

Draft Whole-Genome Sequence of *Haemophilus ducreyi* Strain AUSPNG1, Isolated from a Cutaneous Ulcer of a Child from Papua New Guinea

Dharanesh Gangaiah,^a Georgi K. Marinov,^b Sally A. Roberts,^c Jenny Robson,^d Stanley M. Spinola^{a,e,f,g}

Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana, USA^a; Department of Biology, Indiana University, Bloomington, Indiana, USA^b; Department of Microbiology, Auckland District Health Board, Auckland, New Zealand^c; Department of Microbiology, Sullivan Nicolaides Pathology, Brisbane, Queensland, Australia^d; Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana, USA^e; Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, Indiana, USA^f; Center for Immunobiology, Indiana University School of Medicine, Indianapolis, Indiana, USA^g

***Haemophilus ducreyi* has recently emerged as a leading cause of cutaneous ulcers in the yaws-endemic areas of Papua New Guinea and other South Pacific islands. Here, we report the draft genome sequence of the *H. ducreyi* strain AUSPNG1, isolated from a cutaneous ulcer of a child from Papua New Guinea.**

Received 2 December 2015 Accepted 21 December 2015 Published 4 February 2016

Citation Gangaiah D, Marinov GK, Roberts SA, Robson J, Spinola SM. 2016. Draft whole-genome sequence of *Haemophilus ducreyi* strain AUSPNG1, isolated from a cutaneous ulcer of a child from Papua New Guinea. *Genome Announc* 4(1):e01661-15. doi:10.1128/genomeA.01661-15.

Copyright © 2016 Gangaiah et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Stanley M. Spinola, sspinola@iupui.edu.

Haemophilus ducreyi causes the genital ulcer disease chancroid and has emerged as a leading cause of cutaneous ulcers (CU) in the yaws-endemic regions of the South Pacific islands and equatorial Africa (1–3). By whole-genome sequence analysis, CU strains from Samoa and Vanuatu are almost identical to the genital ulcer (GU) strain 35000HP, and CU strains form a subcluster within the class I clade of GU *H. ducreyi* (4). This study was limited by the lack of CU strains from other countries. Here, we report the draft genome sequence of *H. ducreyi* AUSPNG1, which was isolated in 2013 from a CU of a 12-year-old boy from Papua New Guinea, who was treated in Brisbane, Australia.

AUSPNG1 was grown on GC medium base agar plates (Difco, Becton, Dickinson) supplemented with 1% bovine hemoglobin (Sigma-Aldrich) and 1% GCHI enrichment (Remel, Thermo Fisher) at 33°C with 5% CO₂. Genomic DNA was extracted using the MagNAPure nucleic acid extraction kit (Roche). The libraries were prepared using the NexteraXT DNA library preparation kit (Illumina) and sequenced on the MiSeq platform as paired 250-bp reads. Adapter sequences and low-quality bases were trimmed using Trimmomatic version 0.33 (5). Reads were error-corrected using BayesHammer and assembled using SPAdes version 3.5.0, Edena version 3.131028, and IDBA version 1.1.1; assemblies were then integrated using CISA version 1.3 (6–10). Protein-coding genes were annotated using RAST (11). rRNAs were annotated using RNAmmer version 1.2 and tRNAs using tRNAscan-SE version 1.3.1 (12, 13). Additional noncoding RNAs were annotated using Infernal version 1.1.1 and the Rfam database version 12.0 (14, 15). The contigs were ordered and aligned against the reference genome 35000HP using Mauve (16, 17). Phylogenetic analysis was performed by the maximum likelihood method using Mega version 6.0 (18).

After filtering out potential contaminants, the final assembly consisted of 26 contigs (the largest being 480,771 bp) with a cov-

erage of ~294×, total length of 1,727,680 bp, GC content of 37.5%, *N*₅₀ of 268,281 bp, and *N*₉₀ of 35,093 bp. Annotation resulted in 1,697 protein-coding genes, 48 tRNAs, 7 5S rRNAs, 6 16S rRNAs, 6 23S rRNAs, and 31 other ncRNAs. Phylogenetically, AUSPNG1 belongs to the CU subcluster within the class I clade and is most related to 35000HP among the GU strains and to NZV1 among the CU strains. A circular plasmid with 99% nucleotide identity to pB1000 of *Haemophilus parasuis* and containing the β-lactam resistance gene *bla*ROB-1 was noted; β-lactamase production was confirmed using the BBL Cefinase paper discs (Becton, Dickinson). This is the first report of a CU strain expressing β-lactamase, which is disturbing in that penicillin is frequently used for empirical treatment of CU in the tropics (4, 19, 20).

The availability of the AUSPNG1 genome sequence will facilitate additional studies to better understand the epidemiology, pathogenesis, evolution, and prevention of *H. ducreyi*-associated CU in yaws-endemic regions.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number [LMZZ00000000](https://www.ncbi.nlm.nih.gov/nuccore/LMZZ00000000). The version described in this paper is the first version, LMZZ01000000.

ACKNOWLEDGMENTS

This work was supported by funds from the Indiana University School of Medicine and the Microbiology Education and Research Fund, Department of Microbiology, Auckland District Health Board.

We thank Indira Basu for preparation of DNA. All authors have no relevant financial relationships to disclose.

REFERENCES

- Mitjà O, Lukehart SA, Pokowas G, Moses P, Kapa A, Godornes C, Robson J, Cherian S, Houine W, Kazadi W, Siba P, de Lazzari E, Bassat Q. 2014. *Haemophilus ducreyi* as a cause of skin ulcers in children from a yaws-endemic area of Papua New Guinea: a prospective cohort study.

- Lancet Glob Health 2:e235–e241. [http://dx.doi.org/10.1016/S2214-109X\(14\)70019-1](http://dx.doi.org/10.1016/S2214-109X(14)70019-1).
2. Ghinai R, El-Duah P, Chi KH, Pillay A, Solomon AW, Bailey RL, Agana N, Mabey DC, Chen CY, Adu-Sarkodie Y, Marks M. 2015. A cross-sectional study of “yaws” in districts of Ghana which have previously undertaken azithromycin mass drug administration for trachoma control. *PLoS Negl Trop Dis* 9:e0003496. <http://dx.doi.org/10.1371/journal.pntd.0003496>.
 3. Marks M, Chi KH, Vahi V, Pillay A, Sokana O, Pavluck A, Mabey DC, Chen CY, Solomon AW. 2014. *Haemophilus ducreyi* associated with skin ulcers among children, Solomon Islands. *Emerg Infect Dis* 20:1705–1707. <http://dx.doi.org/10.3201/eid2010.140573>.
 4. Gangaiah D, Webb KM, Humphreys TL, Fortney KR, Toh E, Tai A, Katz SS, Pillay A, Chen CY, Roberts SA, Munson RS, Jr, Spinola SM. 2015. *Haemophilus ducreyi* cutaneous ulcer strains are nearly identical to class I genital ulcer strains. *PLoS Negl Trop Dis* 9:e0003918. <http://dx.doi.org/10.1371/journal.pntd.0003918>.
 5. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <http://dx.doi.org/10.1093/bioinformatics/btu170>.
 6. Lin SH, Liao YC. 2013. CISA: contig integrator for sequence assembly of bacterial genomes. *PLoS One* 8:e60843. <http://dx.doi.org/10.1371/journal.pone.0060843>.
 7. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <http://dx.doi.org/10.1089/cmb.2012.0021>.
 8. Peng Y, Leung HC, Yiu SM, Chin FY. 2012. IDBA-UD: a *de novo* assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics* 28:1420–1428. <http://dx.doi.org/10.1093/bioinformatics/bts174>.
 9. Hernandez D, François P, Farinelli L, Osterås M, Schrenzel J. 2008. *De novo* bacterial genome sequencing: millions of very short reads assembled on a desktop computer. *Genome Res* 18:802–809. <http://dx.doi.org/10.1101/gr.072033.107>.
 10. Nikolenko SI, Korobeynikov AI, Alekseyev MA. 2013. BayesHammer: Bayesian clustering for error correction in single-cell sequencing. *BMC Genomics* 14(suppl 1):S7. <http://dx.doi.org/10.1186/1471-2164-14-S1-S7>.
 11. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formisano 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, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
 12. Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 35:3100–3108. <http://dx.doi.org/10.1093/nar/gkm160>.
 13. Schattner P, Brooks AN, Lowe TM. 2005. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. *Nucleic Acids Res* 33:W686–W689. <http://dx.doi.org/10.1093/nar/gki366>.
 14. Nawrocki EP, Eddy SR. 2013. Infernal 1.1: 100-fold faster RNA homology searches. *Bioinformatics* 29:2933–2935. <http://dx.doi.org/10.1093/bioinformatics/btt509>.
 15. Burge SW, Daub J, Eberhardt R, Tate J, Barquist L, Nawrocki EP, Eddy SR, Gardner PP, Bateman A. 2013. Rfam 11.0: 10 years of RNA families. *Nucleic Acids Res* 41:D226–D232. <http://dx.doi.org/10.1093/nar/gks1005>.
 16. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 5:e11147. <http://dx.doi.org/10.1371/journal.pone.0011147>.
 17. Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT. 2009. Reordering contigs of draft genomes using the mauve aligner. *Bioinformatics* 25:2071–2073. <http://dx.doi.org/10.1093/bioinformatics/btp356>.
 18. Tamura K, Stecher G, Peterson D, Filipitski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729. <http://dx.doi.org/10.1093/molbev/mst197>.
 19. Ussher JE, Wilson E, Campanella S, Taylor SL, Roberts SA. 2007. *Haemophilus ducreyi* causing chronic skin ulceration in children visiting Samoa. *Clin Infect Dis* 44:e85–e87. <http://dx.doi.org/10.1086/515404>.
 20. Marckmann P, Højbjerg T, von Eyben FE, Christensen I. 1989. Imported pedal chancroid: case report. *Genitourin Med* 65:126–127. <http://dx.doi.org/10.1136/sti.65.2.126>.