York.
14. **Kirkbride, C. A., C. E. Gates, J. E. Collins, and M. S. Ritchie.** 1985. Ovine abortion associated with an anaerobic bacterium. J. Am. Vet. Med. Res. **186:**789–791.
15. **Kusukawa, N., T. Uemori, K. Asada, and I. Kato.** 1990. Rapid and reliable protocol for direct sequencing of material amplified by the polymerase chain reaction. BioTechniques **9:**66–72.
16. **Lee, A.** 1991. Spiral organisms: what are they? A microbiologic introduction to *Helicobacter pylori*. Scand. J. Gastroenterol. (Suppl. 187)**26:**9–22.
17. **Lee, A., and J. O'Rourke.** 1993. Gastric bacteria other than *Helicobacter pylori*. Gastroenterol. Clin. N. Am. **22:**21–42.
18. **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.
19. **Lockard, V. G., and R. K. Boler.** 1970. Ultrastructure of a spiraled microorganism in the gastric mucosa of dogs. Am. J. Vet. Res. **31:**1453–1462.
20. **Marshall, B. J., and J. R. Warren.** 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet **i:**1311–1314.
21. **Parsonnet, J., G. D. Friedman, D. P. Vandersteen, J. H. Chang, J. H. Vogelman, N. Orentriech, and R. K. Sibley.** 1991. *Helicobacter pylori* infection and the risk of gastric adenocarcinoma. N. Engl. J. Med. **325:**1127–1131.
22. **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.
23. **Queiroz, D. M. M., C. Contigli, R. S. Coimbra, A. M. M. F. Nogueira, E. N. Mendes, G. A. Rocha, and S. B. Moura.** 1992. Spiral bacterium associated with gastric, ileal and caecal mucosa of mice. Lab. Anim. **26:**288–294.
24. **Queiroz, D. M. M., E. N. Mendes, and G. A. Rocha.** 1987. Indicator medium for isolation of *Campylobacter pylori*. J. Clin. Microbiol. **25:**2378–2379.
25. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
26. **Schauer, D. B., N. Ghori, and S. Falkow.** 1993. Isolation and characterization of "*Flexispira rappini*" from laboratory mice. J. Clin. Microbiol. **31:**2709–2714.
27. **Solnick, J. V., J. O'Rourke, A. Lee, B. J. Paster, F. E. Dewhirst, and L. S. Tompkins.** 1993. Uncultured gastric spiral organism is a newly identified *Helicobacter* in humans. J. Infect. Dis. **168:**379–385.
28. **Stanley, J., D. Linton, A. P. Burnens, F. E. Dewhirst, S. L. W. On, A. Porter, R. J. Owen, and M. Costas.** 1994. *Helicobacter pullorum* sp. nov.: polyphasic

description of a new species from poultry which infects humans. Microbiology **140:**3441–3449.

29. **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.
30. **Studier, J., and K. Keppler.** 1988. A note on the neighbor-joining algorithm of Saitou and Nei. Mol. Biol. Evol. **5:**729–731.
31. **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 fennelliae* (sp. nov.): two new *Campylobacter* species associated with enteric disease in homosexual men. J. Infect. Dis. **151:**131–139.
32. **Vandamme, P., E. Falsen, R. Rossau, B. Hoste, P. Segers, R. Tytgat, and J. Deley.** 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.
33. **Wotherspoon, A. C., C. Doglioni, T. C. Diss, P. Langxing, A. Moschini, M. de Boni, and P. G. Isaacson.** 1993. Regression of primary low-grade B-cell gastric lymphoma of mucosa associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet **342:**575–577.