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Helicobacter rodentium sp. nov., a Urease-Negative *Helicobacter* Species Isolated from Laboratory Mice

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A spiral-shaped bacterium with bipolar, single, nonsheathed flagella was isolated from the intestines of laboratory mice. The organism grew at 37 and 42°C under microaerobic and anaerobic conditions, did not hydrolyze urea, was weakly positive for catalase and oxidase, reduced nitrate to nitrite, did not hydrolyze indoxyl acetate or hippurate, and was resistant to cephalothin and nalidixic acid. This is the first urease-negative, murine *Helicobacter* spp. isolated from intestines. Also, *Helicobacter pullorum* and this bacterium are unique among the genus *Helicobacter* in having nonsheathed flagella. The new bacterium appears to be part of the normal intestinal flora; although its pathogenic potential is unknown, this organism was also isolated from *scid* mice with diarrhea that were co-infected with *Helicobacter bilis*. On the basis of 16S rRNA gene sequence analysis data and biochemical and phenotypic criteria, the new organism is classified as a novel helicobacter, for which we propose the name *Helicobacter rodentium*. The type strain is MIT 95-1707 (= ATCC 700285).

Helicobacter spp. that possess different ultrastructural characteristics are common inhabitants of the gastrointestinal tracts of both humans and animals (8). The type species of the genus, *Helicobacter pylori*, causes chronic gastritis and peptic ulcer disease in humans and has recently been linked to the development of gastric adenocarcinoma and gastric mucosa-associated lymphoma (4, 17, 18). Other nonhuman *Helicobacter* spp., namely, *Helicobacter felis*, *Helicobacter mustelae*, and *Helicobacter acinonyx*, have been associated with gastritis in their respective hosts (3, 5, 6).

Helicobacters infect several animal hosts, as well as colonize different anatomical regions of the gastrointestinal system. Six formally named *Helicobacter* spp. capable of colonizing the intestinal tracts of rodents have been characterized by phenotypic, biochemical, and molecular analyses. *Helicobacter muridarum* colonizes the cecum and ileum and induces a gastritis following colonization of the gastric mucosa in older rodents (15, 20). "*Flexispira rappini*," a helicobacter based on 16S rRNA data but formally unnamed, which has been linked to abortion in sheep, necrotic liver foci in aborted sheep fetuses, and diarrheal disease in humans, has also been isolated recently from the feces of mice (1, 22). *Helicobacter cinaedi*, a normal intestinal inhabitant of hamsters, also has been isolated from homosexual men with enteritis, proctocolitis, and asymptomatic rectal infections (12, 25). Two other *Helicobacter* spp., *Helicobacter bilis* and *Helicobacter hepaticus*, have been isolated from livers, ceca, and colons of mice, and both of these species have also been isolated from the livers of animals with hepatitis (7, 9, 10). Most recently, *Helicobacter trogontum* has been isolated from intestines of asymptomatic rats (16).

During routine health surveillance for *H. hepaticus* in mice, we isolated a urease-negative, helicobacter-like bacterium that differed from the previously described *Helicobacter* species in other phenotypic characteristics. Although other urease-negative helicobacters have been described, this was the first urease-negative helicobacter-like organism isolated from the mouse intestine. Consequently, we hypothesized that this organism was a novel species. In this paper, we provide biochemical, phenotypic, and phylogenetic data which confirm that this bacterium is distinct from previously recognized *Helicobacter* species, and we propose the name *Helicobacter rodentium* for it.

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MATERIALS AND METHODS

Animals. The novel murine *Helicobacter* species was recovered from 74 mice housed in an Association for Accreditation and Assessment of Laboratory Animal Care-accredited animal facility. The mice either were housed in a barrier facility and originated from a single vendor or were rederived by embryo transfer. Some mice were animals that came from other academic institutions and therefore were housed in a quarantine facility. Although the bacterium was originally identified in asymptomatic mice, the organism was subsequently recognized in diarrheic *scid* mice infected with *H. bilis*. Mice housed in the barrier facility were free of all other recognized murine *Helicobacter* species.

Bacterial culture. Freshly pooled fecal samples and cecum, colon, liver, and uterine specimens were aseptically collected from each mouse. Samples from each site were homogenized in 1.0 ml of phosphate-buffered saline. Each slurry was gently passed through a 0.45-μm-pore-size syringe filter, and the homogenate was streaked onto a Columbia blood agar (5% sheep blood) plate (Remel Laboratories, Lenexa, Kans.). The cultures were then incubated at 37°C under microaerophilic conditions in vented jars containing N_2, H_2, and CO_2 (90:5:5).

Electron microscopy. The following two isolates were examined by electron microscopy: type strain MIT 95-1707 (= ATCC 700285) and strain MIT 96-2160 (= ATCC 700286). Cells grown in Trypticase soy broth for 24 h were centrifuged and gently suspended in 10 mM Tris-HCl buffer (pH 7.4) at a concentration of about 10^8 cells per ml. Samples were negatively stained with 1% (wt/vol) phosphotungstic acid (pH 6.5) for 20 to 30 s. Specimens were examined with a JEOL model JEM-1200EX transmission electron microscope operating at 100 kV.

Biochemical characterization. A detailed biochemical characterization analysis was performed with 9 of 74 isolates as previously described by Fox et al. (10). The nine isolates represented mice from five different sources. For the remaining 65 strains, motility, Gram staining, oxidase, catalase, and urease assays were performed, and sensitivities to nalidixic acid and cephalothin were determined.

Extraction of DNA for sequencing. Bacteria were cultured on blood agar plates, and cells were harvested and washed twice with 1 ml of double-distilled H_2O. The pellets were suspended in STET buffer (8% sucrose, 50 mM EDTA, 0.1% Triton X-100, 50 mM Tris-HCl [pH 8.0]), and lysozyme (hen egg white; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was added to a final concentration of 3 mg/ml. The suspension was incubated for 12 min at 37°C and was then lysed with 1% sodium dodecyl sulfate. RNase A (bovine pancreas; Boehringer Mannheim) was added to a final concentration of 0.05 mg/ml, and the solution was incubated for 1 h at 37°C. Then 0.1 volume of a 5% CTAB (cetyltrimethylammonium bromide)–0.5 M NaCl solution (Sigma Chemical Co., St. Louis, Mo.) was added, and the solution was gently mixed and incubated at 65°C for 10 min. DNA was extracted with an equal volume of phenol-chloroform (1:1, vol/vol), precipitated overnight in 0.3 M sodium acetate with 2 volumes of absolute ethanol at −20°C, and pelleted by centrifugation at 13,000 × *g* for 1 h at 4°C. The ethanol was decanted, and the pellet was air dried and suspended in sterile distilled water.

TABLE 1. Sources and accession numbers of the strains studied

Taxon	Strain[a]	Other designation(s)[b]	GenBank accession no.[c]
New Isolates			
Helicobacter rodentium	MIT 95-1707^T	ATCC 700285^T	U96296
Helicobacter rodentium	MIT 95-1708		U96296
Helicobacter rodentium	MIT 95-2160	ATCC 700286	U96297
Helicobacter rodentium	MIT 96-1312		U96297
Helicobacter sp.	MIT 95-2011		U96298
Helicobacter sp.	Eaton 94-536		U96299
Reference organisms			
Arcobacter cryaerophilus	CCUG 17801^T	ATCC 43158^T	LI4624
Campylobacter jejuni	CCUG 11284^T		L04315
"*Flexispira rappini*"	ATCC 43968		U96300
"*Flexispira rappini*"	ATCC 43879		M88138
"*Gastrospirillum hominis*" 1[d]			L10079
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	M57389
Helicobacter fennelliae	CCUG 18820^T	ATCC 35684^T	M88154
Helicobacter hepaticus	Fox Hh-2^T	ATCC 51448^T	U07574
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, ATCC 51478^T	M88147
Helicobacter pullorum	NCTC 12824^T		L36141
Helicobacter pullorum	NCTC 12826		L36143
Helicobacter pylori	ATCC 43504^T		M88157
Helicobacter sp. strain CLO 3	CCUG 14564		M88151
Helicobacter sp. strain Bird B	Seymour B10^T	CCUG 29256^T, ATCC 51480^T	M88139
Helicobacter sp. strain Bird C	Seymour B52^T	CCUG 29561^T, ATCC 51482^T	M88144
Helicobacter trogontum	LRB 8581^T	ATCC 700114	U65103
Wolinella succinogenes	Tanner 602W^T	ATCC 29543^T	M88159

[a] Strains whose sequences were determined were obtained from the following individuals or culture collections: K. A. Eaton, Department of Veterinary Pathobiology, Ohio State University, Columbus; J. G. Fox, Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge; A. Lee, Department of Microbiology and Immunology, University of New South Wales, Sydney; Australia; C. Seymour, Department of Microbiology, Boston University School of Medicine, Boston, Mass.; A. Tanner, Department of Microbiology, Forsyth Dental Center, Boston, Mass.; American Type Culture Collection (ATCC), Rockville, Md.; CCUG, Culture Collection of the University of Göteborg (CCUG), Göteborg, Sweden; and National Collection of Type Cultures (NCTC), London, United Kingdom.

[b] Alternate culture collection sources of strains.

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

[d] This organism is uncultivable.

16S rRNA gene sequencing. Sequences of the 16S rRNA genes of four *H. rodentium* strains, two closely related but unnamed *Helicobacter* sp. strains isolated from mice, and "*F. rappini*" ATCC 43968 were determined. The strains used are listed in Table 1. For amplification of 16S rRNA citrons, 16S rRNA gene sequencing, and 16S rRNA data analysis we used the methods described by Fox et al. (10). Briefly, primers C70 and B37 (10) were used to amplify the 16S rRNA genes. The 1,507-bp amplicons were purified and directly sequenced by using a TAQuence cycle sequencing kit (U.S. Biochemicals, Cleveland, Ohio). The 16S rRNA gene sequences were entered into RNA, a program for analysis of 16S rRNA data in Microsoft Quickbasic for use with IBM PC-compatible computers, and were aligned as previously described (19). The database used contains approximately 100 *Helicobacter*, *Wolinella*, *Arcobacter*, and *Campylobacter* sequences and more than 900 sequences for other bacteria. Similarity matrices were constructed from the aligned sequences by using only those base positions for which 90% of the strains had data and were corrected for multiple base changes by the method of Jukes and Cantor (14). Phylogenetic trees were constructed by the neighbor-joining method (21).

Identification of strains by PCR with specific primers. Species-specific PCR primers selected from 16S rRNA were designed for identification of *H. rodentium*. The forward primer, D86 (5′-GTC CTT AGT TGC TAA CTA TT), and the reverse primer, D87 (5′-AGA TTT GCT CCA TTT CAC AA), produced an amplified 166-bp product. A 16S rRNA-based primer set that is genus specific for all *Helicobacter* spp. was also used. Primer C97 (5′-GCT ATG ACG GGT ATC C) and primer C98 (5′-GAT TTT ACC CCT ACA CCA) produced an amplified 422-bp product.

Bacterial DNA was isolated by using an InstaGene Matrix (Bio-Rad, Hercules, Calif.) as recommended by the manufacturer. Briefly, 200 μl of InstaGene Matrix was added to cell pellets that had been washed twice with double-distilled H_2O. The mixture was incubated at 56°C for 30 min and then vortexed for 10 s at high speed. The samples were placed in a boiling water bath for 10 min, vortexed again, and centrifuged at 12,000 rpm for 5 min. Then 20 μl of the DNA preparation was added to 100 μl (final volume) of reaction mixture containing 1× *Taq* polymerase buffer (containing $MgCl_2$ at a final concentration of 2.25 mM), each of the two primers at a concentration of 0.5 μM, each deoxynucleotide at a concentration of 200 μM, and 200 μg of bovine serum albumin per ml. Samples were heated at 94°C for 4 min, briefly centrifuged, and cooled to 58°C. Then 2.5 U of *Taq* polymerase (Boehringer Mannheim) and 1 U of the polymerase enhancer Perfect Match (Stratagene, La Jolla, Calif.) were added, and this was followed by addition of an overlay consisting of 100 μl of mineral oil. A total of 35 cycles, each consisting of 1 min at 94°C, 90 s at 58°C, and 2 min at 72°C, were performed. The same cycling conditions were used for both sets of primers. Amplified products were electrophoresed on a 6% Visigel separation matrix (Stratagene) by using previously described methods (23).

Nucleotide sequence accession numbers. The GenBank accession numbers for the strains examined are given in Table 1. The strains whose sequences were determined in this study have been deposited under the following GenBank accession numbers: *H. rodentium* MIT 95-1707^T, U96296; *H. rodentium* MIT 95-2160, U96297; *Helicobacter* sp. strain MIT 95-2011, U96298; *Helicobacter* sp. strain Eaton 94-536, U96299; and "*F. rappini*" ATCC 43968, U96300.

RESULTS

Isolation and growth characteristics. After 4 to 7 days of incubation, growth on agar surfaces appeared as thin, spreading films. Occasionally, isolated colonies that were 1 to 2 mm in

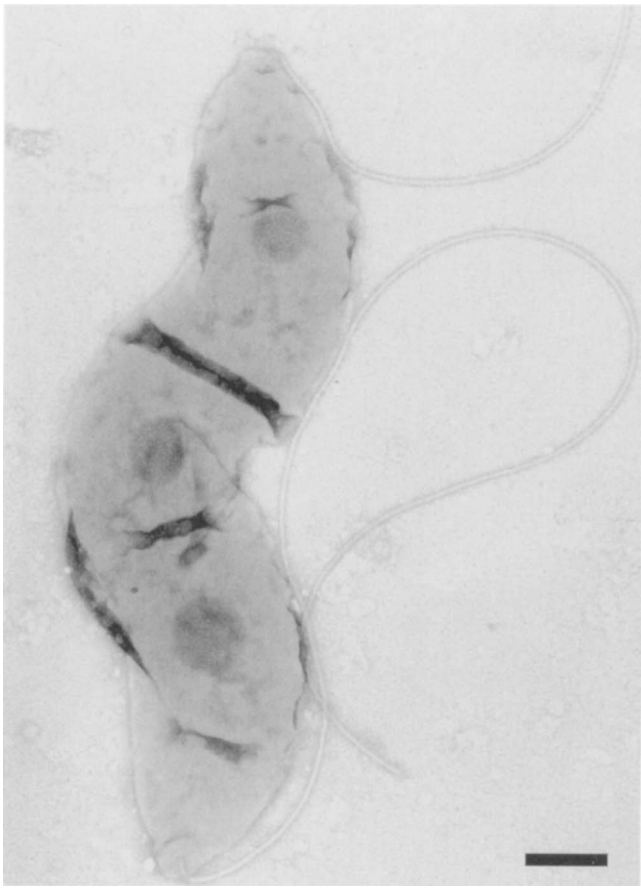

FIG. 1. Transmission electron micrograph of a negatively stained cell of *H. rodentium*. The typical spiral cell has a single nonsheathed, terminal flagellum at each end. Note the doughnut-shaped structure at each point of flagellar insertion. Bar = 0.2 μm.

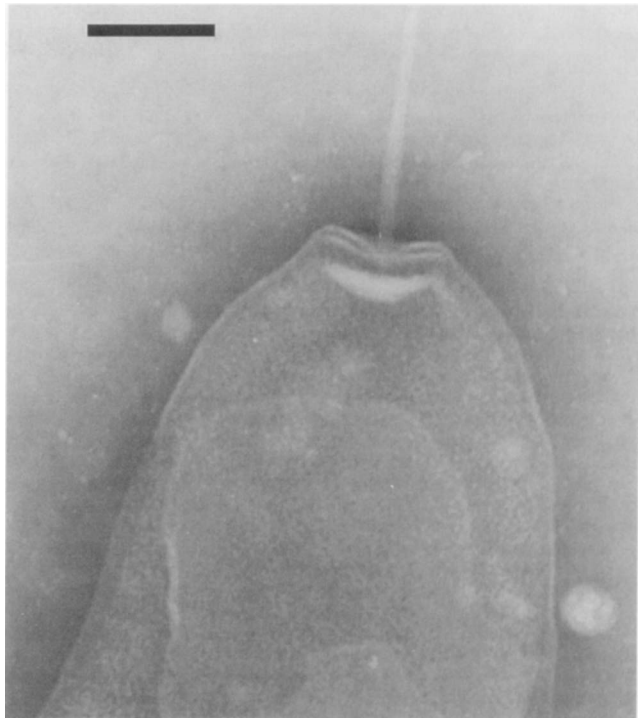

FIG. 3. Transmission electron micrograph of an end of a negatively stained cell of *H. rodentium*. The flagellum is clearly nonsheathed. Bar = 0.5 μm.

diameter were observed. Strains were routinely isolated from feces and intestinal scrapings by using microaerobic conditions at 37°C. The bacteria grew under microaerobic and anaerobic conditions at 37 and 42°C, but not 25°C. Organisms with this phenotype were not isolated from liver or uterine samples.

Ultrastructure. *H. rodentium* cells were motile, curved or spiral, and 0.3 by 1.5 to 5 μm (Fig. 1 and 2). They possessed single, bipolar, nonsheathed flagella (Fig. 3), each of which was

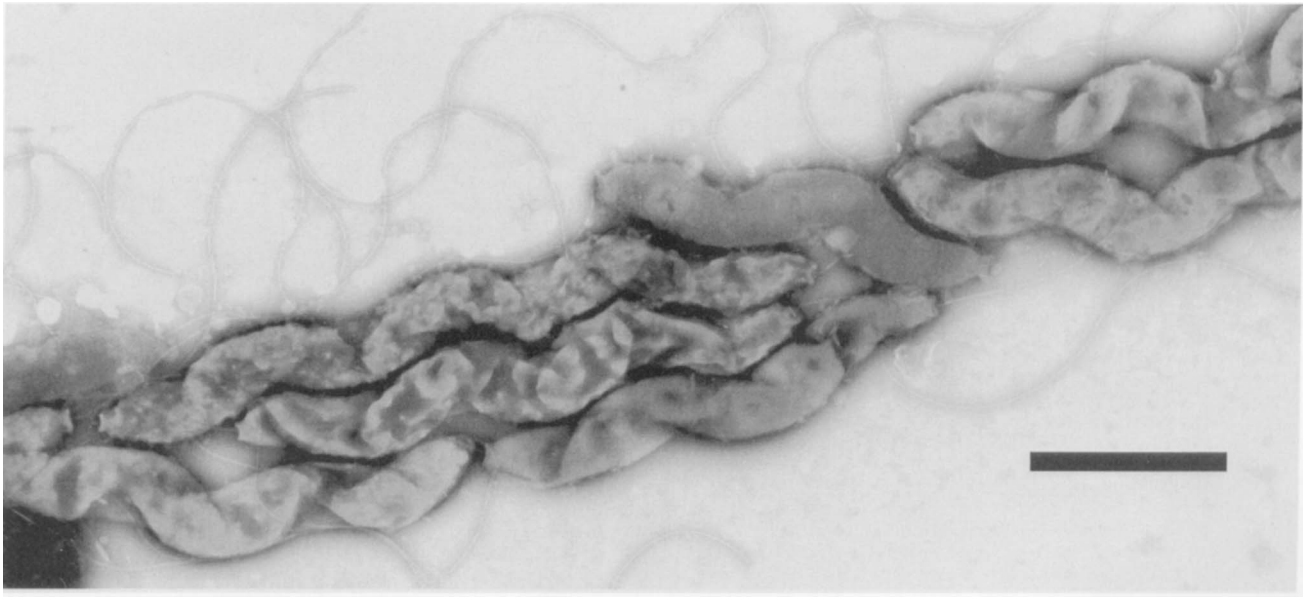

FIG. 2. Transmission electron micrograph of negatively stained cells of *H. rodentium*, showing the morphological variation of cells (e.g., variation in the number of spirals and cell length). Bar = 1 μm.

TABLE 2. Characteristics which differentiate *H. rodentium* from other *Helicobacter* species[a]

Taxon	Catalase production	Nitrate reduction	Alkaline phosphatase hydrolysis	Urease	Indoxyl acetate hydrolysis	γ-Glutamyl transpeptidase	Growth at 42°C	Growth with 1% glycine	Susceptibility to:		Peri-plasmic fibers	No. of flagella	Distribution of flagella	G+C content (mol%)
									Nalidixic acid (30-μg disc)	Cephalothin (30-μg disc)				
H. rodentium	+ (9/9)[b]	+ (9/9)	− (0/9)	− (0/9)	− (0/9)	− (0/9)	+ (7/9)	+ (9/9)	R (9/9)	R (9/9)	−	2	Bipolar	ND
H. pullorum	+	+	−	−	−	ND	+	ND	R	S	−	1	Monopolar	34-35
Helicobacter sp. strain CLO-3	+	−	+	−	+	−	+	+	I	R	−		ND	45
H. pylori	+	−	+	+	−	+	−	−	R	S	−	4-8	Bipolar	35-37
H. nemestrinae	+	−	+	+	−	ND	+	−	R	S	−	4-8	Bipolar	24
H. acinonyx	+	−	+	+	−	+	−	−	R	S	−	2-5	Bipolar	30
H. felis	+	+	+	+	−	+	+	−	R	S	+	14-20	Bipolar	42
H. fennelliae	+	−	+	−	+	−	−	+	S	S	−	2	Bipolar	35
H. trogontum	+	+	−	+	ND	+	+	ND	R	R	+	5-7	Bipolar	ND
H. muridarum	+	−	+	+	+	+	−	−	R	R	+	10-14	Bipolar	34
H. hepaticus	+	+	ND	+	+	ND	−	+	R	R	−	2	Bipolar	ND
H. canis	−	−	+	−	+	ND	+	ND	S	I	−	2	Bipolar	48
H. bilis	+	+	ND	+	−	ND	+	+	R	R	+	3-14	Bipolar	ND
"*F. rappini*"	+	−	−	+	ND	+	+	−	R	R	+	10-20	Bipolar	34
H. cinaedi	+	+	−	−	−	−	−	+	S	I	−	1-2	Bipolar	37-38
H. 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
H. mustelae	+	+	+	+	+	+	+	−	S	R	−	4-8	Peritrichous	36
H. bizzozeronii	+	+	+	+	+	+	+	−	R	S	−	10-20	Bipolar	ND

[a] Data were obtained from references 10, 13, 16, and 24 and this study. +, positive reaction; −, negative reaction; S, susceptible; R, resistant; I, intermediate; ND, not determined.
[b] The numbers in parentheses are number of strains positive or resistant/number of strains tested.

TABLE 3. Similarity matrix based on 16S rRNA sequence comparisons

Taxon[a]	% Similarity or % difference[b]																										
	Helicobacter rodentium MIT 95-1707^T	*Helicobacter rodentium* MIT 96-1312	*Helicobacter* sp. strain MIT 95-2011	*Helicobacter* sp. strain Eaton 94-536	"*Flexispira rappini*" ATCC 43968	*Helicobacter pullorum* NCTC 12824	*Helicobacter pullorum* NCTC 12826	*Helicobacter* sp. strain CLO-3	*Helicobacter pylori*	*Helicobacter nemestrinae*	*Helicobacter acinonyx*	"*Gastrospirillum hominis*" 1	*Helicobacter felis*	*Helicobacter fennelliae*	*Helicobacter trogontum*	*Helicobacter muridarum*	*Helicobacter hepaticus*	*Helicobacter canis*	*Helicobacter bilis*	"*Flexispira rappini*" ATCC 43879	*Helicobacter cinaedi*	*Helicobacter pametensis*	*Helicobacter* sp. strain Bird C	*Helicobacter* sp. strain Bird B	*Helicobacter mustelae*	*Wolinella succinogenes*	*Campylobacter jejuni*	*Arcobacter cryaerophilus*
Helicobacter rodentium MIT 95-1707^T		99.9	98.3	96.8	96.0	96.1	96.6	95.1	93.9	93.5	93.1	92.2	92.7	94.7	95.1	94.6	95.0	95.5	95.8	95.4	95.4	95.9	95.3	95.4	94.9	93.3	87.4	86.1
Helicobacter rodentium MIT 96-1312	0.1		98.3	96.9	96.1	96.2	96.6	95.1	93.8	93.5	93.1	92.2	92.6	94.8	95.2	94.7	95.1	95.6	95.9	95.5	95.5	95.9	95.4	95.5	95.0	93.2	87.4	86.1
Helicobacter sp. MIT 95-2011	1.7	1.7		97.0	96.7	96.5	96.2	95.6	93.9	93.4	92.9	93.2	93.2	95.6	95.1	94.3	94.6	94.8	95.1	95.1	94.8	95.9	95.0	94.7	94.4	92.0	87.2	85.6
Helicobacter sp. Eaton 94-536	3.3	3.2	3.1		95.6	95.5	95.8	94.1	93.9	93.4	92.8	91.5	91.6	94.0	95.4	94.7	94.5	94.1	94.6	94.4	94.8	95.8	95.6	94.6	94.3	92.5	86.2	84.8
"*Flexispira rappini*" ATCC 43968	4.1	4.0	3.3	4.6		97.1	96.9	95.1	93.6	93.9	93.2	92.9	93.7	95.5	95.9	95.1	95.4	95.5	95.7	95.6	95.6	96.3	95.7	95.6	95.4	92.8	86.9	85.5
Helicobacter pullorum NCTC 12824	4.0	3.9	3.6	4.7	2.9		99.4	96.3	94.8	94.1	94.2	93.2	94.3	95.9	96.0	95.2	95.8	96.4	96.3	96.1	95.7	97.4	96.2	96.0	95.5	92.8	87.1	86.1
Helicobacter pullorum NCTC 12826	3.5	3.5	3.9	4.4	3.2	0.6		95.8	94.8	94.4	94.4	93.0	94.1	95.6	95.6	95.7	96.3	96.9	96.7	96.5	96.3	97.6	96.5	96.3	95.6	93.1	87.3	85.9
Helicobacter sp. CLO-3	5.1	5.0	4.6	6.2	5.1	3.8	4.3		93.8	93.2	93.1	92.8	93.2	94.6	95.4	94.3	95.1	96.0	95.4	95.5	95.3	95.1	95.4	95.2	95.3	92.2	86.1	85.6
Helicobacter pylori	6.4	6.5	6.3	6.4	6.7	5.4	5.4	6.5		98.2	97.3	94.8	95.5	92.9	92.6	93.0	93.2	93.7	93.4	93.1	92.7	94.4	94.0	93.7	93.8	90.8	85.4	84.6
Helicobacter nemestrinae	6.8	6.8	6.9	6.9	6.4	6.1	5.8	7.1	1.8		96.5	94.6	95.5	92.6	92.7	92.9	93.4	93.6	93.5	93.2	93.1	94.4	94.3	93.8	93.9	91.1	85.7	85.0
Helicobacter acinonyx	7.2	7.3	7.4	7.6	7.1	6.0	5.8	7.3	2.7	3.5		94.8	96.4	92.3	92.3	92.5	93.1	93.2	92.7	92.5	92.4	94.0	93.5	93.5	93.5	90.4	85.2	84.3
"*Gastrospirillum hominis*" 1	8.2	8.2	7.1	9.1	7.4	7.1	7.3	7.6	5.3	5.6	5.4		96.6	92.6	91.6	91.9	92.2	92.3	92.0	92.2	92.0	92.5	92.2	92.2	92.1	89.2	84.5	83.6
Helicobacter felis	7.6	7.7	7.2	8.9	6.6	5.9	6.1	7.1	4.7	4.7	3.7	3.5		93.0	92.7	92.6	93.1	93.2	92.7	92.6	92.6	93.9	93.2	93.4	93.4	90.2	84.8	83.7
Helicobacter fennelliae	5.5	5.4	4.6	6.3	4.7	4.2	4.5	5.6	7.4	7.8	8.2	7.8	7.4		95.8	95.1	95.3	95.4	95.8	95.4	95.8	95.5	94.6	94.6	94.4	92.3	86.1	84.5
Helicobacter trogontum	5.0	4.9	5.1	4.8	4.3	4.1	4.5	4.8	7.7	7.6	8.2	8.9	7.7	4.3		96.3	97.1	96.1	96.1	95.5	96.2	95.9	96.0	96.8	96.3	93.4	85.8	85.8
Helicobacter muridarum	5.6	5.5	5.9	5.5	5.1	5.0	4.4	5.9	7.4	7.5	8.0	8.6	7.8	5.1	3.8		97.7	96.5	96.3	96.1	95.9	95.7	96.4	96.1	95.7	93.2	86.0	85.0
Helicobacter hepaticus	5.2	5.1	5.6	5.7	4.7	4.3	3.8	5.1	7.1	6.9	7.3	8.3	7.2	4.8	3.0	2.3		97.2	97.2	97.3	96.8	96.2	96.4	96.4	96.2	92.7	86.2	85.0
Helicobacter canis	4.7	4.6	5.4	6.1	4.6	3.7	3.1	4.2	6.5	6.7	7.1	8.1	7.2	4.7	4.0	3.6	2.9		98.8	98.1	97.8	96.5	96.9	96.9	96.4	93.2	86.9	85.5
Helicobacter bilis	4.3	4.3	5.0	5.6	4.4	3.8	3.4	4.8	7.0	6.8	7.7	8.4	7.7	4.3	4.0	3.7	2.8	1.3		98.8	98.4	96.7	96.6	96.3	96.0	93.2	86.6	85.3
"*Flexispira rappini*" ATCC 43879	4.7	4.6	5.1	5.8	4.5	4.1	3.6	4.6	7.3	7.1	7.9	8.3	7.8	4.8	4.6	4.0	2.8	2.0	1.2		98.8	96.5	96.4	95.9	95.8	92.9	86.9	85.1
Helicobacter cinaedi	4.7	4.7	5.4	5.4	4.6	4.5	3.8	4.9	7.7	7.3	8.0	8.4	7.8	4.3	3.9	4.2	3.2	2.2	1.6	1.2		95.6	95.6	95.4	95.2	92.8	86.3	85.1
Helicobacter pametensis	4.3	4.2	4.2	4.3	3.8	2.6	2.5	5.1	5.9	5.8	6.3	7.9	6.4	4.7	4.3	4.4	3.9	3.6	3.4	3.6	4.6		98.1	97.9	97.1	94.0	87.4	86.1
Helicobacter sp. Bird C	4.9	4.8	5.2	4.6	4.4	3.9	3.6	4.7	6.2	6.0	6.8	8.2	7.2	5.6	4.1	3.7	3.7	3.1	3.4	3.6	4.5	2.0		98.3	97.9	94.5	87.4	85.6
Helicobacter sp. Bird B	4.7	4.6	5.5	5.6	4.5	4.1	3.8	5.0	6.6	6.4	6.8	8.2	6.9	5.6	3.3	4.0	3.7	3.2	3.8	4.2	4.7	2.1	1.7		98.6	94.0	87.1	86.2
Helicobacter mustelae	5.2	5.2	5.9	5.9	4.7	4.7	4.5	4.9	6.5	6.4	6.8	8.3	6.9	5.9	3.8	4.4	3.9	3.7	4.1	4.3	5.0	2.9	2.1	1.4		93.6	86.8	86.1
Wolinella succinogenes	7.1	7.2	8.5	7.9	7.5	7.5	7.3	8.2	9.8	9.5	10.3	11.6	10.6	8.1	6.9	7.1	7.7	7.1	7.2	7.4	7.6	6.2	5.7	6.2	6.7		86.3	85.7
Campylobacter jejuni	13.8	13.8	14.1	15.2	14.3	14.1	13.9	15.4	16.2	15.9	16.5	17.3	17.0	15.3	15.7	15.5	15.2	14.4	14.8	14.4	15.2	13.7	13.8	14.1	14.5	15.2		87.2
Arcobacter cryaerophilus	15.4	15.4	16.0	17.0	16.1	15.4	15.6	16.0	17.2	16.7	17.6	18.5	18.4	17.4	15.8	16.7	16.7	16.2	16.4	16.6	16.6	15.4	15.9	15.2	15.4	15.8	14.0	

[a] Strains belonging to the same species were distinguished by their sequences. Table 1 shows nucleotide sequence accession numbers.

[b] 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 (14).

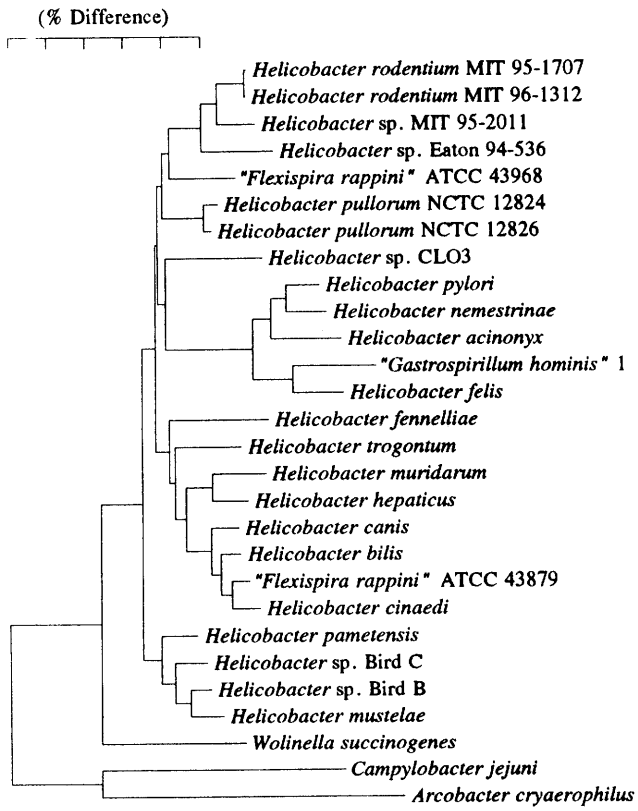

FIG. 4. Phylogenetic tree constructed on the basis of 16S rRNA sequence similarity values. Scale bar = 5% difference in nucleotide sequences as determined by measuring the lengths of the horizontal lines connecting two species.

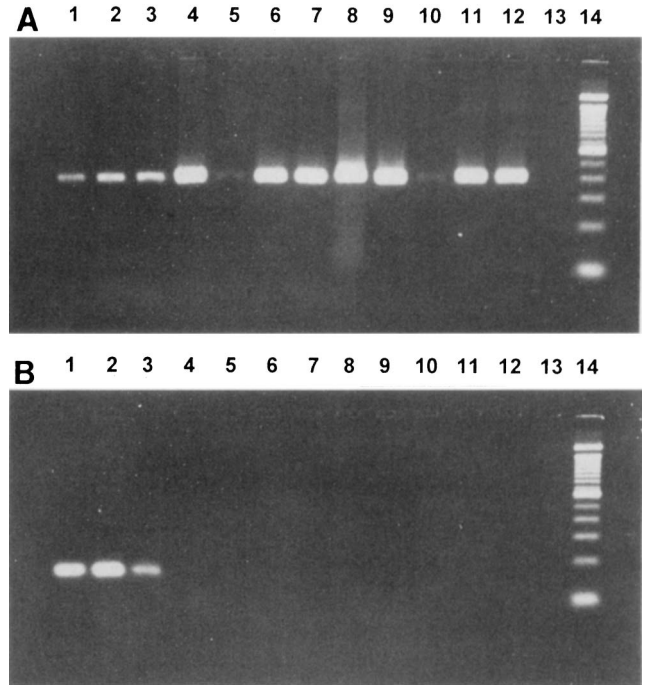

FIG. 5. Electrophoresis of DNA amplified by PCR on a Visigel separation matrix by using *Helicobacter* genus-specific primers (A) and *H. rodentium* species-specific primers (B). Lanes 1 through 3, DNAs isolated from cultures containing presumptively identified *H. rodentium*; lane 4, *H. pullorum*; lane 5, *H. canis*; lane 6, *H. cineadi*; lane 7, *H. fennelliae*; lane 8, *H. muridarum*; lane 9, "*F. rappini*"; lane 10, *H. bilis*; lane 11, *H. hepaticus*; lane 12, *H. pylori*; lane 13, blank; lane 14, 100-bp DNA ladder.

inserted into a doughnut-shaped, disklike structure (Fig. 1 and 3), but did not possess periplasmic fibers. Circular structures containing dark granules were often present inside the organism (Fig. 1). Similar structures, reported to be polyphosphate granules, have been described in other *Helicobacter* species (2).

Biochemical and physiological characteristics. Biochemical and physiological properties of nine *H. rodentium* strains were compared with properties of previously described *Helicobacter* spp. (Table 2). These strains were different from other murine *Helicobacter* spp. since they had no detectable urease activity and were weakly oxidase and catalase positive. Key phenotypic features that differentiated *H. rodentium* from other urease-negative *Helicobacter* species were nitrate reduction and sensitivity to nalidixic acid and cephalothin. The remaining 65 strains were also motile, gram negative, spiral shaped, weakly catalase and oxidase positive, and urease negative. All strains were resistant to both cephalothin and nalidixic acid.

Phylogenetic analysis. Approximately 95% of the total RNA gene sequence (approximately 1,450 bases) was determined for the four murine, urease-negative strains of *H. rodentium*, "*F. rappini*" ATCC 43968, and strain Eaton 94-536. The sequences of isolates MIT 95-1707^T and MIT 95-1708 were identical. The sequences of isolates MIT 95-2160 and 96-1312 were identical and differed from the sequences of the other two strains by only one base. The sequences obtained were compared with the sequences of 23 reference species belonging to the genera *Helicobacter*, *Wolinella*, *Arcobacter*, and *Campylobacter*. A similarity matrix based on these comparisons is shown in Table 3. A phylogenetic tree constructed by the neighbor-joining method is shown in Fig. 4. The closest relative of *H. rodentium* was strain MIT 95-2011 (level of similarity, approximately 98%). *H. rodentium* falls in a separate cluster of helicobacters which includes strain MIT 95-2011, "*F. rappini*" ATCC 43968, strain Eaton 94-536, and two *H. pullorum* strains. The levels of sequence similarity and phenotypic differences discussed above identify *H. rodentium* as a novel species.

PCR identification of strains. PCR amplification with the *Helicobacter* genus-specific primer set produced a 422-base fragment from the 10 *Helicobacter* species tested (Fig. 5). PCR amplification with primers D86 and D87 was species specific since it produced a 166-bp fragment from only the presumptively identified *H. rodentium* strains (Fig. 5). All nine strains tested for their biochemical phenotypes were positive with this set of primers. Using this primer set with other urease-negative helicobacters, urease-positive murine *Helicobacter* spp., or the type strain of *H. pylori* did not result in any amplified product.

DISCUSSION

In this study a urease-negative, spiral-shaped organism was isolated from the intestines of laboratory mice. It was characterized as a member of the genus *Helicobacter* based on its morphology, its biochemical traits, and the results of a molecular analysis of its 16S rRNA gene sequences. Four other murine *Helicobacter* species have been described previously, and it is very likely they all normally colonize the gastrointestinal tract. *H. muridarum* and "*F. rappini*" were originally isolated from mouse gastrointestinal tracts, where their presence normally does not elicit an inflammatory response (15, 20, 22). However, *H. muridarum* can colonize the gastric tissue of mice and induce gastritis (20). *H. bilis* and *H. hepaticus* not only can be isolated from the cecum and colon, but also can colonize the

liver and bile, and in certain strains of mice, these bacteria can induce hepatitis and in one strain of mice, A/JCr, they can cause hepatic cancer (7, 9, 10, 27). Most recently, *H. hepaticus* has been associated with inflammatory bowel disease (11, 26).

There are many strains isolated from different mammalian sources that have been referred to as "*F. rappini*" on the basis of a distinct morphology (i.e., spiral or helical shape, 10 to 20 bipolar flagella, and periplasmic fibers that wrap around the cell). We have previously shown that all of these strains are helicobacters, including *H. bilis* (8, 10). However, not all of the strains are members of a single *Helicobacter* species, as shown by the different phylogenetic positions of *H. bilis*, "*F. rappini*" ATCC 43968, and "*F. rappini*" ATCC 43879 (Fig. 4). On the basis of 16S rRNA sequence comparisons, at least five different *Helicobacter* species are represented in more than 30 "*F. rappini*" strains. These data will be reported elsewhere.

H. rodentium is the first murine helicobacter that is urease negative. By Southern blot analysis we confirmed that whole genomic *H. rodentium* DNA digested with *Hin*dIII did not hybridize with urease PCR gene products from *H. pylori* or *H. hepaticus* (data not shown). Urease is considered necessary for survival in the acidic environment of the mammalian stomach and is characteristic of gastric *Helicobacter* spp. (8). The absence of urease in nongastric *Helicobacter* species might be explained by the possibility that deamination of urea in nonacidic environments may yield significant quantities of toxic end products and thereby elicit inflammatory responses in intestinal mucosa. For example, urease is present in other intestinal *Helicobacter* spp. and in one species, *H. hepaticus*, may cause focal or more generalized inflammatory bowel disease (11, 27). *H. rodentium* can be distinguished from other urease-negative helicobacters based on phenotypic and phylogenetic criteria (Table 2 and Fig. 4). The urease-negative organism *H. pullorum*, which is isolated from the livers, duodena, and ceca of chickens (24), falls in the same phylogenetic cluster as *H. rodentium*. Like *H. rodentium*, *H. pullorum* does not have sheathed flagella, which is characteristic of all other *Helicobacter* species. However, strain Eaton 94-536, which falls in this phylogenetic branch of helicobacters, does possess sheathed flagella (unpublished data). Consequently, it is unlikely that the presence of nonsheathed flagella has any phylogenetic significance. At present, it is not known whether "*F. rappini*" ATCC 43968 and strain MIT 95-2011 also possess sheathed flagella.

The pathogenic potential of *H. rodentium* is still unclear. *H. rodentium* appears to be part of the normal flora of the lower intestinal tract; however, severe diarrhea has been observed in *scid* mice co-infected with *H. rodentium* and another intestinal helicobacter, *H. bilis* (4a).

H. pylori-induced gastritis and gastric adenocarcinoma in humans have received attention by numerous research groups, and many animal models, including mice, have been used to study various aspects of pathogenesis induced by gastric *Helicobacter* spp. (8). It is important to recognize that in addition to urease-positive murine *Helicobacter* spp., a urease-negative helicobacter also colonizes the colons and ceca of mice. We, therefore, recommend bacterial screening of rodents for the presence of all indigenous helicobacters to avoid confounding experimental results, particularly when immunological parameters and vaccine development are being studied.

Description of *Helicobacter rodentium* sp. nov. *Helicobacter rodentium* (ro.den'ti um.L. gen. n. *rodentium*, of gnawing animals, referring to the fact that the organism was first isolated from mice). Cells are slender curved to spiral rods (0.3 by 1.5 to 5 μm) which have one to three spiral turns. They are gram negative and nonsporulating and motile by means of nonsheathed, single, bipolar flagella. Individual colonies are 1 to 2 mm in diameter, but cultures often appear as thin spreading layers on agar media. Cells exhibit microaerobic or anaerobic growth; however, there is no growth aerobically. Growth occurs at 37°C, and most strains (including the type strain) grow at 42°C but not 25°C. The bacteria are urease negative and weakly catalase and oxidase positive, reduce nitrate to nitrite, and do not hydrolyze indoxyl acetate or hippurate. Growth occurs in the presence of 1.5% NaCl, 1% glycine, and 0.04% triphenyltetrazolium chloride, but the bacterium does not produce hemolysis on blood agar or produce pigment. Cells are resistant to cephalothin and nalidixic acid. Cells have been isolated from the feces, colons, and ceca of mice. The type strain is MIT 95-1707 (= ATCC 700285).

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