Genome Sequence of Oxalobacter formigenes Strain HC-1

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ABSTRACT

The lack of Oxalobacter formigenes colonization of the human gut has been correlated with the formation of calcium oxalate kidney stones and also with the number of recurrent kidney stone episodes. Here, we present the genome sequence of HC-1, a human strain isolated from an individual residing in Iowa, USA.

An anaerobe, with a substrate-specificity for oxalate, was isolated from human and other animal feces and a new genus and species Oxalobacter formigenes, was established (1). Individuals forming oxalate kidney stones who are Oxalobacter-negative have significantly higher urinary oxalate and stone episodes correlate with the lack of Oxalobacter (2). Colonization of a mouse model of the genetic disease primary hyperoxaluria, type 1 with Oxalobacter resulted in a normalization of both hyperoxaluria and hyperoxalemia exhibited in noncolonized counterparts (3, 4). Since a human strain, HC-1, was tested in some small human clinical trials (5–7), the present study was undertaken to determine the complete genome sequence of the HC-1 strain which was archived in the Hatch laboratory, notated as HC-1MH, since 2011.

A genomic DNA library was prepared following the protocol specified by Pacific Biosciences (Menlo Park, CA). Briefly, genomic DNA was sheared to an average fragment length of 20 kb, using the SAGE ELF (Sage Science, Beverly, MA), end-repaired, and single-molecule real-time (SMRT) bell oligonucleotide adaptors blunt-end ligated to construct a DNA fragment library for sequencing on the Pacific Biosciences RSII platform. A single SMRT cell produced a total of 1.58 Gb in 93,480 polymerase reads having an N50 of 19.7 kb and a subread N50 is 9.9 kb. The HC-1 genome was assembled using HGAP version 3 (8), and annotated using RAST (http://rast.nmpdr.org) (9–11).

The complete HC-1MH genome contains a single contig of 2,468,871 bp and has an average G+C content of 49.6%. A total of 2,599 genes were annotated by RAST, including 47 tRNAs, 7 ribosomal RNAs, and 2,545 predicted coding sequences (CDSs). RAST annotation assigns 1,062 (42%) of the 2,545 HC-1 CDSs as members of 336 categorized subsystems. Subsystems are defined as a set of functional roles implementing specific biological process or structure (12). In general, subsystems may be considered biological pathways. The most abundant subsystem classifications include 203 genes involved in protein metabolism; 169 involved in metabolism of cofactors, vitamins, prosthetic groups, and pigments; 205 in amino acid and derivative metabolism; and 108 in carbohydrate metabolism. A total of 1,483 CDSs (58%) are not assigned to specific subsystems.

The annotated HC-1MH genome was compared to O. formigenes CC13 (NCBI accession no. NZ_ACDQ00000000) and O. formigenes HOxBLS (accession no. NZ_ACDP00000000). At the protein level, 2,473 of 2,545 (97%) HC-1 CDSs have greater than 99% identity with CDSs identified in CC13. The genome of HC-1MH contains 54 CDSs not present in CC13, the majority of which (42 CDSs) are identified as hypothetical proteins. The remaining 12 CDSs identified in HC-1MH but absent from CC13 largely represent phage-associated proteins, primarily clustered in a ~35 kb region of the

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HC-1MH genome. Only 260 (10%) HC-1MH CDSs share greater than 90% amino acid identity with HOxBLS CDSs. Compared to HOxBLS, 713 CDSs appear exclusively in the HC-1MH genome, of which 533 are annotated as hypothetical proteins and the remaining 180 CDS annotations include proteins characterized as ABC and other transporters, bacteriophage-related proteins, transcriptional regulators, large subunit ribosomal proteins, and a small cluster of clustered regularly interspaced short palindromic repeat (CRISPR)-associated proteins.

**Accession number(s).** This genome sequencing project was deposited in GenBank under accession no. CP018787. The version described is the first version.

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**REFERENCES**

1. Allison MJ, Dawson KA, Mayberry WR, Foss JG. 1985. Oxalobacter formigenes gen. nov., sp. nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract. Arch Microbiol 141:1–7. [https://doi.org/10.1007/BF00446731](https://doi.org/10.1007/BF00446731).

2. Hatch M, Freel RW. 2008. The roles and mechanisms of intestinal oxalate transport in oxalate homeostasis. Semin Nephrol 28:143–151. [https://doi.org/10.1016/j.semnephrol.2008.01.007](https://doi.org/10.1016/j.semnephrol.2008.01.007).

3. Hatch M, Gmyrhiska A, Salido EC, Allison MJ, Freel RW. 2011. Enteric oxalate elimination is induced and oxalate is normalized in a mouse model of primary hyperoxaluria following intestinal colonization with Oxalobacter. Am J Physiol Gastrointest Liver Physiol 300:G461–G469. [https://doi.org/10.1152/ajpgi.00434.2010](https://doi.org/10.1152/ajpgi.00434.2010).

4. Hatch M, Freel RW. 2013. A human strain of Oxalobacter (HC-1) promotes enteric oxalate secretion in the small intestine of mice and reduces urinary oxalate excretion. Urothiasis 41:379–384. [https://doi.org/10.1007/s00240-013-0601-8](https://doi.org/10.1007/s00240-013-0601-8).

5. Hoppe B, von Unruh G, Hesse A, Hesse A, Sidhu H. 2005. Oxalate degrading bacteria: new treatment option for patients with primary and secondary hyperoxaluria? Urol Res 33:372–375. [https://doi.org/10.1007/s00240-005-0497-z](https://doi.org/10.1007/s00240-005-0497-z).

6. Hoppe B, Beck B, Gatter N, von Unruh G, Tischer A, Hesse A, Laube N, Kauf P, Sidhu H. 2006. Oxalobacter formigenes: a potential tool for the treatment of primary hyperoxaluria type 1. Kidney Int 70:1305–1311. [https://doi.org/10.1038/sj.ki.5001707](https://doi.org/10.1038/sj.ki.5001707).

7. Hoppe B, Groothoff JW, Hulten SA, Cochet P, Niaudet P, Kemper MJ, Deschênes G, Unwin R, Milliner D. 2011. Efficacy and safety of Oxalobacter formigenes to reduce urinary oxalate in primary hyperoxaluria. Nephrol Dial Transplant 26:3609–3615. [https://doi.org/10.1093/ndt/gfr107](https://doi.org/10.1093/ndt/gfr107).

8. Li X, Ellis ML, Knight J. 2015. Oxalobacter formigenes colonization and oxalate dynamics in a mouse model. Appl Environ Microbiol 81:5048–5054. [https://doi.org/10.1128/AEM.01313-15](https://doi.org/10.1128/AEM.01313-15).

9. Overbeek R, Olson R, Busch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42:D206–D214. [https://doi.org/10.1093/nar/gkt1226](https://doi.org/10.1093/nar/gkt1226).

10. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Busch GD, Shukla M, Thomason JA, III, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. [https://doi.org/10.1038/srep08365](https://doi.org/10.1038/srep08365).

11. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formisna K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Busch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnotico O. 2008. The RAST Server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. [https://doi.org/10.1186/1471-2164-9-75](https://doi.org/10.1186/1471-2164-9-75).

12. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang HY, Cohoon M, de Crécy-Lagard V, Diaz N, Disz T, Edwards R, Fonstein M, Frank ED, Gerdes S, Glass EM, Goesmann A, Hanson A, Iwata-Reuhl D, Jensen R, Jamshidi N, Krause L, Kubal M, Larsen N, Linke B, McHardy AC, Meyer F, Neuvéglis H, Olsen G, Olson R, Osterman A, Portnoy V, Pusch GD, Rodionov DA, Rütter C, Steijer J, Stevens R, Thiele I, Vassieva O, Ye Y, Zagnotico O, Vonstein V. 2005. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res 33:5691–5702. [https://doi.org/10.1093/nar/gki866](https://doi.org/10.1093/nar/gki866).