



# Draft Genome Sequence of *Pseudomonas aeruginosa* Strain LMG 1272, an Atypical White Line Reaction Producer

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**ABSTRACT** The draft genome sequence of *Pseudomonas aeruginosa* LMG 1272, isolated from mushroom, is reported here. This strain triggers formation of a precipitate (“white line”) when cocultured with *Pseudomonas tolaasii*. However, LMG 1272 lacks the capacity to produce a cyclic lipopeptide that is typically associated with white line formation, suggesting the involvement of a different diffusible factor.

When some *Pseudomonas* strains are cocultured on solid medium, a visible precipitate (“white line”) is formed between two physically separated colonies (1). This phenomenon, designated the white line reaction (WLR), presumably results from the coprecipitation of cyclic lipopeptides (CLPs) secreted by both interacting *Pseudomonas* strains, as the WLR is abolished if CLP production in one of the strains is inactivated (2). Originally, the WLR was considered specific to the interaction of the tolaasin-producing mushroom pathogen *Pseudomonas tolaasii* with “*Pseudomonas re-actans*,” a group representing different species producing white line-inducing principle (WLIP) (3, 4). However, the WLR was also observed between producers of the tolaasin analogue sessilin and producers of orfamide (5). A WLR with *P. tolaasii* was even reported for the mushroom isolate NCPPB 2195, which belongs to *Pseudomonas aeruginosa*, an opportunistic human pathogen not known to produce CLPs (6). Here, we report the draft genome sequence of *P. aeruginosa* strain LMG 1272 (NCPPB 2195). Default parameters were used for all software without exception.

Strain LMG 1272, obtained from the BCCM/LMG culture collection (Belgium), was cultured in one subculture in Trypticase soy broth (TSB) or agar (TSA) medium (BD Biosciences) at 37°C. Genomic DNA was obtained from the pure broth culture using the Genra Puregene Yeast/Bact. kit (Qiagen Benelux B.V., Venlo, The Netherlands). Genomic library preparation and sequencing were outsourced to Macrogen (Seoul, South Korea). Shotgun library preparation was performed using a TruSeq Nano DNA kit with a target insert size of 350 bp (Illumina, San Diego, CA). Paired-end sequencing (2 × 101-bp paired-end reads) was performed by an Illumina HiSeq 2000 system. In total, 2,324,619 paired-end reads were obtained. Based on FastQC version 0.11.5, all plots and reports passed the required threshold, indicating approved quality of sequencing. *De novo* assembly was performed using MaSuRCA version 2.3.2 with default settings for bacteria. Reads were mapped onto the contigs by the EIM pipeline (7) in order to generate a modified contig set. A total of 81 contigs with an  $N_{50}$  value of 314,105 bp (about 68-fold coverage) were generated. The final assembled length comprises 6,449,416 bp with a G+C content of 66.3%, and the longest contig size is 890,303 bp. QUASt version 5.0.2 (8) was used to compare contigs produced by the EIM pipeline and by MaSuRCA. The generated genomes were aligned by QUASt against the

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genome sequence of *P. aeruginosa* strain 19BR. The results show that EIM reduced the average numbers of mismatches and indels per 100 kb from 500.08 to 488.81 and from 12.52 to 12.14, respectively. In addition, the number of IUPAC codes was decreased from 564 to 8. Annotation of the assembled contigs using the NCBI Prokaryotic Genome Annotation Pipeline (9) identified 6,039 coding DNA sequences and 84 tRNA genes.

*P. aeruginosa* LMG 1272 carries homologues of pyocin genes S4 and S11 and also a homologue of F-type tailocin gene cluster (10). Genome analysis using antiSMASH 5.0 (11) to identify biosynthetic gene clusters of secondary metabolites revealed the capacity to produce pyoluteorin (12), pyochelin (13), 2-amino-4-methoxy-*trans*-3-butenic acid (14), and azabicyclene (15). The lack of characteristic CLP biosynthetic genes (16) indicates that a different diffusible factor is involved in the WLR phenotype.

**Data availability.** This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under accession number [VIYL00000000](https://doi.org/10.1101/2020.03.10.388888). The version described in this paper is version VIYL01000000. The raw sequencing data are available from the Sequence Read Archive (SRA) under accession number [PRJNA552675](https://doi.org/10.1101/2020.03.10.388888).

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## REFERENCES

- Wong WC, Preece TF. 1979. Identification of *Pseudomonas tolaasii*: the white line in agar and mushroom tissue block rapid pitting tests. *J Appl Bacteriol* 47:401–407. <https://doi.org/10.1111/j.1365-2672.1979.tb01200.x>.
- Osdaghi E, Martins SJ, Ramos-Sepulveda L, Vieira FR, Pecchia JA, Beyer DM, Bell TH, Yang Y, Hockett KL, Bull CT. 2019. 100 years since Tolaas: bacterial blotch of mushrooms in the 21st century. *Plant Dis* 103:2714–2732. <https://doi.org/10.1094/PDIS-03-19-0589-FE>.
- Rokni-Zadeh H, Li W, Sanchez-Rodriguez A, Sinnaeve D, Rozenski J, Martins JC, De Mot R. 2012. Genetic and functional characterization of cyclic lipopeptide white-line-inducing principle (WLIP) production by rice rhizosphere isolate *Pseudomonas putida* RW1052. *Appl Environ Microbiol* 78:4826–4834. <https://doi.org/10.1128/AEM.00335-12>.
- Rokni-Zadeh H, Li W, Yilma E, Sanchez-Rodriguez A, de Mot R. 2013. Distinct lipopeptide production systems for WLIP (white line-inducing principle) in *Pseudomonas fluorescens* and *Pseudomonas putida*. *Environ Microbiol Rep* 5:160–169. <https://doi.org/10.1111/1758-2229.12015>.
- D'aes J, Kieu NP, Léclère V, Tokarski C, Olorunleke FE, De Maeyer K, Jacques P, Höfte M, Ongena M. 2014. To settle or to move? The interplay between two classes of cyclic lipopeptides in the biocontrol strain *Pseudomonas* CMR12a. *Environ Microbiol* 16:2282–2300. <https://doi.org/10.1111/1462-2920.12462>.
- Munsch P, Alatossava T. 2002. The white-line-in-agar test is not specific for the two cultivated mushroom associated pseudomonads, *Pseudomonas tolaasii* and *Pseudomonas "reactans"*. *Microbiol Res* 157:7–11. <https://doi.org/10.1078/0944-5013-00125>.
- Salari F, Zare-Mirakabad F, Sadeghi M, Rokni-Zadeh H. 2018. Assessing the impact of exact reads on reducing the error rate of read mapping. *BMC Bioinformatics* 19:406. <https://doi.org/10.1186/s12859-018-2432-7>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- 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>.
- Ghequire MGK, De Mot R. 2014. Ribosomally encoded antibacterial proteins and peptides from *Pseudomonas*. *FEMS Microbiol Rev* 38:523–568. <https://doi.org/10.1111/1574-6976.12079>.
- Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res* 47:W81–W87. <https://doi.org/10.1093/nar/gkz310>.
- Gross H, Loper JE. 2009. Genomics of secondary metabolite production by *Pseudomonas* spp. *Nat Prod Rep* 26:1408–1446. <https://doi.org/10.1039/b817075b>.
- Ronnebaum TA, Lamb AL. 2018. Nonribosomal peptides for iron acquisition: pyochelin biosynthesis as a case study. *Curr Opin Struct Biol* 53:1–11. <https://doi.org/10.1016/j.sbi.2018.01.015>.
- Rojas Murcia N, Lee X, Waridel P, Maspoli A, Imker HJ, Chai T, Walsh CT, Reimann C. 2015. The *Pseudomonas aeruginosa* antimetabolite L-2-amino-4-methoxy-*trans*-3-butenic acid (AMB) is made from glutamate and two alanine residues via a thiotemplate-linked tripeptide precursor. *Front Microbiol* 6:170. <https://doi.org/10.3389/fmicb.2015.00170>.
- Patteson JB, Lescallete AR, Li B. 2019. Discovery and biosynthesis of azabicyclene, a conserved nonribosomal peptide in *Pseudomonas aeruginosa*. *Org Lett* 21:4955–4959. <https://doi.org/10.1021/acs.orglett.9b01383>.
- Götze S, Stallforth P. 2020. Structure, properties, and biological functions of nonribosomal lipopeptides from pseudomonads. *Nat Prod Rep*. <https://doi.org/10.1039/C9NP00022D>.