



Improved Draft Genome Sequence of *Bacillus* sp. Strain YF23, Which Has Plant Growth-Promoting Activity

Ye Xia,^a Seth DeBolt,^b Qin Ma,^c Adam McDermaid,^d Cankun Wang,^d Nicole Shapiro,^e Tanja Woyke,^e Nikos C. Kyrpides^e

^aDepartment of Plant Pathology, The Ohio State University, Columbus, Ohio, USA

^bDepartment of Horticulture, University of Kentucky, Lexington, Kentucky, USA

^cDepartment of Biomedical Informatics, The Ohio State University, Columbus, Ohio, USA

^dDepartment of Agronomy, Horticulture, and Plant Science, South Dakota State University, Brookings, South Dakota, USA

eDepartment of Energy Joint Genome Institute, Walnut Creek, California, USA

ABSTRACT We report here the improved draft genome sequence of *Bacillus* sp. strain YF23, a bacterium originally isolated from switchgrass (*Panicum virgatum*) plants and shown to exhibit plant growth-promoting activity. The genome comprised 5.82 Mbp, containing 5,933 genes, with 193 as RNA genes, and a GC content of 35.10%.

Bacillus is a genus of Gram-positive and rod-shaped bacteria in the phylum *Firmicutes. Bacillus* spp. generally produce endospores, which can help the bacteria survive under stress conditions, such as extreme temperature, or under terrestrial soil conditions, which experience periodic drought stress (1–3). Some strains of *Bacillus* have been reported to promote the growth of different plants through diverse mechanisms (4–6). *Bacillus* sp. strain YF23 originated from switchgrass (*Panicum virgatum* L. var. Alamo) plants, one of the most important biofuel crops (7). *Bacillus* sp. YF23 was isolated from the endophytic compartment of switchgrass, which was growing on a reclaimed coal-mining site in western Kentucky. This bacterium showed significant growth-promoting activity on greenhouse-propagated switchgrass plants, indicating its potential to benefit the host plant under certain conditions and increase the yield and/or fitness of the biofuel crop (7). The aim of this study was to generate the genome of *Bacillus* sp. YF23 found in the endophytic compartment, as this may provide clues into its metabolic features and mechanisms for host interaction.

The switchgrass plants were collected from a coal-mining site in Kentucky (7, 8). Then, the shoots and roots of the switchgrass plants were cut into 3- to 5-cm segments and were sterilized with 20 to 30% Clorox bleach for 15 min to kill the surface-localized microbes. The segments were washed with the sterilized water 3 to 5 times. Further, the plant samples were cut into 1- to 1.5-cm segments and put on the plates with the tryptic soy agar medium (Sigma, USA). The plates were incubated in an incubator with a constant temperature of 26°C for 3 to 5 days. Bacterial strains from different tissues were isolated and further purified by growing them on the tryptic soy agar medium plates 2 to 3 times. One of the isolates, Bacillus sp. YF23, was then obtained and further purified (7, 8). For DNA extraction, Bacillus sp. YF23 was first cultured in the tryptic soy broth medium (Sigma) and grown on a shaker at room temperature for 1 to 2 days. Then, the broth containing bacterial cells was centrifuged, and the cell pellets were used for DNA extraction. The genomic DNA was extracted by using the cetyltrimethylammonium bromide (CTAB) approach developed by the Department of Energy Joint Genome Institute (DOE-JGI [9]). The genomic DNA was sequenced at the DOE-JGI using Pacific Biosciences (PacBio) technology. The PacBio SMRTbell library was constructed and sequenced with $86 \times$ depth (10).

Citation Xia Y, DeBolt S, Ma Q, McDermaid A, Wang C, Shapiro N, Woyke T, Kyrpides NC. 2019. Improved draft genome sequence of *Bacillus* sp. strain YF23, which has plant growth-promoting activity. Microbiol Resour Announc 8:e00099-19. https://doi.org/10.1128/ MRA.00099-19.

Editor David Rasko, University of Maryland School of Medicine

Copyright © 2019 Xia et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Ye Xia, xia.374@osu.edu.

Received 29 January 2019 Accepted 18 March 2019 Published 11 April 2019



FIG 1 Circular representation of the *Bacillus* sp. YF23 genome generated using the Circos software. Features include the following: ring 1, 7 contigs of genome sequences; ring 2, Clusters of Orthologous Groups (COG)-annotated coding sequences; ring 3, TIGRFAM-annotated coding sequences; ring 4, KEGG orthology regions; ring 5, Pfam-annotated genes; ring 6, transmembrane helix regions; ring 7, GC content, with blue indicating above and black indicating below the genome average of 35.1%, with a 5-kb window; ring 8, GC skew, with red indicating above and black indicating below zero, with a 5-kb window; ring 9, RNA genes.

A total of 5,820,595 genome sequence reads were generated for *Bacillus* sp. YF23, yielding an assembly of 7 contigs (Fig. 1), by using Circos software analysis with the default settings (11). The code in its entirety, including specific parameters and settings, used to generate Fig. 1 can be found in a GitHub repository (see https://github.com/ Wang-Cankun/Bacillus-sp.-YF23-Circos-scripts). The average read length for raw reads of >5 kb was 7,849 bp. Reads were assembled, quality controlled, and error corrected using HGAP version 2.3.0 with the default settings (12). The scaffold N_{50} value is 2 Mb. The genome annotation was carried out using the JGI Integrated Microbial Genome (IMG) system (13). Genes were identified using Prodigal 2.5 (14). The genome contains a total of 5,933 genes and has 35.10% GC content. The numbers of total protein-coding genes and protein-coding genes with predicted function are 5,740 and 4,670, respectively. The numbers of genes in biosynthetic clusters and genes coding signal peptides are 568 and 268, respectively. A total of 193 RNA genes were identified. Among them, 44 are rRNA genes, 116 are tRNA genes, and 33 are other RNA genes. For the rRNA genes, 14 are 55 rRNA, 14 are 165 rRNA, and 16 are 235 rRNA (Fig. 1).

The genome information provides insight into the functional mechanisms and application of this beneficial bacterium in enhancing switchgrass plant growth and health for biofuel production.

Data availability. The whole-genome sequence has been deposited at DDBJ/EMBL/ GenBank under the accession no. PRJNA243950. The version described in this paper is the first version. The associated sequence data can also be found at the Joint Genome Institute (JGI) portal with the IMG taxon oid no. 2603880214 (https://genome.jgi.doe.gov/portal/ BacillusspYF23_FD/BacillusspYF23_FD.info.html).

ACKNOWLEDGMENTS

The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported under contract no. DE-AC02-

05CH11231. This work is also partially supported by the Hatch Project from grant USDA-NIFA-OHO01392 and OHOA1615 fund from the Ohio Agricultural Research and Development Center (OARDC) and The Ohio State University.

REFERENCES

- 1. Kumar P, Dubey RC, Maheshwari DK. 2012. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiol Res 167:493–499. https://doi.org/10 .1016/j.micres.2012.05.002.
- Sharmin F, Wakelin S, Huygens F, Hargreaves M. 2013. Firmicutes dominate the bacterial taxa within sugar-cane processing plants. Sci Rep 3:3107. https://doi.org/10.1038/srep03107.
- Sella SRBR, Vandenberghe LPS, Soccol CR. 2014. Life cycle and spore resistance of spore-forming *Bacillus atrophaeus*. Microbiol Res 169: 931–939. https://doi.org/10.1016/j.micres.2014.05.001.
- Ortíz-Castro R, Valencia-Cantero E, López-Bucio J. 2008. Plant growth promotion by *Bacillus megaterium* involves cytokinin signaling. Plant Signal Behav 3:263–265. https://doi.org/10.4161/psb.3.4.5204.
- Meldau DG, Long HH, Baldwin IT. 2012. A native plant growth promoting bacterium, *Bacillus* sp. B55, rescues growth performance of an ethyleneinsensitive plant genotype in nature. Front Plant Sci 3:112. https://doi .org/10.3389/fpls.2012.00112.
- Sharma R, Chauhan A, Shirkot CK. 2015. Characterization of plant growth promoting *Bacillus* strains and their potential as crop protectants against *Phytophthora capsici* in tomato. Biol Agric Hortic 31:230–244. https://doi .org/10.1080/01448765.2015.1009860.
- Xia Y, Greissworth E, Mucci C, Williams MA, De Bolt S. 2012. Characterization of culturable bacterial endophytes of switchgrass (*Panicum virgatum* L.) and their capacity to influence plant growth. Glob Change Biol Bioenergy 5:674–682. https://doi.org/10.1111/j.1757-1707.2012.01208.x.
- 8. Xia Y, Amna A, Opiyo SO. 2018. The culturable endophytic fungal communities of switchgrass grown on a coal-mining site and their

effects on plant growth. PLoS One 13:e0198994. https://doi.org/10.1371/journal.pone.0198994.

- Department of Energy Joint Genome Institute. 2012. Bacterial genomic DNA isolation using CTAB. Department of Energy Joint Genome Institute, Walnut Creek, CA. https://lofdmq2n8tc36m6i46scovo2e-wpengine .netdna-ssl.com/wp-content/uploads/2014/02/JGI-Bacterial-DNA -isolation-CTAB-Protocol-2012.pdf.
- Rhoads A, Au KF. 2015. PacBio sequencing and its applications. Genomics Proteomics Bioinformatics 13:278–289. https://doi.org/10.1016/j.gpb .2015.08.002.
- Krzywinski M, Schein J, Birol İ, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. 2009. Circos: an information aesthetic for comparative genomics. Genome Res 19:1639–1645. https://doi.org/10.1101/gr.092759.109.
- Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/nmeth.2474.
- Chen IMA, Markowitz VM, Chu K, Palaniappan K, Szeto E, Pillay M, Ratner A, Huang J, Andersen E, Huntemann M, Varghese N, Hadjithomas M, Tennessen K, Nielsen T, Ivanova NN, Kyrpides NC. 2017. IMG/M: integrated genome and metagenome comparative data analysis system. Nucleic Acids Res 45:D507–D516. https://doi.org/10.1093/nar/gkw929.
- Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119. https://doi.org/10.1186/1471 -2105-11-119.