



Draft Genome Sequence of *Pseudomonas stutzeri* Strain 19, an Isolate Capable of Efficient Degradation of Aromatic Hydrocarbons

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ABSTRACT *Pseudomonas stutzeri* strain 19 is a Gram-negative bacterium capable of degrading aromatic hydrocarbons. The draft genome of *P. stutzeri* 19 is estimated to be 5.1 Mb, containing 4,652 protein-coding genes and a G+C content of 63.3%. Multiple genes responsible for the degradation of aromatics are present in this strain.

Pseudomonas stutzeri strain 19 was isolated from a wastewater sample from Dayton, OH, USA. *P. stutzeri* 19 was shown, through gas chromatography-mass spectrometry (GC-MS) analysis, to efficiently metabolize toluene, xylenes, and 1,2,4-trimethyl benzene. Comparative BLAST analysis (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) of the 16S rRNA gene of *P. stutzeri* 19, identified using RNAmmer (1), showed 99% similarity with *P. stutzeri* DSM 4166 and *P. stutzeri* A1501, while Rapid Annotations using Subsystems Technology (RAST) identified *P. stutzeri* A1501 as the closest neighbor, with a score of 507. The genome of *P. stutzeri* was chosen for sequencing due to its ability to degrade recalcitrant aromatics and grow in harsh hydrocarbon-containing environments.

Whole-genome shotgun sequencing was performed on a Roche 454-GS Junior platform, producing 334,879 reads. Newbler assembly (version 2.9) was used to align reads, creating 136 large (>500-bp) contigs with an average size of 37,500 bp, an N_{50} of 104,286, and an L_{50} of 15. The draft genome sequence was 5,100,040 bp in length, with a G+C content of 63.3%. The largest contig extended for 263,585 bp. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/) predicted 4,895 genes, 4,652 coding sequences (CDSs), and 54 tRNAs. Rapid genome annotations using the RAST server (2) assigned the protein-coding sequences to 512 subsystems, of which amino acids and derivatives ($n = 439$ CDSs), carbohydrates ($n = 368$), cofactors, vitamins, prosthetic groups, and pigments ($n = 332$), protein metabolism ($n = 278$), fatty acids, lipids, and isoprenoids ($n = 161$), RNA metabolism ($n = 204$), nucleosides and nucleotides ($n = 115$), virulence, disease, and defense ($n = 129$), stress response ($n = 176$), respiration ($n = 148$), DNA metabolism ($n = 162$), motility and chemotaxis ($n = 129$), membrane transport ($n = 200$), and cell wall and capsule ($n = 183$) were most abundant.

The NCBI PGAP predicted multiple genes involved in hydrocarbon degradation, including catechol 1,2-dioxygenase, homogentisate 1,2-dioxygenase, phenol monooxygenase, small and large subunits of benzoate 1,2-dioxygenase (*benA* and *benB*), alkane 1-monooxygenase, rubredoxin, alkene reductase, 2-alkenal reductase, P450, and a benzoate transporter protein, among others. BLAST analysis revealed two coding sequences with 99% homology to the alpha and beta subunits of toluene 1,2-

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dioxygenase of *P. putida* MT53 plasmid pWW53. Also, coding sequences with 96% homology to the xylene monooxygenase electron transfer subunit and 98% homology to the xylene monooxygenase hydrolase subunit of *P. putida* MT53 plasmid pWW53 were found. The presence of these enzymes explains the toluene and xylene degradation capacities of *P. stutzeri* 19. The genes for protocatechuate 3,4-dioxygenase, 3-carboxyruconate cycloisomerase, and 4-carboxyruconolactone decarboxylase of the central protocatechuate catabolic pathway for aromatic degradation were also present. A cluster of genes was observed with at least 78% homology to the *ttg2* operon of *P. putida* that encodes an ABC transporter implicated in resistance to toluene (3, 4). The genome of *P. stutzeri* 19 encodes many multidrug and heavy-metal resistance-nodulation-division (RND) efflux transporters, some of which have been associated with hydrocarbon resistance (5). The genome of *P. stutzeri* strain 19 will help to understand the adaptive mechanisms deployed by Gram-negative bacteria for survival and proliferation in hydrocarbons.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [NFZU00000000](https://doi.org/10.1093/nar/gkm160). The version described in this paper is NFZU01000000.

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REFERENCES

1. Lagesen K, Hallin PF, Rødland E, Stærfeldt HH, Ussery DW. 2007. RNAmmer: consistent annotation of rRNA genes in genomic sequences. *Nucleic Acids Res* 35:3100–3108. <https://doi.org/10.1093/nar/gkm160>.
2. 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. <https://doi.org/10.1186/1471-2164-9-75>.
3. Kim K, Lee S, Lee K, Lim D. 1998. Isolation and characterization of toluene-sensitive mutants from the toluene-resistant bacterium *Pseudomonas putida* GM73. *J Bacteriol* 180:3692–3696.
4. Ruiz ON, Brown LM, Striebich RC, Mueller SS, Gunasekera TS. 2015. Draft genome sequence of *Pseudomonas frederiksbergensis* SI8, a psychrotrophic aromatic-degrading bacterium. *Genome Announc* 3(4):e00811-15. <https://doi.org/10.1128/genomeA.00811-15>.
5. Gunasekera TS, Bowen LL, Zhou CE, Howard-Byerly SC, Foley WS, Striebich RC, Dugan LC, Ruiz ON. 2017. Transcriptomic analyses elucidate adaptive differences of closely related strains of *Pseudomonas aeruginosa* in fuel. *Appl Environ Microbiol* 83:e03249-16. <https://doi.org/10.1128/AEM.03249-16>.