



# Whole-Genome Sequencing of the Tropical Marine Bacterium *Nocardiopsis dassonvillei* NCIM 5124, Containing the Ectoine Biosynthesis Gene Cluster *ectABC*

Pratik Kadam,<sup>a</sup> Swapnil Kajale,<sup>b\*</sup> Avinash Sharma,<sup>b</sup> Dhiraj Dhotre,<sup>b</sup> Vitthal Barvkar,<sup>c</sup> Yogesh Shouche,<sup>b§</sup>  Smita Zinjarde<sup>a</sup>

<sup>a</sup>Department of Biotechnology (with jointly merged Institute of Bioinformatics and Biotechnology), Savitribai Phule Pune University, Pune, India

<sup>b</sup>National Center for Microbial Resource (NCMR), National Center for Cell Science (NCCS), Pune, India

<sup>c</sup>Department of Botany, Savitribai Phule Pune University, Pune, India

**ABSTRACT** The genome sequence (7,057,619 bp; GC content, 72.07%) of a tropical marine isolate, *Nocardiopsis dassonvillei* NCIM 5124, containing the biomedically and biotechnologically important gene cluster *ectABC* is reported here.

The genus *Nocardiopsis* of the order *Actinomycetales* was first described by Meyer in 1976 (1). The members of this genus were later included in a new family, *Nocardiopsaceae* (2). *Nocardiopsis* species are aerophilic, Gram-positive, non-acid-fast, catalase-positive actinomycetes, with colony characteristics similar to those of *Nocardia* and *Actinomadura* species (3). *Nocardiopsis* spp. have been isolated from saline habitats and produce a variety of bioactive compounds (4–7). A strain of *Nocardiopsis dassonvillei* isolated from oil-contaminated seawater (deposited in the National Collection of Industrial Microorganisms, India, as NCIM 5124), capable of degrading hydrocarbons, producing proteases, and mediating the synthesis of gold nanoparticles, was used in this study (8–11).

The culture was grown on glucose-yeast-malt extract (GYM) agar medium and incubated at 30°C for 48 h. DNA was extracted using the method described by Yeates et al. (12). The modified method is as follows: a single colony was suspended in 500  $\mu$ L of extraction buffer (100 mM Tris-HCl [pH 8.0], 100 mM Na<sub>2</sub>EDTA [pH 8.0]), followed by bead beating for 2 to 3 min with glass beads. Proteinase K (NitroGen, USA) was added (20 mg/mL), and the culture was incubated at 55°C for 2 h with intermittent shaking. An aliquot (100  $\mu$ L) of NaCl (0.5 M) was added, and the culture was incubated at 72°C for 30 min. DNA was extracted using phenol:chloroform:isoamyl alcohol (25:24:1), washed twice with 70% ethanol, dissolved in 1,000  $\mu$ L Tris-EDTA buffer (pH 8.0), analyzed by electrophoresis (0.8% agarose gel), and visualized by ethidium bromide staining using a UV transilluminator.

Library preparation for Illumina was conducted using the Nextera DNA Flex library preparation kit (Illumina Inc., San Diego, CA, USA). Genomic DNA was sequenced on the Illumina MiSeq platform using paired-end (2  $\times$  250-bp) technology with v2 Illumina chemistry (13, 14). The genome quality was evaluated using the FastQC v0.11.9 tool (15); the raw reads were assembled using the Unicycler v0.4.8 assembler and polished using Pilon v1.23 in the PATRIC v3.6.12 online server (16). Genome finishing was performed using the MeDuSa Web server (17). The quality of the assembly was checked using the tools QUAST v5.1.0rc1 (18) and CheckM v1.2.0 (19). The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v6.1 (20). In all, 1,001,347 reads and 483,998,405 bases were generated, with 70% genome coverage. Default parameters were used for all software unless otherwise noted.

The total length of the genome sequence was 7,057,619 bp; sequencing yielded 35 contigs ( $N_{50}$ , 6,954,860 bp), with a GC content of 72.07% and 97.25% completeness. Among the 6,390 total genes, 6,328 coding sequences and 6,130 proteins were identified. In addition, 57 tRNAs and 2 rRNAs (one 16S rRNA and one 23S rRNA) were found. The genome harbors

**Editor** Irene L. G. Newton, Indiana University, Bloomington

**Copyright** © 2022 Kadam et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](#).

Address correspondence to Smita Zinjarde, [smita@unipune.ac.in](mailto:smita@unipune.ac.in).

\*Present address: Swapnil Kajale, Institute of Soil, Water and Environmental Sciences, Rishon LeZion, Israel.

§Present address: Yogesh Shouche, Azim Premji University, Sarjapura, Bengaluru, India.

The authors declare no conflict of interest.

**Received** 6 May 2022

**Accepted** 10 September 2022

**Published** 26 September 2022

genes coding for CRISPR arrays, virulence factors, transporters, drug targets, antibiotic resistance, and ectoine biosynthesis. The genome shows the presence of the *ectABC* gene cluster, which is involved in the synthesis of ectoine, a commercially valuable compatible solute (21–25).

**Data availability.** This whole-genome shotgun sequencing project has been deposited at DDBJ/ENA/GenBank under the accession number [JALPTI000000000](https://doi.org/10.1099/00207713-26-4-487). The version described in this paper is version [JALPTI000000000.1](https://doi.org/10.1099/00207713-26-4-487). The associated BioProject and Sequence Read Archive accession numbers are [PRJNA818875](https://doi.org/10.1099/00207713-26-4-487) and [SRR19025777](https://doi.org/10.1099/00207713-26-4-487).

## ACKNOWLEDGMENTS

We thank the Savitribai Phule Pune University (SPPU) University Grants Commission (UGC) and the SPPU J.R.D. Tata Ph.D. program for funds to carry out this research. We also thank the National Center for Microbial Resource (NCMR) at the National Center for Cell Science (NCCS) for support with genome sequencing.

## REFERENCES

- Meyer J. 1976. *Nocardiopsis*, a new genus of the order Actinomycetales. *Int J Syst Bacteriol* 26:487–493. <https://doi.org/10.1099/00207713-26-4-487>.
- Rainey FA, Ward-Rainey N, Kroppenstedt RM, Stackebrandt E. 1996. The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* 46:1088–1092. <https://doi.org/10.1099/00207713-46-4-1088>.
- Cook AE, Meyers PR. 2003. Rapid identification of filamentous actinomycetes to the genus level using genus-specific 16S rRNA gene restriction fragment patterns. *Int J Syst Evol Microbiol* 53:1907–1915. <https://doi.org/10.1099/ijs.0.02680-0>.
- Bennur T, Kumar AR, Zinjarde S, Javdekar V. 2015. *Nocardiopsis* species: incidence, ecological roles and adaptations. *Microbiol Res* 174:33–47. <https://doi.org/10.1016/j.micres.2015.03.010>.
- Bennur T, Kumar AR, Zinjarde S, Javdekar V. 2014. *Nocardiopsis* species as potential sources of diverse and novel extracellular enzymes. *Appl Microbiol Biotechnol* 98:9173–9185. <https://doi.org/10.1007/s00253-014-6111-y>.
- Bennur T, Kumar AR, Zinjarde SS, Javdekar V. 2016. *Nocardiopsis* species: a potential source of bioactive compounds. *J Appl Microbiol* 120:1–16. <https://doi.org/10.1111/jam.12950>.
- Ibrahim AH, Desoukey SY, Fouad MA, Kamel MS, Gulder TAM, Abdelmohsen UR. 2018. Natural product potential of the genus *Nocardiopsis*. *Mar Drugs* 16:147. <https://doi.org/10.3390/md16050147>.
- Dixit VS, Pant A. 2000. Comparative characterization of two serine endopeptidases from *Nocardiopsis* sp. NCIM 5124. *Biochim Biophys Acta* 1523:261–268. [https://doi.org/10.1016/S0304-4165\(00\)00132-X](https://doi.org/10.1016/S0304-4165(00)00132-X).
- Dixit VS, Pant A. 2000. Hydrocarbon degradation and protease production by *Nocardiopsis* sp. NCIM 5124. *Lett Appl Microbiol* 30:67–69. <https://doi.org/10.1046/j.1472-765x.2000.00665.x>.
- Rohamare SB, Dixit V, Nareddy PK, Sivaramakrishna D, Swamy MJ, GaiKWad SM. 2013. Polyproline fold—in imparting kinetic stability to an alkaline serine endopeptidase. *Biochim Biophys Acta* 1834:708–716. <https://doi.org/10.1016/j.bbapap.2012.12.007>.
- Bennur T, Javdekar V, Tomar GB, Zinjarde S. 2020. Gold nanoparticles biosynthesized by *Nocardiopsis dassonvillei* NCIM 5124 enhance osteogenesis in gingival mesenchymal stem cells. *Appl Microbiol Biotechnol* 104:4081–4092. <https://doi.org/10.1007/s00253-020-10508-z>.
- Yeates C, Gillings MR, Davison AD, Altavilla N, Veal DA. 1997. PCR amplification of crude microbial DNA extracted from soil. *Lett Appl Microbiol* 25:303–307. <https://doi.org/10.1046/j.1472-765x.1997.00232.x>.
- Kajale S, Deshpande N, Pali S, Shouche Y, Sharma A. 2020. *Natrialba swarupiae* sp. nov., a halophilic archaeon isolated from a hypersaline lake in India. *Int J Syst Evol Microbiol* 70:1876–1881. <https://doi.org/10.1099/ijsem.0.003986>.
- Jani K, Kajale S, Shetye M, Palkar S, Sharma A. 2021. *Marisediminicola senii* sp. nov. isolated from Queen Maud Land, Antarctica. *Int J Syst Evol Microbiol* 71. <https://doi.org/10.1099/ijsem.0.004641>.
- Wingett SW, Andrews S. 2018. FastQ Screen: a tool for multi-genome mapping and quality control. *F1000Res* 7:1338. <https://doi.org/10.12688/f1000research.15931.2>.
- Davis JJ, Wattam AR, Aziz RK, Brettn T, Butler R, Butler RM, Chlenski P, Conrad N, Dickerman A, Dietrich EM, Gabbard JL, Gerdes S, Guard A, Kenyon RW, Machi D, Mao C, Olson DM, Nguyen M, Nordberg EK, Olsen GJ, Olson RD, Overbeek JC, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomas C, VanOeffelen M, Vonstein V, Warren AS, Xia F, Xie D, Yoo H, Stevens R. 2020. The PATRIC Bioinformatics Resource Center: expanding data and analysis capabilities. *Nucleic Acids Res* 48:D606–D612. <https://doi.org/10.1093/nar/gkz943>.
- Bosi E, Donati B, Galardini M, Brunetti S, Sagot MF, Lió P, Crescenzi P, Fani R, Fondi M. 2015. MeDuSa: a multi-draft based scaffold. *Bioinformatics* 31:2443–2451. <https://doi.org/10.1093/bioinformatics/btv171>.
- 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>.
- 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>.
- Li W, O'Neill KR, Haft DH, DiCuccio M, Chetvertnin V, Badretdin A, Coulouris G, Chitsaz F, Derbyshire MK, Durkin AS, Gonzales NR, Gwadz M, Lanczycki CJ, Song JS, Thanki N, Wang J, Yamashita RA, Yang M, Zheng C, Marchler-Bauer A, Thibaud-Nissen F. 2021. RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. *Nucleic Acids Res* 49:D1020–D1028. <https://doi.org/10.1093/nar/gkaa1105>.
- Pastor JM, Salvador M, Argandoña M, Bernal V, Reina-Bueno M, Csonka LN, Iborra JL, Vargas C, Nieto JJ, Cánovas M. 2010. Ectoines in cell stress protection: uses and biotechnological production. *Biotechnol Adv* 28:782–801. <https://doi.org/10.1016/j.biotechadv.2010.06.005>.
- Liu M, Liu H, Shi M, Jiang M, Li L, Zheng Y. 2021. Microbial production of ectoine and hydroxyectoine as high-value chemicals. *Microb Cell Fact* 20:76. <https://doi.org/10.1186/s12934-021-01567-6>.
- Mustakhimov II, Reshetnikov AS, Glukhov AS, Khmelenina VN, Kalyuzhnaya MG, Trotsenko YA. 2010. Identification and characterization of Ectr1, a new transcriptional regulator of the ectoine biosynthesis genes in the halotolerant methanotroph *Methylomicrobium alcaliphilum* 20Z. *J Bacteriol* 192:410–417. <https://doi.org/10.1128/JB.00553-09>.
- Gregory GJ, Morreale DP, Carpenter MR, Kalburge SS, Boyd EF. 2019. Quorum sensing regulators AphA and OpaR control expression of the operon responsible for biosynthesis of the compatible solute ectoine. *Appl Environ Microbiol* 85:e01543-19. <https://doi.org/10.1128/AEM.01543-19>.
- Czech L, Höppner A, Kobus S, Seubert A, Riclea R, Dickschat JS, Heider J, Smits SHJ, Bremer E. 2019. Illuminating the catalytic core of ectoine synthase through structural and biochemical analysis. *Sci Rep* 9:364. <https://doi.org/10.1038/s41598-018-36247-w>.