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Draft Genome Sequence of *Pseudomonas azotifigens* Strain DSM 17556^T (6H33b^T), a Nitrogen Fixer Strain Isolated from a Compost Pile

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***Pseudomonas azotifigens* strain 6H33b^T is a nitrogen fixer isolated from a hyperthermal compost pile in 2005 by Hatayama and collaborators. Here we report the draft genome, which has an estimated size of 5.0 Mb, exhibits an average G+C content of 66.73%, and is predicted to encode 4,536 protein-coding genes and 100 RNA genes.**

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Stain 6H33b^T was isolated by Hatayama and collaborators from a hyperthermal compost pile in Japan because of its ability to grow diazotrophically (1). It was proposed as the type and unique strain of *Pseudomonas azotifigens* (1). Phylogenetic analysis indicated that *Pseudomonas indica* (98% identity in 16S rRNA genes) in the *Pseudomonas aeruginosa* group was its closest neighbor. A multilocus sequence analysis of four housekeeping genes later demonstrated that it belongs to the *Pseudomonas stutzeri* phylogenetic group (2). The nitrogen-fixing ability within the genus *Pseudomonas* has been debated for many years (3), and the only strains recognized to fix nitrogen are *P. azotifigens* and some members of *P. stutzeri*.

The whole-genome shotgun sequence of strain 6H33b^T (DSM 17556^T) was obtained in the context of the Genomic Encyclopedia of Type Strains (4). The draft genome of *P. azotifigens* DSM 17556^T was generated at the Department of Energy (DOE) Joint Genome Institute (JGI) using the Illumina technology (5). An Illumina standard shotgun library was constructed and sequenced using the Illumina HiSeq 2000 platform, which generated 12,314,136 reads totaling 1,847.1 Mb. Illumina sequencing and library artifacts were removed using Duk filtering (L. Mingkun, A. Copeland, and H. J. Duk, unpublished data). Filtered Illumina reads were assembled using Velvet (version 1.1.04) (6), simulated paired-end reads were created from Velvet contigs using wgsim (<https://github.com/lh3/wgsim>), and simulated read pairs were reassembled using Allpaths-LG (version r42328) (7), resulting in an assembly of 5.0 Mb in 59 scaffolds (96 contigs) with an average 122.8× coverage of the genome.

Protein-coding genes were identified using Prodigal (8); protein product names were assigned by the DOE-JGI Microbial An-

notation Pipeline (9) based on the hits to the TIGRFam, Pfam, KEGG, COG, and InterPro databases. Noncoding RNAs were identified using the DOE-JGI Microbial Annotation Pipeline (9). Additional gene prediction analysis and manual functional annotation were performed within the Integrated Microbial Genomes (IMG) platform (10). The G+C mole percent is 66.73%, with 4,636 genes (4,536 protein-coding genes) with function prediction for 3,477 of them. A total of 100 RNA genes were detected. A complete set of nitrogen-fixation genes was found, as well as other genes characteristic for the genus and species (details are given in the IMG database) (10).

Whole-genome sequences of 14 *P. stutzeri* strains are publicly available, and 5 of them are considered nitrogen fixers (11–19). Genome analysis confirmed that *P. azotifigens* exhibited overall similarity to the previously sequenced *P. stutzeri* strains of genovars 1, 2, 3, 8, and 19. *P. azotifigens* showed ANIb values (20) of 77.67% to 80.57% with *P. stutzeri* strains, which demonstrate the close genetic relationship between both species. Together with phenotypic properties, the absence of denitrification genes and the high GC content confirmed the previous proposal of Hatayama and collaborators that *P. azotifigens* represents a distinct species (1). Comparative genomics of *P. azotifigens* and *P. stutzeri* will facilitate the understanding of the phylogeny of the *nif* operon in *Pseudomonas* species and related genera.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number **AUDU00000000**. The version described in this paper is the first version, AUDU01000000.

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REFERENCES

1. Hayatama K, Kawai S, Shoun H, Ueda Y, Nakamura A. 2005. *Pseudomonas azotifigens* sp. nov., a novel nitrogen-fixing bacterium isolated from a compost pile. *Int. J. Syst. Evol. Microbiol.* 55:1539–1544.
2. Mulet M, Lalucat J, García-Valdés E. 2010. DNA sequence-based analysis of the *Pseudomonas* species. *Environ. Microbiol.* 12:1513–1530.
3. Lalucat J, Bennasar A, Bosch R, García-Valdés E, Palleroni NJ. 2006. Biology of *Pseudomonas stutzeri*. *Microbiol. Mol. Biol. Rev.* 70:510–547.
4. Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ, Hooper SD, Pati A, Lykidis A, Spring S, Anderson IJ, D’haesleer P, Zemla A, Singer M, Lapidus A, Nolan M, Copeland A, Han C, Chen F, Cheng JF, Lucas S, Kerfeld C, Lang E, Gronow S, Chain P, Bruce D, Rubin EM, Kyripies NC, Klenk HP, Eisen JA. 2009. A phylogeny-driven genomic encyclopaedia of *Bacteria* and *Archaea*. *Nature* 462:1056–1060.
5. Bennett S. 2004. Solexa Ltd. *Pharmacogenomics* 5:433–438.
6. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* 18:821–829.
7. Gnerre S, MacCallum I. 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. *Proc. Natl. Acad. Sci. U. S. A.* 108:1513–1518.
8. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119.
9. Mavromatis K, Ivanova NN, Chen I-MA, Szeto E, Markowitz VM, Kyripies NC. 2009. The DOE-JGI standard operating procedure for the annotations of microbial genomes. *Stand. Genomic Sci.* 1:63–67.
10. Markowitz VM, Chen I-MA, Palaniappan K, Chu K, Szeto E, Grechkin Y, Ratner A, Jacob B, Huang J, Williams P, Huntemann M, Anderson I, Mavromatis K, Ivanova NN, Kyripies NC. 2012. IMG: the integrated microbial genomes database and comparative analysis system. *Nucleic Acids Res.* 40:D115–D122.
11. Busquets A, Peña A, Gomila M, Bosch R, Nogales B, García-Valdés E, Lalucat J, Bennasar A. 2012. Genome sequence of *Pseudomonas stutzeri* strain JM300 (DSM 10701) a soil isolate and model organism for natural transformation. *J. Bacteriol.* 194:5477–5478.
12. Busquets A, Peña A, Gomila M, Mayol J, Bosch R, Nogales B, García-Valdés E, Bennasar A, Lalucat J. 2013. Draft genome sequence of *Pseudomonas stutzeri* B1SMN1, a nitrogen-fixing and naphthalene-degrading strain isolated from wastewater. *Genome Announc.* 1(4):e00584-13. doi: [10.1128/genomeA.00584-13](https://doi.org/10.1128/genomeA.00584-13).
13. Brunet-Galmés I, Busquets A, Peña A, Gomila M, Nogales B, García-Valdés E, Lalucat J, Bennasar A, Bosch R. 2012. Complete genome sequence of the naphthalene-degrading bacterium *Pseudomonas stutzeri* AN10 (CCUG 29243). *J. Bacteriol.* 194:6642–6643.
14. Chen M, Yan Y, Zhang W, Lu W, Wang J, Ping S, Lin M. 2011. Complete genome sequence of the type strain *Pseudomonas stutzeri* CGMCC 1.1803. *J. Bacteriol.* 193:6095. doi: [10.1128/JB.06061-11](https://doi.org/10.1128/JB.06061-11).
15. Grigoryeva TV, Laikov AV, Naumova RP, Manolov AI, Larin AK, Karpova IY, Semashko TA, Alexeev DG, Kostryukova ES, Müller R, Govorun VM. 2013. Draft genome of the nitrogen-fixing bacterium *Pseudomonas stutzeri* strain KOS6 isolated from industrial hydrocarbon sludge. *Genome Announc.* 1(1):e00072-12. doi: [10.1128/genomeA.00072-12](https://doi.org/10.1128/genomeA.00072-12).
16. Peña A, Busquets A, Gomila M, Bosch R, Nogales B, García-Valdés E, Lalucat J, Bennasar A. 2012. Draft genome of *Pseudomonas stutzeri* strain ZoBell (CCUG 16156), a marine isolate and model organism for denitrification studies. *J. Bacteriol.* 194:1277–1278.
17. Peña A, Busquets A, Gomila M, Mayol J, Bosch R, Nogales B, García-Valdés E, Bennasar A, Lalucat J. 2013. Draft genome of *Pseudomonas stutzeri* strain NF13, a nitrogen fixer isolated from the Galapagos rift hydrothermal vent. *Genome Announc.* 1(2):e00113-13. doi: [10.1128/genomeA.00113-13](https://doi.org/10.1128/genomeA.00113-13).
18. Yan Y, Yang J, Dou Y, Chen M, Ping S, Peng J, Lu W, Zhang W, Yao Z, Li H, Liu W, He S, Geng L, Zhang X, Yang F, Yu H, Zhan Y, Li D, Lin Z, Wang Y, Elmerich C, Lin M, Jin Q. 2008. Nitrogen fixation island and rhizosphere competence traits in the genome of root-associated *Pseudomonas stutzeri* A1501. *Proc. Natl. Acad. Sci. U. S. A.* 105:7564–7569.
19. Yu H, Yuan M, Lu W, Yang J, Dai S, Li Q, Yang Z, Dong J, Sun L, Deng Z, Zhang W, Chen M, Ping S, Han Y, Zhan Y, Yan Y, Jin Q, Lin M. 2011. Complete genome sequence of the nitrogen-fixing and rhizosphere-associated bacterium *Pseudomonas stutzeri* strain DSM 4166. *J. Bacteriol.* 193:3422–3423.
20. Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl. Acad. Sci. U. S. A.* 106: 19126–19131.