




Draft Genome Sequence of Calcium-Dependent *Novosphingobium* sp. Strain TCA1, Isolated from a Hot Spring Containing a High Concentration of Calcium Ions

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ABSTRACT Calcium-dependent *Novosphingobium* sp. strain TCA1 was newly isolated from a water sample from a hot spring containing a high concentration of calcium ions. Here, we report the draft genome sequence of this bacterium, which may be the basis for research on calcium ion homeostasis.

We report the draft genome sequence of *Novosphingobium* sp. strain TCA1, which shows calcium-dependent growth. We collected a hot spring sample containing a high concentration of calcium from Tsurumaki-onsen in Japan (35.387668 N, 139.277898 E).

The sample was spread on Tris medium agar plates (pH 7.7) containing 5 mM CaCl₂ and incubated for 2 days at 30°C. Tris medium contained 30 mM Tris base, 7 mM citric acid monohydrate, 0.05% (wt/vol) yeast extract, 50 mM glucose, and 1% (vol/vol) trace elements (1). Dozens of colonies were randomly selected and streaked onto Tris medium agar plates (pH 7.7) with and without 5 mM CaCl₂. Finally, one strain that did not grow on the Tris medium plate but grew on a Tris medium plate containing 5 mM CaCl₂ was successfully isolated. Chromosomal DNA was prepared using the UltraClean microbial DNA isolation kit (Mo Bio Laboratories). A partial 16S rRNA gene of this bacterium was amplified using primers 27F and 1492R (2, 3) and KOD FX Neo DNA polymerase (Toyobo). The amplified 1,366-bp PCR product was ligated with SmaI-digested pGEM-7zf(+) (Promega) and transformed into *Escherichia coli* DH5α competent cells. Sequencing of the positive plasmid was performed using universal primers T7 and SP6. This bacterium appeared to be most closely related to *Novosphingobium guangzhouense* strain SA925, based on partial 16S rRNA gene sequence identity (4), and was named *Novosphingobium* sp. strain TCA1.

Strain TCA1 was grown for 18 h at 30°C in LB containing 5 mM CaCl₂, and genomic DNA was purified using the DNeasy blood and tissue kit (Qiagen), according to the manufacturer's instructions.

For sequencing, a library was constructed using the Nextera DNA library preparation kit (Illumina), with an insert length of approximately 300 bp; the library was then subjected to 100-bp paired-end sequencing on the Illumina HiSeq 2500 system (2 × 100 bp). Adapter sequences and low-quality data (Q score, ≥30; read length, ≥10 bases) were trimmed using Trimmomatic v.0.36 (5); 35 million paired-end reads, with an average length of 100 bases, were obtained. The draft genome sequence of *Novosphingobium* sp. strain TCA1 was 6,173,804 bp, with a GC content of 64.3% for the total length, and was composed of 136 large contigs (>500 bp), as assembled by SPAdes v.3.12.0. The N₅₀ value was 174,423 bp. Automatic annotation was performed using

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Annotated at DFAST (<https://dfast.nig.ac.jp>) (6), which predicted a total of 5,649 protein-coding genes. The product names of the predicted protein-coding genes were revised by DFAST. The rRNAs were predicted by Barrnap, which predicted a total of 3 rRNAs, and tRNAs were predicted by ARAGORN (7), which predicted a total of 53 tRNAs. Software analyses were conducted using default parameter settings.

Data availability. The GenBank accession number for the draft genome sequence of *Novosphingobium* sp. strain TCA1 is [BLJH00000000](https://ncbi.nlm.nih.gov/GenBank/BLJH00000000). Raw sequencing data were deposited in the DDBJ Sequence Read Archive under accession number [DRA009695](https://ncbi.nlm.nih.gov/SRA/DRA009695) (GenBank BioProject number [PRJDB9061](https://ncbi.nlm.nih.gov/BioProject/PRJDB9061) and BioSample number [SAMD00197004](https://ncbi.nlm.nih.gov/BioSample/SAMD00197004)).

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REFERENCES

1. Imazawa R, Takahashi Y, Aoki W, Sano M, Ito M. 2016. A novel type bacterial flagellar motor that can use divalent cations as a coupling ion. *Sci Rep* 6:19773. <https://doi.org/10.1038/srep19773>.
2. Turner S, Pryer KM, Miao VP, Palmer JD. 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *J Eukaryot Microbiol* 46:327–338. <https://doi.org/10.1111/j.1550-7408.1999.tb04612.x>.
3. Lane DJ. 1991. 16S/23S rRNA sequencing, p 115–175. *In* Stackebrandt E, Goodfellow M (ed), *Nucleic acid techniques in bacterial systematics*. John Wiley & Sons, New York, NY.
4. Sha S, Zhong J, Chen B, Lin L, Luan T. 2017. *Novosphingobium guangzhouense* sp. nov., with the ability to degrade 1-methylphenanthrene. *Int J Syst Evol Microbiol* 67:489–497. <https://doi.org/10.1099/ijsem.0.001669>.
5. 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>.
6. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. *Bioinformatics* 34:1037–1039. <https://doi.org/10.1093/bioinformatics/btx713>.
7. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res* 32:11–16. <https://doi.org/10.1093/nar/gkh152>.