Complete Genome Sequence of *Pseudomonas stutzeri* Type Strain SGAir0442, Isolated from Singapore Air Samples

Carmon Kee,a Ana Carolina M. Junqueira,a* Akira Uchida,a Rikky W. Purbojati,a James N. I. Houghton,a Caroline Chénard,b Anthony Wong,a Sandra Kolundžija,a Megan E. Clare,a Kawita K. Kushwaha,a Deepa Panicker,a Alexander Putra,a Nicolas E. Gaultier,a Balakrishnan N. V. Premkrishnan,a Cassie E. Heinele,a Vineeth Kodengil Vettath,a Daniela I. Drautz-Moses,a and Stephan C. Schuster*a

a Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore
b Asian School of the Environment, Nanyang Technological University, Singapore

ABSTRACT *Pseudomonas stutzeri* strain SGAir0442 was isolated from tropical air samples collected in Singapore. It is a Gram-negative denitrifying bacterium and an opportunistic human pathogen. Its complete genome consists of one chromosome of 4.52 Mb, containing 4,129 protein-coding genes, 12 rRNA subunits, and 62 tRNAs.

*Pseudomonas stutzeri* is a rod-shaped, single polar flagellated bacterium (1) belonging to the class *Gammaproteobacteria*. *P. stutzeri* has been well documented to have been isolated from a wide range of natural environments, such as soil (2, 3), marine water (4), and sediments (3). It is an active denitrifying Gram-negative bacterium and an opportunistic human pathogen with low virulence that causes diseases such as urinary tract infection (5), meningitis (6, 7), and pneumonia (8, 9).

An air sample was collected in Singapore (Global Positioning System [GPS] coordinates of 1.345N, 103.679E) using an Andersen single-stage Impactor (SKC BioStage). Strain SGAir0442 was isolated from trypticase soy agar (TSA) (Becton Dickinson) incubated at 30°C after impacting airborne microorganisms onto agar. The strain was streaked several times to obtain an axenic culture. The isolated strain was cultured in Luria-Bertani (LB) broth overnight at room temperature prior to DNA extraction. Genomic DNA of the strain was extracted using a Wizard genomic DNA purification kit (Promega) based on the manufacturer’s recommended protocol. A SMRTBell template prep kit 1.0 (Pacific Biosciences) was used to construct a whole-genome shotgun (WGS) library, followed by single-molecule real-time (SMRT) sequencing conducted on a PacBio RS II (Pacific Biosciences) sequencer. Additional WGS libraries were constructed using a TruSeq Nano DNA library preparation kit (Illumina), and 300-bp paired-end short reads were generated on an Illumina MiSeq sequencer.

The PacBio RS II platform provided 52,081 long reads (122.49-fold coverage), while the total number of short reads obtained with the Illumina MiSeq sequencer was 829,385 (110.35-fold coverage). The Hierarchical Genome Assembly Process version 3 (HGAP3) (10), implemented in the PacBio SMRT Analysis algorithm version 2.3.0, was used for de novo genome assembly of long reads. The complete processing of the assembly includes preassembly, de novo assembly with HGAP3, polishing with Quiver (10), and error correction using Pilou version 1.16 (11) with Illumina reads to improve accuracy. The consensus assembly generated one contig with a chromosome size of 4,524,660 bp with an average G+C content of 64.03%.

The complete genome presented 98.3% identity with the *Pseudomonas* genus, as predicted by the Phylum-specific Automated Phylogenetic Inference Pipeline (Phyla_AMPHORA) (12). Further taxonomical identification was conducted using the average nucleotide identity (ANI) method with Microbial Species Identifier (MiSI) (13), which

Received 15 April 2018 Accepted 17 April 2018 Published 31 May 2018

Citation Kee C, Junqueira ACM, Uchida A, Purbojati RW, Houghton JNI, Chénard C, Wong A, Kolundžija S, Clare ME, Kushwaha KK, Panicker A, Gaultier NE, Premkrishnan BNV, Heinele CE, Vettath VK, Drautz-Moses DI, Schuster SC. 2018. Complete genome sequence of *Pseudomonas stutzeri* type strain SGAir0442, isolated from Singapore air samples. Genome Announc 6:e00424-18. https://doi.org/10.1128/genomeA.00424-18.

Copyright © 2018 Kee et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Stephan C. Schuster, scschuster@ntu.edu.sg.

* Present address: Ana Carolina M. Junqueira, Departamento De Genética, Instituto De Biologia, Universidade Federal Do Rio De Janeiro, Rio De Janeiro, Brazil.

C.K. and A.C.M.J. contributed equally to this work.
revealed a 98.2% similarity with the available reference genome *Pseudomonas stutzeri* DSM4166 (GenBank assembly accession number GCA_000195105).

Annotation of the genome was performed with NCBI’s Prokaryotic Genome Annotation Pipeline (PGAP) version 4.2 (14). The complete genome was predicted to contain 4,309 genes, of which there were 4,129 protein-coding genes (PCGs), 12 rRNA subunits (5S, 16S, and 23S), 62 tRNAs, and 4 noncoding RNAs. The genome also contains 102 predicted pseudogenes. Functional annotation based on Rapid Annotations using Subsystems Technology (RAST) (15–17) classified 117 genes associated with virulence, disease, and defense. The same number of genes was found to have a role in motility and chemotaxis, and 107 genes were found to be involved in nitrogen metabolism, reflecting the nitrogen-fixing characteristic of this bacterium.

**Accession number(s).** The complete genome sequence of *Pseudomonas stutzeri* strain SGAir0442 has been deposited in DDBJ/EMBL/GenBank under the accession CP025149.

**ACKNOWLEDGMENT**

This work was supported by a Singapore Ministry of Education Academic Research Fund tier 3 grant (MOE2013-T3-1-013).

**REFERENCES**

1. Lalucat J, Bennasar A, Bosch R, Garcia-Valdes E, Palleroni NJ. 2006. Biology of *Pseudomonas stutzeri*. Microbiol Mol Biol Rev 70:510–547. https://doi.org/10.1128/MMBR.00047-05.
2. Bennasar A, Guasp C, Lalucat J. 1998. Molecular methods for the detection and identification of *Pseudomonas stutzeri* in pure culture and environmental samples. Microb Ecol 35:22–23.
3. Sikorski J, Mohle M, Wackernagel W. 2002. Identification of complex composition, strong strain diversity and directional selection in local *Pseudomonas stutzeri* populations from marine sediments and soils. Environ Microbiol 4:465–476. https://doi.org/10.1046/j.1462-2920.2002.00325.x.
4. Romanenko LA, Uchino M, Falsen E, Lysenko AM, Zhukova NV, Mikhailov VV. 2005. *Pseudomonas xanthomarina* sp. nov., a novel bacterium isolated from marine ascidian. J Gen Appl Microbiol 51:65–71. https://doi.org/10.2323/jgam.51.65.
5. Tan RJ, Lim EW, Sakazaki R. 1977. Unusual cause of urinary tract infection by *Pseudomonas stutzeri* in Singapore. Jpn J Exp Med 47:311–313.
6. Roig P, Ortiz A, Navarro V. 1996. Meningitis due to *Pseudomonas stutzeri* in a patient infected with human immunodeficiency virus. Clin Infect Dis 22:587–588. https://doi.org/10.1093/clinids/22.3.587.
7. Tasdelen FN, Acuner IC, Coban AY, Fisgin T, Birinci A, Durupinar B. 2004. Meningitis due to *Pseudomonas stutzeri*: a case report. Mikrobiyol Bul 38:261–264.
8. Cannata J, Salazar A, Mascaro J, Santin M. 1992. Community-acquired pneumonia due to *Pseudomonas stutzeri*. Clin Infect Dis 14:792. https://doi.org/10.1093/clinids/14.3.792.
9. Ostergaard K, Andersen PL. 1993. Etiology of community-acquired pneumonia: evaluation by transtracheal aspiration, blood, culture and serology. Chest 104:1400–1407.
10. Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korfach J. 2013. nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/nmeth.2474.
11. Walker BJ, Abeel T, Shea T, Priest M, Abouelli A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963.
12. Wang Z, Wu M. 2013. A phyllum-level bacterial phylogenetic marker database. Mol Biol Evol 30:1258–1262. https://doi.org/10.1093/molbev/mst059.
13. Varghese NJ, Mukherjee S, Ivanova N, Konstantinidis KT, Mavrommatis K, Kyrpides NC, Patti A. 2015. Microbial species delineation using whole genome sequences. Nucleic Acids Res 43:6761–6771. https://doi.org/10.1093/nar/gkv657.
14. Tatusova T, Dicuccio M, Badretdin A, Chetverin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Fisgin T, Birinci A, Durupinar B. 2004. Meningitis due to *Pseudomonas stutzeri*: a case report. Mikrobiyol Bul 38:261–264.
15. Aziz RK, Bartels D, Best AA, Delongh M, Disz T, Edwards RA, Formisno K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Pazcian T, Arrello B, Posch GD, Reich C, Stevens R, Sussieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
16. Overbeek RA, McNeil LK, Paarmann D, Pazcian T, Arrello B, Posch GD, Reich C, Stevens R, Sussieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
17. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Posch GD, 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/gkt226.
18. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Posch GD, Shukla M, Thomason JA, 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.