



Complete Genome Sequence of *Aurantimicrobium minutum* Type Strain KNC^T, a Planktonic Ultramicrobacterium Isolated from River Water

Ryosuke Nakai,^{a,b} Takatomo Fujisawa,^c Yasukazu Nakamura,^{c,d} Hiroyo Nishide,^e Ikuo Uchiyama,^{e,f} Tomoya Baba,^g Atsushi Toyoda,^c Asao Fujiyama,^{c,d,h} Takeshi Naganuma,ⁱ Hironori Niki^{a,d}

Genetic Strains Research Center, National Institute of Genetics, Mishima, Japan^a; Superlative Postdoctoral Research Fellow of the Japan Society for the Promotion of Science, Tokyo, Japan^b; Center for Information Biology, National Institute of Genetics, Mishima, Japan^c; The Graduate University for Advanced Studies (SOKENDAI), Mishima, Japan^d; Data Integration and Analysis Facility, National Institute for Basic Biology, National Institutes of Natural Sciences, Okazaki, Japan^e; Laboratory of Genome Informatics, National Institute for Basic Biology, National Institutes of Natural Sciences, Okazaki, Japan^e; Tansdisciplinary Research Integration Center, Research Organization of Information and Systems, Tokyo, Japan^e; Principles of Informatics Research Division, National Institute of Informatics, Tokyo, Japan^h; Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japanⁱ

Aurantimicrobium minutum type strain KNC^T is a planktonic ultramicrobacterium isolated from river water in western Japan. Strain KNC^T has an extremely small, streamlined genome of 1,622,386 bp comprising 1,575 protein-coding sequences. The genome annotation suggests that strain KNC^T has an actinorhodopsin-based photometabolism.

Received 10 May 2016 Accepted 17 May 2016 Published 30 June 2016

Citation Nakai R, Fujisawa T, Nakamura Y, Nishide H, Uchiyama I, Baba T, Toyoda A, Fujiyama A, Naganuma T, Niki H. 2016. Complete genome sequence of Aurantimicrobium minutum type strain KNC^T, a planktonic ultramicrobacterium isolated from river water. Genome Announc 4(3):e00616-16. doi:10.1128/genomeA.00616-16. Copyright © 2016 Nakai et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license. Address correspondence to Ryosuke Nakai, rnakai@nig.ac.jp.

The phylum Actinobacteria includes members that are often numerically dominant (up to 60%) in the freshwater bacterial communities (1, 2). The actinobacterial Luna2 lineage, currently classified into the acIII lineage (2), has a cosmopolitan distribution in inland freshwater ecosystems (3). The cell morphology of this lineage is typical of ultramicrobacteria (cell volume <0.1 μ m³ [4]). Aurantimicrobium minutum type strain KNC^T was the first described strain with a validly published name within the acIII lineage of Actinobacteria (5) and was isolated from a 0.2 μ m filtrate of river water in western Japan (6). Here, we report the whole-genome sequence of strain KNC^T, with the aim of elucidating the physiological traits that have facilitated such a wide distribution pattern.

The genomic DNA of strain KNC^T was extracted from cells grown in nutrient broth, soytone, yeast extract (NSY) liquid medium (7) using Qiagen Genomic-Tip 100/G columns. Genomic shotgun and fosmid-end sequences were determined using an ABI3730xl sequencer. De novo assembly was conducted with phrap version 1.080812, resulting in a single chromosome with 22.2-fold genome coverage. The total length of the complete genome was 1,622,386 bp with a G+C content of 52.8 mol%. Strain KNC^T has an extremely small, streamlined genome that is consistent with other ultramicrobacteria such as "Candidatus Pelagibacter ubique" (8) (Alphaproteobacteria) and Rhodoluna lacicola (9) (Actinobacteria). The phylum Actinobacteria is generally considered to be comprised of high-G+C Gram-positive bacteria. However, the genomes of freshwater and marine actinobacteria were recently reported to have unusually low G+C contents (9, 10). The genomic G+C content of freshwater strain KNC^T is also quite low.

The genome annotation by the Microbial Genome Annotation

Pipeline online server (11) predicted one copy of 16S-23S-5S rRNA operon, 42 tRNAs, and 1,575 protein-coding sequences. The genome encoded a putative rhodopsin, known as actinorhodopsin (12), suggesting that strain KNC^T has an ability to utilize light energy for supplemental energy generation. Conversely, genes encoding a cytochrome *bd* complex, a respiratory quinol:O₂ oxidoreductase found in many prokaryotes and expressed under oxygen-limited conditions (13), were missing within the genome. Comparative genomic analysis by MBGD (14) revealed that the genomic traits described above are shared with the genome of another ultramicrosized actinobacterium *R. lacicola* (9). In summary, we sequenced the complete genome of a cosmopolitan freshwater ultramicrobacterium. This will provide new insight into genome streamlining and related missing genes of ultramicrobacteria.

Nucleotide sequence accession number. The complete genome sequence of *A. minutum* KNC^T has been deposited in the DDBJ/ENA/GenBank database under the accession no. AP017457. Annotated genome data were deposited with the help of the Genome Refine web service (http://genome.annotation.jp /genomerefine/).

ACKNOWLEDGMENTS

This work was supported by the Systematic Analysis for Global Environmental Change and Life on Earth project promoted by the Transdisciplinary Research Integration Center, under the umbrella of the Research Organization of Information and Systems, Japan. R.N. was supported by a Japan Society for the Promotion of Science (JSPS) Postdoctoral Fellowship for Young Scientists (13J03441). This work was partially funded by a JSPS Grant-in-Aid for Challenging Exploratory Research (15K14424 to R.N.).

Computational resources were partially provided by the NIG super-

computer at ROIS National Institute of Genetics and the Data Integration and Analysis Facility, National Institute for Basic Biology.

FUNDING INFORMATION

This work, including the efforts of Ryosuke Nakai, was funded by Japan Society for the Promotion of Science (JSPS) (13J03441 and 15K14424).

REFERENCES

- Allgaier M, Grossart HP. 2006. Diversity and seasonal dynamics of Actinobacteria populations in four lakes in northeastern Germany. Appl Environ Microbiol 72:3489-3497. http://dx.doi.org/10.1128/ AEM.72.5.3489-3497.2006.
- Newton RJ, Jones SE, Eiler A, McMahon KD, Bertilsson S. 2011. A guide to the natural history of freshwater lake bacteria. Microbiol Mol Biol Rev 75:14–49. http://dx.doi.org/10.1128/MMBR.00028-10.
- 3. Hahn MW, Pöckl M. 2005. Ecotypes of planktonic *Actinobacteria* with identical 16S rRNA genes adapted to thermal niches in temperate, sub-tropical, and tropical freshwater habitats. Appl Environ Microbiol 71: 766–773. http://dx.doi.org/10.1128/AEM.71.2.766-773.2005.
- Duda VI, Suzina NE, Polivtseva VN, Boronin AM. 2012. Ultramicrobacteria: formation of the concept and contribution of ultramicrobacteria to biology [in Russian]. Mikrobiologiia 81:415–427. http://dx.doi.org/ 10.1134/S0026261712040054.
- Nakai R, Baba T, Niki H, Nishijima M, Naganuma T. 2015. Aurantimicrobium minutum gen. nov., sp. nov., a novel ultramicrobacterium of the family Microbacteriaceae, isolated from river water. Int J Syst Evol Microbiol 65:4072–4079. http://dx.doi.org/10.1099/ijsem.0.000541.
- Nakai R, Shibuya E, Justel A, Rico E, Quesada A, Kobayashi F, Iwasaka Y, Shi G-Y, Amano Y, Iwatsuki T, Naganuma T. 2013. Phylogeographic analysis of filterable bacteria with special reference to *Rhizobiales* strains that occur at cryospheric habitats. Antarct Sci 25:219–228. http:// dx.doi.org/10.1017/S0954102012000831.

- Hahn MW, Lünsdorf H, Wu Q, Schauer M, Höfle MG, Boenigk J, Stadler P. 2003. Isolation of novel ultramicrobacteria classified as *Actinobacteria* from five freshwater habitats in Europe and Asia. Appl Environ Microbiol 69:1442–1451. http://dx.doi.org/10.1128/AEM.69.3.1442 -1451.2003.
- Giovannoni SJ, Tripp HJ, Givan S, Podar M, Vergin KL, Baptista D, Bibbs L, Eads J, Richardson TH, Noordewier M, Rappé MS, Short JM, Carrington JC, Mathur EJ. 2005. Genome streamlining in a cosmopolitan oceanic bacterium. Science 309:1242–1245. http://dx.doi.org/10.1126/ science.1114057.
- Hahn MW, Schmidt J, Taipale SJ, Doolittle WF, Koll U. 2014. *Rhodo-luna lacicola* gen. nov., sp. nov., a planktonic freshwater bacterium with stream-lined genome. Int J Syst Evol Microbiol 64:3254–3263. http://dx.doi.org/10.1099/ijs.0.065292-0.
- Kang I, Lee K, Yang SJ, Choi A, Kang D, Lee YK, Cho JC. 2012. Genome sequence of "*Candidatus* Aquiluna" sp. strain IMCC13023, a marine member of the *Actinobacteria* isolated from an arctic fjord. J Bacteriol 194:3550–3551. http://dx.doi.org/10.1128/JB.00586-12.
- 11. Sugawara H, Ohyama A, Mori H, Kurokawa K. 2009. Microbial genome annotation pipeline (MiGAP) for diverse users, Poster s001-1-2. In 20th Int. Conf. Genome Informatics (GIW2009). Yokohama, Japan.
- 12. Sharma AK, Sommerfeld K, Bullerjahn GS, Matteson AR, Wilhelm SW, Jezbera J, Brandt U, Doolittle WF, Hahn MW. 2009. Actinorhodopsin genes discovered in diverse freshwater habitats and among cultivated freshwater *Actinobacteria*. ISME J 3:726–737. http://dx.doi.org/10.1038/ismej.2009.13.
- 13. Borisov VB, Gennis RB, Hemp J, Verkhovsky MI. 2011. The cytochrome *bd* respiratory oxygen reductases. Biochim Biophys Acta **1807**:1398–1413. http://dx.doi.org/10.1016/j.bbabio.2011.06.016.
- Uchiyama I, Mihara M, Nishide H, Chiba H. 2015. MBGD update 2015: microbial genome database for flexible ortholog analysis utilizing a diverse set of genomic data. Nucleic Acids Res 43:D270–D276. http://dx.doi.org/ 10.1093/nar/gku1152.