genomeA
Journals.ASM.org

Draft Genome Sequence of a Novel Nicotine-Degrading Bacterium, *Pseudomonas plecoglossicida* TND35

Gurusamy Raman,[a] Natarajan Sakthivel,[b] SeonJoo Park[a]

Department of Life Sciences, Yeungnam University, Gyeongsan, Gyeongbuk, Republic of Korea[a]; Department of Biotechnology, School of Life Sciences, Pondicherry University, Puducherry, India[b]

***Pseudomonas plecoglossicida* TND35 is a potent nicotine-degrading bacterium. The draft genome sequence of strain TND35 contains 6,209,227 bp, 5,511 coding genes, and a G+C content of 62.3%. It encompasses genes related to catabolism of nicotine, *N*-heterocyclic aromatic compounds, heavy metal degradation, and butanol biosynthesis.**

Received 30 September 2014 **Accepted** 9 October 2014 **Published** 16 July 2015

Citation Raman G, Sakthivel N, Park S. 2015. Draft genome sequence of a novel nicotine-degrading bacterium, *Pseudomonas plecoglossicida* TND35. Genome Announc. 3(4): e01162-14. doi:10.1128/genomeA.01162-14.

Address correspondence to SeonJoo Park, sjpark01@ynu.ac.kr, or Natarajan Sakthivel, puns2005@gmail.com.

Tobacco wastes that contain the principle alkaloid nicotine are harmful to human health and pollute the environment (1). These wastes are largely accumulated during the tobacco manufacturing process (2). Biological methods efficiently clean up toxic and hazardous wastes in the contaminated sites. A specific group of nicotine-degrading bacteria is involved in the degradation of nicotine (3). A novel nicotine-degrading bacterium, *Pseudomonas plecoglossicida* TND35, was isolated, identified, and deposited at the bacterial culture collection center, Department of Biotechnology, Pondicherry University, India. The 16S rRNA sequence of strain TND35 was deposited at GenBank database under accession no. JQ660543. This bacterium utilizes nicotine and employs a variant of the pyrrolidine pathway of nicotine biodegradation (1). However, the genome sequence of strain TND35 has not been sequenced yet. The genome sequence will provide insight into the molecular mechanism of its nicotine biodegradation.

The genome sequence of strain TND35 was determined by Illumina High-Seq 2000 (Macrogen, Inc., South Korea). A total of 31,406,636 reads were generated from a 100-bp paired-end library (total reads, 14,082,296; ~100-fold coverage) and 8-kb mate-pair libraries (total reads, 17,324,340; ~100-fold coverage). Sequence trimming and *de novo* assembly were performed using CLC-Genomics-Workbench, v7.0.4 (CLC-Bio, Denmark) and generated 69 contigs (>1000 bp) with an N_{50} length of 272,736 bp, a maximum contig size of 573,487, and an average size of 90,008 bp. These contigs were ordered using CONTIGuator v2.3 (4) with its closely related genome *Pseudomonas monteilii* SB3078 as a reference (GenBank accession no. CP006978.1). Forty-seven contigs contained in the 6.15-Mb genome were aligned with the reference genome. Sequences not mapped with the reference genome corresponding to 22 contigs, which are comprised of 58,201 nucleotides, were added later to the draft genome of strain TND35. These scaffolds were used for annotation using the RAST server (5) and the NCBI Prokaryotic Genomes and Automatic Annotation Pipeline, v2.7 (6). The scaffolds were searched against the KEGG database to analyze metabolic pathways and gene functions (7). Glimmer3 and GeneMarkS were used for the prediction of structural genes (8). RNAmmer and tRNAscan-SE were used to identify rRNAs and tRNAs (9, 10).

The draft genome sequence of strain TND35 contains 6,209,227 bp with a G+C content of 62.3%. It encodes 5,511 genes which include 5,397 predicted coding sequences (CDS), 49 pseudogenes, 60 tRNAs, 4 rRNAs, one non-coding RNA (ncRNA), and 36 frameshifted genes. The genome covers a total of 530 subsystems, which includes 4,106 CDS in total. The genome includes genes related to catabolism of *N*-heterocyclic aromatic compounds. The nicotine-degrading gene *hsp* was identified in the genome sequence of strain TND35. This key gene is very important in the conversion of 6-hydroxy-3-succinoyl pyridine to 2,5-dihydroxypyridine of the pyrrolidine pathway of *Pseudomonas* bacteria (11–13). Based on these findings, the strain TND35 follows the pyrrolidine pathway of nicotine degradation. The announcement of this genome information will allow further studies on the molecular mechanisms of nicotine-degrading genes and other genes related to *N*-heterocyclic aromatic compound degradation.

Nucleotide sequence accession number. This draft sequence has been deposited at DDBJ/EMBL/GenBank under accession no. JOJY00000000. The version described here is the first version.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea (KRF) grant funded by the Korea Government (KRF, No. 2012R1A1A2004996), South Korea and University Grants Commission (UGC), Government of India, New Delhi, India.

REFERENCES

1. **Raman G, Mohan K, Manohar V, Sakthivel N.** 2014. Biodegradation of nicotine by a novel nicotine-degrading bacterium, *Pseudomonas plecoglossicida* TND35 and its new biotransformation intermediates. Biodegradation **25**:95–107. http://dx.doi.org/10.1007/s10532-013-9643-4.
2. **Briski F, Horgas N, Vukovic M, Gomzi Z.** 2003. Aerobic composting of tobacco industry solid waste-simulation of the process. Clean Technol Env. **5**:295–301. http://dx.doi.org/10.1007/s10098-003-0218-7.
3. **Raman G, Sakthivel N.** 2013. Current status on biochemistry and molec-

ular biology of microbial degradation of nicotine. Sci. World J. **2013:**1–15. http://dx.doi.org/10.1155/2013/125385.

4. **Galardini M, Biondi EG, Bazzicalupo M, Mengoni A.** 2011. CONTIGuator: a bacterial genome finishing tool for structural insights on draft genomes. Source Code Biol Med **6:**11. http://dx.doi.org/10.1186/1751-0473-6-11.
5. **Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O.** 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics **9:**75. http://dx.doi.org/10.1186/1471-2164-9-75.
6. **Pruitt KD, Tatusova T, Klimke W, Maglott DR.** 2009. NCBI reference sequences: current status, policy and new initiatives. Nucleic Acids Res **37:**32–36. http://dx.doi.org/10.1093/nar/gkn721.
7. **Kanehisa M, Goto S.** 2000. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res **28:**27–30. http://dx.doi.org/10.1093/nar/28.1.27.
8. **Delcher AL, Harmon D, Kasif S, White O, Salzberg SL.** 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res **27:**4636–4641. http://dx.doi.org/10.1093/nar/27.23.4636.
9. **Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res **35:**3100–3108. http://dx.doi.org/10.1093/nar/gkm160.
10. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res **25:**955–964. http://dx.doi.org/10.1093/nar/25.5.0955.
11. **Tang H, Wang S, Ma L, Meng X, Deng Z, Zhang D, Ma C, Xu P.** 2008. A novel gene, encoding 6-hydroxy-3-succinoylpyridine hydroxylase, involved in nicotine degradation by *Pseudomonas putida* strain S16. Appl Environ Microbiol **74:**1567–1574. http://dx.doi.org/10.1128/AEM.02529-07.
12. **Tang H, Wang L, Meng X, Ma L, Wang S, He X, Wu G, Xu P.** 2009. Novel nicotine oxidoreductase-encoding gene involved in nicotine degradation by *Pseudomonas putida* strain S16. Appl Environ Microbiol **75:**772–778. http://dx.doi.org/10.1128/AEM.02300-08.
13. **Tang H, Yao Y, Zhang D, Meng X, Wang L, Yu H, Ma L, Xu P.** 2011. A novel NADH-dependent and FAD-containing hydroxylase is crucial for nicotine degradation by *Pseudomonas putida*. J Biol Chem **286:**39179–39187. http://dx.doi.org/10.1074/jbc.M111.283929.