



Complete Genome Sequence of *Achromobacter* Strain ES-001, a Betaproteobacterium Associated with a Cellulolytic Soil Community

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ABSTRACT The genome sequence of the soilborne bacterium *Achromobacter* strain ES-001, assembled from Illumina NextSeq and Nanopore MinION reads, is rich in genes predicted to encode iron, arsenic, and hydrocarbon metabolism, as well as type 6 secretion components. The sequenced genome will aid in determining the roles of noncellulolytic species in cellulose-enriched environments.

The Gram-negative genus *Achromobacter* is composed of motile, facultative aerobes that generally inhabit soils and water (1), with some species also acting as opportunistic pathogens (2–4). *Achromobacter* strain ES-001 is associated with microbial communities of cellulose-rich soils and can be maintained on carboxymethylcellulose (CMC) (5). Cellulolytic activity, however, has not been previously observed in the genus. To better understand its metabolic contributions to cellulolytic soil communities, the complete genome sequence of *Achromobacter* strain ES-001 is presented here.

Soil-water suspensions (1:10) from leaf-mulched soil in east central Kansas, USA (38.43 N, 96.21 W) were plated onto M9 agar (Sigma-Aldrich, St. Louis, MO, USA) with 1% CMC (Sigma-Aldrich) as the carbon source (5). After 10 passages to fresh M9/CMC agar at 30°C, single colonies of an unknown species, designated ES-001, were isolated from the streak plates to M9/CMC plates and maintained thereafter on LB agar (6) at 30°C. DNA from cultures grown at 30°C at 250 rpm in LB broth was isolated using the Quick-DNA fungal/bacterial miniprep kit (Zymo Research, Irvine, CA, USA). The University of Kansas Genome Sequencing Core Laboratory (Lawrence, KS, USA) prepared a genomic library using the NEBNext Ultra II DNA library prep kit (New England Biolabs, Ipswich, MA, USA), targeting 200-bp fragments. Illumina NextSeq 550 sequencing with a 300-cycle midoutput kit v2.5 yielded 23,450,192 paired-end reads, for a total of 3.542 Gb of sequence with a mean quality score of Q34.54. The Oxford Nanopore ligation sequencing kit (SQK-LSK109, Oxford Nanopore, Oxford, UK) was used to prepare a second library from the same DNA as above, which was sequenced with the Nanopore MinION platform to generate 40,000 reads with an average length of 6,638 bp. The Illumina and Nanopore reads were uploaded to the public [usegalaxy.org](https://www.usegalaxy.org) server (7) for quality analysis and processing using fastp v0.19.5+galaxy1 and Porechop v0.2.3 tools (8, 9) with default settings. Unicycler v0.4.8.0 (10) was used to assemble 3.38 Gb of paired Illumina reads (516.8-fold coverage), with 25,303 high-quality MinION reads (170.38 Mb, 26.0-fold coverage) as scaffolds, to generate a single, circular 6,544,928-bp contig with 64.5% GC content. Nanopore reads spanning the ends of the assembled contig confirmed circularity.

The assembled ES-001 16S rRNA gene, when used as the query in BLASTN searches of the NCBI 16S rRNA database and in Needleman-Wunsch alignments, is 100% identical to *Achromobacter spanius* NTCT13519 (GenBank accession number [LR134302.1](https://www.ncbi.nlm.nih.gov/nuccore/LR134302.1)). However, assessment of the average nucleotide identity of orthologs using the Rapid Annotation Subsystems Technology (RAST) v2.0 SEED (11) and OrthoANIu (12) servers

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showed that ES-001 is 87.9% identical to *A. spanius* NTCT13519, well below the proposed species cutoff of 96% (13). Therefore, *Achromobacter* strain ES-001 may be a distinct species.

NCBI PGAP (Prokaryotic Genome Annotation Pipeline) annotation (14) predicted 5,766 protein-coding genes, 74 RNA-coding genes, and 95 pseudogenes. Predicted genes for auxin synthesis, iron acquisition, hydrocarbon metabolism, arsenic/copper/heavy metal regulation, and a complete type 6 secretion system (T6SS) (15) suggest that *Achromobacter* strain ES-001 performs roles in stimulating plant growth, protecting against environmental toxins, and regulating microbial community composition, similar to the environmental roles of other *Achromobacter* species (16–20).

Data availability. The *Achromobacter* strain ES-001 genome sequence has been deposited in DDBJ/ENA/GenBank under accession number [CP079940](https://doi.org/10.1093/nar/gkt1226). Raw sequence data used for assembly are deposited in DDBJ/ENA/GenBank under SRA accession number [SAMN20284516](https://doi.org/10.1093/nar/gkw569). The assembly described in this paper is the first version, [CP079940.1](https://doi.org/10.1093/nar/gkw569).

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REFERENCES

- Bergey DH, Harrison FC, Breed RS, Hammer BW, Huntoon FM (ed). 1923. Bergey's manual of determinative bacteriology, 1st ed. The Williams & Wilkins Co, Baltimore, MD.
- Aisenberg G, Rolston KV, Safdar A. 2004. Bacteremia caused by *Achromobacter* and *Alcaligenes* species in 46 patients with cancer (1989–2003). *Cancer* 101:2134–2140. <https://doi.org/10.1002/cncr.20604>.
- Amoureux L, Bador J, Bounoua Zouak F, Chapuis A, de Curraize C, Neuwirth C. 2016. Distribution of the species of *Achromobacter* in a French cystic fibrosis centre and multilocus sequence typing analysis reveal the predominance of *A. xylooxidans* and clonal relationships between some clinical and environmental isolates. *J Cyst Fibros* 15:486–494. <https://doi.org/10.1016/j.jcf.2015.12.009>.
- Isler B, Kidd TJ, Stewart AG, Harris P, Paterson DL. 2020. *Achromobacter* infections and treatment options. *Antimicrob Agents Chemother* 64: e01025–20. <https://doi.org/10.1128/AAC.01025-20>.
- Yeoh HH, Khew E, Lim G. 1985. A simple method for screening cellulolytic fungi. *Mycologia* 77:161–162. <https://doi.org/10.2307/3793263>.
- Bertani G. 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. *J Bacteriol* 62:293–300. <https://doi.org/10.1128/jb.62.3.293-300.1951>.
- Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Cech M, Chilton J, Clements D, Coraor N, Grüning BA, Guerler A, Hillman-Jackson J, Hiltmann S, Jalili V, Rasche H, Soranzo N, Goecks J, Taylor J, Nekrutenko A, Blankenberg D. 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Res* 46: W537–W544. <https://doi.org/10.1093/nar/gky379>.
- Loman NJ, Quinlan AR. 2014. Poretools: a toolkit for analyzing Nanopore sequence data. *Bioinformatics* 30:3399–3401. <https://doi.org/10.1093/bioinformatics/btu555>.
- Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ pre-processor. *Bioinformatics* 34:i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 42:D206–D214. <https://doi.org/10.1093/nar/gkt1226>.
- Yoon S-H, Ha S, Lim J, Kwon S, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek* 110:1281–1286. <https://doi.org/10.1007/s10482-017-0844-4>.
- Ciufo S, Kannan S, Sharma S, Badretin A, Clark K, Turner S, Brover S, Schoch CL, Kimchi A, DiCuccio M. 2018. Using average nucleotide identity to improve taxonomic assignments in prokaryotic genomes at the NCBI. *Int J Syst Evol Microbiol* 68:2386–2392. <https://doi.org/10.1099/ijssem.0.002809>.
- Tatusova T, DiCuccio M, Badretin 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>.
- Coulthurst S. 2019. The type VI secretion system: a versatile bacterial weapon. *Microbiology (Reading)* 165:503–515. <https://doi.org/10.1099/mic.0.000789>.
- Mohapatra B, Satyanarayana T, Sar P. 2018. Molecular and eco-physiological characterization of arsenic (As)-transforming *Achromobacter* sp. KAs 3-5^T from As-contaminated groundwater of West Bengal, India. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 53:915–924. <https://doi.org/10.1080/10934529.2018.1462897>.
- Wang P, Gao J, Zhao Y, Zhang M, Zhou S. 2021. Biodegradability of di-(2-ethylhexyl) phthalate by a newly isolated bacterium *Achromobacter* sp. RX. *Sci Total Environ* 755:142476. <https://doi.org/10.1016/j.scitotenv.2020.142476>.
- Xia Z-Y, Zhang L, Zhao Y, Yan X, Li S-P, Gu T, Jiang J-D. 2017. Biodegradation of the herbicide 2,4-dichlorophenoxyacetic acid by a new isolated strain of *Achromobacter* sp. LZ35. *Curr Microbiol* 74:193–202. <https://doi.org/10.1007/s00284-016-1173-y>.
- Castanheira N, Dourado AC, Alves PI, Cortés-Pallero AM, Delgado-Rodríguez AI, Prazeres Á, Borges N, Sánchez C, Barreto Crespo MT, Fareleira P. 2014. Annual ryegrass-associated bacteria with potential for growth promotion. *Microbiol Res* 169:768–779. <https://doi.org/10.1016/j.micres.2013.12.010>.
- Soares MA, Li H-Y, Kowalski KP, Bergen M, Torres MS, White JF. 2016. Functional role of bacteria from invasive *Phragmites australis* in promotion of host growth. *Microb Ecol* 72:407–417. <https://doi.org/10.1007/s00248-016-0793-x>.