## The Complete Genome Sequence of *Helicobacter pylori* Strain $G27^{\nabla}$

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*Helicobacter pylori* is a gram-negative pathogen that colonizes the stomachs of over half the world's population and causes a spectrum of gastric diseases including gastritis, ulcers, and gastric carcinoma. The *H. pylori* species exhibits unusually high levels of genetic variation between strains. Here we announce the complete genome sequence of *H. pylori* strain G27, which has been used extensively in *H. pylori* research.

Helicobacter pylori was the first organism for which the genome sequence of multiple isolates was determined (1), revealing a great deal of genetic variation at both the sequence and gene content levels. This sequence variation poses challenges for researchers using H. pylori strains for which the full genome sequence has not been determined. H. pylori strain G27, which was originally isolated from an endoscopy patient from Grosseto Hospital (Tuscany, Italy) (7), has been used extensively in H. pylori research. It is readily transformable and therefore amenable to gene disruption (6). In addition, it efficiently delivers the translocated effector protein CagA to cells in culture, facilitating the cell biological analysis of this important virulence factor (2, 8, 9, 12, 13). The strain has also been subjected to multiple experimental adaptations to new environments, including growth on canine kidney epithelial cells (2), serial passage through the mouse stomach (3, 5), and adaptation to in vitro growth in the presence or absence of a functional natural transformation system (4). Determination of the complete genome sequence of G27 will facilitate research with this strain and provide a foundation for molecular evolution studies of the genetic basis for its adaptation to new environments.

The complete genome sequence of *H. pylori* strain G27 was determined at the Washington University School of Medicine Genome Sequencing Center. Genomic DNA from an isolate of strain G27 that had been minimally passaged in the laboratory was purified by CsCl gradient centrifugation as previously described (11). The genomic DNA was used to generate plasmid and fosmid libraries, both of which were subjected to whole shotgun Sanger sequencing (4,609 total reads). In addition, the

genomic DNA was analyzed to 20-fold coverage by 454 pyrosequencing (10,752 total reads). The combined data were assembled to generate a draft sequence of the G27 genome. Gaps in the assembly were then filled in by targeted Sanger sequencing. Total Q20 coverage per base for the finished assembly is  $9.2\times$ . Protein coding regions were identified by comparing outputs from both the Genemark and Glimmer programs with a minimum cutoff size of 50 amino acids.

The G27 genome consists of a single circular 1,652,983-bp chromosome that is AT rich (61.1%), contains 1,515 open reading frames (ORFs), and is similar in size and composition to the other published H. pylori genomes of strains 26695, J99, and HPAG (1, 10, 14). G27 also contains one 10,032-bp ATrich (65.2%) plasmid that encodes 11 genes and resembles the plasmid found in strain HPAG (10). The G27 cag pathogenicity island contains a transposon, but this does not disrupt any of the open reading frames and is not predicted to interfere with the type IV secretion system delivery of CagA into host cells. Similar to strains J99 and HPAG but in contrast to 26695, G27 has a single plasticity region, between HPG27 ORFs 927 and 985, which contains many H. pylori-specific genes that are variably present between strains. G27 contains 58 genes that are not found in 26695, J99, or HPAG, as defined by a blastp hit of 1e-5. The majority of these G27-specific genes are predicted to encode hypothetical proteins.

**Nucleotide sequence accession numbers.** The GenBank accession number for the *H. pylori* strain G27 chromosome is CP001173, and that for the G27 plasmid is CP00174. The G27 genome sequence can be interrogated and compared with the other sequenced *Helicobacter* genomes by using the University of California Santa Cruz Microbial Genome Browser at http: //hpylori.ucsc.edu.

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## REFERENCES

- Alm, R. A., L. S. Ling, D. T. Moir, B. L. King, E. D. Brown, P. C. Doig, D. R. Smith, B. Noonan, B. C. Guild, B. L. deJonge, G. Carmel, P. J. Tummino, A. Caruso, M. Uria-Nickelsen, D. M. Mills, C. Ives, R. Gibson, D. Merberg, S. D. Mills, Q. Jiang, D. E. Taylor, G. F. Vovis, and T. J. Trust. 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature **397**:176–180.
- Amieva, M. R., R. Vogelmann, A. Covacci, L. S. Tompkins, W. J. Nelson, and S. Falkow. 2003. Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA. Science 300:1430–1434.
- Baldwin, D. N., B. Shepherd, P. Kraemer, M. K. Hall, L. K. Sycuro, D. M. Pinto-Santini, and N. R. Salama. 2007. Identification of *Helicobacter pylori* genes that contribute to stomach colonization. Infect. Immun. 75:1005–1016.
- Baltrus, D. A., K. Guillemin, and P. C. Phillips. 2008. Natural transformation increases the rate of adaptation in the human pathogen *Helicobacter pylori*. Evolution 62:39–49.
- Castillo, A. R., S. S. Arevalo, A. J. Woodruff, and K. M. Ottemann. 2008. Experimental analysis of *Helicobacter pylori* transcriptional terminators suggests this microbe uses both intrinsic and factor-dependent termination. Mol. Microbiol. 67:155–170.
- Censini, S., C. Lange, Z. Xiang, J. E. Crabtree, P. Ghiara, M. Borodovsky, R. Rappuoli, and A. Covacci. 1996. *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. USA 93:14648–14653.
- Covacci, A., S. Censini, M. Bugnoli, R. Petracca, D. Burroni, G. Macchia, A. Massone, E. Papini, Z. Xiang, N. Figura, and R. Rappuoli. 1993. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter*

*pylori* associated with cytotoxicity and duodenal ulcer. Proc. Natl. Acad. Sci. USA **90:**5791–5795.

- El-Etr, S. H., A. Mueller, L. S. Tompkins, S. Falkow, and D. S. Merrell. 2004. Phosphorylation-independent effects of CagA during interaction between *Helicobacter pylori* and T84 polarized monolayers. J. Infect. Dis. 190:1516– 1523.
- Guillemin, K., N. R. Salama, L. S. Tompkins, and S. Falkow. 2002. Cag pathogenicity island-specific responses of gastric epithelial cells to *Helico*bacter pylori infection. Proc. Natl. Acad. Sci. USA 99:15136–15141.
- Oh, J. D., H. Kling-Backhed, M. Giannakis, J. Xu, R. S. Fulton, L. A. Fulton, H. S. Cordum, C. Wang, G. Elliott, J. Edwards, E. R. Mardis, L. G. Engstrand, and J. I. Gordon. 2006. The complete genome sequence of a chronic atrophic gastritis *Helicobacter pylori* strain: evolution during disease progression. Proc. Natl. Acad. Sci. USA 103:9999–10004.
- Salama, N., K. Guillemin, T. K. McDaniel, G. Sherlock, L. Tompkins, and S. Falkow. 2000. A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. Proc. Natl. Acad. Sci. USA 97:14668–14673.
- Segal, E. D., J. Cha, J. Lo, S. Falkow, and L. S. Tompkins. 1999. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. Proc. Natl. Acad. Sci. USA 96:14559– 145564.
- Stein, M., R. Rappuoli, and A. Covacci. 2000. Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after *cag*-driven host cell translocation. Proc. Natl. Acad. Sci. USA 97:1263–1268.
- Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, E. K. Hickey, D. E. Berg, J. D. Gocayne, T. R. Utterback, J. D. Peterson, J. M. Kelley, M. D. Cotton, J. M. Weidman, C. Fujii, C. Bowman, L. Watthey, E. Wallin, W. S. Hayes, M. Borodovsky, P. D. Karp, H. O. Smith, C. M. Fraser, and J. C. Venter. 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature 388: 539–547.