



Draft Genome Sequence of *Xanthobacter aminoxidans* ATCC BAA-299^T

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ABSTRACT *Xanthobacter aminoxidans* is a Gram-negative pleomorphic and diazotrophic Knallgas bacillus that undergoes asymmetric budding of V-shaped branched cells during cell division. Like other *Xanthobacter* spp., cells are yellow from production of zeaxanthine dirhamnoside. We sequenced strain 14a^T (= ATCC BAA-299^T) and report a genome size of 5,829,486 bp with a G+C content of 67.9%.

The family *Xanthobacteraceae* of the class *Alphaproteobacteria*, which is found in freshwater, wetlands, soils, marine sediments, and plant roots and in waste treatment systems and polluted sites, contains aerobic chemoheterotrophs, although facultative chemolithoautotrophy utilizing hydrogen is also commonly found (1–13). *Xanthobacter aminoxidans* (strain 14a^T = VKM B-2254^T = ATCC BAA-299^T) was isolated from the activated sludge of a sewage purification system at the Baikal paper mill in Russia in 1979 (8). *X. aminoxidans* cells are Gram-negative pleomorphic rods that branch into V-shaped cells in asymmetric cell division (8, 10). *Xanthobacter aminoxidans* has varied metabolic capabilities, including growing autotrophically in H₂ plus O₂ plus CO₂, reducing nitrates to nitrites, and utilizing many carbon sources, including glutamine and methanol; the latter function is potentially useful to combat methanol pollution in wastewater treatment (10, 12, 14). Additionally, *X. aminoxidans*, like other *Xanthobacter* species, is capable of fixing N₂ under low-oxygen conditions (10, 12). While some *Xanthobacter* strains have been sequenced (12, 15–17), *X. aminoxidans* was still not sequenced, which led to the sequencing effort described below.

Xanthobacter aminoxidans ATCC BAA-299^T was obtained from ATCC (Manassas, VA, USA) in lyophilized form. Bacteria from an isolated colony were cultured in tryptic soy broth for 24 h at 30°C. A QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) was employed to extract non-size-selected genomic DNA (gDNA), and the KAPA HyperPlus kit (KR1145, v.5.19 [KK8515]; Kapa Biosystems, Wilmington, MA, USA) was then used to create the sequencing library by enzymatic fragmentation with HyperPlus end repair. The DNA library was sequenced on an Illumina HiSeq 2500 instrument by the Hubbard Center for Genome Studies at the University of New Hampshire (Durham, NH, USA), generating 250-bp paired-end fragments. The resulting reads were trimmed by Trimmomatic v.0.38 (settings: paired-end mode with a window size of 4, quality requirement of 15, and minimum read length of 36) (18). SPAdes v.3.13.0 (19) assembled 23,117,624 trimmed short reads with default bacterial parameters. After removal of small (<500 bp) and low-coverage (<65×) contigs, gene prediction and annotation for the remaining 78 *X. aminoxidans* contigs were completed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.6.1 (settings: best-placed reference protein set and GeneMarkS-2+) (20). The largest contig was 596,261 bp long, with a genome *N*₅₀ value of 242,177 bp. The total genome length was 5,829,267 bp, with a G+C content of 67.9%, similar to other members of the genus, such as *Xanthobacter oligotrophicus* 29k^T (5,313,426 bp, with a G+C content of 67.9%) (12). A total of 5,542 total genes were identified, of which 5,415 were protein-coding genes. The genome contained 54 RNA genes (1 complete

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copy of each rRNA, 47 tRNAs, and 4 noncoding RNAs) and 73 pseudogenes. The genome assembly was estimated to contain 100% of the expected highly conserved essential genes by benchmarking universal single-copy orthologs (BUSCO) v.5.2.2 analysis (default bacterial lineage settings, with no duplicated BUSCOs detected) (21–23), with an average genome coverage of 1,156×.

Consistent with known metabolism, we found by RAST analysis (24) genetic signatures of diverse types of nitrogen metabolism (from N₂ fixation to ammonia assimilation), carbohydrate metabolism (including C1 and C2 metabolic functions), and catabolism of diverse aromatic compounds. Interestingly, we also found a number of phage/prophage elements within the genome sequence, as well as 17 flagellar genes, although *X. aminoxidans* is described as a nonmotile species (8, 10).

Data availability. The *Xanthobacter aminoxidans* ATCC BAA-299^T whole-genome sequencing (WGS) project was deposited in DDBJ/ENA/GenBank under accession number [JAMJXC00000000](https://doi.org/10.1007/BF00446318). The raw data from BioProject accession number [PRJNA509625](https://doi.org/10.1099/00207713-28-4-573) were submitted to the NCBI Sequence Read Archive (SRA) under accession number [SRX15392594](https://doi.org/10.1099/ijsem.0.004972).

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REFERENCES

- Baumgarten J, Reh M, Schlegel HG. 1974. Taxonomic studies on some Gram-positive coryneform hydrogen bacteria. *Arch Microbiol* 100:207–217. <https://doi.org/10.1007/BF00446318>.
- Wiegel J, Wilke D, Baumgarten J, Opitz R, Schlegel HG. 1978. Transfer of the nitrogen-fixing hydrogen bacterium *Corynebacterium autotrophicum* Baumgarten et al. to *Xanthobacter* gen. nov. *Int J Syst Evol Microbiol* 28:573–581. <https://doi.org/10.1099/00207713-28-4-573>.
- Malik KA, Claus D. 1979. *Xanthobacter flavus*, a new species of nitrogen-fixing hydrogen bacteria. *Int J Syst Evol Microbiol* 29:283–287. <https://doi.org/10.1099/00207713-29-4-283>.
- Jenni B, Aragno M. 1987. *Xanthobacter agilis* sp. nov., a motile, dinitrogen-fixing, hydrogen-oxidizing bacterium. *Syst Appl Microbiol* 9:254–257. [https://doi.org/10.1016/S0723-2020\(87\)80030-9](https://doi.org/10.1016/S0723-2020(87)80030-9).
- Reding HK, Hartel PG, Wiegel J. 1991. Effect of *Xanthobacter*, isolated and characterized from rice roots, on growth of wetland rice. *Plant Soil* 138:221–229. <https://doi.org/10.1007/BF00012249>.
- Spiess E, Sommer C, Görisch H. 1995. Degradation of 1,4-dichlorobenzene by *Xanthobacter flavus* 14p1. *Appl Environ Microbiol* 61:3884–3888. <https://doi.org/10.1128/aem.61.11.3884-3888.1995>.
- Padden AN, Rainey FA, Kelly DP, Wood AP. 1997. *Xanthobacter tagetidis* sp. nov., an organism associated with *Tagetes* species and able to grow on substituted thiophenes. *Int J Syst Bacteriol* 47:394–401. <https://doi.org/10.1099/00207713-47-2-394>.
- Doronina NV, Trotsenko YA. 2003. Reclassification of ‘*Blastobacter viscosus*’ 7d and ‘*Blastobacter aminoxidans*’ 14a as *Xanthobacter viscosus* sp. nov. and *Xanthobacter aminoxidans* sp. nov. *Int J Syst Evol Microbiol* 53:179–182. <https://doi.org/10.1099/ijsem.0.02231-0>.
- Hirano S, Kitauchi F, Haruki M, Imanaka T, Morikawa M, Kanaya S. 2004. Isolation and characterization of *Xanthobacter polyaromaticivorans* sp. nov. 127W that degrades polycyclic and heterocyclic aromatic compounds under extremely low oxygen conditions. *Biosci Biotechnol Biochem* 68:557–564. <https://doi.org/10.1271/bbb.68.557>.
- Wiegel J. 2006. The genus *Xanthobacter*, p 290–314. In Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (ed), *The prokaryotes*, vol 5. *Proteobacteria: alpha and beta subclasses*, 3rd ed. Springer, New York, NY.
- Oren A. 2014. The family *Xanthobacteraceae*, p 709–726. In Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (ed), *The prokaryotes: alphaproteobacteria and betaproteobacteria*, 4th ed. Springer, Berlin, Germany.
- Tikhonova EN, Grouzdev DS, Kravchenko IK. 2021. *Xanthobacter oligotrophicus* sp. nov., isolated from paper mill sewage. *Int J Syst Evol Microbiol* 71:e004972. <https://doi.org/10.1099/ijsem.0.004972>.
- Gilbride KA, Fulthorpe RR. 2004. A survey of the composition and diversity of bacterial populations in bleached kraft pulp-mill wastewater secondary treatment systems. *Can J Microbiol* 50:633–644. <https://doi.org/10.1139/w04-031>.
- Li E, Jin X, Lu S. 2018. Microbial communities in biological denitrification system using methanol as carbon source for treatment of reverse osmosis concentrate from coking wastewater. *J Water Reuse Desalination* 8:360–371. <https://doi.org/10.2166/wrd.2017.024>.
- Fatima SA, Goen AE, MacLea KS. 2019. Draft genome sequence of *Xanthobacter tagetidis* ATCC 700314T. *Microbiol Resour Announc* 8:e00242-19. <https://doi.org/10.1128/MRA.00242-19>.
- Pelletier DA, Li Z, Lu T-YS, Zhang L, Hu Z, Morris GP, Glavina del Rio T, Wang D, Chen J-G, Pan C. 2020. Genome sequences of 42 bacteria isolated from *Sorghum bicolor* roots. *Microbiol Resour Announc* 9:e00736-20. <https://doi.org/10.1128/MRA.00736-20>.
- Wang Y, Ma F, Yang J, Guo H, Su D. 2021. *Xanthobacter dioxanivorans* sp. nov., a 1,4-dioxane-degrading bacterium. *Int J Syst Evol Microbiol* 71:e005139. <https://doi.org/10.1099/ijsem.0.005139>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- 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>.
20. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Ciufu S, Li W. 2013. Prokaryotic Genome Annotation Pipeline: the NCBI handbook, 2nd ed. National Center for Biotechnology Information, Bethesda, MD. <https://www.ncbi.nlm.nih.gov/books/NBK174280>.
 21. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210–3212. <https://doi.org/10.1093/bioinformatics/btv351>.
 22. Manni M, Berkeley MR, Seppey M, Simão FA, Zdobnov EM. 2021. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Mol Biol Evol* 38:4647–4654. <https://doi.org/10.1093/molbev/msab199>.
 23. Manni M, Berkeley MR, Seppey M, Zdobnov EM. 2021. BUSCO: assessing genomic data quality and beyond. *Curr Protoc* 1:e323.
 24. 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>.