

Draft Genome Sequence of Bacillus altitudinis Lc5, a Biocontrol and Plant Growth-Promoting Endophyte Strain Isolated from Indigenous Black Rice of Manipur

Momota Potshangbam,a Dinabandhu Sahoo,a Preveen Verma,c Sandhya Verma,c Mohan Chandra Kalita,b Sarangthem Indira Devia

genomeA_{nnouncements™}

aMicrobial Resources Division, Institute of Bioresources and Sustainable Development (IBSD), Department of Biotechnology, Government of India, Imphal, Manipur, India ^bDepartment of Biotechnology, Gauhati University, Guwahati, Assam, India c National Institute of Plant Genome Research, Jawaharlal Nehru University Campus, New Delhi, India

ABSTRACT We report here the 3.6-Mb draft genome of Bacillus altitudinis Lc5, a potential plant growth promoter and an active antagonistic endophyte of black rice. This genome study will provide better insights into the strain's mechanisms for plant growth promotion and biocontrol, thus facilitating its application in organic agriculture.

Acillus altitudinis Lc5 was isolated by conventional microbial isolation techniques from an effectively sterilized leaf tissue of healthy indigenous black rice of Manipur, India. To our knowledge, this is the first Bacillus altitudinis endophyte strain reported from black rice. The strain could tolerate abiotic stress when grown in different experimental conditions and produce major defensive enzymes and growth-promoting factors like beta-1,3-glucanase, siderophore, protease, chitinase, cellulase, P-solubilizing ability, indole acetic acid, nitrogen-fixing ability, and antagonistic activity against the phytopathogens Fusarium oxysporum, Rhizoctonia solani, Sclerotium oryzae, Pyricularia oryzae, and Pythium ultimun. At present, a total of 14 genome sequences of B. altitudinis strains with various functional activities, including biocontrol and plant growthpromoting traits [\(1](#page-1-0)[–](#page-1-1)[3\)](#page-1-2), have been deposited in GenBank.

The genome sequencing was performed on the Illumina HiSeq 2500 platform. Libraries were prepared with Illumina technology, and the adapter sequences were removed using Cutadapt version 1.8 [\(4\)](#page-1-3). All low-quality ($Q < 30$) data were filtered out using Sickle version 1.33 [\(5\)](#page-1-4), and the cleaned reads were subjected to KmerGenie [\(6\)](#page-1-5) to predict the optimal k value and assembly size, which were found to be 27 and 3,760,383 bp, respectively. De novo assembly was performed using ABySS version 2.0.1 [\(7\)](#page-1-6). The draft genome assembly obtained after trimming the paired-end reads comprised 91 scaffolds, with a total length of 3,741,201 bp in 91 scaffolds, with a largest contig size of 885,254 bp, a contig N_{50} size of 691,681 bp, and a G+C content of 41.89%. Genes were predicted from the ABySS-assembled contigs using Glimmer version 3.02 [\(8\)](#page-1-7). The number of predicted genes was 3,950, and the number of genes with a significant BLASTx match (E value $\leq 1 \times 10^{-5}$ and similarity score \geq 40%) was 3,286. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [\(https://www.ncbi.nlm.nih.gov/](https://www.ncbi.nlm.nih.gov/genome/annotation_prok) [genome/annotation_prok\)](https://www.ncbi.nlm.nih.gov/genome/annotation_prok) was used for automatic annotation and prediction of the assembled contigs, which predicted 69 contigs ($L_{50} = 6$), 3,824 genes, 3,679 proteincoding sequences, 74 tRNAs, 24 rRNAs, and 145 pseudogenes.

Annotation of the Lc5 genome revealed several genes and biosynthetic pathways, including antioxidant enzymes, such as catalase, peroxidase, and superoxide dismutase, that support the strain's potential role as a biocontrol agent against factors such as

AMERICAN SOCIETY FOR MICROBIOLOGY

> **Received** 30 May 2018 **Accepted** 30 May 2018 **Published** 28 June 2018

Citation Potshangbam M, Sahoo D, Verma P, Verma S, Kalita MC, Indira Devi S. 2018. Draft genome sequence of Bacillus altitudinis Lc5, a biocontrol and plant growth-promoting endophyte strain isolated from indigenous black rice of Manipur. Genome Announc 6: e00601-18. [https://doi.org/10.1128/genomeA](https://doi.org/10.1128/genomeA.00601-18) [.00601-18.](https://doi.org/10.1128/genomeA.00601-18)

Copyright © 2018 Potshangbam et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0](https://creativecommons.org/licenses/by/4.0/) [International license.](https://creativecommons.org/licenses/by/4.0/)

Address correspondence to Sarangthem Indira Devi, [sidevi1@yahoo.co.in.](mailto:sidevi1@yahoo.co.in)

temperature stress, salinity stress, drought stress, and oxidative stress. Also present in the annotation were gene clusters and pathways related to phosphorous solubilization, iron uptake, cellulose degradation, chitinolytic activity, glucanase, acetoin dehydrogenase, protease, trehalose metabolism, exopolysaccharides, cytokinin, and tryptophan biosynthesis, as well as nitrogen-fixing proteins and genes responsible for sporulation, motility, chemotaxis, and quorum sensing.

Antibiotics and secondary metabolites were analyzed using antiSMASH version 4.1.0 [\(9\)](#page-1-8). Also predicted were 26 gene cluster types comprising a terpene-siderophore, type III polyketide synthase, bacteriocin, nonribosomal peptide synthetase, sactipeptide, and biosynthetic gene clusters of bacilysin, pseudomonine, fengycin, surfactin, and lichenysin. Thus, we conclude that B. altitudinis Lc5 harbors important putative genes, and further exploration will lead to significant contributions toward its sustainable application in organic agricultural practices.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. [QCWN00000000.](https://www.ncbi.nlm.nih.gov/nuccore/QCWN00000000) The version described in this paper is the first version, QCWN01000000.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial support of the Department of Science and Technology and the Department of Biotechnology, Government of India.

The AgriGenome Labs Private Limited, Kerela, India, provided technical assistance for the genome sequencing.

REFERENCES

- 1. Kumaravel S, Thankappan S, Raghupathi S, Uthandi S. 2018. Draft genome sequence of plant growth-promoting and drought-tolerant Bacillus altitudinis FD48, isolated from rice phylloplane. Genome Announc 6(9): e00019-18. [https://doi.org/10.1128/genomeA.00019-18.](https://doi.org/10.1128/genomeA.00019-18)
- 2. Chen Y, Rekha P, Arun A, Shen F, Lai W-A, Young C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33– 41. [https://doi.org/10.1016/j](https://doi.org/10.1016/j.apsoil.2005.12.002) [.apsoil.2005.12.002.](https://doi.org/10.1016/j.apsoil.2005.12.002)
- 3. Budiharjo A, Jeong H, Wulandari D, Lee S, Ryu C-M. 2017. Complete genome sequence of Bacillus altitudinis P-10, a potential bioprotectant against Xanthomonas oryzae pv. oryzae, isolated from rice rhizosphere in Java, Indonesia. Genome Announc 5(48):e01388-17. [https://doi.org/10](https://doi.org/10.1128/genomeA.01388-17) [.1128/genomeA.01388-17.](https://doi.org/10.1128/genomeA.01388-17)
- 4. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17:10 –17.
- 5. Joshi N, Fass J. 2011. Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files, version 1.33. [https://github.com/najoshi/](https://github.com/najoshi/sickle) [sickle.](https://github.com/najoshi/sickle)
- 6. Chikhi R, Medvedev P. 2014. Informed and automated k-mer size selection for genome assembly. Bioinformatics 30:31–37. [https://doi.org/10.1093/](https://doi.org/10.1093/bioinformatics/btt310) [bioinformatics/btt310.](https://doi.org/10.1093/bioinformatics/btt310)
- 7. Jackman SD, Vandervalk BP, Mohamadi H, Chu J, Yeo S, Hammond SA, Jahesh G, Khan H, Coombe L, Warren RL, Birol I. 2017. ABySS 2.0: resourceefficient assembly of large genomes using a Bloom filter. Genome Res 27:768 –777. [https://doi.org/10.1101/gr.214346.116.](https://doi.org/10.1101/gr.214346.116)
- 8. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673– 679. [https://doi.org/10.1093/bioinformatics/btm009.](https://doi.org/10.1093/bioinformatics/btm009)
- 9. 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://](https://doi.org/10.1093/nar/gkv437) [doi.org/10.1093/nar/gkv437.](https://doi.org/10.1093/nar/gkv437)