



Complete Genome Sequences of *Pseudomonas lundensis* Strains M101 and M105, Isolated from 1% Pasteurized Milk

Keerthikka Ravi,^a John R. Erb-Downward,^b Natalie K. Gammon,^b Nicole R. Falkowski,^b  Gary B. Huffnagle^{a,b,c,d}

^aDepartment of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, Michigan, USA

^bDivision of Pulmonary and Critical Care Medicine, University of Michigan Medical School, Ann Arbor, Michigan, USA

^cDepartment of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan, USA

^dMary H. Weiser Food Allergy Center, University of Michigan, Ann Arbor, Michigan, USA

ABSTRACT Here, we report the complete genome sequences of two strains of *Pseudomonas lundensis*, M101 and M105, which were isolated from 1% pasteurized milk. Long-read sequencing was performed using a MinION sequencer, and reads were assembled into circular chromosomes of 4,842,187 bp and 4,814,486 bp for M101 and M105, respectively. Both strains had additional plasmid sequences.

Pseudomonas lundensis is a Gram-negative, ubiquitous psychrotrophic bacterium that belongs to the *Pseudomonas fluorescens* complex of species (1, 2). It can thrive in cold environments, such as Antarctic melt ponds, and it is also a well-known cause of cold food spoilage, including milk and dairy products (3). Interestingly, the bacterium has also been isolated from sputum samples from patients with cystic fibrosis (2). To date, very little is known about any source-specific genomic features of *P. lundensis*. Here, two strains of *P. lundensis* (M101 and M105) were isolated from 1% pasteurized milk (purchased at a U.S. supermarket) by selective outgrowth for 3 weeks at 4°C. A 16S rRNA gene-specific colony PCR assay, a *P. lundensis*-specific (*P. lundensis* ExoU) PCR assay, and average nucleotide identity analysis with BLAST were used to confirm the identities of the strains as *P. lundensis* (4). Here, we present the complete genome sequences of these two milk isolates of *P. lundensis*.

Frozen (−80°C) glycerol stocks of the isolated strains were grown in LB medium at room temperature, and DNA isolation for whole-genome sequencing was performed using the DNeasy blood and tissue kit (Qiagen) without RNase treatment.

Sequencing libraries were prepared using the rapid barcoding kit (SQK-RBK001) and sequenced on an R9.4.1 flow cell for 4 h using a MinION sequencer (Oxford Nanopore Technologies). Base calling for the raw reads was performed using Guppy v4.2.3 in high-accuracy mode. A total of 79,480 and 58,783 reads, with N_{50} values of 5,855 and 6,460 bp, respectively, were obtained after base calling for M101 and M105, respectively. The genome sequences were assembled *de novo* using Flye v2.8.1 (5) with default parameters. The assembly was further polished through two rounds with Racon v1.4.20 (6) using default parameters for all except scores for matching and mismatching bases (-match 8 -mismatch -6). A final round of polishing was performed using Medaka v1.2.3 (7), once again using default parameters.

M101 (genome coverage, 53×) and M105 (genome coverage, 44×) were assembled into three and two circular contigs, respectively (Table 1). The assembled contigs were confirmed as nonviral contigs using ViralVerify (8). plasmidVerify (9) identified the 4.8-Mb contigs in both M101 and M105 as chromosomes and the two 24-kb contigs, as well as the 3-kb contig, as plasmid sequences. Sequence annotation for both strains was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (10).

Citation Ravi K, Erb-Downward JR, Gammon NK, Falkowski NR, Huffnagle GB. 2021. Complete genome sequences of *Pseudomonas lundensis* strains M101 and M105, isolated from 1% pasteurized milk. Microbiol Resour Announc 10:e00711-21. <https://doi.org/10.1128/MRA.00711-21>.

Editor David A. Baltrus, University of Arizona

Copyright © 2021 Ravi 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 Gary B. Huffnagle, ghuff@umich.edu.

Received 20 July 2021

Accepted 23 September 2021

Published 21 October 2021

TABLE 1 Genome sequence data for *Pseudomonas lundensis* isolates M101 and M105

Assembly accession no.	Strain	SRA accession no.	GenBank accession no.	Contig	Genome size	GC content (%)
GCA_018449005.1	M101	SRR15403967	CP075177.1	Chromosome	4.8 Mb	58.69
			CP075178.1	pPL-3mi	3 kb	60.4
			CP075179.1	pPL-24mi	24 kb	55.4
GCA_018449025.1	M105	SRR15403966	CP075180.1	Chromosome	4.8 Mb	58.69
			CP075181.1	pPL-24mi	24 kb	55.4

Data availability. The raw sequence data and assembled sequences are deposited in NCBI under the BioProject accession number [PRJNA729724](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA729724). The accession numbers for the deposited sequences are listed in Table 1.

ACKNOWLEDGMENTS

This work was supported in part by funding provided by NIH grants NHLBI R01HL121774 (G.B.H.) and NIAID R01AI138348 (G.B.H.), the Mary H. Weiser Food Allergy Center (MHWFAC) (G.B.H.), the Nina and Jerry D. Luptak Endowment of the MHWFAC (G.B.H.), and the Molecular, Cellular, and Developmental Biology Graduate Program of the University of Michigan (K.R.).

REFERENCES

- Molin G, Ternstrom A, Ursing J. 1986. *Pseudomonas lundensis*, a new bacterial species isolated from meat. *Int J Syst Bacteriol* 36:339–342. <https://doi.org/10.1099/00207713-36-2-339>.
- Scales BS, Erb-Downward JR, Falkowski NR, LiPuma JJ, Huffnagle GB. 2018. Genome sequences of 12 *Pseudomonas lundensis* strains isolated from the lungs of humans. *Genome Announc* 6:e01461-17. <https://doi.org/10.1128/genomeA.01461-17>.
- Marchand S, Heylen K, Messens W, Coudijzer K, De Vos P, Dewettinck K, Herman L, De Block J, Heyndrickx M. 2009. Seasonal influence on heat-resistant proteolytic capacity of *Pseudomonas lundensis* and *Pseudomonas fragi*, predominant milk spoilers isolated from Belgian raw milk samples. *Environ Microbiol* 11:467–482. <https://doi.org/10.1111/j.1462-2920.2008.01785.x>.
- Thompson N. 2018. Identification of novel strains of *Pseudomonas lundensis* from food samples, p 47. University of Michigan, Ann Arbor, MI.
- Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. *Nat Biotechnol* 37:540–546. <https://doi.org/10.1038/s41587-019-0072-8>.
- Vaser R, Sović I, Nagarajan N, Šikić M. 2017. Fast and accurate de novo genome assembly from long uncorrected reads. *Genome Res* 27:737–746. <https://doi.org/10.1101/gr.214270.116>.
- Oxford Nanopore Technologies. 2018. Medaka: sequence correction provided by ONT Research. <https://github.com/nanoporetech/medaka>.
- Antipov D, Raiko M, Lapidus A, Pevzner PA. 2020. Metaviral SPAdes: assembly of viruses from metagenomic data. *Bioinformatics* 36:4126–4129. <https://doi.org/10.1093/bioinformatics/btaa490>.
- Antipov D, Raiko M, Lapidus A, Pevzner PA. 2019. Plasmid detection and assembly in genomic and metagenomic data sets. *Genome Res* 29:961–968. <https://doi.org/10.1101/gr.241299.118>.
- 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>.