AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Draft Genome Sequence of the Microcystin-Degrading Bacterium *Novosphingobium* sp. Strain MD-1

Kunihiro Okano,[a] Kazuya Shimizu,[b] Takeshi Saito,[c] Hideaki Maseda,[d] Motoo Utsumi,[b,e] Tomoaki Itayama,[f] Norio Sugiura[b,g]

[a]Department of Biological Environment, Faculty of Bioresource Sciences, Akita Prefectural University, Akita, Japan
[b]Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan
[c]R&D Biological Science Research, Kao Corporation, Wakayama, Japan
[d]National Institute of Advanced Industrial Science and Technology, Osaka, Japan
[e]Microbiology Research Center for Sustainability, University of Tsukuba, Ibaraki, Japan
[f]Graduate School of Engineering, Nagasaki University, Nagasaki, Japan
[g]Malaysia-Japan International Institute of Technology, Universiti Technologi Malaysia, Kuala Lumpur, Malaysia

ABSTRACT This report describes the whole-genome sequence of a microcystin-degrading bacterium, *Novosphingobium* sp. strain MD-1, isolated from a lake in Japan. The *Novosphingobium* sp. strain MD-1 genome had a total length of 4,617,766 bp. Moreover, strain MD-1 showed a conserved microcystin-degrading gene cluster (*mlrA* to *mlrF*), similar to *Sphingopyxis* sp. strain C-1.

Harmful algal blooming cyanobacteria such as *Microcystis* spp. produce microcystin, which is a potent hepatotoxin. Microcystin is very stable (1, 2) but can be degraded by specific enzymes (MlrA, MlrB, MlrC, and MlrD) in microcystin-degrading bacteria (3–6). Lakes and reservoirs that have harmful algal blooms generally have highly alkaline pH (7), and alkaline conditions provide the optimal pH for most microcystin-degrading bacteria (8). However, the microcystin-degrading bacterium *Novosphingobium* sp. strain MD-1 (formerly classified as *Sphingomonas* sp. strain MD-1) prefers a neutral pH (9, 10). Therefore, the whole gene structure may differ greatly from that of other microcystin-degrading bacteria. Here, we report the whole-genome sequence of *Novosphingobium* sp. strain MD-1.

Novosphingobium sp. strain MD-1 was isolated from Lake Kasumigaura in 1999 (9). *Novosphingobium* sp. strain MD-1 has been maintained in a glycerol stock at −80°C. Culturing was conducted using 1:5 peptone-yeast extract (PY) at 28°C, and then total DNA was extracted in the late logarithmic growth phase. Genomic DNA was prepared as a paired-end library (350 bp) with the TruSeq Nano DNA low-throughput (LT) sample preparation kit (Illumina, San Diego, CA, USA) and as a mated-pair library (8 to 10 kb) with a Nextera mate pair sample preparation kit (Illumina). Whole-genome sequencing was carried out using an Illumina HiSeq 2500 system with a paired-end library and a mated-pair library. A total of 22,852,894 reads, averaging 99 bp long, were obtained, for a total of 2,262,436,506 bases of the sequence. The final coverage (depth) of the filtered genome sequence was 146×. All reads were assembled *de novo* using Velvet v1.2.08 (11). Gaps between the resultant 292 contigs were closed using Platanus v1.2.1 (12). The whole genome was annotated using the RAST server (http://rast.nmpdr.org/rast.cgi), which predicted protein-coding sequences (CDSs). Default parameters were used for all software.

The *Novosphingobium* sp. strain MD-1 genome consisted of 34 contigs, with an N_{50} value of 406,083 bp, and had a total length of 4,617,766 bp. The draft genome had a G+C content of 65.80%, with 4,178 CDSs and 60 RNA-coding genes (i.e., 3 sets of rRNA genes and 51 tRNA genes). The annotation revealed that 3,060 CDSs exhibited homol-

Citation Okano K, Shimizu K, Saito T, Maseda H, Utsumi M, Itayama T, Sugiura N. 2020. Draft genome sequence of the microcystin-degrading bacterium *Novosphingobium* sp. strain MD-1. Microbiol Resour Announc 9:e01413-19. https://doi.org/10.1128/MRA.01413-19.

Editor J. Cameron Thrash, University of Southern California

Address correspondence to Kunihiro Okano, k_okano@akita-pu.ac.jp.

Received 5 December 2019
Accepted 28 February 2020
Published 19 March 2020

ogy to genes with known functions; the remaining 1,118 CDSs encoded hypothetical proteins with unknown functions.

Okano et al. reported that microcystin is degraded by six enzymes in *Sphingopyxis* sp. strain C-1, namely, MlrA, MlrB, MlrC, MlrD, MlrE, and MlrF (13). The *mlrF*, *mlrE*, *mlrB*, *mlrD*, *mlrA*, and *mlrC* genes of *Novosphingobium* sp. strain MD-1 were designated with locus tags NMD1_03009 to NMD1_03013, respectively (positions 426855 to 435216), in the genome (GenBank accession number BBXA01000030). MlrA, MlrB, MlrC, MlrD, MlrE, and MlrF had 93.3, 95.7, 93.7, 95.3, 96.8, and 90.7% identities, respectively, to those of *Sphingopyxis* sp. strain C-1 on an amino acid basis, using the Align Sequences Protein BLAST. Therefore, it was suggested that the six genes participated in microcystin degradation independent of the suitable pH for growth.

Data availability. This whole-genome shotgun project was deposited in DDBJ/EMBL/GenBank under accession number BBXA00000000. Raw HiSeq data were deposited in the DDBJ Sequence Read Archive under accession number DRA009321.

ACKNOWLEDGMENTS

This work was supported by the Kakenhi Challenge Project of Akita Prefectural University and a Grant-in-Aid for Scientific Research (B) from JSPS KAKENHI (grant 18H03396).

REFERENCES

1. Tsuji K, Watanuki T, Kondo F, Watanabe MF, Suzuki S, Nakazawa H, Suzuki M, Uchida H, Harada KI. 1995. Stability of microcystins from cyanobacteria–II. Effect of UV light on decomposition and isomerization. Toxicon 33:1619–1631. https://doi.org/10.1016/0041-0101(95)00101-8.
2. Okano K, Maseda H, Sugita K, Saito T, Utsumi M, Maekawa T, Kobayashi M, Sugiura N. 2006. Biochemical characteristics of microcystin LR degradation by typical protease. J Jpn Soc Water Treat Biol 42:27–35. https://doi.org/10.2521/jswtb.42.27.
3. Bourne DG, Riddles P, Jones GJ, Smith W, Blakeley RL. 2001. Characterisation of a gene cluster involved in bacterial degradation of the cyanobacterial toxin microcystin LR. Environ Toxicol 16:523–534. https://doi.org/10.1002/tox.10013.
4. Shimizu K, Maseda H, Okano K, Kurashima T, Kawauchi Y, Xue Q, Utsumi M, Zhang Z, Sugiura N. 2012. Enzymatic pathway for biodegrading microcystin LR in *Sphingopyxis* sp. C-1. J Biosci Bioeng 114:630–634. https://doi.org/10.1016/j.jbiosc.2012.07.004.
5. Kormas KA, Lymperopoulou DS. 2013. Cyanobacterial toxin degrading bacteria: who are they? Biomed Res Int 2013:463894. https://doi.org/10.1155/2013/463894.
6. Maseda H, Shimizu K, Doi Y, Inamori Y, Utsumi M, Sugiura N, Kobayashi M. 2012. MlrA located in the inner membrane is essential for initial degradation of microcystin in *Sphingopyxis* sp. C-1. J Jpn Soc Water Treat Biol 48:99–107. https://doi.org/10.2521/jswtb.48.99.
7. López-Archilla AI, Moreira D, López-García P, Guerrero C. 2004. Phytoplankton diversity and cyanobacterial dominance in a hypereutrophic shallow lake with biologically produced alkaline pH. Extremophiles 8:109–115. https://doi.org/10.1007/s00792-003-0369-9.
8. Okano K, Shimizu K, Kawauchi Y, Maseda H, Utsumi M, Zhang Z, Neilan BA, Sugiura N. 2009. Characteristics of a microcystin-degrading bacterium under alkaline environmental conditions. J Toxicol 2009:954291. https://doi.org/10.1155/2009/954291.
9. Saito T, Sugiura N, Itayama T, Inamori Y, Matsumura M. 2003. Degradation characteristics of microcystins by isolated bacteria from Lake Kasumigaura. J Water Supply Res Technol-Aqua 52:13–18. https://doi.org/10.2166/aqua.2003.0002.
10. Saito T, Okano K, Park HD, Itayama T, Inamori Y, Neilan BA, Burns BP, Sugiura N. 2003. Detection and sequencing of the microcystin LR-degrading gene, *mlrA*, from new bacteria isolated from Japanese lakes. FEMS Microbiol Lett 229:271–276. https://doi.org/10.1016/S0378-1097(03)00847-4.
11. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18:821–829. https://doi.org/10.1101/gr.074492.107.
12. Kajitani R, Toshimoto K, Noguchi H, Toyoda A, Ogura Y, Okuno M, Yabana M, Harada M, Nagayasu E, Maruyama H, Kohara Y, Fujiyama A, Hayashi T, Itoh T. 2014. Efficient de novo assembly of highly heterozygous genomes from whole-genome shotgun short reads. Genome Res 24:1384–1395. https://doi.org/10.1101/gr.170720.113.
13. Okano K, Shimizu K, Maseda H, Kawauchi Y, Utsumi M, Itayama T, Zhang Z, Sugiura N. 2015. Whole-genome sequence of the microcystin-degrading bacterium *Sphingopyxis* sp. strain C-1. Genome Announc 3:e00838-15. https://doi.org/10.1128/genomeA.00838-15.