



# Draft Genome Sequence of *Nocardioides alcanivorans* NGK65<sup>T</sup>, a Hexadecane-Degrading Bacterium

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**ABSTRACT** The Gram-positive bacterium *Nocardioides alcanivorans* NGK65<sup>T</sup> was isolated from plastic-polluted soil and cultivated on medium with polyethylene as the single carbon source. Nanopore sequencing revealed the presence of candidate enzymes for the biodegradation of polyethylene. Here, we report the draft genome of this newly described member of the terrestrial plastisphere.

The accumulation of plastic debris in aqueous and terrestrial environments has become one of the major challenges of the 21st century and significantly contributes to a growing global pollution problem (1). While plastic debris can generally be degraded slowly by nature through photooxidative and thermooxidative depolymerization processes, the scientific focus has shifted toward the investigation of plastic-associated microorganisms with the ability to break down plastic polymers (2, 3).

*Nocardioides alcanivorans* NGK65<sup>T</sup> is a recently described Gram-negative bacterial strain that was isolated from plastic-polluted soil from an abandoned landfill in eastern Germany (52°02'58.8"N, 12°39'34.8"E) (4). Microbial isolates were enriched in slurries with plastic debris from the landfill and minimal salt medium (MSM) containing cycloheximide (1% of a 1 M cycloheximide solution) but no additional carbon source according to a protocol described by Burd (5). After 3 to 5 days at 27°C, slurries were plated on solid MSM containing 0.1% powdered and UV-weathered polyethylene as an additional carbon source. Single colonies were transferred from polyethylene-containing MSM to MSM with 1% *n*-hexadecane as the only carbon source. In addition, growth on hexadecane as the sole carbon source was verified by monitoring the optical density (600 nm) in liquid MSM. After cultivation on 1/2x LB medium, genomic DNA of strain NGK65<sup>T</sup> was extracted from cell material resuspended in extraction buffer using the UltraClean microbial DNA isolation kit (MoBio, Carlsbad, CA, USA).

High-molecular-weight DNA was prepared without specific size selection using the rapid barcoding sequencing kit (Oxford Nanopore Technologies [ONT], Oxford, UK) and was cleaned using AMPure XP beads (Beckman Coulter, Pasadena, CA). The resulting sequencing library was sequenced using the MinION platform (ONT) and the FLO-MIN106 flow cell. The sequencing ran for 72 h, and the quality was monitored using the MinKNOW interface v21.06.0 (ONT). Default parameters were used for all software unless otherwise specified. Raw sequencing data were base called and demultiplexed with high accuracy using guppy v4.4.2 + 9623c1626 (ONT), resulting in 244,068 raw reads with a median length of 3,758 bp. Assembly and polishing were performed with Flye v2.8.2-b1689 (6) (parameters: –plasmid –meta). Draft genome quality was assessed using the lineage\_wf workflow, and full-length 16S rRNA sequences were recovered using the ssu\_finder tool of CheckM v1.0.13 (7). Draft genome characteristics were assessed using QAST v5.0.2 (8).

The resulting draft genome had a size of 4,918,204 bp across 57 contigs, with a GC content of 68.2%, an  $N_{50}$  value of 125,013 bp, and coverage of 139×. The draft genome was found to be 91.7% complete and 4.5% contaminated. The taxonomic affiliation was inferred

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using GTDB-Tk v1.5.0 (9), which confirmed that the isolate belonged to the genus *Nocardioidea*. The recovered draft genome was compared to other *Nocardioidea* genomes by calculating the average nucleotide identity (ANI) using the JSpeciesWS online tool (10; <https://jspecies.ribohost.com/jspeciesws/>) (accessed 21 September 2021). Results suggested that the strain NGK65<sup>T</sup> draft genome was most closely related to *Nocardioidea daejeonensis* MJ31<sup>T</sup> (QHKZ00000000), which was isolated from a sewage disposal plant (11) (ANI<sub>b</sub>, 78.29%; ANI<sub>m</sub>, 84.75%). A full-length 16S rRNA sequence comparison using NCBI BLAST (12) revealed 98.6% sequence identity to *Nocardioidea daejeonensis* strain MJ31<sup>T</sup> across 1,468 bp.

The annotation of the recovered draft genome using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (13) indicated the presence of putative genes thought to be essential for the microbial breakdown of polyethylene (2, 14–16). The following putative genes were found in the reconstructed *Nocardioidea alcanivorans* NGK65<sup>T</sup> draft genome: alkane 1-monooxygenase (EC 1.14.15.3 [locus tag L1066\_RS06220]) and flavin-dependent monooxygenases (EC 1.14.14 [locus tags L1066\_RS12955, L1066\_RS06710, and L1066\_RS10445]).

**Data availability.** The draft genome of *Nocardioidea alcanivorans* NGK65<sup>T</sup> was deposited in GenBank with the accession number [JAKGRW000000000](https://www.ncbi.nlm.nih.gov/nuclseq/JAKGRW000000000) and the BioProject accession number [PRJNA797358](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA797358). The gene annotation is available via the accession number [NZ\\_JAKGRW010000000](https://www.ncbi.nlm.nih.gov/nuclseq/NZ_JAKGRW010000000). Raw reads are accessible via the accession number [ERR7416542](https://www.ncbi.nlm.nih.gov/nuclseq/ERR7416542), and the 16S rRNA sequence is available via the accession number [ON063448](https://www.ncbi.nlm.nih.gov/nuclseq/ON063448). The strain is available at the German Collection of Microorganisms and Cell Cultures (DSM 113112<sup>T</sup>) and the Netherlands Culture Collection of Bacteria (NCCB 100846<sup>T</sup>).

## REFERENCES

- Zhang K, Hamidian AH, Tubić A, Zhang Y, Fang JKH, Wu C, Lam PKS. 2021. Understanding plastic degradation and microplastic formation in the environment: a review. *J Environ Pollut* 274:116554. <https://doi.org/10.1016/j.envpoll.2021.116554>.
- Mohan N, Montazer Z, Sharma PK, Levin DB. 2020. Microbial and enzymatic degradation of synthetic plastics. *Front Microbiol* 11:580709. <https://doi.org/10.3389/fmicb.2020.580709>.
- MacLean J, Mayanna S, Benning LG, Horn F, Bartholomäus A, Wiesner Y, Wagner D, Liebner S. 2021. The terrestrial plastisphere: diversity and polymer-colonizing potential of plastic-associated microbial communities in soil. *Microorganisms* 9:1876. <https://doi.org/10.3390/microorganisms9091876>.
- Mitzscherling J, MacLean J, Lipus D, Bartholomäus A, Mangelsdorf K, Lipski A, Roddatis V, Liebner S, Wagner D. 2022. *Nocardioidea alcanivorans* sp. nov., a novel hexadecane-degrading species isolated from plastic waste. *Int J Syst Evol Microbiol* 72:005319. <https://doi.org/10.1099/ijsem.0.005319>.
- Burd D. 2008. Plastic not fantastic. *Proj Rep Canada Wide Sci Fair*. [https://www.academia.edu/26009308/Burd\\_Report](https://www.academia.edu/26009308/Burd_Report)
- Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. *Nat Biotechnol* 37:540–546. <https://doi.org/10.1038/s41587-019-0072-8>.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. 2020. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* 36:1925–1927. <https://doi.org/10.1093/bioinformatics/btz848>.
- Richter M, Rosselló-Móra R, Glöckner FO, Peplis J. 2016. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32:929–931. <https://doi.org/10.1093/bioinformatics/btv681>.
- Woo S-G, Srinivasan S, Yang J, Jung Y-A, Kim MK, Lee M. 2012. *Nocardioidea daejeonensis* sp. nov., a denitrifying bacterium isolated from sludge in a sewage-disposal plant. *Int J Syst Evol Microbiol* 62:1199–1203. <https://doi.org/10.1099/ijso.033308-0>.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Tatusova T, Dicuccio M, Badretdin 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>.
- Jeon HJ, Kim MN. 2016. Comparison of the functional characterization between alkane monooxygenases for low-molecular-weight polyethylene biodegradation. *Int Biodeterior Biodegradation* 114:202–208. <https://doi.org/10.1016/j.ibiod.2016.06.012>.
- Ribicic D, Netzer R, Hazen TC, Techtmann SM, Drabløs F, Brakstad OG. 2018. Microbial community and metagenome dynamics during biodegradation of dispersed oil reveals potential key-players in cold Norwegian seawater. *Mar Pollut Bull* 129:370–378. <https://doi.org/10.1016/j.marpolbul.2018.02.034>.
- Landry Z, Swa BK, Herndl GJ, Stepanauskas R, Giovannoni SJ. 2017. SAR202 genomes from the dark ocean predict pathways for the oxidation of recalcitrant dissolved organic matter. *mBio* 8:e00413-17. <https://doi.org/10.1128/mBio.00413-17>.