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Helicobacter hepaticus sp. nov., a Microaerophilic Bacterium Isolated from Livers and Intestinal Mucosal Scrapings from Mice

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A bacterium with a spiral shape and bipolar, single, sheathed flagella was isolated from the livers of mice with active, chronic hepatitis. The bacteria also colonized the cecal and colonic mucosae of mice. The bacterium grew at 37°C under microaerophilic and anaerobic conditions, rapidly hydrolyzed urea, was catalase and oxidase positive, reduced nitrate to nitrite, and was resistant to cephalothin and nalidixic acid but sensitive to metronidazole. On the basis of 16S rRNA gene sequence analysis, the organism was classified as a novel helicobacter, *Helicobacter hepaticus*. This new helicobacter, like two other murine *Helicobacter* species, *H. muridarum* and "*H. rappini*," is an efficient colonizer of the gastrointestinal tract, but in addition, it has the pathogenic potential to elicit persistent hepatitis in mice.

During the last decade, microaerophilic spiral-to-curve-shaped bacteria isolated from the stomachs of humans and animals have been the focus of considerable research because of their association with gastric disease (24). These microorganisms are recognized as belonging to the genus *Helicobacter*. It is now known that the type species *Helicobacter pylori* causes active, chronic gastritis and peptic ulcer disease in humans (14, 21, 24). This microorganism has also been recently linked to the development of gastric adenocarcinoma and gastric mucosa-associated lymphoma (11, 25, 27, 41). Several additional *Helicobacter* species have been isolated from the stomachs of various mammalian species (2, 5, 12, 22) and have been shown to cause various degrees of gastritis in their hosts (9, 10, 13). Additional *Helicobacter* species have been isolated from the intestinal tracts of mammals (6, 35, 37, 38) and birds (4). One of them, *H. muridarum*, primarily colonizes the ilea and ceca of rodents but can also apparently elicit gastritis after colonizing the gastric mucosae of older rodents (23, 31). Also, "*Helicobacter* (*Flexispira*) *rappini*," which has periplasmic fibers like *H. muridarum* and *H. felis* but is distinguishable by the shape of its protoplasmic cylinder, has been associated with abortion in sheep and intestinal disease in animals and humans (1, 16, 32). Experimental inoculation of "*H. rappini*" into guinea pigs also causes abortion (3). Most recently, "*H. rappini*" has been isolated from the colons and ceca of mice (34). Thus, as the genus expands, it is clear that *Helicobacter* species can infect several animal species as well as colonize different anatomical regions of the gastrointestinal system (13).

A spiral-to-curved bacterium was observed with Steiner's silver stains in livers of barrier-maintained mice suffering from multifocal necrotic hepatitis (39, 40). Because of the microaerophilic nature of the bacterium, its morphology, and its strong urease activity, we hypothesized that this bacterium may be another helicobacter. In this paper, we provide biochemical, ultrastructural, and molecular data confirming that this bacterium represents a new *Helicobacter* species, *H. hepaticus*.

MATERIALS AND METHODS

Fifteen strains (Hh-1 through Hh-15) of *H. hepaticus* were isolated from the livers of SCID/NCr mice. In addition, seven strains of *H. hepaticus* (Hh-16 through Hh-22) were isolated from either colonic or cecal mucosal scrapings from SCID/NCr or A/JCr mice. Briefly, the original isolate, Hh-1, was isolated from the liver of a mouse with multifocal hepatitis by streaking liver tissue onto moist Columbia blood agar plates (Remel Labs, Lenexa, Kans.) and incubating it at 37°C under anaerobic conditions (GasPak system; BBL Microbiology Systems, Cockeysville, Md.). Subsequent isolations of bacteria from infected livers were performed on either moist Trypticase soy agar blood agar plates or brucella blood agar with TVP (trimethoprim, vancomycin, polymyxin) (Remel Labs) at 37°C

TABLE 1. Oligonucleotide primers used for PCR amplification and sequencing of 16S rDNA

Type	Sequence (5′→3′)[a]	Position[b]	Orientation
PCR	AGAGTTTGATYCTGGCT	8–24	Forward
PCR	TACGGYTACCTTGTTACGACT	1493–1513	Reverse
Sequencing	ACTGCTGCCTCCCGT	344–358	Reverse
Sequencing	GTRTTACCGCGGCTGCTG	519–536	Reverse
Sequencing	CTACCAGGGTATCTAATC	786–804	Reverse
Sequencing	GGTTGCGCTCGTTGCGGG	1096–1113	Reverse
Sequencing	GGAATCGCTAGTAATCG	1337–1353	Forward
Sequencing	CCCGGGAACGTATTCACCG	1369–1387	Reverse

[a] Base codes are standard International Union of Biochemistry codes for bases and ambiguity.
[b] Numbering based on that of *Escherichia coli*.

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TABLE 2. Sources and accession numbers of strains studied

Organism	Strain examined[a]	Culture collection no.[b]	GenBank accession no.[c]
Helicobacter hepaticus isolate	Fox Hh-1	ATCC 51449	U07573
	Fox Hh-2^T	ATCC 51448^T	U07574
	Fox Hh-3	ATCC 51450	U07575
Reference species			
Arcobacter cryaerophila	CCUG 17801^T	ATCC 43158^T	L14624
Arcobacter butzleri	CCUG 10373		L14626
Arcobacter skirrowii	CCUG 10374^T		L16625
Campylobacter coli	CCUG 11238^T	ATCC 33559^T	L04312
Campylobacter concisus	Tanner 484^T	ATCC 33237^T	L04322
Campylobacter fetus subsp. *fetus*	ATCC 27374^T		M65012
Campylobacter lari	CCUG 23947^T	ATCC 35221^T	L04316
Campylobacter rectus	Tanner 371^T	ATCC 33238^T	L04317
"*Flexispira rappini*"	NADC 1893^T	ATCC 43966^T	M88137
"*Gastrospirillum hominis* 1"			L10079
"*Gastrospirillum hominis* 2"			L10080
Helicobacter acinonyx	Eaton 90-119-3^T	ATCC 51101^T, CCUG 29263^T	M88148
Helicobacter canis	NCTC 12739^T		L13464
Helicobacter cinaedi	CCUG 18818^T	ATCC 35683^T	M88150
Helicobacter felis	Lee CS1^T	ATCC 49179^T	M37642
Helicobacter fennelliae	CCUG 18820^T	ATCC 35684^T	M88154
Helicobacter mustelae	Fox R85-13-6^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 pylori	ATCC 43504^T		M88157
Helicobacter sp. strain Bird-B	Seymour B10^T	CCUG 29256^T	M88139
Helicobacter sp. strain Bird-C	Seymour B52^T	CCUG 29561^T	M88144
Helicobacter sp. strain CLO-3	CCUG 14564	LMG 7792	M88151
Wolinella succinogenes	Tanner 602W^T	ATCC 29543^T	M88159

[a] Sources of the strains from which sequences were determined were as follows: Eaton, K. A. Eaton, Department of Veterinary Pathobiology, Ohio State University, Columbus; Fox, J. G. Fox, Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge; Lee, A. Lee, Department of Microbiology and Immunology, University of New South Wales, Sydney, Australia; Seymour, C. Seymour, Department of Microbiology, Boston University School of Medicine, Boston, Mass.; Tanner, A. Tanner, Department of Microbiology, Forsyth Dental Center, Boston, Mass.; ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; LMG, Laboratorium voor Microbiologie en microbielle Genetica, Ghent, Belgium; NADC, National Animal Disease Center, Ames, Iowa; and NCTC, National Collection of Type Cultures, London, United Kingdom.

[b] Alternate culture collection sources for sequenced strains.

[c] 16S rRNA sequences for these strains are available for electronic retrieval from GenBank under the accession numbers shown above. Through cross-distribution of data bases, these sequences should also be available from the EMBL and DDBJ data bases.

under microaerophilic conditions in vented jars containing N_2, H_2, and CO_2 (90:5:5:).

Electron microscopy. Cells grown on Trypticase soy agar blood agar plates 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 15 to 20 s. Specimens were examined with a JEOL model JEM-1200EX transmission electron microscope operating at 100 kV.

Biochemical characterization. Detailed biochemical characterization of strains Hh-1 through Hh-10 (Table 1) was performed as previously described (23, 29). In the remaining 12 strains, motility, Gram stain reaction, oxidase, catalase, and urease activities and sensitivity to nalidixic acid, cephalothin, and metronidazole were determined.

Crude DNA isolation. Bacteria were cultured on Trypticase soy agar blood agar plates. A loopful of cells was harvested and suspended in 100 μl of lysis buffer (50 mM Tris-HCl [pH 7.6], 1 mM EDTA, 0.5% Tween 20, 200 μg of proteinase K per ml) and incubated at 55°C for 2 h. The proteinase K was inactivated by being heated to 95°C for 10 min. Crude DNA was then precipitated with 2 volumes of cold absolute ethanol.

Amplification of 16S rRNA cistrons. The 16S rRNA cistrons were amplified with the top two primers in Table 1. PCRs were performed in thin-walled tubes with a Perkin-Elmer 480 thermal cycler. Ten microliters of the crude DNA and 1 μM (each) primers were added to the reaction mixture, which had a final volume of 82 μl. Ampliwax PCR Gem100s (Perkin-Elmer) were used in a hot-start protocol as suggested by the manufacturer. The following conditions were used 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 an additional 5 s added for each cycle. A total of 25 cycles were performed, 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% agarose gel (FMC Bioproducts). DNA was stained with ethidium bromide and viewed under short-wavelength UV light.

Purification of PCR products. The amplified DNA was purified by precipitation with polyethylene glycol 8000 (20). After removal of Ampliwax, 0.6 volume of 20% polyethylene glycol 8000 (Sigma) in 2.5 M NaCl was added, and the mixture was incubated at 37°C for 10 min. The sample was centrifuged for 15 min at 15,000 × *g*, and the pellet was washed with 80% ethanol and pelleted as before. The pellet was air dried, dissolved in 30 μl of distilled water, and used for cycle sequencing as described below.

Sequencing methods. The DNA sample from PCR was directly sequenced with a cycle-sequencing kit (TAQuence cycle sequencing kit; United States Biochemical Corp.). The

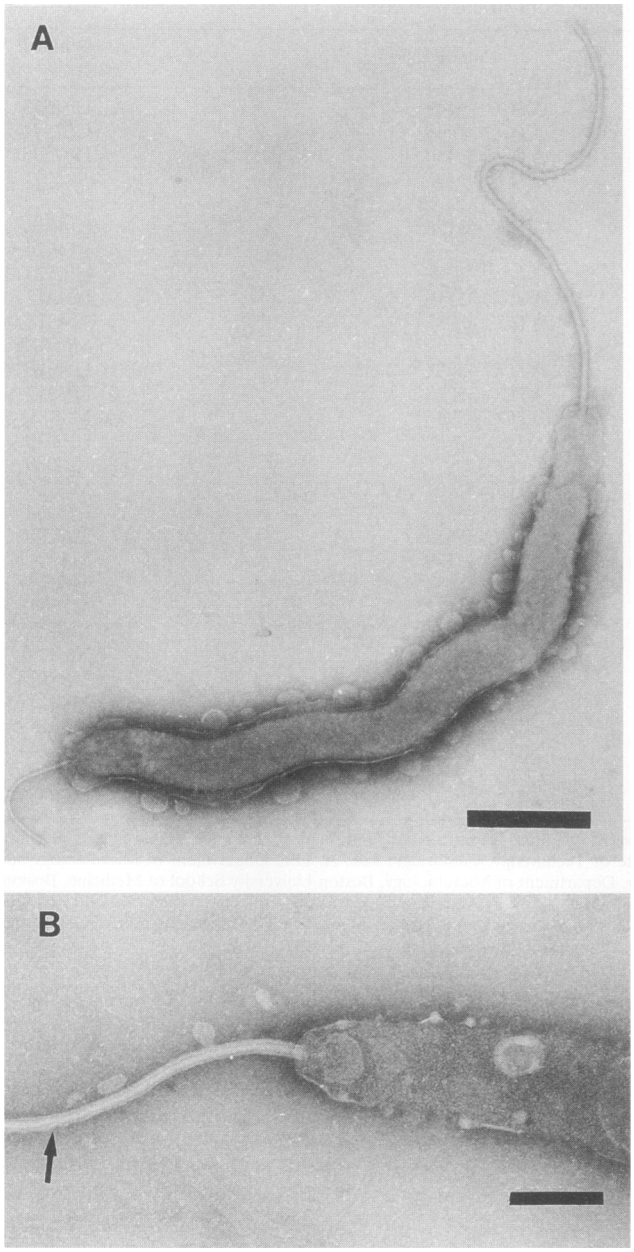

FIG. 1. Negatively stained preparation of *H. hepaticus* Hh-2. (A) Typical cell with a single subterminal flagellum at either end. Bar, 500 nm. (B) Sheathed flagellum (arrow). Bar, 200 nm.

manufacturer's protocol was followed. The six sequencing primers are given in Table 1. 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 used for sequencing. Reaction products were loaded onto 8% polyacrylamide–urea gels, electrophoresed, and detected by exposure to X-ray film for 24 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 (28). RNA sequences were entered and aligned as previously described (28). Our sequence data base contains approximately 300 sequences determined in our laboratory, and another 200 were obtained from GenBank or the Ribosomal Database Project (26). Reference strains used in the 16S rRNA analysis are given in Table 2. Similarity matrices were constructed from the aligned sequences by using only those sequence positions for which 90% of the strains had data. The similarity matrices were corrected for multiple base changes at single positions by the method of Jukes and Cantor (15). Phylogenetic trees were constructed by the neighbor-joining method of Saitou and Nei (33, 36).

GenBank accession number. The GenBank and culture collection accession numbers for the strains examined in this report are given in Table 2.

RESULTS

Isolation, morphology, and growth characteristics. After a 3- to 7-day incubation under anaerobic or microaerophilic conditions, a thin spreading film was noted on the agar surface. Examination with dark-field and phase microscopy revealed the presence of spiral, motile bacteria. While the bacteria grow anaerobically, microaerophilic conditions were routinely used to isolate bacteria from the liver and are considered the optimal environmental conditions for growth of *H. hepaticus*. The bacteria were gram negative, curved to spiral, and 1.5 to 5.0 μm long and 0.2 to 0.3 μm wide.

Ultrastructure. The organism had a smooth surface and lacked periplasmic fibers, which are found in the two mouse intestinal helicobacters "*H. rappini*" and *H. muridarum* (3, 23). The organism varied in shape and size from curved to spiral, having one to several spirals (Fig. 1A). The bacteria were characterized by bipolar sheathed flagella (one at each end) (Fig. 1B).

Biochemical and physiological characteristics. Ten strains of *H. hepaticus* were subjected to a number of tests to distinguish biochemical and physiological properties which were then compared with those of known *Helicobacter* species. Like *H. muridarum* and "*H. rappini*," *H. hepaticus* has strong urease activity and was oxidase and catalase positive. *H. hepaticus* strains consistently produced H_2S by using lead acetate and reduced nitrate to nitrite. The bacteria grew microaerophilically at 37°C but not at 25 or 42°C. The bacteria also grew in 1.5% NaCl, 1% glycine, 0.04% triphenyltetrazolium chloride, and 0.1% trimethylamine *N*-oxide (anaerobically). *H. hepaticus* hydrolyzed indoxyl acetate but did not hydrolyze hippurate, nor did it produce hemolysis in blood agar or produce yellow pigment. The remaining 12 strains were also gram negative, curved to spiral, and motile, as well as catalase, oxidase, and strongly urease positive. All of the strains were resistant to both cephalothin and nalidixic acid but sensitive to metronidazole.

Phylogenetic analysis. We analyzed approximately 95% of the total RNA sequence for strain Hh-1 and partial sequences for Hh-2 and Hh-3. The sequences for the three mouse liver strains were identical. Comparison of the consensus sequence with those of other bacteria in our data base indicated that the *H. hepaticus* sequence was most closely related to that of *H. muridarum* (97.8% similarity). This degree of sequence difference clearly identifies *H. hepaticus* as a novel species. Strain Hh-2 was compared with 26 reference species in the genera *Helicobacter*, *Wolinella*, *Arcobacter*, and *Campylobacter*. The similarity matrix for these comparisons is presented in Table 3. A phylogenetic tree constructed with the neighbor-joining method is presented in Fig. 2. *H. hepaticus* falls in a cluster of

TABLE 3. Similarity matrix on the basis of 16S rRNA sequence comparisons[a]

	H.he	*H.mr*	*H.ca*	*F.ra*	*H.ci*	*H.fe*	*H.s3*	*H.py*	*H.ne*	*H.ac*	*G.h1*	*G.h2*	*H.fe*	*H.pa*	*H.sC*	*H.sB*	*H.ms*	*W.su*	*A.cr*	*A.sk*	*A.bu*	*C.re*	*C.co*	*C.fe*	*C.he*	*C.la*	*C.je*
Helicobacter hepaticus	.	97.8	97.3	97.4	97.0	95.5	95.2	93.5	93.6	93.2	92.3	92.8	93.3	96.3	96.5	96.5	96.4	92.8	84.9	84.6	85.8	85.5	87.1	86.1	86.6	86.6	86.1
Helicobacter muridarum	2.3	.	96.5	96.1	95.9	95.1	94.3	93.1	93.1	92.5	91.9	92.4	92.6	95.7	96.3	96.0	95.8	93.4	85.0	84.6	85.8	84.8	86.4	85.6	86.4	86.2	85.8
Helicobacter canis	2.8	3.6	.	98.0	97.8	95.4	96.0	93.9	93.8	93.2	92.3	92.7	93.2	96.5	96.9	96.8	96.4	93.4	85.4	85.0	85.9	85.6	87.2	86.5	87.2	87.1	86.6
"Flexispira rappini"	2.6	4.0	2.0	.	98.8	95.4	95.5	93.2	93.4	92.5	92.2	92.3	92.6	96.5	96.4	95.8	95.8	93.1	85.1	84.7	85.5	85.9	87.4	86.7	87.1	87.2	86.7
Helicobacter cinaedi	3.1	4.2	2.3	1.2	.	95.9	95.3	92.8	93.3	92.4	92.0	92.4	92.6	95.5	95.6	95.4	95.2	92.9	85.0	84.6	85.4	85.8	87.2	86.4	86.3	86.5	86.0
Helicobacter fennelliae	4.6	5.1	4.7	4.8	4.3	.	94.8	93.1	92.8	92.3	92.6	92.8	93.0	95.5	94.6	94.6	94.4	92.5	84.5	84.3	85.6	85.4	87.0	86.1	86.0	86.8	86.2
Helicobacter sp. CLO-3	4.9	5.9	4.1	4.6	4.9	5.4	.	93.9	93.4	93.0	92.7	92.8	93.2	95.1	95.5	95.2	95.2	92.4	85.5	85.1	86.3	85.2	86.8	85.9	85.9	86.6	85.9
Helicobacter pylori	6.8	7.2	6.4	7.1	7.5	7.3	6.4	.	98.2	97.4	94.9	95.1	95.4	94.5	94.2	93.8	93.9	90.9	84.5	84.0	85.3	84.1	85.1	85.1	85.6	85.6	85.4
Helicobacter nemestrinae	6.7	7.3	6.4	6.9	7.0	7.6	6.9	1.8	.	96.7	94.7	94.9	95.5	94.6	94.5	94.0	94.0	91.0	85.0	84.4	85.6	84.8	86.0	85.7	86.2	86.0	85.7
Helicobacter acinonyx	7.1	8.0	7.1	7.9	8.0	8.1	7.4	2.6	3.3	.	94.7	96.0	96.4	94.0	93.5	93.5	93.5	90.6	84.2	83.9	85.1	83.6	84.8	84.6	85.2	85.2	85.0
"Gastrospirillum hominis" 1	8.1	8.6	8.1	8.3	8.4	7.8	7.6	5.3	5.5	5.5	.	96.5	96.6	92.6	92.2	92.2	92.0	89.3	83.5	83.2	83.7	83.2	84.6	84.5	85.0	84.9	84.4
"Gastrospirillum hominis" 2	7.6	8.0	7.6	8.1	8.0	7.6	7.5	5.1	5.3	4.1	3.6	.	98.8	93.7	92.9	93.0	93.2	90.0	83.7	83.2	84.1	82.9	84.5	84.4	85.0	85.1	84.6
Helicobacter felis	7.0	7.8	7.1	7.7	7.8	7.3	7.1	4.7	4.7	3.7	3.4	1.3	.	93.9	93.2	93.4	93.4	90.2	83.5	83.3	84.2	83.0	84.5	84.5	85.0	85.1	84.6
Helicobacter pametensis	3.8	4.5	3.6	3.6	4.6	4.6	5.0	5.7	5.6	6.3	7.8	6.6	6.3	.	98.1	97.9	97.2	94.2	86.0	85.6	86.5	85.9	87.6	86.5	87.2	87.7	87.2
Helicobacter sp. Bird-C	3.6	3.8	3.2	3.7	4.5	5.6	4.7	6.1	5.7	6.8	8.2	7.5	7.1	2.0	.	98.3	98.0	94.6	85.6	85.1	86.3	85.5	86.8	86.6	87.3	87.7	87.1
Helicobacter sp. Bird-B	3.6	4.1	3.2	4.3	4.8	5.6	5.0	6.5	6.2	6.8	8.2	7.3	6.9	2.1	1.8	.	98.7	94.2	86.1	85.9	86.9	85.1	86.5	86.2	86.9	87.2	86.9
Helicobacter mustelae	3.7	4.4	3.7	4.3	5.0	5.8	4.9	6.4	6.2	6.8	8.4	7.1	6.9	2.9	2.1	1.3	.	93.8	86.0	85.6	86.5	84.8	86.1	85.8	86.5	87.2	86.6
Wolinella succinogenes	7.6	6.9	6.9	7.3	7.4	7.9	8.0	9.8	9.5	10.1	11.5	10.7	10.5	6.1	5.6	6.1	6.4	.	85.6	85.2	86.1	86.3	86.3	85.9	86.0	86.8	86.4
Arcobacter cryaerophilus	16.8	16.8	16.2	16.7	16.7	17.4	16.1	17.4	16.8	17.7	18.7	18.4	18.7	15.4	16.0	15.3	15.4	15.9	.	99.0	97.4	86.1	87.6	86.1	86.5	87.2	87.3
Arcobacter skirrowii	17.3	17.2	16.7	17.2	17.3	17.6	16.6	18.0	17.5	18.1	19.1	19.0	18.9	16.0	16.6	15.6	16.0	16.4	1.0	.	97.3	85.6	87.6	86.0	86.2	86.9	87.2
Arcobacter butzleri	15.8	15.7	15.6	16.1	16.2	16.0	15.1	16.3	16.0	16.6	18.3	17.9	17.7	14.9	15.2	14.4	14.9	15.3	2.6	2.8	.	86.6	88.0	86.1	86.4	86.7	86.9
Campylobacter rectus	16.2	17.0	16.0	15.6	15.8	16.2	16.5	17.8	17.0	18.5	19.1	19.4	19.3	15.6	16.1	16.6	16.9	15.1	15.4	16.0	14.8	.	96.0	94.0	91.8	92.5	92.9
Campylobacter concisus	14.2	15.1	14.0	13.8	14.0	14.3	14.6	16.6	15.5	17.0	17.2	17.4	17.3	13.6	14.5	14.9	15.4	15.1	13.6	13.6	13.1	4.1	.	95.6	92.3	93.9	93.8
Campylobacter fetus	15.3	16.0	14.9	14.6	15.0	15.4	15.6	16.6	15.9	17.3	17.4	17.4	17.4	14.9	14.7	15.3	15.7	15.6	15.4	15.5	15.3	6.2	4.6	.	93.3	94.2	94.5
Campylobacter helveticus	14.8	15.0	14.0	14.1	15.1	15.5	15.6	16.0	15.3	16.5	16.7	16.7	16.7	14.0	13.9	14.4	14.9	15.5	14.9	15.3	15.0	8.6	8.1	7.0	.	96.7	97.1
Campylobacter lari	14.7	15.2	14.1	14.1	14.8	14.6	14.8	16.0	15.5	16.4	16.8	16.7	16.6	13.4	13.5	14.0	14.1	14.5	14.0	14.4	14.7	7.9	6.4	6.0	3.4	.	98.7
Campylobacter jejuni	15.4	15.8	14.7	14.7	15.5	15.2	15.7	16.2	15.8	16.7	17.5	17.2	17.2	14.0	14.1	14.4	14.7	15.1	13.9	14.1	14.4	7.5	6.5	5.7	3.0	1.3	.

[a] Numbers above the diagonal represent uncorrected percentages of similarity, and those below the diagonal are percentages of difference corrected for multiple base changes by the method of Jukes and Cantor (15).

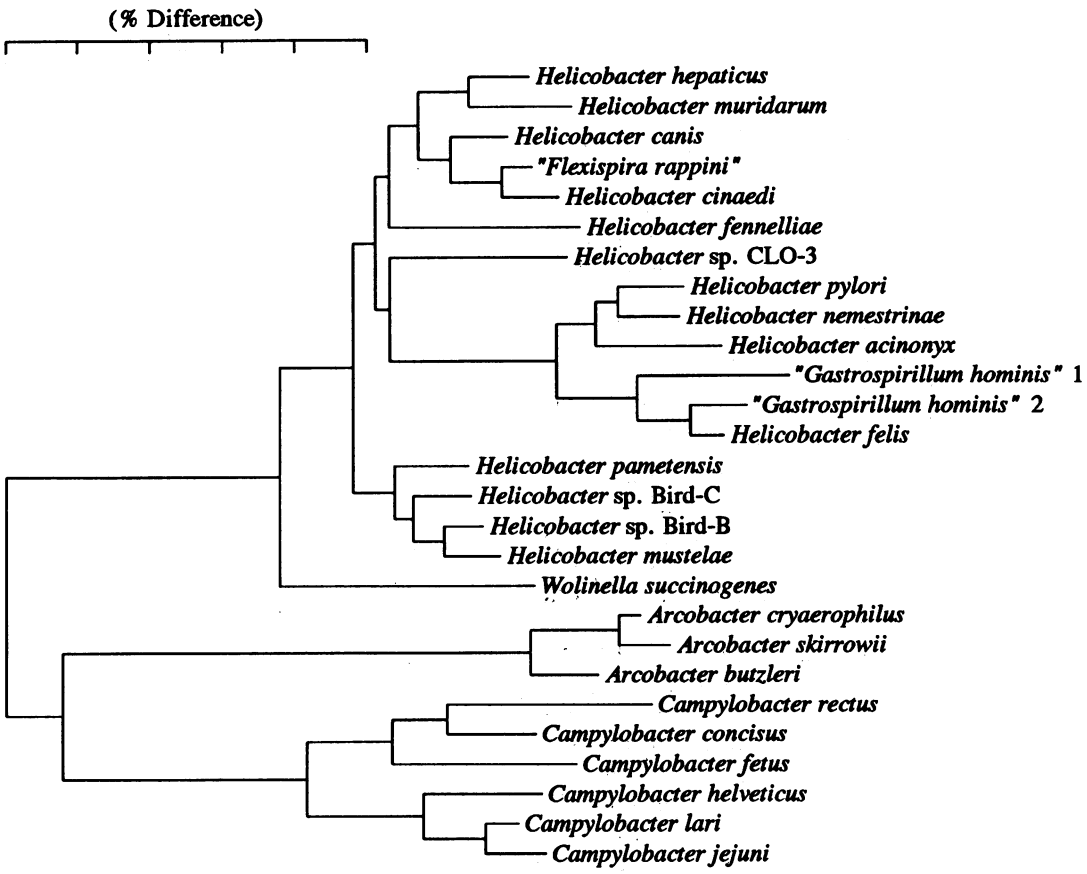

FIG. 2. Phylogenetic tree for 27 strains of *Helicobacter*, *Wolinella*, *Arcobacter*, and *Campylobacter* species on the basis of 16S rRNA sequence similarity. The scale bar represents a 5% difference in nucleotide sequence as determined by measuring the length of horizontal lines connecting any two species.

intestinal helicobacters which includes *H. muridarum*, *H. canis*, "*H. rappini*," and *H. cinaedi*.

Differentiation of *H. hepaticus* from other *Helicobacter* species. The phenotypic characteristics which differentiate *H. hepaticus* from other named and described *Helicobacter* species are presented in Table 4.

DISCUSSION

An active, chronic hepatitis was detected during 1991 to 1993 in several inbred strains of mice originating from the Frederick Cancer Center barrier-maintained facility. As described by Ward et al. (39, 40), *H. hepaticus* was associated with acute, focal nonsuppurative necrotizing hepatitis in young mice which progressed to chronic, active hepatitis. This chronic hepatitis was characterized by minimal necrosis, hepatocytomegaly, oval cell hyperplasia, and cholangitis. With Steiner stain, helical organisms were seen between hepatocytes adjacent to areas of necrosis in young mice and throughout the livers of older mice with chronic hepatitis. The bacteria were observed by electron microscopy in bile canaliculi (39, 40). To date, the chronic, active hepatitis has been diagnosed in at least 15 strains of mice in 16 buildings located at the Frederick Cancer Center premises. In addition, the hepatic lesions have been associated with a high incidence of hepatic tumors in A/JCr mice, which normally have a low frequency of hepatic tumors (40). The hepatitis has also been successfully reproduced in germ-free mice by oral inoculation of *H. hepaticus* (8).

The epizootiology of the disease at present is unknown. However, like that of other *Helicobacter* species, its normal ecological niche is probably the gastrointestinal tract. Prior to the association of *H. hepaticus* with hepatitis in mice, two other helicobacters, *H. muridarum* and "*H. rappini*," had been cultured from the mouse gastrointestinal tract (23, 30, 34). Both are natural colonizers of the lower gastrointestinal tract, where their presence doesn't apparently elicit an inflammatory response (30, 34). *H. muridarum*, under circumstances not completely understood but probably related to subtle pH changes in the stomachs of rodents as they age, can colonize gastric tissue in both mice and rats and can induce gastritis (31). On the basis of our preliminary culture results, *H. hepaticus* can be isolated from the ceca and colons of mice with and without *H. hepaticus*-associated liver lesions (8). *H. hepaticus* is actively motile because of the single sheathed flagellum at one or each end of the bacteria. As for the two other intestinal colonizers of mice, *H. muridarum* and "*H. rappini*," the presence of the flagella is probably important in colonization of mucus in the intestinal crypts.

Even though all three mouse helicobacters colonize the lower gastrointestinal tract, *H. hepaticus* can be differentiated from the two other helicobacters of mice biochemically by the ability of *H. hepaticus* to reduce nitrate and to grow in 1% glycine. Also, ultrastructurally, the single bipolar sheathed flagellum and curved-to-slightly spiral shape of *H. hepaticus* easily distinguish this organism from the two other known

rodent helicobacters, which have multiple bipolar flagella and periplasmic fibrils. Why some inbred mouse strains appear more susceptible to developing liver lesions is unknown but may be related to the host genotype. It is well-known that the mouse haplotype is often one of the determinants in conferring resistance or susceptibility to a number of infectious agents. Alternatively, different strains of *H. hepaticus* may exist with various degrees of pathogenic potential. There is precedence for *Helicobacter* species causing hepatitis under certain circumstances: "*H. rappini*" can cross the placenta of pregnant sheep, induce abortions, and cause acute hepatic necrosis in sheep fetuses (3, 16). Also, an organism closely resembling "*H. rappini*" was observed in the common bile duct of rats experimentally infected with the liver fluke, *Fasciola hepatica* (7). The authors speculated that the fluke infection altered the biochemical properties of the rats' bile and allowed the bacteria to colonize this normally bacteriostatic milieu. Thus, *H. hepaticus* is similar to all other known *Helicobacter* species in being an efficient colonizer of the gastrointestinal tract, but in addition it has the pathogenic potential to elicit hepatitis in several strains of mice, and in one strain, A/JCr, *H. hepaticus* is strongly associated with hepatic cancer (39, 40).

While little has been done experimentally regarding the role of *Helicobacter* species in hepatic disease, there has been substantial research with *Campylobacter jejuni*-associated hepatitis in mice (17–19). Focal necrotic hepatitis in the absence of diarrhea also has been noted in mice 1 to 2 months after oral dosing with selected strains of *C. jejuni* (17, 19). The liver lesions persisted, and by 4 months postinoculation, *C. jejuni* was still cultured and inflammation in both the parenchyma and portal triads became extensive. Ten micrograms of the purified hepatotoxic factor isolated from selected strains (in 4 of 20 tested) of *C. jejuni* reproductively caused acute hepatic necrosis in specific-pathogen-free dd-Y mice when inoculated intravenously. Interestingly, when the hepatotoxic factor was given intravenously at two time points 14 days apart, a diffuse mononuclear inflammatory hepatitis developed which mimicked the chronic hepatopathy noted in mice orally infected with *C. jejuni* 10 months previously (18). The authors suggested that the increasing intensity of mononuclear inflammation was due to a persistent host response to the active moiety of the hepatotoxic factor—a possible consequence of an immunopathological tissue response (18). Although further studies are required to test this hypothesis, these findings may provide important clues to the pathogenesis of the hepatotoxic hepatitis noted in mice infected with *H. hepaticus*.

With the ability to isolate and characterize this new murine pathogen, the prevalence of *H. hepaticus* in other mice colonies as well as its pathogenic potential can be determined. Furthermore, we can begin to explore whether the organism can colonize other hosts, including humans, and elicit a pathologic response.

Description of *H. hepaticus* sp. nov. *H. hepaticus* (he.pa'ti.cus. Gr. adj. *hepatikos*, relating to the liver). Cells are slender curved-to-spiral rods (0.2 to 0.3 μm by 1.5 to 5.0 μm) which form one to three spiral turns. They are gram negative and nonsporulating and are motile by means of sheathed, single, bipolar flagella. Colonies are pinpoint, but cultures often appear as a thin spreading layer on agar media. Cells exhibit microaerophilic or anaerobic growth, but there is no growth aerobically. There is growth at 37°C but not at 25 or 42°C. There is growth in 1.5% NaCl, 1% glycine, 0.4% triphenyltetrazolium chloride, and 0.1% trimethylamine *N*-oxide (anaerobically). *H. hepaticus* produces urease, catalase, and oxidase activity. Nitrate is reduced. H_2S is detected on lead acetate discs. Indoxyl acetate is hydrolyzed, whereas hippurate

TABLE 4. Characteristics which differentiate *H. hepaticus* from other *Helicobacter* species[a]

Taxon	Catalase production	Nitrate reduction	Alkaline phosphatase hydrolysis	Urease	γ-glutamyl transpeptidase	Indoxyl acetate hydrolysis	Growth at 42°C	Growth on 1% glycine	Susceptibility to:		Periplasmic fibers	No. of flagella	Distribution of flagella	G+C content (mol%)
									Nalidixic acid (30-μg disc)	Cephalothin (30-μg disc)				
Helicobacter hepaticus[b]	+	+	ND	+	ND	+	−	+	R	R	−	2	Bipolar	ND
Helicobacter muridarum	+	−	+	+	+	+	−	−	R	R	+	10–14	Bipolar	34
Helicobacter canis	−	−	+	−	ND	+	+	ND	S	I	−	2	Bipolar	48
"*Flexispira rappini*"	+	−	−	+	+	ND	+	−	R	R	+	10–20	Bipolar	34
Helicobacter cinaedi	+	+	−	−	−	−	−	+	S	I	−	1–2	Bipolar	37–38
Helicobacter fennelliae	+	−	+	−	−	+	−	+	S	S	−	2	Bipolar	35
Helicobacter sp. strain CLO-3	+	−	+	−	−	+	+	+	I	R	−			45
Helicobacter pylori	+	−	+	+	+	−	−	−	R	S	−	4–8	Bipolar	35–37
Helicobacter nemestrinae	+	−	+	+	ND	−	+	−	R	S	−	4–8	Bipolar	24
Helicobacter acinonyx	+	−	+	+	+	−	−	−	R	S	−	2–5	Bipolar	30
Helicobacter felis	+	+	+	+	+	−	+	−	R	S	+	14–20	Bipolar	42
Helicobacter pametensis	+	+	+	−	−	−	+	+	S	S	−	2	Bipolar	38
Helicobacter sp. strain Bird-C	+	+	+	+	−	+	+	+	S	R	−	2	Bipolar	30
Helicobacter sp. strain Bird-B	+	+	+	+	−	+	+	+	S	R	−	2	Bipolar	31
Helicobacter mustelae	+	+	+	+	+	+	+	−	S	R	−	4–8	Peritrichous	36

[a] Data were obtained from references 5 and 35 and this study. +, positive reaction; −, negative reaction; S, susceptible; R, resistant; I, intermediate; ND, not determined.
[b] All 10 strains had identical biochemical test results.

is not. Cells are resistant to cephalothin and nalidixic acid but sensitive to metronidazole. Cells have been isolated from the colons and ceca of mice, as well as from the livers of mice with active, chronic hepatitis. The type strain Hh-2 was isolated from the liver of a mouse with active, chronic hepatitis. The type strain has been deposited with the American Type Culture Collection as ATCC 51448. The essentially complete 16S rRNA sequence of the type strain is available for electronic retrieval from the GenBank, EMBL, and DDBJ data bases under accession no. U07574.

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