

Draft Genome Sequences of Eight Enterohepatic *Helicobacter* Species Isolated from Both Laboratory and Wild Rodents

Alexander Sheh,^a Zeli Shen,^a James G. Fox^{a,b}

Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA^a; Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA^b

A.S. and Z.S. are co-first authors.

The draft genome sequences of eight enterohepatic *Helicobacter* species, *H. muridarum*, *H. trogontum*, *H. typhlonius*, and five unnamed helicobacters, are presented here. Using laboratory mice pervasively infected with helicobacters, we characterized the presence of known virulence factors.

Received 9 October 2014 Accepted 16 October 2014 Published 26 November 2014

Citation Sheh A, Shen Z, Fox JG. 2014. Draft genome sequences of eight enterohepatic *Helicobacter* species isolated from both laboratory and wild rodents. *Genome Announc.* 2(6):e01218-14. doi:10.1128/genomeA.01218-14.

Copyright © 2014 Sheh et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to James G. Fox, jgfox@mit.edu.

Enterohepatic *Helicobacter* species (EHS) are Gram-negative, microaerophilic, spiral-shaped bacteria that colonize the mucosa of the gastrointestinal tract and/or the livers of mammals, including humans, and birds (1, 2). Natural enterohepatic *Helicobacter* sp. infection is prevalent in 88% of research mouse colonies worldwide (3). Our previous work reported the high prevalence of *Helicobacter hepaticus*, *Helicobacter rodentium*, *Helicobacter bilis*, and *Helicobacter typhlonius* in research mouse facilities (3). Previously, we have sequenced multiple EHS, including *H. bilis*, *Helicobacter pullorum*, *H. hepaticus*, *Helicobacter cinaedi*, and *Helicobacter canadensis* (4, 5). While most infected mice develop minimal pathological changes, susceptible strains exhibit typhlocolitis and hepatitis, which can progress to colon cancer and hepatocellular carcinoma (6). Previous studies have shown that *Helicobacter* infections can affect experimental outcomes in cancer studies and confound study results (7–9).

Furthermore, studies have highlighted the potential zoonotic nature of EHS species, as EHS isolated in rodents or birds, such as *H. cinaedi*, *H. canadensis*, *H. bilis*, and *H. pullorum*, have been identified in patients with diarrhea, cholecystitis, and biliary neoplasia (10–12), and it is well-documented that EHS can also infect

other animal species, such as dogs, cats, geese, rhesus macaques, hamsters, gerbils, guinea fowl, and chickens (13–31).

In this report, we announce the whole-genome sequencing of eight EHS, including *Helicobacter muridarum* ST1, *Helicobacter trogontum*, *H. typhlonius*, as well as unnamed *Helicobacter* species (Massachusetts Institute of Technology [MIT] strains 01-6451, 03-1614, 03-1616, 05-5293, and 11-5569). These isolates were obtained from cecal, colon, and fecal samples of either laboratory or wild mice and rats. The isolates were sequenced using Illumina MiSeq sequencing technology, as described previously (32). The 250-bp paired-end sequencing reads generated by MiSeq were assembled into contigs using Velvet (33). The sequences were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (34). The G+C contents ranged from 33 to 39%, and between 1,922 and 2,520 genes were annotated per genome (Table 1).

Due to the ability of EHS to interfere with biomedical research involving rodents, we evaluated the presence of known *Helicobacter* virulence determinants, such as gamma-glutamyl transpeptidase (*ggt*), cytolethal distending toxin subunit B (*cdtB*), and components of both the type IV and type VI secretion systems. Both

TABLE 1 Genome characteristics and accession numbers of eight rodent helicobacters

Strain	GenBank accession no.	Host	Fold coverage	G+C content (%)	Estimated genome length (bp) using Velvet	No. of contigs using PGAP	No. of genes using PGAP
<i>H. muridarum</i> ST1	JRPD00000000	Mouse	56	33	2,354,445	92	2,351
<i>H. trogontum</i> ("Flexispira rappini taxon 6") ATCC 700114	JRPL00000000	Rat	48	34	2,762,714	129	1,922
<i>H. typhlonius</i> MIT strain 97-6810	JRPF00000000	Mouse	62	38.5	1,899,179	25	2,520
<i>Helicobacter</i> sp. MIT strain 01-6451	JRMQ00000000	Mouse	89	37.5	2,056,937	48	2,064
<i>Helicobacter</i> sp. MIT strain 03-1614	JRMS00000000	Mouse	36	37.5	1,927,676	172	2,057
<i>Helicobacter</i> sp. MIT strain 03-1616	JROY00000000	Mouse	37	39	1,890,582	176	1,974
<i>Helicobacter</i> sp. MIT strain 05-5293	JROZ00000000	Wild mouse	65	38	2,016,563	101	2,097
<i>Helicobacter</i> sp. MIT strain 11-5569	JRPB00000000	Mouse	80	35	2,024,356	83	2,135

H. muridarum and *H. trogonum* ATCC 700144 possess *ggt*, a *Helicobacter pylori* virulence factor that leads to cell cycle arrest, necrosis, and apoptosis (35). *cdtB* is present in *H. muridarum*, *H. typhlonius*, and the unnamed MIT strains 01-6451, 03-1614, 03-1616, and 05-5293. The entire *cdtABC* cluster was found in *H. muridarum* and the unnamed MIT strains 01-6451, 03-1614, and 05-5293. Multiple type IV secretion genes (*virB2-virB11* or *virD4*) were found in all species presented, excluding *H. muridarum* and MIT strain 01-6451. Type VI genes (*hcp*, *icmF*, *vasD*, and *vgrG*), associated with pathogenicity (36, 37), were less common. *icmF*, *vasD*, and *vgrG* were found in *H. trogonum* ATCC 700114 and the unnamed species MIT strain 03-1614. *vgrG* was found in *H. typhlonius* and several unnamed species (01-6451, 03-1616, and 11-5569).

Nucleotide sequence accession numbers. The genome sequences have been submitted to GenBank under the accession numbers listed in Table 1.

ACKNOWLEDGMENTS

This project has been funded in part with federal funds from the National Institutes of Health, under grants R01CA067529, R01OD011141, P01CA26731, and P30ES002109 (all to J.G.F.).

REFERENCES

- Solnick JV, Schauer DB. 2001. Emergence of diverse *Helicobacter* species in the pathogenesis of gastric and enterohepatic diseases. *Clin. Microbiol. Rev.* 14:59–97. <http://dx.doi.org/10.1128/CMR.14.1.59-97.2001>.
- Fox JG. 2002. The non-*H. pylori* helicobacters: their expanding role in gastrointestinal and systemic diseases. *Gut* 50:273–283. <http://dx.doi.org/10.1136/gut.50.2.273>.
- Taylor NS, Xu S, Nambiar P, Dewhirst FE, Fox JG. 2007. Enterohepatic *Helicobacter* species are prevalent in mice from commercial and academic institutions in Asia, Europe, and North America. *J. Clin. Microbiol.* 45:2166–2172. <http://dx.doi.org/10.1128/JCM.00137-07>.
- Shen Z, Sheh A, Young SK, Abouelliel A, Ward DV, Earl AM, Fox JG. 2014. Draft genome sequences of six enterohepatic *Helicobacter* species isolated from humans and one from rhesus macaques. *Genome Announc.* 2(5):e00857-14. <http://dx.doi.org/10.1128/genomeA.00857-14>.
- Suerbaum S, Josenhans C, Sterzenbach T, Drescher B, Brandt P, Bell M, Droge M, Fartmann B, Fischer HP, Ge Z, Horster A, Holland R, Klein K, König J, Macko L, Mendz GL, Nyakatura G, Schauer DB, Shen Z, Weber J, Frosch M, Fox JG. 2003. The complete genome sequence of the carcinogenic bacterium *Helicobacter hepaticus*. *Proc. Natl. Acad. Sci. U. S. A.* 100:7901–7906. <http://dx.doi.org/10.1073/pnas.1332093100>.
- Whary MT, Fox JG. 2004. Natural and experimental *Helicobacter* infections. *Comp. Med.* 54:128–158.
- Ward JM, Fox JG, Anver MR, Haines DC, George CV, Collins MJ, Jr, Gorelick PL, Nagashima K, Gonda MA, Gilden RV, et al. 1994. Chronic active hepatitis and associated liver tumors in mice caused by a persistent bacterial infection with a novel *Helicobacter* species. *J. Natl. Cancer Inst.* 86:1222–1227. <http://dx.doi.org/10.1093/jnci/86.16.1222>.
- Fox JG, Dewhirst FE, Tully JG, Paster BJ, Yan L, Taylor NS, Collins MJ, Jr, Gorelick PL, Ward JM. 1994. *Helicobacter hepaticus* sp. nov., a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. *J. Clin. Microbiol.* 32:1238–1245.
- Hailey JR, Haseman JK, Bucher JR, Radovsky AE, Malarkey DE, Miller RT, Nyska A, Maronpot RR. 1998. Impact of *Helicobacter hepaticus* infection in B6C3F1 mice from twelve National Toxicology Program two-year carcinogenesis studies. *Toxicol. Pathol.* 26:602–611.
- Fox JG, Dewhirst FE, Shen Z, Feng Y, Taylor NS, Paster BJ, Ericson RL, Lau CN, Correa P, Araya JC, Roa I. 1998. Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. *Gastroenterology* 114:755–763. [http://dx.doi.org/10.1016/S0016-5085\(98\)70589-X](http://dx.doi.org/10.1016/S0016-5085(98)70589-X).
- Matsukura N, Yokomuro S, Yamada S, Tajiri T, Sundo T, Hadama T, Kamiya S, Naito Z, Fox JG. 2002. Association between *Helicobacter bilis* in bile and biliary tract malignancies: *H. bilis* in bile from Japanese and Thai patients with benign and malignant diseases in the biliary tract. *Jpn. J. Cancer Res.* 93:842–847. <http://dx.doi.org/10.1111/j.1349-7006.2002.tb01327.x>.
- Fox JG, Chien CC, Dewhirst FE, Paster BJ, Shen Z, Melito PL, Woodward DL, Rodgers FG. 2000. *Helicobacter canadensis* sp. nov. isolated from humans with diarrhea as an example of an emerging pathogen. *J. Clin. Microbiol.* 38:2546–2549.
- Kiehlauch JA, Brenner DJ, Cameron DN, Steigerwalt AG, Makowski JM, Baker CN, Patton CM, Wachsmuth IK. 1995. Genotypic and phenotypic characterization of *Helicobacter cinaedi* and *Helicobacter fennelliae* strains isolated from humans and animals. *J. Clin. Microbiol.* 33:2940–2947.
- Burnens AP, Stanley J, Schaad UB, Nicolet J. 1993. Novel *Campylobacter*-like organism resembling *Helicobacter fennelliae* isolated from a boy with gastroenteritis and from dogs. *J. Clin. Microbiol.* 31:1916–1917.
- Foley JE, Marks SL, Munson L, Melli A, Dewhirst FE, Yu S, Shen Z, Fox JG. 1999. Isolation of *Helicobacter canis* from a colony of Bengal cats with endemic diarrhea. *J. Clin. Microbiol.* 37:3271–3275.
- Stanley J, Linton D, Burnens AP, Dewhirst FE, On SL, Porter A, Owen RJ, Costas M. 1994. *Helicobacter pullorum* sp. nov. genotype and phenotype of a new species isolated from poultry and from human patients with gastroenteritis. *Microbiology* 140:3441–3449. <http://dx.doi.org/10.1099/13500872-140-12-3441>.
- Waldenström J, On SL, Ottvall R, Hasselquist D, Harrington CS, Olsen B. 2003. Avian reservoirs and zoonotic potential of the emerging human pathogen *Helicobacter canadensis*. *Appl. Environ. Microbiol.* 69:7523–7526. <http://dx.doi.org/10.1128/AEM.69.12.7523-7526.2003>.
- Goto K, Jiang W, Zheng Q, Oku Y, Kamiya H, Itoh T, Ito M. 2004. Epidemiology of *Helicobacter* infection in wild rodents in the Xinjiang-Uygur autonomous region of China. *Curr. Microbiol.* 49:221–223. <http://dx.doi.org/10.1007/s00284-004-4287-6>.
- Van den Bulck K, Decostere A, Baelle M, Marechal M, Ducatelle R, Haesebrouck F. 2006. Low frequency of *Helicobacter* species in the stomachs of experimental rabbits. *Lab. Anim.* 40:282–287. <http://dx.doi.org/10.1258/00236770677611424>.
- Nebbia P, Tramuta C, Ortoffi M, Bert E, Cerruti Sola S, Robino P. 2007. Identification of enteric *Helicobacter* in avian species. *Schweiz. Arch. Tierheilkd.* 149:403–407. <http://dx.doi.org/10.1024/0036-7281.149.9.403>.
- Flores BM, Fennell CL, Kuller L, Bronsdon MA, Morton WR, Stamm WE. 1990. Experimental infection of pig-tailed macaques (*Macaca nemestrina*) with *Campylobacter cinaedi* and *Campylobacter fennelliae*. *Infect. Immun.* 58:3947–3953.
- Fox JG, Drolet R, Higgins R, Messier S, Yan L, Coleman BE, Paster BJ, Dewhirst FE. 1996. *Helicobacter canis* isolated from a dog liver with multifocal necrotizing hepatitis. *J. Clin. Microbiol.* 34:2479–2482.
- Greiter-Wilke A, Scanziani E, Soldati S, McDonough SP, McDonough PL, Center SA, Rishniw M, Simpson KW. 2006. Association of *Helicobacter* with cholangiohepatitis in cats. *J. Vet. Intern. Med.* 20:822–827. <http://dx.doi.org/10.1111/j.1939-1676.2006.tb01792.x>.
- Shomer NH, Dangler CA, Marini RP, Fox JG. 1998. *Helicobacter bilis/ Helicobacter rodentium* co-infection associated with diarrhea in a colony of scid mice. *Lab. Anim. Sci.* 48:455–459.
- Shomer NH, Dangler CA, Schrenzel MD, Fox JG. 1997. *Helicobacter bilis*-induced inflammatory bowel disease in scid mice with defined flora. *Infect. Immun.* 65:4858–4864.
- Franklin CL, Beckwith CS, Livingston RS, Riley LK, Gibson SV, Besch-Williford CL, Hook RR, Jr. 1996. Isolation of a novel *Helicobacter* species, *Helicobacter cholecystus* sp. nov., from the gallbladders of Syrian hamsters with cholangiofibrosis and centrilobular pancreatitis. *J. Clin. Microbiol.* 34:2952–2958.
- Haines DC, Gorelick PL, Battles JK, Pike KM, Anderson RJ, Fox JG, Taylor NS, Shen Z, Dewhirst FE, Anver MR, Ward JM. 1998. Inflammatory large bowel disease in immunodeficient rats naturally and experimentally infected with *Helicobacter bilis*. *Vet. Pathol.* 35:202–208. <http://dx.doi.org/10.1177/030098589803500305>.
- Eaton KA, Dewhirst FE, Paster BJ, Tzellas N, Coleman BE, Paola J, Sherding R. 1996. Prevalence and varieties of *Helicobacter* species in dogs from random sources and pet dogs: animal and public health implications. *J. Clin. Microbiol.* 34:3165–3170.
- Fox JG, Yan LL, Dewhirst FE, Paster BJ, Shames B, Murphy JC, Hayward A, Belcher JC, Mendes EN. 1995. *Helicobacter bilis* sp. nov., a novel *Helicobacter* species isolated from bile, livers, and intestines of aged, inbred mice. *J. Clin. Microbiol.* 33:445–454.
- Fox JG, Boutin SR, Handt LK, Taylor NS, Xu S, Rickman B, Marini RP,

- Dewhirst FE, Paster BJ, Motzel S, Klein HJ. 2007. Isolation and characterization of a novel *Helicobacter* species, “*Helicobacter macacae*,” from rhesus monkeys with and without chronic idiopathic colitis. *J. Clin. Microbiol.* 45:4061–4063. <http://dx.doi.org/10.1128/JCM.01100-07>.
31. Marini RP, Muthupalani S, Shen Z, Buckley EM, Alvarado C, Taylor NS, Dewhirst FE, Whary MT, Patterson MM, Fox JG. 2010. Persistent infection of rhesus monkeys with “*Helicobacter macacae*” and its isolation from an animal with intestinal adenocarcinoma. *J. Med. Microbiol.* 59: 961–969. <http://dx.doi.org/10.1099/jmm.0.019117-0>.
 32. Sheh A, Chaturvedi R, Merrell DS, Correa P, Wilson KT, Fox JG. 2013. Phylogeographic origin of *Helicobacter pylori* determines host-adaptive responses upon coculture with gastric epithelial cells. *Infect. Immun.* 81: 2468–2477. <http://dx.doi.org/10.1128/IAI.01182-12>.
 33. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res.* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
 34. Klimke W, Agarwala R, Badretdin A, Chetvernin S, Ciufu S, Fedorov B, Kiryutin B, O’Neill K, Resch W, Resenchuk S, Schafer S, Tolstoy I, Tatusova T. 2009. The National Center for Biotechnology Information’s Protein Clusters Database. *Nucleic Acids Res.* 37:D216–D223. <http://dx.doi.org/10.1093/nar/gkn734>.
 35. Ricci V, Giannouli M, Romano M, Zarrilli R. 2014. *Helicobacter pylori* gamma-glutamyl transpeptidase and its pathogenic role. *World J. Gastroenterol.* 20:630–638. <http://dx.doi.org/10.3748/wjg.v20.i3.630>.
 36. Lertpiriyapong K, Gamazon ER, Feng Y, Park DS, Pang J, Botka G, Graffam ME, Ge Z, Fox JG. 2012. *Campylobacter jejuni* type VI secretion system: roles in adaptation to deoxycholic acid, host cell adherence, invasion, and *in vivo* colonization. *PLoS One* 7:e42842. <http://dx.doi.org/10.1371/journal.pone.0042842>.
 37. Bartonickova L, Sterzenbach T, Nell S, Kops F, Schulze J, Venzke A, Brenneke B, Bader S, Gruber AD, Suerbaum S, Josenhans C. 2013. Hcp and VgrG1 are secreted components of the *Helicobacter hepaticus* type VI secretion system and VgrG1 increases the bacterial colitogenic potential. *Cell. Microbiol.* 15:992–1011. <http://dx.doi.org/10.1111/cmi.12094>.