Draft Genome Sequence of *Pseudomonas aeruginosa* Strain N002, Isolated from Crude Oil-Contaminated Soil from Geleky, Assam, India

Abhjit Sarma Roy, Reshita Baruah, Dhrubajyoti Gogoi, Maina Borah, Anil Kumar Singh, Hari Prasanna Deka Boruah

Department of Biotechnology, Council of Scientific and Industrial Research (CSIR)-North East Institute of Science and Technology, Jorhat, Assam, India

Here, we report the draft genome sequence of crude oil-degrading *Pseudomonas aeruginosa* strain N002, isolated from a crude oil-polluted soil sample from Geleky, Assam, India. Multiple genes potentially involved in crude oil degradation were identified.

Received 8 November 2012 Accepted 5 December 2012 Published 7 February 2013

Citation Roy AS, Baruah R, Gogoi D, Borah M, Singh AK, Deka Boruah HP. 2013. Draft genome sequence of *Pseudomonas aeruginosa* strain N002, isolated from crude oilcontaminated soil from Geleky, Assam, India. Genome Announc. 1(1):e00104-12. doi:10.1128/genomeA.00104-12.

Copyright © 2013 Roy et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Anil Kumar Singh, 1010anil@gmail.com, or Hari Prasanna Deka Boruah, dekaboruah@yahoo.com.

nvironmental pollution by petroleum hydrocarbons has become a global issue, with particular importance in places such as Kuwait (1-3), India (4), Libva (5), China (6, 7), and the United States (8). Crude oil contamination occurs quite often as a result of exploration, production, maintenance, transportation, storage, and accidental release, leading to significant ecological impacts (9, 10). Reducing hydrocarbons in a contaminated environment is a significant challenge. Various conventional techniques, e.g., mechanical and chemical, have been utilized in the cleanup of oil spills (11). These techniques require site restoration and are expensive. Consequently, environment-friendly techniques, like microbial degradation that will provide remediation for the degraded landmass and bring about ecorestoration, have been adapted. Many microorganisms capable of degrading crude oil have been reported to date (4, 12, 13). The majority of the crude oil bioremediation reported to date has been carried out with single or mixed bacterial strains that have the ability to grow on crude oil as their sole carbon source (14–17).

Here, we isolated the strain N002 from crude oil-contaminated soil from Geleky, Assam, India, using an enrichment culture method in M1 mineral medium with 2% crude oil as the sole carbon source. In order to isolate pure hydrocarbon-degrading bacteria, a serially diluted inoculum was spread onto solid medium and morphologically distinct colonies were purified in M1 solid agar. Further, the degradation ability of N002 was verified, and good growth was observed to occur independently in all crude oil components, i.e., aliphatic, aromatic, and asphaltene fractions. The N002 strain was confirmed to be *Pseudomonas aeruginosa* (corresponding to accession no. JX035794.1) using 16S rRNA gene PCR and sequencing.

The complete genome sequence of *P. aeruginosa* N002 was obtained by whole-genome shotgun sequencing using the Ion Torrent method. Sequencing was carried out as per the Ion 316 chip sequencing protocol provided in the Ion sequencing kit v.2.0 user guide. The genome sequence was assembled using Genome Sequencer (GS) *de novo* assembler v.2.6. The total number of reads generated using a reference-based approach was 1,074,106, with a mean length of 123 bp. The mapping of Ion Torrent 2.0 highquality reads on the reference genome was performed using Torrent Mapping Alignment Program (TMAP) v.0.0.28 to get a consensus sequence. The open reading frames (ORFs) were predicted using Glimmer (18). tRNAscan-SE (19) and RNAmmer (20) were utilized to predict tRNAs and rRNAs, respectively. The G+C content was determined using GeneMark v.2.5 (21).

The draft genome sequence of *P. aeruginosa* N002 constituted 20.32-fold coverage, comprising 132.42 Mbp with 5,499 annotated genes and representing total genome coverage of 93.34%. The assembled genome sequence consists of 235 *de novo* assembly-based contigs with an average contig size of 2,574 bp and G+C contents ranging from 65 to 70%. The genome contains 11,038 ORFs and 5,429 protein-coding genes, 62 tRNA genes, and 12 rRNA genes. The strain N002 showed 100% homology to *P. aeruginosa* JN661695. However, the genome of strain N002 contained different hydrocarbon degradation-related genes, e.g., genes for alcohol dehydrogenase, alkane 1-monooxygenase, alkane sulfonate monooxygenase, and catecol 1,2-dioxygenase.

Nucleotide sequence accession numbers. The Whole Genome Shotgun project has been deposited in DDBJ/EMBL/GenBank under the accession no. ALBV00000000. The version described in this article is the first version, ALBV01000000.

ACKNOWLEDGMENTS

We thank P. G. Rao, director, North East Institute of Science and Technology (NEIST), Jorhat, Assam, for his encouragement and support.

The work was supported by funding from the CSIR and the Department of Biotechnology (DBT), New Delhi, India.

REFERENCES

- Al-Sarawi M, Massoud M. 1998. Preliminary assessment of oil contamination levels in soils contaminated with oil lakes in the greater Burgan oil fields, Kuwait. Water Air Soil Pollut. 106:493–504.
- Al-Hashem MA, Brain PF, Omar SA. 2007. Effects of oil pollution at Kuwait's greater Al-Burgan oil field on polycyclic aromatic hydrocarbon concentrations in the tissues of the desert lizard *Acanthodactylus scutellatus* and their ant prey. Ecotoxicology 16:551–555.
- 3. ud Din S, Al Dousari A, Literathy P . 2008. Evidence of hydrocarbon contamination from the Burgan oil field, Kuwait: interpretations from thermal remote sensing data. J. Environ. Manage. 86:605–615.
- Gogoi B, Dutta N, Goswami P, Mohan T. 2003. A case study of bioremediation of petroleum-hydrocarbon contaminated soil at a crude oil spill site. Adv. Environ. Res. 7:767–782.

- Abdol Hamid H, Kassim WM, El Hishir A, El-Jawashi SA. 2008. Risk assessment and remediation suggestion of impacted soil by produced water associated with oil production. Environ. Monit. Assess. 145:95–102.
- Xiong ZT, Hu HX, Wang YX, Fu GH, Tan ZQ, Yan GA. 1997. Comparative analyses of soil contaminant levels and plant species diversity at developing and disused oil well sites in Qianjiang oilfield, China. Bull. Environ. Contam. Toxicol. 58:667–672.
- Liang Y, Li G, Van Nostrand JD, He Z, Wu L, Deng Y, Zhang X, Zhou J. 2009. Microarray-based analysis of microbial functional diversity along an oil contamination gradient in oil field. FEMS Microbiol. Ecol. 70:324–333.
- Lundegard P, Johnson P. 2006. Source zone natural attenuation at petroleum hydrocarbon spill sites-II: application to a former oil field. Ground Water Monit. R. 26:93–106.
- Allen JP, Atekwana EA, Atekwana EA, Duris JW, Werkema DD, Rossbach S. 2007. The microbial community structure in petroleumcontaminated sediments corresponds to geophysical signatures. Appl. Environ. Microbiol. 73:2860–2870.
- Paissé S, Coulon F, Goñi-Urriza M, Peperzak L, McGenity TJ, Duran R. 2008. Structure of bacterial communities along a hydrocarbon contamination gradient in a coastal sediment. FEMS Microbiol. Ecol. 66:295–305.
- 11. Frick CM, Farrell RE, Germida JJ. 1999. Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated sites. Petroleum Technology Alliance Canada, Calgary, Canada.
- 12. Das K, Mukherjee AK. 2005. Characterization of biochemical properties and biological activities of biosurfactants produced by *Pseudomonas aeruginosa* mucoid and non-mucoid strains isolated from hydrocarboncontaminated soil samples. Appl. Microbiol. Biotechnol. **69**:192–199.

- Silva RMP, Rodriguez AA, Montes de Oca JMG, Moreno DC. 2006. Biodegradation of crude oil by *Pseudomonas aeruginosa* AT18 strain. Technología Química 1:70–77.
- Solanas AM, Parés R, Bayona JM, Albaigés J. 1984. Degradation of aromatic petroleum hydrocarbons by pure microbial cultures. Chemosphere 13:593–601.
- Palittapongarnpim M, Pokethitiyook P, Upatham ES, Tangbanluekal L. 1998. Biodegradation of crude oil by soil microorganisms in the tropic. Biodegradation 9:83–90.
- Viñas V, Grifoll M, Solanas SJ. 2002. Biodegradation of a crude oil by three microbial consortia of different origins and metabolic capabilities. J. Ind. Microbiol. Biotechnol. 28:252–260.
- 17. Gunn A, Braathen K, Børresen M, Engene B, Kolstad P. 2003. In situ biodegradation of petroleum hydrocarbons in frozen Arctic soils. Cold Regions Sci. Technol. 37:97–120.
- Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res. 27: 4636–4641.
- 19. Schattner P, Brooks AN, Lowe TM. 2005. The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. Nucleic Acids Res. 33:686–689.
- Lagesen K, Hallin P, Rødland EA, Stærfeldt H-H, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100–3108.
- 21. Borodovsky M, McIninch J. 1993. GeneMark: parallel gene recognition for both DNA strands. Comput. Chem. 17:123–133.