




Draft Genome Sequence of Phenol-Degrading *Variovorax boronicumulans* Strain c24

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ABSTRACT We report the draft genome sequence of *Variovorax boronicumulans* strain c24, which was isolated from a soil-inoculated chemostat culture amended with phenol as a sole carbon and energy source. The genome data will provide insights into phenol and other xenobiotic compound degradation mechanisms for bioremediation applications.

The genus *Variovorax* belongs to the *Betaproteobacteria* and lives in soil and water and on plants (1, 2). A chemostat culture was amended with phenol as the sole carbon and energy source and trichloroethene (TCE)-contaminated aquifer soil as the inoculum (3). Strain c24 was isolated and purified aerobically from the chemostat culture using a plating method with MP medium (4) containing 0.5 mM phenol (MP0.5phe). The purity of strain c24 was confirmed by sequencing PCR-amplified 16S rRNA genes (3). Strain c24 is one of the strains exhibiting the highest affinity for TCE (3).

Genomic DNA of strain c24 cultivated in MP0.5phe medium was extracted by phenol-chloroform extraction (5) and fragmented using a Covaris M220 instrument according to the manufacturer's protocol for a 550-bp fragment. A genomic library was constructed using a TruSeq DNA PCR-free library preparation kit (Illumina) according to the manufacturer's instructions and sequenced on the Illumina MiSeq platform to generate 302-bp paired-end reads. The raw reads were cleaned up for quality using Trimmomatic v0.36 (6) by trimming adapter sequences, the 1 or 2 bases off the ends of the reads, low-quality ends with a quality score of less than 15, and reads of less than 150 bp. High-quality sequence fragments (1,071,488 paired-end reads, total of 491.7 Mb, and 70.5-fold coverage of the genome) were then assembled using SPAdes v.3.13.0 (7) with a default set of *k*-mer sizes and options (–careful, –only-assembler, and –cov-cutoff 20), and the contigs smaller than 200 bp were removed.

The draft genome sequence of strain c24 consists of 29 contigs with a total length of 6,973,636 bp, an N_{50} value of 1,080,070 bp, and a G+C content of 68.2%. Average nucleotide identity (ANI) analysis of the strain c24 whole genome using JSpeciesWS (8) showed the highest ANI value (96.8%) with the whole genome of *Variovorax boronicumulans* strain J1 (GenBank accession no. CP023284), suggesting that strain c24 belongs to *V. boronicumulans*. The numbers of protein-coding genes (CDS) and tRNA genes in the genome were predicted to be 6,493 and 63, respectively, by DFAST-core v1.2.0 (9). Sequencing coverages of the contigs were calculated by mapping high-quality reads to all contigs using the Burrows-Wheeler Aligner MEM algorithm (BWA-MEM) v0.7.12-r1039 (10) with default settings and analysis using QualiMap v2.2.1 (11). As a result, Contig20 (GenBank accession no. BKDI01000020.1), occupied by an rRNA gene operon (90%), shows 168.6-fold coverage, which is 2.4 times higher than the

Citation Abdul Aziz FA, Suzuki K, Amano K, Moriuchi R, Dohra H, Tashiro Y, Futamata H. 2020. Draft genome sequence of phenol-degrading *Variovorax boronicumulans* strain c24. *Microbiol Resour Announc* 9:e00597-20. <https://doi.org/10.1128/MRA.00597-20>.

Editor David Rasko, University of Maryland School of Medicine

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Received 22 May 2020

Accepted 21 August 2020

Published 10 September 2020

average coverage of the other contigs (69.9-fold). No other rRNA operons were found on extant contigs. This suggested that strain c24 contained two rRNA gene operons.

Strain c24 contained a multicomponent phenol hydroxylase (12–15), with *ortho*-cleavage metabolic pathways for catechol via catechol 1,2-dioxygenase (16). From the results of annotation, genes related to degradation of aromatic compounds (e.g., naphthalene, several kinds of benzoate compounds, functionally unknown monooxygenase, and dioxygenase) and heavy metal (e.g., Cu, Ag, Ni, As, and Co) transport systems were found.

Data availability. The complete genome sequence of *Variovorax boronicumulans* strain c24 has been deposited in DDBJ/ENA/GenBank under the accession no. [BKDI00000000](https://doi.org/10.1093/bioinformatics/btx713), and the raw sequencing reads have been deposited under the accession no. [DRR189179](https://doi.org/10.1093/bioinformatics/btp698).

ACKNOWLEDGMENTS

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI grant no. 19K22927. It was also supported partially by the JSPS RONPAKU program.

REFERENCES

- Barbara S, Wübbeler JH, Steinbüchel A. 2013. Metabolic characteristics of the species *Variovorax paradoxus*. *Appl Microbiol Biotechnol* 97: 541–560. <https://doi.org/10.1007/s00253-012-4585-z>.
- Willems A, De Ley J, Gillis M, Kersters K. 1991. NOTES: *Comamonadaceae*, a new family encompassing the *Acidovorans* rRNA complex, including *Variovorax paradoxus* gen. nov., comb. nov., for *Alcaligenes paradoxus* (Davis 1969). *Int J Syst Bacteriol* 41:445–450. <https://doi.org/10.1099/00207713-41-3-445>.
- Futamata H, Nagano Y, Watanabe K, Hiraishi A. 2005. Unique kinetic properties of phenol-degrading *Variovorax* strains responsible for efficient trichloroethylene degradation in a chemostat enrichment culture. *Appl Environ Microbiol* 71:904–911. <https://doi.org/10.1128/AEM.71.2.904-911.2005>.
- Watanabe K, Yamamoto S, Hino S, Harayama S. 1998. Population dynamics of phenol-degrading bacteria in activated sludge determined by *gyrB*-targeted quantitative PCR. *Appl Environ Microbiol* 64:1203–1209. <https://doi.org/10.1128/AEM.64.4.1203-1209.1998>.
- Futamata H, Harayama S, Watanabe K. 2001. Group-specific monitoring of phenol hydroxylase genes for a functional assessment of phenol-stimulated trichloroethylene bioremediation. *Appl Environ Microbiol* 67:4671–4677. <https://doi.org/10.1128/aem.67.10.4671-4677.2001>.
- 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>.
- Bankevich A, Nurk S, Antipov D, Gurevich A, Dvorkin M, Kulikov AS, Lesin V, Nikolenko S, Pham S, Prjibelski A, Pyshkin A, Sirotkin A, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Richter M, Rosselló-Móra R, Glöckner FO, Peplies 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>.
- 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>.
- Li HX, Durbin R. 2010. Fast and accurate long-read alignment with Burrows–Wheeler transform. *Bioinformatics* 26:589–595. <https://doi.org/10.1093/bioinformatics/btp698>.
- García-Alcalde F, Okonechnikov K, Carbonell J, Cruz LM, Götz S, Tarazona S, Dopazo J, Meyer TF, Conesa A. 2012. QualiMap: evaluating next-generation sequencing alignment data. *Bioinformatics* 28:2678–2679. <https://doi.org/10.1093/bioinformatics/bts503>.
- Nordlund I, Powlowski J, Shingler V. 1990. Complete nucleotide sequence and polypeptide analysis of multicomponent phenol hydroxylase from *Pseudomonas* sp. strain CF600. *J Bacteriol* 172:6826–6833. <https://doi.org/10.1128/jb.172.12.6826-6833.1990>.
- Suzuki K, Aziz FAA, Inuzuka Y, Tashiro Y, Futamata H. 2016. Draft genome sequence of *Pseudomonas* sp. LAB-08 isolated from trichloroethene-contaminated aquifer soil. *Genome Announc* 4:e00948-16. <https://doi.org/10.1128/genomeA.00948-16>.
- Azwani F, Suzuki K, Honjo M, Tashiro Y, Futamata H. 2017. Draft genome sequence of *Comamonas testosteroni* R2, consisting of aromatic compound degradation genes for phenol hydroxylase. *Genome Announc* 5:e00875-17. <https://doi.org/10.1128/genomeA.00875-17>.
- Suzuki K, Fatma AA, Honjo M, Nishimura T, Masuda K, Minoura A, Kudo Y, Moriuchi R, Dohra H, Tashiro Y, Futamata H. 2018. Draft genome sequence of the phenol-degrading bacterium *Cupriavidus* sp. strain P-10, isolated from trichloroethene-contaminated aquifer soil. *Microbiol Resour Announc* 7:e01009-18. <https://doi.org/10.1128/MRA.01009-18>.
- Tuan NN, Hsieh H-C, Lin Y-W, Huang S-L. 2011. Analysis of bacterial degradation pathways for long-chain alkylphenols involving phenol hydroxylase, alkylphenol monooxygenase and catechol dioxygenase genes. *Bioresour Technol* 102:4232–4240. <https://doi.org/10.1016/j.biortech.2010.12.067>.