AMERICAN SOCIETY FOR MICROBIOLOGY
Microbiology Resource Announcements

GENOME SEQUENCES

Draft Genome Sequences of Two *Bacillus* spp. and an *Oceanobacillus* sp. Strain Isolated from Marine Macroalgae

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ABSTRACT We present the draft genome sequences of *Bacillus flexus* strain DMTMMB08, *Bacillus licheniformis* strain DMTMMB10, and *Oceanobacillus picturae* strain DMTMMB24, isolated from marine macroalgae.

In marine environments, *Bacillus* sp. strains are common inhabitants and are known to produce a variety of natural products (1, 2). *Bacillus flexus*, *Bacillus licheniformis*, and *Oceanobacillus picturae* strains are known as alkali-halotolerant bacteria which produce bacteriocins, glycopeptides, exopolysaccharides, serine proteases, cellulases, and polyhydroxyalkanoates, and they are also known for their phosphate-solubilizing capabilities (3–11).

Bacillus flexus strain DMTMMB08 and *Bacillus licheniformis* strain DMTMMB10 were isolated from surface-sterilized tissues of *Sargassum polycystum* L., and *Oceanobacillus picturae* strain DMTMMB24 was isolated from surface-sterilized tissues of *Acanthophora spicifera* L. following the method of Stoltzfus et al. (12) and using Zobell marine agar (Himedia). After 72 h of incubation, individual bacterial colonies were transformed to liquid medium (Zobell marine broth) for overnight growth and then subjected to genomic DNA (gDNA) extraction and purification using a genomic DNA isolation kit (Qiagen). The sequencing library was set up with a TruSeq Nano DNA library preparation kit (Illumina) with gDNA, following the manufacturer's instructions, and sequenced using a NextSeq 500 with 2 × 150-bp read chemistry (Eurofins Genomics, India). The sequenced raw data were processed to obtain high-quality clean reads using Trimmomatic version 0.38; reads with more than 10% quality threshold (QV) $<$20 Phred score and those $<$100 nucleotides (nt) in length were eliminated (13). After removing the adapters and low-quality sequences, 3,049,932 paired-end reads totaling 906,971,477 bases remained for *Bacillus flexus* strain DMTMMB08, 6,368,973 paired-end reads totaling 1,896,742,518 bases remained for *Bacillus licheniformis* strain DMTMMB10, and 6,532,483 paired-end reads totaling 1,945,935,542 bases remained for *Oceanobacillus picturae* strain DMTMMB24. *De novo* genome assembly was performed using the SPAdes genome assembler version 3.11.1 with default parameters (14). The resulting draft genome sequence consists of 189 contigs with a total genome size of 3,463,842 bp (38.1% GC content) for *Bacillus flexus* strain DMTMMB08, 24 contigs with a total genome size of 4,265,203 bp (45.9% GC content) for *Bacillus licheniformis* strain DMTMMB10, and 49 contigs with a total genome size of 3,663,112 bp (39.2% GC content) for *Oceanobacillus picturae* strain DMTMMB24, with average coverages of 257-fold, 416-fold, and 488-fold, respectively. The draft genome sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (15). A total of 3,638 (3,517 protein-coding genes), 4,469 (4,300 protein-coding genes), and 3,678 (3,563 protein-coding genes) genes were predicted in *Bacillus flexus* strain DMTMMB08, *Bacillus licheniformis* strain DMTMMB10, and *Oceanobacillus picturae* strain DMTMMB24, respectively.

Biosynthetic gene cluster (BGC) analysis was carried out using all analytical features

Citation Subramanian M, Maruthamuthu M. 2019. Draft genome sequences of two *Bacillus* spp. and an *Oceanobacillus* sp. strain isolated from marine macroalgae. Microbiol Resour Announc 8:e01417-18. https://doi.org/10.1128/MRA.01417-18.

Editor David A. Baltrus, University of Arizona

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Received 16 October 2018
Accepted 16 January 2019
Published 14 February 2019

of antiSMASH version 4.2.0 (16). Twenty-five BGCs from *Bacillus flexus* DMTMMB08, 33 BGCs from *Bacillus licheniformis* DMTMMB10, and 29 BGCs from *Oceanobacillus picturae* strain DMTMMB24 were predicted, respectively. All three bacteria were found to have the signature of known and putative BGCs, like type 3 polyketides (T3PKs), bacteriocins, siderophore synthases, terpenes, nonribosomal peptides (NRPs), polysaccharides, and fatty acids within them. In *Bacillus flexus* strain DMTMMB08, the novel hydroxamate siderophore signature was noted in the predicted BGCs. The presence of an ectoine BGC, with the capability of extreme osmotic tolerance, and a novel kijanimicin-like antibiotic BGC were found in *Oceanobacillus picturae* strain DMTMMB10. Further insights into these draft genome sequences will help guide natural product discovery from these strains in the future.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers QWLS00000000, QWLT00000000, and QWLU00000000 and the SRA accession numbers SRR8039988, SRR8039989, and SRR8039987 for *Bacillus flexus* DMTMMB08, *Bacillus licheniformis* DMTMMB10, and *Oceanobacillus picturae* strain DMTMMB24, respectively.

ACKNOWLEDGMENTS

This work was funded by UGC-BSR and DST-PURSE, Ministry of Science and Technology, India.

REFERENCES

1. Pakpitcharoen A, Potivejkul K, Kanjanavas P, Areekit S, Chansiri K. 2008. Biodiversity of thermotolerant *Bacillus* sp. producing biosurfactants, biocatalysts, and antimicrobial agents. Sci Asia 3:424–431. https://doi.org/10.2306/scienceasia1513-1874.2008.34.424.
2. Ivanova EP, Vysotskii MV, Svetashev VI, Nedashkovskaya OI, Gorshkova NM, Mikhailov VV, Yumoto N, Shigeri Y, Taguchi T, Yoshikawa S. 1999. Characterization of Bacillus strains of marine origin. Int Microbiol 2:267–271.
3. Trivedi N, Gupta V, Kumar M, Kumari P, Reddy CRK, Jha B. 2011. An alkali-halotolerant cellulase from *Bacillus flexus* isolated from green seaweed *Ulva lactuca*. Carbohydr Polym 83:891–897. https://doi.org/10.1016/j.carbpol.2010.08.069.
4. Divyashree MS, Rastogi NK, Shamala TR. 2009. A simple kinetic model for growth and biosynthesis of polyhydroxyalkanoate in *Bacillus flexus*. New Biotechnol 26:92–98. https://doi.org/10.1016/j.nbt.2009.04.004.
5. Maruthiah T, Esakkiraj P, Immanuel G, Palavesam A. 2014. Alkaline serine protease from marine *Bacillus flexus* APCMST-RS2P: purification and characterization. Curr Biotechnol 3:238–243. https://doi.org/10.2174/22113529401666140528002909.
6. Tareq FS, Kim JH, Lee MA, Lee H-S, Lee YJ, Lee JS, Shin HJ. 2012. Ieodoglucomides A and B from a marine-derived bacterium *Bacillus licheniformis*. Org Lett 14:1464–1467. https://doi.org/10.1021/ol300202z.
7. Cladera-Olivera F, Caron GR, Brandelli A. 2004. Bacteriocin-like substance production by *Bacillus licheniformis* strain P40. Lett Appl Microbiol 38:251–256. https://doi.org/10.1111/j.1472-765X.2004.01478.x.
8. Arena A, Maugeri TL, Pavone B, Iannello D, Gugliandolo C, Bisignano G. 2006. Antiviral and immunoregulatory effect of a novel exopolysaccharide from a marine thermotolerant *Bacillus licheniformis*. Int Immunopharmacol 6:8–13. https://doi.org/10.1016/j.intimp.2005.07.004.
9. Singh RP, Shukla MK, Mishra A, Kumari P, Reddy CRK, Jha B. 2011. Isolation and characterization of exopolysaccharides from seaweed associated bacteria *Bacillus licheniformis*. Carbohydr Polym 84:1019–1026. https://doi.org/10.1016/j.carbpol.2010.12.061.
10. Lee J-S, Lim J-M, Lee KC, Lee J-C, Park Y-H, Kim C-J. 2006. *Virgibacillus koreensis* sp. nov., a novel bacterium from a salt field, and transfer of *Virgibacillus picturae* to the genus *Oceanobacillus* as *Oceanobacillus picturae* comb. nov. with emended descriptions. Int J Syst Evol Microbiol 56:251–257. https://doi.org/10.1099/ijs.0.63734-0.
11. El-Tarabily KA, Youssef T. 2010. Enhancement of morphological, anatomical and physiological characteristics of seedlings of the mangrove *Avicennia marina* inoculated with a native phosphate-solubilizing isolate of *Oceanobacillus picturae* under greenhouse conditions. Plant Soil 332:147–162. https://doi.org/10.1007/s11104-010-0280-y.
12. Stoltzfus JR, So RM, Malarvithi PP, Ladha JK, de Bruijn FJ. 1997. Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. Plant Soil 194:25–36. https://doi.org/10.1023/a:1004298921641.
13. 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.
14. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, 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.
15. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt K, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.
16. Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH. 2015. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 43:W237–W243. https://doi.org/10.1093/nar/gkv437.