Complete Genome Sequence of *Pseudomonas putida* Strain TS312, Harboring an HdtS-Type *N*-Acyl-Homoserine Lactone Synthase, Isolated from a Paper Mill

Ayaka Hosoe,a Toshikazu Suenaga,b Takumi Sugi,c Taro Iizumi,d Naohiro Nagai,d Akihiko Teradaa,b

---

**ABSTRACT** We report the complete genome sequence of *Pseudomonas putida* strain TS312, in the class of *Gammaproteobacteria*. The strain, isolated from a paper mill, harbors the *hdts* gene, encoding *N*-acyl-homoserine lactone synthase. Deciphering the genome contributes to revealing the mechanisms of quorum sensing and associated biofilm formation in engineered systems.

Cooling water, paper mills, and water reclamation systems are sites where microbial biofilms grow. The formation of a biofilm in these engineered systems affects thermal efficiency (1), product quality (2), and water filtration (3), requiring the mitigation of biofilm formation. In engineered systems in which biofilms are detrimental, antibacterial reagents have been broadly applied. However, care should be taken because the discharged residual reagents may have harmful environmental effects (4). Quorum sensing (QS) is a bacterial cell-cell communication mechanism in which bacterial cells sense others via a signal molecule whose external concentration depends on the cell population density. Exceeding a threshold concentration of a signal compound triggers the expression of specific genes associated with virulence, fluorescence, biofilm formation, or other processes (5). *N*-Acyl-homoserine lactone (AHL)-mediated QS is often used by diverse bacteria belonging to *Gammaproteobacteria*, e.g., *Aeromonas hydrophila* and *Pseudomonas aeruginosa* (6).

*Pseudomonas putida* strain TS312, reported in this study, was isolated from process water (white water) in a Japanese paper mill. Reportedly, *P. putida* produces AHLs to regulate biofilm formation (7–9). However, the in-depth metabolic potential and AHL-dependent QS mechanisms of *P. putida* strain TS312 have yet to be deciphered. Understanding the physiological traits of *P. putida* strain TS312 may provide clues to suppressing biofouling in engineered systems where biofilms are unwanted.

White water was taken from the paper mill, serially diluted, and spread onto an agar medium consisting of 1 g/liter of polypeptide, 1 g/liter of yeast extract, 0.5 g/liter of NaCl, and 15 g/liter of agar. After incubation at 30°C for 7 days, colonies were picked up, followed by identification based on the 16S rRNA gene. A single colony of the isolate, *P. putida* strain TS312, was suspended, grown aerobically in R2A broth (DAIGO; Fujifilm Holdings Corp., Tokyo, Japan), and subsequently harvested as a cell pellet by centrifugation. Genomic DNA was extracted by a phenol-chloroform extraction method (10) and subsequently purified with cetyltrimethylammonium bromide. RNA contaminating the genomic DNA was degraded with RNase A (TaKaRa Bio, Inc., Shiga, Japan). The DNA library was prepared as reported previously (11, 12). In brief, the library was prepared using a 1D ligation sequencing kit (SQK-LSK-109; Oxford Nanopore Technologies Ltd., Oxford, UK), without a fragmentation procedure, and sequenced on the MinION Mk1B.
system using an R.10 flow cell (FLO-MIN110; Oxford Nanopore Technologies). The sequence data were base called by Guppy (version 3.3.2) using the high-accuracy mode. The sequences produced included 397,900 reads, consisting of 5.73 Gbp in total, with an N_{50} value of 26.21 kbp. The sequence quality was confirmed by NanoPlot (13), and the adaptor sequences, low-quality reads (score of <Q10), and short reads (<1,000 bp) were removed using Porechop as a tool for trimming adapters. The quality control-passed sequences totaled 806 Mbp and were used for further analysis. The acquired sequences were assembled using Canu (version 1.8) (14), and only 1 suggested circular contig was generated, with 141-fold coverage of the genome. The consensus sequence was polished by medaka (version 0.10.1), and the completeness, as assessed by BUSCO (version 1) (15), was 100%. The parameters used for the bioinformatics software were the default values unless otherwise noted. The complete genome sequence was annotated using the DDBJ Fast Annotation and Submission Tool (16). The genome consists of 5,681,150 bp in 1 contig, with a G+C content of 66.2%, containing 5,110 protein-coding DNA sequences, 22 rRNAs, and 75 tRNAs.

Based on a BLAST search, *P. putida* strain TS312 harbors typical functional genes encoding AHL acylase, i.e., PvdQ and QuiP (17, 18). Strain TS312 also contains a third type of AHL synthase, HtdS, a member of the lysophosphatic acid acyl transferase family possessed by *Pseudomonas fluorescens* (19). The HtdS family performs both acylation of lysophosphatic acid and synthesis of AHLs; however, the implication of HtdS in the synthesis of AHLs has not been fully clarified (20). Further research to elucidate the roles of HtdS in biofilm formation by *P. putida* strain TS312 could lead to the development of a strategy to combat unwanted biofilm formation.

**Data availability.** The complete genome sequence of *P. putida* strain TS312 has been deposited in GenBank under accession number AP022324 and BioProject number PRJDB9166. The raw data are available in the DDBJ Sequence Read Archive (DRA) under accession number DRA009539. The data addressed in this paper represent the first version.

**ACKNOWLEDGMENT**

The genome assembly was performed on the NIG supercomputer at ROIS National Institute of Genetics.

**REFERENCES**

1. Nebot E, Casanueva JF, Casanueva T, Fernández-Bastón MM, Sales D. 2006. In situ experimental study for the optimization of chlorine dosage in seawater cooling systems. Appl Therm Eng 26:1893–1900. [https://doi.org/10.1016/j.applthermaleng.2006.02.001](https://doi.org/10.1016/j.applthermaleng.2006.02.001).

2. Flemming HC, Meier M, Schild T. 2013. Mini-review: microbial problems in paper production. Biofouling 29:683–696. [https://doi.org/10.1080/08927014.2013.798865](https://doi.org/10.1080/08927014.2013.798865).

3. Guo W, Ngo HH, Li J. 2012. A mini-review on membrane fouling. Biore- sour Technol 122:27–34. [https://doi.org/10.1016/j.biortech.2012.04.089](https://doi.org/10.1016/j.biortech.2012.04.089).

4. Allonier AS, Khalanski M, Camel V, Bermond A. 1999. Characterization of chlorination by-products in cooling effluents of coastal nuclear power stations. Mar Pollut Bull 38:1232–1241. [https://doi.org/10.1016/S0025-326X(99)00168-X](https://doi.org/10.1016/S0025-326X(99)00168-X).

5. Waters CM, Bassler BL. 2005. Quorum sensing: cell-to-cell communica- tion in bacteria. Annu Rev Cell Dev Biol 21:319–346. [https://doi.org/10.1146/annurev.cellbio.21.012704.131001](https://doi.org/10.1146/annurev.cellbio.21.012704.131001).

6. Williams P, Winzer K, Chan WC, Cámara M. 2007. Look who’s talking: communication and quorum sensing in the bacterial world. Philos Trans R Soc Lond B Biol Sci 362:1119–1134. [https://doi.org/10.1098/rstb.2007.2039](https://doi.org/10.1098/rstb.2007.2039).

7. Steidle A, Alleslen-Holm M, Riedel K, Berg G, Givskov M, Molin S, Eberl L. 2002. Identification and characterization of an N-acylhomoserine lactone-dependent quorum-sensing system in *Pseudomonas putida* strain Isol. Appl Environ Microbiol 68:6371–6382. [https://doi.org/10.1128/AEM.68.12.6371-6382.2002](https://doi.org/10.1128/AEM.68.12.6371-6382.2002).

8. Dubem JF, Lugtenberg BJJ, Bloemberg GV. 2006. The *ppuI- rsaI-gpur* quorum-sensing system regulates biofilm formation of *Pseudomonas putida* PCL1445 by controlling biosynthesis of the cyclic lipopeptides putisolvins I and II. J Bacteriol 188:2898–2906. [https://doi.org/10.1128/JB.188.8.2898-2906.2006](https://doi.org/10.1128/JB.188.8.2898-2906.2006).

9. Rampioni G, Bertani I, Pillai CR, Venturi V, Zennaro E, Leoni L. 2012. Functional characterization of the quorum sensing regulator RsaI in the plant-beneficial strain *Pseudomonas putida* WCS358. Appl Environ Microbiol 78:726–734. [https://doi.org/10.1128/AEM.06442-11](https://doi.org/10.1128/AEM.06442-11).

10. Butler JM. 2012. Advanced topics in forensic DNA typing: methodology, p 29–47. Academic Press, New York, NY.

11. Bouchez V, Baines SL, Guillot S, Brisse S. 2018. Complete genome sequences of *Bordetella pertussis* clinical isolate FRS810 and reference strain Tohama from combined Oxford Nanopore and Illumina sequencing. Microbiol Resour Announc 7:e01207-18. [https://doi.org/10.1128/MRA.01207-18](https://doi.org/10.1128/MRA.01207-18).

12. Valcek A, Overballe-Petersen S, Hansen F, Dolejska M, Hasman H. 2019. Complete genome sequence of *Escherichia coli* MT102, a plasmid-free recipient resistant to rifampin, azide, and streptomycin, used in conjugation experiments. Microbiol Resour Announc 8:e00383-19. [https://doi.org/10.1128/MRA.00383-19](https://doi.org/10.1128/MRA.00383-19).

13. De Coster W, D’Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: visualizing and processing long-read sequencing data. Bioinformatics 34:2666–2669. [https://doi.org/10.1093/bioinformatics/bty149](https://doi.org/10.1093/bioinformatics/bty149).

14. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Genome assembly weighting and repeat separation. Genome Res 27:722–736. [https://doi.org/10.1101/gr.215087.116](https://doi.org/10.1101/gr.215087.116).

15. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation complete-
ness with single-copy orthologs. Bioinformatics 31:3210–3212. https://doi.org/10.1093/bioinformatics/btv351.

16. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039. https://doi.org/10.1093/bioinformatics/btx713.

17. Huang JJ, Han JI, Zhang LH, Leadbetter JR. 2003. Utilization of acyl-homoserine lactone quorum signals for growth by a soil pseudomonad and Pseudomonas aeruginosa PAO1. Appl Environ Microbiol 69:5941–5949. https://doi.org/10.1128/aem.69.10.5941-5949.2003.

18. Huang JJ, Petersen A, Whiteley M, Leadbetter JR. 2006. Identification of QuiP, the product of gene PA1032, as the second acyl-homoserine lactone acylase of Pseudomonas aeruginosa PAO1. Appl Environ Microbiol 72:1190–1197. https://doi.org/10.1128/AEM.72.2.1190-1197.2006.

19. Laue BE, Jiang Y, Chhabra SR, Jacob S, Stewart G, Hardman A, Downie JA, O’Gara F, Williams P. 2000. The biocontrol strain Pseudomonas fluorescens F113 produces the Rhizobium small bacteriocin, N-(3-hydroxy-7-cis-tetradecenoyl) homoserine lactone, via HdtS, a putative novel N-acylhomoserine lactone synthase. Microbiology 146:2469–2480. https://doi.org/10.1099/00221287-146-10-2469.

20. Churchill MEA, Chen L. 2011. Structural basis of acyl-homoserine lactone-dependent signaling. Chem Rev 111:68–85. https://doi.org/10.1021/cr1000817.