



Genome announcement

Complete genome sequence of opine-utilizing *Variovorax* sp. strain PAMC28711 isolated from an Antarctic lichen



So-Ra Han^a, Joo-Ho Lee^a, Seunghyun Kang^b, Hyun Park^{b,c,*}, Tae-Jin Oh^{a,**}

^a Department of BT-Convergent Pharmaceutical Engineering, SunMoon University, #100, Kalsan-ri, Tangjeong-myeon, Asansi, Chungnam 31460, Republic of Korea

^b Korea Polar Research Institute (KOPRI), 213-3, Songdo-dong, Yeonsu-gu, Incheon 21990, Republic of Korea

^c Polar Sciences, University of Science & Technology, Yuseong-gu, Daejeon 34113, Republic of Korea

ARTICLE INFO

Article history:

Received 18 March 2016

Accepted 22 March 2016

Available online 23 March 2016

Keywords:

Variovorax sp. PAMC28711

Complete genome sequence

Opine

Octopine

Antarctica lichen *Himantormia* sp.

ABSTRACT

We report the complete genome sequence of *Variovorax* sp. strain PAMC28711 isolated from the Antarctic lichen *Himantormia* sp. Whole genome sequencing revealed opine oxidase- and octopine dehydrogenase-related gene clusters that are involved in octopine utilization. These data will lead to future genetic and biochemical studies on the unusual catabolic traits of opine and octopine utilization in extremely cold environments.

© 2016 Elsevier B.V. All rights reserved.

Opines are a unique source of carbon rarely used by microorganisms. In plants, opines are produced in neoplasias as a result of infection with soil-inhabiting *Agrobacterium* species, including *Agrobacterium tumefaciens*, *Allorhizobium vitis*, and *Rhizobium rhizogenes* (Savka et al., 2002). These species have been used to study the Opine Concept in *Agrobacterium*-plant interactions (Savka and Farrand, 1997) and for genetic research on opine catabolism (Dessaux et al., 1987). Betaproteobacteria of the genus *Variovorax* are found in many different habitats (Willems et al., 2005), and some strains of *Variovorax paradoxus* have been shown to confer advantages to the ambient flora and a wide variety of catabolic pathways (Maimaiti et al., 2007; Satola et al., 2013). *V. paradoxus* strain TBEA6, which utilizes 3,3'-thiodipropionic acid as its sole source of carbon and energy, has been used as a model organism (Bruland et al., 2009). *V. paradoxus* strain B4, which was first isolated from contaminated soil near an industrial plant, utilizes 2-mercaptosuccinate as its sole source of carbon, sulfur, and energy (Carbajal-Rodriguez et al., 2011). Watanabe et al. (2015) recently reported a role for flavin-containing octopine dehydrogenases in

opine catabolism in several bacterial species. However, to date, there have been no reports on the utilization of opines as a source of carbon and energy in the genus *Variovorax*. Here, we report the complete genome sequence of *Variovorax* sp. strain PAMC28711. These whole genome data may lead to the discovery of key genes involved in opine biosynthesis and increase our understanding of *Variovorax*-lichen interactions.

Variovorax sp. strain PAMC28711 (deposited as PAMC28711 in the Polar and Alpine Microbial Collection, Korea Polar Research Institute, Incheon, Korea) was isolated from *Himantormia* sp., an Antarctic lichen species collected on Barton Peninsula, King George Island, Antarctica (62°13'S, 58°47'W). Genomic DNA was extracted from *Variovorax* sp. PAMC28711 using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA), and the quantity and purity were determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Genome sequencing was performed using PacBio RS II single-molecule real-time (SMRT) sequencing technology (Pacific Biosciences, Menlo Park, CA). Ten-kilobase SMRTbell library inserts were sequenced using SMRT cells. Raw sequence data were generated from 57,392 reads and 747,626,052 bp that were *de novo* assembled using the hierarchical genome-assembly process (HGAP) protocol (Chin et al., 2013) and RS HGAP Assembly 2 in SMRT analysis version 2.3 software (Pacific Biosciences; <https://github.com/PacificBiosciences/SMRT-Analysis>). The complete circular chromosome was 4,316,152 bp with a G + C content

* Corresponding author at: Korea Polar Research Institute (KOPRI), 213-3, Songdo-dong, Yeonsu-gu, Incheon 21990, Republic of Korea.

** Corresponding author.

E-mail addresses: hpark@kopri.re.kr (H. Park), tjoh3782@sunmoon.ac.kr (T.-J. Oh).

Table 1
Genome features of *Variovorax* sp. PAMC28711.

Features	Value
Genome size (bp)	4,316,152
Contig numbers	1
G + C content (%)	65.97
Protein coding genes	4103
rRNA genes	6
tRNA genes	46

of 65.97%. Coding sequences (CDSs) were predicted and annotated with the NCBI Prokaryotic Genome Annotation Pipeline and Rapid Annotation using the Subsystem Technology (RAST) server (Aziz et al., 2008). Predicted gene sequences were translated and searched against the National Center for Biotechnology Information (NCBI) non-redundant database, Clusters of Orthologous Groups (COG), and Kyoto Encyclopedia of Genes and Genomes (KEGG). A total of 4103 CDSs were predicted; the coding region accounted for 92.56% of the *Variovorax* sp. PAMC28711 genome. In addition, 46 tRNA and 6 rRNA were predicted in the complete genome (Table 1).

Sequence analyses revealed that octopine dehydrogenase-related biosynthesis genes were localized as a cluster that included genes encoding 2-oxoglutarate dehydrogenase (*odhA*, AX767_00820), dihydrolipoamide S-succinyltransferase (*odhB*, AX767_00815), and dihydrolipoamide dehydrogenase (*odhC*, AX767_00810). Additionally, genes encoding opine oxidase subunit A (*ooxA*, AX767_04565), opine ABC transporter (AX767_14135), ornithine cyclodeaminase (*ocd*, AX767_17695), and arginase (*arc*, AX767_08420) were identified. An analysis of the whole genome sequence of *Variovorax* sp. PAMC28711 will lead to a better understanding of the Opine Concept in microorganism-lichen interactions in extremely cold environments.

Nucleotide sequence accession numbers

The complete genome information of *Variovorax* sp. PAMC28711 was deposited in GenBank under the accession number CP014517.

Acknowledgement

This work was supported by the Antarctic organisms: Cold-Adaptation Mechanisms and its application grant (PE16070) funded by the Korea Polar Research Institute.

References

- Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Glass, E.M., Kubal, M., Meyer, F., Olsen, G.J., Olson, R., Osterman, A.L., Overbeek, R.A., McNeil, L.K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G.D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., Zagnitko, O., 2008. The RAST Server: rapid annotations using subsystems technology. *BMC Genom.* 9, 75.
- Bruland, N., Wübbeler, J.H., Steinbüchel, A., 2009. 3-Mercaptopropionate dioxygenase, a cysteine dioxygenase homologue, catalyzes the initial step of 3-mercaptopropionate catabolism in the 3,3-thiodipropionic acid-degrading bacterium *Variovorax paradoxus*. *J. Biol. Chem.* 284, 660–672.
- Carbajal-Rodríguez, I., Stöveken, N., Satola, B., Wübbeler, J.H., Steinbüchel, A., 2011. Aerobic degradation of mercaptosuccinate by the Gram-negative bacterium *Variovorax paradoxus* strain B4. *J. Bacteriol.* 193, 527–539.
- Chin, C.-S., Alexander, D.H., Marks, P., Klammer, A.A., Drake, J., Heiner, C., Clum, A., Copeland, A., Huddleston, J., Eichler, E.E., 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat. Methods* 10, 563–569.
- Dessaux, Y., Tempé, J., Farrand, S., 1987. Genetic analysis of mannityl opine catabolism in octopine-type *Agrobacterium tumefaciens* strain 15955. *Mol. Genet. Genom.* 208, 301–308.
- Maimaiti, J., Zhang, Y., Yang, J., Cen, Y.-P., Layzell, D.B., Peoples, M., Dong, Z., 2007. Isolation and characterization of hydrogen-oxidizing bacteria induced following exposure of soil to hydrogen gas and their impact on plant growth. *Environ. Microbiol.* 9, 435–444.
- Satola, B., Wübbeler, J.H., Steinbüchel, A., 2013. Metabolic characteristics of the species *Variovorax paradoxus*. *Appl. Microbiol. Biotechnol.* 97, 541–560.
- Savka, M.A., Farrand, S.K., 1997. Modification of rhizobacterial populations by engineering bacterium utilization of a novel plant-produced resource. *Nat. Biotechnol.* 15, 363–368.
- Savka, M.A., Dessaux, Y., Oger, P., Rossbach, S., 2002. Engineering bacterial competitiveness and persistence in the phytosphere. *Mol. Plant Microbe Interact.* 15, 866–874.
- Watanabe, S., Sueda, R., Fukumori, F., Watanabe, Y., 2015. Characterization of flavin-containing opine dehydrogenase from bacteria. *PLoS One* 10, e0138434.
- Willems, A., Mergaert, J., Swings, J., 2005. Genus X. *Variovorax*. In: Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity, G.M. (Eds.), *Bergey's Manual of Systematic Bacteriology*, Second Edition Volume Two: The Proteobacteria. Part C: The Alpha-, Beta-, Delta and Epsilonproteobacteria. Springer Verlag, Berlin, pp. 732–735.