Complete Genome Sequence of *Achromobacter denitrificans* PR1

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ABSTRACT *Achromobacter denitrificans* strain PR1 was isolated from an enrichment culture able to use sulfamethoxazole as an energy source. Here, we describe the complete genome of this strain sequenced by Illumina MiSeq and Oxford Nanopore MinION.

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*A*chromobacter denitrificans is a Gram-negative rod-shaped bacterium commonly found in soil and occasionally in human infections (1, 2). Members of this species have previously been linked to the degradation of xenobiotics (3–6), highlighting their potential for bioremediation. Here, we describe the complete genome sequence of *A. denitrificans* strain PR1, originally obtained from enriched activated sludge and able to use sulfamethoxazole (SMX) as an energy source (7).

Strain PR1 was incubated overnight at 30°C in mineral medium B (8) with ammonium sulfate (0.54 g/liter), succinate (0.83 g/liter), yeast extract (0.2 g/liter), and SMX (0.15 g/liter). Genomic DNA extraction was performed with GenElute bacterial genomic DNA kit (Sigma) and sequenced using MiSeq (Illumina) and MinION (Oxford Nanopore). For MiSeq paired-end sequencing (2 × 300 bp), two libraries were independently prepared from 1 µg of DNA with the TruSeq DNA LT sample prep kit (library 1 [lib1]) from Illumina or the Kapa HyperPrep kit (library 2 [lib2]) from Kapa Biosystems. The MinION library was prepared from 1 µg of DNA, sheared into 5-kb fragments with a g-TUBE (Covaris), prepared with the genomic DNA sequencing kit (SQK-MAP-103), and sequenced using a flow cell with R7 chemistry (Oxford Nanopore). The library was loaded in the beginning and after 24 h to coincide with the g1-to-g2 pore switch (9).

MiSeq sequencing generated 2.5 million (lib1) and 0.3 million (lib2) paired-end raw reads. All reads were screened for PhiX contamination and adapter and quality trimmed (>Q20) with the BBDuk tool (https://sourceforge.net/projects/bbmap). MinION sequencing generated 12,591 2D reads (10) (>Q9) that were converted to fastq format with Poretools version 0.5.1 (11). Hybrid *de novo* assembly was done with SPAdes version 3.10.0 (12) with the options -careful and -nanopore. Contigs with <1× coverage were removed from the assembly, resulting in a single scaffold. Circularization was performed with PCR and Sanger sequencing, generating a single circular chromosome of 6,929,205 bp with 46-fold average coverage and 67.4% G+C content.

Analysis with the Rapid Annotations using Subsystems Technology (RAST) server version 2.0 (13) predicted 6,425 protein-coding sequences (CDSs), 4 copies of the rRNA operon, and 59 tRNAs. Functional prediction of the CDSs was further refined by aligning protein sequences against the Gene Ontology (GO) database (14) with InterProScan (15) and BLASTp (16) in Blast2GO version 4.1 (17). Of the total CDSs, 5,210 (81.1%) had a functional prediction, and, from these, 2,939 (45.7%) had catalytic activity (891 hydrolases and 746 oxidoreductases). ResFinder (18) analysis identified multiple antibiotic

resistance genes (*sul1*, *sul2*, and *tetC*), with some (*cmlA1h* and *aadA2*) within the new class I integron In1410 (19). Average nucleotide identity (ANI) analysis (20) and *in silico* DNA-DNA hybridization (DDH) analysis (21, 22) with the *A. denitrificans* type strain genome (GenBank accession number BCTQ00000000) showed that strain PR1 belongs to the same species (ANI, 99.33%; DDH, 94.60%; difference in %G+C content, 0.19).

The genome of strain PR1 will provide further insights into sulfamethoxazole metabolism in this microbial consortium and into the species versatility and potential for xenobiotic degradation.

Accession number(s). This complete genome sequence has been deposited in GenBank under the accession no. CP020917. The version described in this paper is the first version.

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REFERENCES

1. Coenye T, Vancanneyt M, Cnockaert MC, Falsen E, Swings J, Vandamme P. 2003. *Kerstersia gyiorum* gen. nov., sp. nov., a novel *Alcaligenes faecalis*-like organism isolated from human clinical samples, and reclassification of *Alcaligenes denitrificans* Ruger and Tan 1983 as *Achromobacter denitrificans* comb. nov. Int J Syst Evol Microbiol 53:1825–1831. https://doi.org/10.1099/ijs.0.02609-0.

2. Kersters K, De Ley J. 1984. Genus *Alcaligenes* Castellani and Chalmers 1919, 936AL, p 361–373. *In* Krieg NR, Holt JG (ed), Bergey's manual of systematic bacteriology. Williams & Wilkins, Baltimore, MD.

3. Benjamin S, Kamimura N, Takahashi K, Masai E. 2016. *Achromobacter denitrificans* SP1 efficiently utilizes 16 phthalate diesters and their downstream products through protocatechuate 3,4-cleavage pathway. Ecotoxicol Environ Saf 134:172–178. https://doi.org/10.1016/j.ecoenv.2016.08.028.

4. Mawad AMM, Hesham AE-l, Mostafa YM, Shoriet A. 2016. Pyrene degrading *Achromobacter denitrificans* ASU-035: growth rate, enzymes activity, and cell surface properties. Rend Fis Acc Lincei 27:557–563. https://doi.org/10.1007/s12210-016-0521-y.

5. Pradeep S, Josh MK, Binod P, Devi RS, Balachandran S, Anderson RC, Benjamin S. 2015. *Achromobacter denitrificans* strain SP1 efficiently remediates di(2-ethylhexyl)phthalate. Ecotoxicol Environ Saf 112:114–121. https://doi.org/10.1016/j.ecoenv.2014.10.035.

6. Sałek K, Zgoła-Grześkowiak A, Kaczorek E. 2013. Modification of surface and enzymatic properties of *Achromobacter denitrificans* and *Stenotrophomonas maltophilia* in association with diesel oil biodegradation enhanced with alkyl polyglucosides. Colloids Surf B Biointerfaces 111:36–42. https://doi.org/10.1016/j.colsurfb.2013.05.021.

7. Reis PJM, Reis AC, Ricken B, Kolvenbach BA, Manaia CM, Corvini PFX, Nunes OC. 2014. Biodegradation of sulfamethoxazole and other sulfonamides by *Achromobacter denitrificans* PR1. J Hazard Mater 280:741–749. https://doi.org/10.1016/j.jhazmat.2014.08.039.

8. Barreiros L, Nogales B, Manaia CM, Ferreira AC, Pieper DH, Reis MA, Nunes OC. 2003. A novel pathway for mineralization of the thiocarbamate herbicide molinate by a defined bacterial mixed culture. Environ Microbiol 5:944–953. https://doi.org/10.1046/j.1462-2920.2003.00492.x.

9. Ip CLC, Loose M, Tyson JR, de Cesare M, Brown BL, Jain M, Leggett RM, Eccles DA, Zalunin V, Urban JM, Piazza P, Bowden RJ, Paten B, Mwaigwisya S, Batty EM, Simpson JT, Snutch TP, Birney E, Buck D, Goodwin S, Jansen HJ, O'Grady J, Olsen HE, MinION Analysis and Reference Consortium. 2015. Phase 1 data release and analysis [version 1; referees: 2 approved]. F1000Res 4:1075. https://doi.org/10.12688/f1000research.7201.1.

10. Lu H, Giordano F, Ning Z. 2016. Oxford Nanopore MinION sequencing and genome assembly. Genomics Proteomics Bioinformatics 14:265–279. https://doi.org/10.1016/j.gpb.2016.05.004.

11. Loman NJ, Quinlan AR. 2014. Poretools: a toolkit for analyzing nanopore sequence data. Bioinformatics 30:3399–3401. https://doi.org/10.1093/bioinformatics/btu555.

12. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.

13. 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.

14. The Gene Ontology Consortium. 2015. Gene Ontology Consortium: going forward. Nucleic Acids Res 43:D1049–D1056. https://doi.org/10.1093/nar/gku1179.

15. Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R. 2005. InterProScan: protein domains identifier. Nucleic Acids Res 33:W116–W120. https://doi.org/10.1093/nar/gki442.

16. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. https://doi.org/10.1093/nar/25.17.3389.

17. Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M, Robles M. 2005.

Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21:3674–3676. https://doi .org/10.1093/bioinformatics/bti610.

18. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi .org/10.1093/jac/dks261.

19. Moura A, Soares M, Pereira C, Leitao N, Henriques I, Correia A. 2009. INTEGRALL: a database and search engine for integrons, integrases and gene cassettes. Bioinformatics 25:1096–1098. https://doi.org/10.1093/ bioinformatics/btp105.

20. Rodriguez-R LM, Konstantinidis KT. 2016. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metag-enomes. PeerJ 4:e1900v1. https://peerj.com/preprints/1900v1/.

21. Clarke GDP, Beiko RG, Ragan MA, Charlebois RL. 2002. Inferring genome trees by using a filter to eliminate phylogenetically discordant se-quences and a distance matrix based on mean normalized BLASTP scores. J Bacteriol 184:2072–2080. https://doi.org/10.1128/JB.184.8.2072 -2080.2002.

22. Meier-Kolthoff JP, Klenk HP, Göker M. 2014. Taxonomic use of DNA G+C content and DNA–DNA hybridization in the genomic age. Int J Syst Evol Microbiol 64:352–356. https://doi.org/10.1099/ijs.0.056994-0.