



## Draft Genome Sequence of *Pseudomonas saudiphocaensis* Strain AGROB56, Isolated from a Dairy Farm in New Zealand

Tanushree B. Gupta,<sup>a</sup> Alexis N. Risson,<sup>a</sup> Ruy Jauregui,<sup>b</sup> Paul Maclean,<sup>b</sup> Gale Brightwell<sup>a</sup>

<sup>a</sup>Food Assurance Team, Hopkirk Research Institute, AgResearch, Palmerston North, New Zealand <sup>b</sup>Knowledge and Analytics, Grasslands Research Centre, AgResearch, Palmerston North, New Zealand

**ABSTRACT** Here, we report the draft genome sequence of a new *Pseudomonas* saudiphocaensis strain, AGROB56, with lipolytic potential, isolated from a sheep dairy farm in New Zealand. The genome is 3.61 Mbp, with a GC content of 61.1%, and the genome sequence was found closely related to *Pseudomonas* saudiphocaensis 20 BN<sup>T</sup>.

P seudomonas species are ubiquitously found in environments, from soil to humans and plants (1–5). This genus was first proposed in 1894 and so far consists of around 254 species (6). They are Gram-negative bacteria, usually aerobic, but can be facultative anaerobes (7–9). While some *Pseudomonas* species can be associated with pathogenicity, some species have been applied as bioremediation agents (5, 10–14), and some are known for their dairy spoilage potential, such as *Pseudomonas fragi*, *P. lundensis*, and *P. fluorescens* (15, 16).

A new species of *Pseudomonas*, *P. saudiphocaensis* (type strain 20 BN), was isolated in 2012 from air samples in the city environment of Makkah, Saudi Arabia. As yet, limited information is available on this strain, including any beneficial or pathogenic traits (17).

Here, we report the whole-genome sequence of a new *Pseudomonas saudipho-caensis* strain, AGROB56, that was isolated from woodchip bedding samples from a sheep dairy farm in North Island of New Zealand. The new strain showed lipolytic activity, indicating it to be a food spoilage bacterium. The sequences obtained will be used to compare genomic data of the new strain to those of the type strain available.

Samples were processed using the methodology, with slight modifications, described previously by Gupta and Brightwell (18). Briefly, woodchip bedding material (25 g) was weighed in a stomacher bag, suspended in 100 ml of phosphate buffer (PB), and homogenized well using a stomacher. The suspension was centrifuged at  $3,466 \times g$  for 1 h, and the pellet was resuspended in 25 ml of PB. One milliliter of the suspension was serially diluted, plated onto cetrimide-fucidin-cephalosporin (CFC) agar plates, and incubated at 25°C (Fort Richard, New Zealand) to isolate *Pseudomonas* strain AGROB56 (19). Lipolytic activity was preliminarily investigated by visualizing a fluorescent halo, under UV light, around the colony grown for 48 h at 25°C on rhodamine B plates (20). The new strain was found to be lipolytic, indicating a potential food/dairy spoilage bacterium. Genomic DNA from the pure culture grown in tryptic soy broth at 25°C was extracted using the phenol-chloroform extraction method (21). Quality and concentration of DNA were determined using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, USA).

The whole genome of *Pseudomonas* species strain AGROB56 was prepared via the NuGEN Celero DNA enzymatic library and sequenced using the Illumina MiSeq sequencing platform version 3 (Massey Genome Services, Palmerston North, New Zealand) to produce 628,743 paired-end reads of 301 base pairs, giving a coverage Maclean P, Brightwell G. 2021. Draft genome sequence of *Pseudomonas saudiphocaensis* strain AGROB56, isolated from a dairy farm in New Zealand. Microbiol Resour Announc 10:e01258-20. https://doi.org/10.1128/MRA .01258-20. **Editor** Catherine Putonti, Loyola University

Citation Gupta TB, Risson AN, Jauregui R,

Editor Catherine Putonti, Loyola University Chicago

**Copyright** © 2021 Gupta et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Tanushree B. Gupta, Tanushree.gupta@agresearch.co.nz.

Received 2 November 2020 Accepted 7 December 2020 Published 7 January 2021 of 104-fold. The reads were quality trimmed, filtered, and assembled via A5-miseq pipeline version 20160825 with default settings (22). The assembly produced 20 contigs with a total genome size of 3.6 Mb, an  $N_{50}$  value of 481 kb, and a GC content of 61.1%. A BUSCO version 3.0.2 (23) test using the bacterial reference produced a completeness score of 99.3%.

Comparative genomic analysis was performed with the genome sequences of the new strain and *Pseudomonas saudiphocaensis* 20 BN<sup>T</sup> using *in silico* DNA-DNA hybridization (dDDH) via the type (strain) genome server (TYGS; https://tygs.dsmz.de/) (24). A dDDH (d<sub>6</sub>) value of 92.8% was obtained, indicating the same species but with probable differences at strain level. Further studies are required to investigate these differences. A two-way average nucleotide identity test (http://enve-omics.ce.gatech.edu/ani/) was carried out as well, producing a 98% value matching with *Pseudomonas saudiphocaensis* 20 BN<sup>T</sup> (25).

As part of the submission process, NCBI annotated the genomic scaffolds with PGAP version 4.13 (26), resulting in 3,396 genes being annotated in total.

**Data availability.** This whole-genome shotgun project has been deposited at DDBJ/ ENA/GenBank under the accession number JADDOO000000000. The version described in this paper is the first version, JADDOO010000000. The raw sequencing data have been deposited at the SRA under BioProject accession number PRJNA670249.

## ACKNOWLEDGMENTS

This work was funded by Strategic Science Investment Fund (SSIF), AgResearch Ltd., New Zealand.

We acknowledge the help of sheep dairy farmers in sample collection.

## REFERENCES

- 1. Sørensen J, Nybroe O. 2004. Pseudomonas in the soil environment, vol 1. Springer, Boston, MA.
- Hardalo C, Edberg SC. 1997. Pseudomonas aeruginosa: assessment of risk from drinking water. Crit Rev Microbiol 23:47–75. https://doi.org/10.3109/ 10408419709115130.
- Preston GM. 2004. Plant perceptions of plant growth-promoting *Pseudo-monas*. Philos Trans R Soc Lond B Biol Sci 359:907–918. https://doi.org/10 .1098/rstb.2003.1384.
- Vogt R, LaRue D, Parry M, Brokopp C, Klaucke D, Allen J. 1982. Pseudomonas aeruginosa skin infections in persons using a whirlpool in Vermont. J Clin Microbiol 15:571–574. https://doi.org/10.1128/JCM.15.4 .571-574.1982.
- Seidler D, Griffin M, Nymon A, Koeppen K, Ashare A. 2016. Throat swabs and sputum culture as predictors of P aeruginosa or S. aureus lung colonization in adult cystic fibrosis patients. PLoS One 11:e0164232. https://doi .org/10.1371/journal.pone.0164232.
- Migula W. 1895. Über ein neues System der Bakterien. Arb Bakteriol Inst Karlsruhe 1: 235–238.
- Ballard R, Palleroni N, Doudoroff M, Stanier R, Mandel M. 1970. Taxonomy of the aerobic pseudomonads: *Pseudomonas cepacia*, *P. marginata*, *P. alliicola* and *P. caryophylli*. J Gen Microbiol 60:199–214. https://doi.org/10 .1099/00221287-60-2-199.
- Arai H. 2011. Regulation and function of versatile aerobic and anaerobic respiratory metabolism in *Pseudomonas aeruginosa*. Front Microbiol 2:103. https://doi.org/10.3389/fmicb.2011.00103.
- Peng J-S, Liu Y, Yan L, Hou T-T, Liu H-C, Zhou Y-G, Liu Z-P. 2019. Pseudomonas nitrititolerans sp. nov., a nitrite-tolerant denitrifying bacterium isolated from a nitrification/denitrification bioreactor. Int J Syst Evol Microbiol 69:2471–2476. https://doi.org/10.1099/ijsem.0.003516.
- Streeter K, Katouli M. 2016. *Pseudomonas aeruginosa*: a review of their pathogenesis and prevalence in clinical settings and the environment. Infect Epidemiol Med 2:25–32.
- Arnold DL, Preston GM. 2019. Pseudomonas syringae: enterprising epiphyte and stealthy parasite. Microbiology (Reading) 165:251–253. https:// doi.org/10.1099/mic.0.000715.
- O'Mahony MM, Dobson ADW, Barnes JD, Singleton I. 2006. The use of ozone in the remediation of polycyclic aromatic hydrocarbon contaminated soil. Chemosphere 63:307–314. https://doi.org/10.1016/j.chemosphere.2005.07 .018.

- Samuel MS, Sivaramakrishna A, Mehta A. 2014. Bioremediation of p-nitrophenol by *Pseudomonas putida* 1274 strain. J Environ Health Sci Eng 12:53. https://doi.org/10.1186/2052-336X-12-53.
- Shimada K, Itoh Y, Washio K, Morikawa M. 2012. Efficacy of forming biofilms by naphthalene degrading *Pseudomonas stutzeri* T102 toward bioremediation technology and its molecular mechanisms. Chemosphere 87:226–233. https://doi.org/10.1016/j.chemosphere.2011.12 .078.
- Mallet A, Guéguen M, Kauffmann F, Chesneau C, Sesboué A, Desmasures N. 2012. Quantitative and qualitative microbial analysis of raw milk reveals substantial diversity influenced by herd management practices. Int Dairy J 27:13–21. https://doi.org/10.1016/j.idairyj.2012.07 .009.
- Ribeiro Júnior JC, Teider Junior PI, Oliveira ALM, Rios EA, Tamanini R, Beloti V. 2018. Proteolytic and lipolytic potential of *Pseudomonas* spp. from goat and bovine raw milk. Pesq Vet Bras 38:1577–1583. https://doi .org/10.1590/1678-5150-pvb-5645.
- Azhar El, Papadioti A, Bibi F, Ashshi AM, Raoult D, Angelakis E. 2017. "Pseudomonas saudimassiliensis" sp. nov. a new bacterial species isolated from air samples in the urban environment of Makkah, Saudi Arabia. New Microbes New Infect 16:43–44. https://doi.org/10.1016/j.nmni .2016.12.021.
- Gupta TB, Brightwell G. 2017. Farm level survey of spore-forming bacteria on four dairy farms in the Waikato region of New Zealand. Microbiologyopen 6:e00457. https://doi.org/10.1002/mbo3.457.
- Mead GC. 1985. Enumeration of pseudomonads using cephaloridine-fucidin-cetrimide agar (CFC). Int J Food Microbiol 2:21–26. https://doi.org/10 .1016/0168-1605(85)90052-2.
- Kouker G, Jaeger K-E. 1987. Specific and sensitive plate assay for bacterial lipases. Appl Environ Microbiol 53:211–213. https://doi.org/10.1128/AEM .53.1.211-213.1987.
- Gupta SK, Haigh BJ, Seyfert H-M, Griffin FJ, Wheeler TT. 2017. Bovine milk RNases modulate pro-inflammatory responses induced by nucleic acids in cultured immune and epithelial cells. Dev Comp Immunol 68:87–97. https://doi.org/10.1016/j.dci.2016.11.015.
- Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. https://doi.org/10.1093/bioinformatics/btu661.
- 23. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM.

2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210–3212. https://doi.org/10.1093/bioinformatics/btv351.

- 24. Meier-Kolthoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:1–10. https://doi.org/10.1038/s41467-019-10210-3.
- 25. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P,

Tiedje JM. 2007. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. https://doi.org/10.1099/ijs.0.64483-0.

 Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/ 10.1093/nar/gkw569.