



Draft Genome Sequence of Pseudomonas sp. nov. H2

Wesley Loftie-Eaton,^{a,b} Haruo Suzuki,^c Kelsie Bashford,^{a*} Holger Heuer,^{a*} Pieter Stragier,^d Paul De Vos,^d Matthew L. Settles,^{a,b} Eva M. Top^{a,b}

Department of Biological Sciences, University of Idaho, Moscow, Idaho, USA^a; Institute for Bioinformatics and Evolutionary Studies (IBEST), University of Idaho, Moscow, Idaho, USA^b; Graduate School of Science and Engineering, Yamaguchi University, Yamaguchi, Japan^c; Laboratory of Microbiology, Ghent University, Ghent, Belgium^d

We report the draft genome sequence of *Pseudomonas* sp. nov. H2, isolated from creek sediment in Moscow, ID, USA. The strain is most closely related to *Pseudomonas putida*. However, it has a slightly smaller genome that appears to have been impacted by horizontal gene transfer and poorly maintains IncP-1 plasmids.

Received 20 February 2015 Accepted 23 February 2015 Published 2 April 2015

Citation Loftie-Eaton W, Suzuki H, Bashford K, Heuer H, Stragier P, De Vos P, Settles ML, Top EM. 2015. Draft genome sequence of *Pseudomonas* sp. nov. H2. Genome Announc 3(2):e00241-15. doi:10.1128/genomeA.00241-15.

Copyright © 2015 Loftie-Eaton et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license. Address correspondence to Eva M. Top, evatop@uidaho.edu.

Pseudomonas sp. nov. H2 is a member of the Gammaproteobacteria. The strain was isolated from creek sediment in Moscow, ID, as a transconjugant after a plate mating of a sediment sample with an auxotrophic donor of the IncP-1 β plasmid pB10. Transconjugants were selected on M9 medium supplemented with 0.1% gluconic acid and the antibiotics tetracycline (20 mg/liter) and amoxicillin (150 mg/liter) (1). Based on its 16S rRNA sequence, it was provisionally called *P. putida* H2. While *P. putida* strains are generally quite capable of maintaining IncP-1 plasmids, strain H2 was reported as an unfavorable host to broadhost-range IncP-1 plasmids, and as such has been used in several plasmid-host evolution and persistence studies (1–4).

Genomic DNA (gDNA) was isolated using a GenElute bacterial genomic DNA kit (Sigma-Aldrich) as per manufacturer's instructions. The gDNA was sequenced using a whole-genome shotgun approach with paired 150 bp reads generated on MiSeq (Illumina) at the IBEST Genomics Resources Core at the University of Idaho, ID, USA. Sequencing adapters and low-quality bases were trimmed using custom scripts, and reads were assembled using Newbler v2.6. A total of 95 contigs >500 bp were produced (total number of contigs is 132). Of these, the largest was 281,547 bp and the N_{50} contig size was 127,674 bp.

Strain H2 was identified as a potential new *Pseudomonas* species using an in-house, four-gene (*glnA*, *gyrB*, *rpoB*, and *rpoD*) multilocus sequence analysis (MLSA) scheme. The *glnA*, *gyrB*, and *rpoD* genes of H2 were highly similar to those of *P. putida* LMG 14676. Previously, strain LMG 14676 was regarded as a member of *P. putida* biotype A based on phenotypic data (5) and SDS PAGE profiles of whole cell proteins (6), but it clearly separates from the *P. putida* type strain on the basis of ribopatterning (7). The *rpoB* gene sequence of strain H2 was incongruent to the *glnA*, *gyrB*, and *rpoD* gene sequences. Comparison among the 11 completely sequenced *P. putida* chromosomes showed that the H2 genome, estimated at 5.79 Mb, is with one exception, smaller than all *P. putida* genomes (the median for *P. putida* is 6.03 Mb, and the smallest and largest genomes are 5.73 and 6.87 Mb). Unsurprisingly, the H2 genome contains fewer predicted protein coding

sequences (CDSs) (4,985) than most *P. putida* genomes (the median number of CDSs is 5,321, and the minimum and maximum are 4,960 and 6,357). Finally, the G+C contents of the H2 genome vary between 28 and 75%, with a median of 62.6% (500-bp sliding window). Combined with the MLSA results, these data suggest that the H2 genome has been considerably impacted by horizontal gene transfer. Because of the few clear distinctions from *P. putida*, we define strain H2 here as a *Pseudomonas* sp. nov.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. JRPO00000000. The version described here is version JRPO01000000. Strain H2 is available from the LMG culture collection (http://bccm.belspo.be/about/lmg.php, LMG 28719).

ACKNOWLEDGMENTS

This work was funded by NIH grant R01 AI084918 from the National Institute of Allergy and Infectious Diseases (NIAID), with additional support from COBRE NIH grants P20 RR16448 and P20 GM103397 and the Idaho INBRE Program, NIH grants P20 RR016454 and P20 GM103408, through the IBEST Genomics and Computational Resources Cores. K.B. was also supported by an Undergraduate Research Fellowship from the University of Idaho Department of Biological Sciences.

REFERENCES

- Heuer H, Fox RE, Top EM. 2007. Frequent conjugative transfer accelerates adaptation of a broad-host-range plasmid to an unfavorable *Pseudomonas* putida host. FEMS Microbiol Ecol 59:738–748. http://dx.doi.org/10.1111/ j.1574-6941.2006.00223.x.
- De Gelder L, Ponciano JM, Joyce P, Top EM. 2007. Stability of a promiscuous plasmid in different hosts: no guarantee for a long-term relationship. Microbiology 153:452–463. http://dx.doi.org/10.1099/mic.0.2006/001784-0.
- De Gelder L, Williams JJ, Ponciano JM, Sota M, Top EM. 2008. Adaptive plasmid evolution results in host-range expansion of a broad-host-range plasmid. Genetics 178:2179–2190. http://dx.doi.org/10.1534/genetics.107.084475.
- Loftie-Eaton W, Tucker A, Norton A, Top EM. 2014. Flow cytometry and real-time quantitative PCR as tools for assessing plasmid persistence. Appl Environ Microbiol 80:5439–5446. http://dx.doi.org/10.1128/AEM.00793-14.
- 5. Van Canneyt M, Torck U, Dewettinck D, Vaerewijck M, Kersters K.

^{*} Present address: Kelsie Bashford, 2702 East 55th Avenue, Spokane, Washington, USA; Holger Heuer, Julius Kühn-Institut, Braunschweig, Germany.

- 1996. Grouping of pseudomonads by SDS PAGE of whole cell proteins. Syst Appl Microbiol 4:556–568. http://dx.doi.org/10.1016/S0723-2020(96)80027-0.
- Grimont PAD, Vancanneyt M, Lefèvre M, Vandemeulebroecke K, Vauterin L, Brosch R, Kersters K, Grimont F. 1996. Ability of biolog and biotype-100 systems to reveal the taxonomic diversity of the pseudomon-
- ads. Syst Appl Microbiol 19:510–527. http://dx.doi.org/10.1016/S0723 -2020(96)80024-5.
- 7. Brosch R, Lefèvre M, Grimont F, Grimont PAD. 1996. Taxonomic diversity of pseudomonads revealed by computer-interpretation of ribotyping data. Syst Appl Microbiol 19:541–555. http://dx.doi.org/10.1016/S0723 -2020(96)80026-9.