**GENOME SEQUENCES** 





## Draft Genome Sequence of the Phosphate-Solubilizing Rhizobacterium *Burkholderia pseudomultivorans* Strain MPSB1, Isolated from a Copper Mined-Out Site

Berna Lou L. Aba-Regis,<sup>a,b</sup> Kristel Mae P. Oliveros,<sup>a</sup> Cherry Ibarra-Romero,<sup>c</sup> Asuncion K. Raymundo,<sup>a,d</sup> Nelly S. Aggangan,<sup>e</sup> Teofila O. Zulaybar,<sup>e</sup> Albert Remus R. Rosana<sup>c</sup>

<sup>a</sup>Microbiology Division, Institute of Biological Sciences, University of the Philippines Los Baños, College, Laguna, Philippines
<sup>b</sup>Department of Biological Sciences, Visayas State University, Baybay City, Leyte, Philippines
<sup>c</sup>Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada
<sup>d</sup>National Academy of Science and Technology Philippines, Bicutan, Taguig City, Philippines
<sup>e</sup>National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, College, Laguna, Philippines

**ABSTRACT** *Burkholderia pseudomultivorans* MPSB1 was isolated from a copper minedout soil sample collected from Mogpog, Marinduque, Philippines. Here, we report the draft genome sequence with predicted gene inventories supporting rhizosphere bioremediation, such as heavy metal tolerance, phosphate solubilization, and siderophore production.

Plant growth-promoting rhizobacteria were isolated and characterized from bioremediated and heavy metal-contaminated environments such as mine sites in Philippines (1–3). Members of the *Burkholderia* species that promote plant growth activities and are normally isolated not from infected patients but from environmental sources have increased remarkably (4). Here, we report the draft genome sequence of *Burkholderia pseudomultivorans* strain MPSB1, isolated from a phytoremediated copper mined-out site in Mogpog, Marinduque, Philippines. The strain was sequenced to provide insights to its potential utilization as a bioremediating inoculum in heavy metal postmining sites in Philippines. A soil sample was taken from a 20-cm depth 10 cm from *Pterocarpus indicus*, the plant used for bioremediation (5).

MPSB1 was isolated by spreading 0.1 ml of 10-fold serial dilutions on National Botanical Research Institutes phosphate (NBRIP) medium containing insoluble tricalcium phosphate (6). The plates were incubated at 37°C for 14 days. Colonies showing halo zones were purified by extensive subculturing on NBRIP medium (7), generating axenic isolate MPSB1. The purity of the isolate was assessed by routine Gram staining, 16S rRNA gene sequencing, and electron microscopy analyses (transmission electron microscopy [TEM] and scanning electron microscopy [SEM]). Genomic DNA was purified from a 48-h culture grown in tryptic soy broth (37°C with shaking at 200 rpm) using the ZymoResearch Quick-DNA fungal/bacterial kit and quantified using NanoDrop and Qubit v2.0. Nextera XT DNA libraries were created and sequenced using the NextSeq reagent kit ( $2 \times 250$  bp) (Illumina, San Diego, CA). FastQC v0.11.8 was used to inspect the quality of the sequences, and quality trimming was based on Phred quality scoring 20 and SolexaQA v3.0 (8). Trimmed reads were de novo assembled using IDBA-UD v1.1.1 (9) implemented in the Microbial Genome Atlas (MiGA) pipeline v0.3.6.2 (10). Genome completeness was assessed using BUSCO v4.1.4 (11). The draft genome sequence was annotated using the NCBI PGAP v4.8 (12). Taxonomic classification was established using the Type (Strain) Genome v0.90 (13) and Microbial Genome Atlas servers, calculating for the digital DNA:DNA hybridization (dDDH) (14) and average nucleotide identity (ANI) (15), respectively.

Romero C, Raymundo AK, Aggangan NS, Zulaybar TO, Rosana ARR. 2021. Draft genome sequence of the phosphate-solubilizing rhizobacterium *Burkholderia pseudomultivorans* strain MPSB1, isolated from a copper mined-out site. Microbiol Resour Announc 10:e01304-20. https://doi.org/10.1128/MRA.01304-20. Editor Kenneth M. Stedman. Portland State

Citation Aba-Regis BLL, Oliveros KMP, Ibarra-

University

**Copyright** © 2021 Aba-Regis et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Berna Lou L. Aba-Regis, bernalou.regis@vsu.edu.ph, Nelly S. Aggangan, nsaggangan@up.edu.ph, or Albert Remus R. Rosana, rosana@ualberta.ca.

Received 14 November 2020 Accepted 10 December 2020 Published 7 January 2021

Predicted biosynthetic metabolite	Contig no.	Coordinates within the contig (nucleotide position)	Similarity to known BGC <sup>a</sup> (%)
Siderophore	14	16571-80539	100 (ornibactin BGC)
Homoserine lactone	15	65458-86066	100 (homoserine lactone BGC)
Bacteriocin	24	6191–17003	50 (linocin BGC)
Terpene	70	19499-39037	50 ( <i>N</i> -acyloxyacyl glutamine BGC)
Arylpolyene	87	1770-25741	50 (polyhydroxyalkanoate BGC)
Phosphonate	103	1–19037	15 (dehydroformidomycin BGC)

<sup>a</sup> BGC, biosynthetic gene cluster.

Secondary bioactive metabolites were predicted using antiSMASH v5.0 (16). Default parameters were used for all software unless otherwise specified.

Paired-end sequencing yielded 1,097,695 reads at  $165 \times$  coverage. The draft genome represented in 145 contigs ( $N_{50}$ , 88,373 bp) has a G+C content of 67.38% and an estimated size of 7,742,780 bp. The genome of MPSB1 showed an ANI of 95.45% with the closest type strain, *Burkholderia pseudomultivorans* DSM 105103<sup>T</sup>.

Genome annotation detected 7,021 coding sequences, 2 rRNA genes, and 59 tRNAs. The genome contains predicted genes supporting rhizosphere-associated processes, including metal scavenging by siderophores, quorum sensing by homoserine lactone, heavy metal efflux pumps, and phosphate solubilization. Table 1 highlights 6 of the 14 predicted biosynthetic gene clusters. The genome also contains predicted biosynthetic gene clusters for the production of bacteriocin (17), biopolymers (polyhydroxyalkanoate and exopolysaccharides) (17, 18), and several terpenoids.

**Data availability.** The whole-genome project for *Burkholderia pseudomultivor ans* MPSB1 has been deposited in DDBJ/ENA/GenBank under accession number JADKRM000000000. The version described in this paper is the first version (JADKRM010000000), under BioProject number PRJNA674354, BioSample number SAMN16631211, and Sequence Read Archive (SRA) number SRR13060793.

## **ACKNOWLEDGMENTS**

B.L.L.A.-R. was supported by a Visayas State University Fellowship and a Commission on Higher Education (CHED) K-to-12 grant, Philippines. K.M.P.O. was supported by a UPLB basic research grant, and A.K.R. received a research fellowship from the National Academy of Science and Technology (NAST), Philippines. N.S.A. and T.O.Z. were both supported by the National Institute of Molecular Biology and Biotechnology (BIOTECH). N.S.A. is also supported by the National Research Council of the Philippines, Department of Science and Technology (NRCP-DOST), through the project Greening mined out areas in the Philippines. C.I.-R. is supported by a Ph.D. fellowship from the Consejo Nacional de Ciencia y Tecnologia (CONACYT), Mexico. A.R.R.R. was supported by a Vanier Canada Graduate Scholarship, Alberta Innovates-Technology Future, and Dorothy and Izaak Walton Killam Memorial Scholarships, Canada.

## REFERENCES

- Llamado AL, Raymundo AK, Aggangan NS, Pampolina NM, Cadiz NM. 2013. Enhanced rhizosphere bacterial population in an abandoned copper mined-out area planted with *Jatropha* interspersed with selected indigenous tree species. J Environ Sci Manag 6:45–55.
- Villegas LC, Llamado AL, Catsao KV, Raymundo AK. 2018. Removal of heavy metals from aqueous solution by biofilm-forming bacteria isolated from mined-out soil in Mogpog, Marinduque, Philippines. Philipp Sci Lett 11:19–27.
- Zarate JT, Aquino GM, Cruz JM, Villa NO, Rosana AR. 2019. Draft genome sequence of *Enterobacter* sp. strain AD2-3, isolated from a postmining site in Benguet, Philippines. Microbiol Resour Announc 8:e00563-19. https:// doi.org/10.1128/MRA.00563-19.
- 4. Eberl L, Vandamme P. 2016. Members of the genus Burkholderia: good and

bad guys. F1000Res 5:1007–1010. https://doi.org/10.12688/f1000research .8221.1.

- Aggangan N, Cadiz N, Llamado A, Raymundo A. 2017. Jatropha curcas for bioenergy and bioremediation in mine tailing area in Mogpog, Marinduque, Philippines. Energy Proc 110:471–478. https://doi .org/10.1016/j.egypro.2017.03.171.
- Kapri A, Tewari L. 2010. Phosphate solubilization potential and phosphatase activity of rhizospheric *Trichoderma* spp. Braz J Microbiol 41:787–795. https://doi.org/10.1590/S1517-83822010005000001.
- Mehta S, Nautiyal CS. 2001. An efficient method for qualitative screening of phosphate-solubilizing bacteria. Curr Microbiol 43:51–56. https://doi .org/10.1007/s002840010259.
- 8. Cox MP, Peterson DA, Biggs PJ. 2010. SolexaQA: at-a-glance quality

assessment of Illumina second-generation sequencing data. BMC Bioinformatics 11:485. https://doi.org/10.1186/1471-2105-11-485.

- Peng Y, Leung HC, Yiu SM, Chin FY. 2012. IDBA-UD: a *de novo* assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28:1420–1428. https://doi.org/10.1093/bioinformatics/bts174.
- Rodriguez-R LM, Gunturu S, Harvey WT, Rosselló-Mora R, Tiedje JM, Cole JR, Konstantinidis KT. 2018. The Microbial Genomes Atlas (MiGA) webserver: taxonomic and gene diversity analysis of Archaea and Bacteria at the whole genome level. Nucleic Acids Res 46:W282–W288. https://doi .org/10.1093/nar/gky467.
- Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210–3212. https://doi.org/ 10.1093/bioinformatics/btv351.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/ 10.1093/nar/gkw569.
- Meier-Kolthoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-3.

- Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. 2013. Genome sequencebased species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. https://doi.org/10.1186/1471 -2105-14-60.
- Yoon SH, Ha SM, Lim JM, Kwon SJ, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286. https://doi.org/10.1007/s10482-017-0844-4.
- Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res 47:W81–W87. https://doi.org/10 .1093/nar/gkz310.
- McClean S, Healy ME, Collins C, Carberry S, O'Shaughnessy L, Dennehy R, Adams Á, Kennelly H, Corbett JM, Carty F, Cahill LA, Callaghan M, English K, Mahon BP, Doyle S, Shinoy M. 2016. Linocin and OmpW are involved in attachment of the cystic fibrosis-associated pathogen *Burkholderia cepacia* complex to lung epithelial cells and protect mice against infection. Infect Immun 84:1424–1437. https://doi.org/10.1128/IAI.01248-15.
- Daas MS, Rosana AR, Acedo JZ, Nateche F, Kebbouche-Gana S, Vederas JC, Case RJ. 2017. Draft genome sequences of *Bacillus cereus* E41 and *Bacillus anthracis* F34 isolated from Algerian salt lakes. Genome Announc 5:e00383-17. https://doi.org/10.1128/genomeA.00383-17.