



Complete Genome Sequence of Plant Growth-Promoting *Bacillus pumilus* TUAT1

 Shin Okazaki,^a Naoto Sano,^{b*} Tetsuya Yamada,^a Kazuo Ishii,^{a*} Katsuhiko Kojima,^c Salem Djedidi,^c María D. Artigas Ramírez,^d Kun Yuan,^d Motoki Kanekatsu,^a Naoko Ohkama-Ohtsu,^{a*} Yuu Hirose,^e Kenshiro Oshima,^f Masahira Hattori,^f Tadashi Yokoyama^a

^aInstitute of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan

^bGraduate School of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan

^cFaculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

^dInstitute of Global Innovation Research, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan

^eDepartment of Environmental and Life Sciences, Toyohashi University of Technology, Aichi, Japan

^fCenter of Omics and Bioinformatics, Graduate School of Frontier Sciences, University of Tokyo, Tokyo, Japan

ABSTRACT *Bacillus pumilus* TUAT1 was isolated from soil in a university research field. Strain TUAT1 has the ability to promote the growth of plants, including that of rice, and has been commercialized as a biofertilizer. Here, we sequenced and annotated the genome of TUAT1 to understand the molecular mechanisms underlying its plant growth promotion.

We screened plant growth-promoting strains from field soils at the Tokyo University of Agriculture and Technology (Tokyo, Japan) and isolated a strain, TUAT1, which was identified as *Bacillus pumilus* by 16S rRNA gene analysis. TUAT1 promotes the growth of several plants, including that of rice (*Oryza sativa* L.) and *Brassica* species (1–5). An inoculant was developed using TUAT1 that has been commercialized in Japan since 2017. So far, the growth-promoting traits of TUAT1 have not been elucidated. Here, we performed whole-genome sequencing of TUAT1 to understand the molecular mechanisms responsible for the strain's plant growth-promoting ability and effective use as a biofertilizer.

TUAT1 was grown on LB medium (6) at 37°C overnight. The cell pellets were lysed in sodium dodecyl sulfate (final concentration, 1.5%) and proteinase K (final concentration, 1 mg/ml) at 50°C for 1 h, followed by extraction with cetyl trimethyl ammonium bromide (CTAB)-NaCl as described by Wilson (7). Whole-genome sequencing was performed using a 454FLX+ instrument (Roche). A shotgun library and an 8-kb paired-end library were prepared using a GS FLX+ library preparation kit (Roche) and GS FLX paired-end kit (Roche), respectively. These libraries were sequenced on the GS FLX+ instrument, yielding 566,529 shotgun reads (259 Mb) and 188,461 paired-end reads (71 Mb). GS FLX data processing was performed using the Roche GS FLX software (version 2.7). Base-called reads were trimmed and filtered for quality. These reads were assembled using Newbler version 2.8 (Roche) with default parameters, resulting in two scaffolds of 3.7 Mb and 3.0 kbp containing 18 contigs (>500 bp) with an N_{50} contig size of 499 kbp. The assembled data were further polished using sequencing data produced by Gene Analyzer Ix (Illumina, CA), namely, 51,850,857 reads of 102 bp. The remaining 21 gaps were closed by Sanger sequencing.

The length of the whole genome obtained was 3,723,433 bp with a G+C content of 41.4%. Genome annotations were done using the Prokaryotic Genome Annotation Pipeline (PGAP) algorithm of the National Center for Biotechnology Information (NCBI) (8) using default parameters. The annotated genome contained 3,888 genes, with 3,778

Citation Okazaki S, Sano N, Yamada T, Ishii K, Kojima K, Djedidi S, Artigas Ramírez MD, Yuan K, Kanekatsu M, Ohkama-Ohtsu N, Hirose Y, Oshima K, Hattori M, Yokoyama T. 2019. Complete genome sequence of plant growth-promoting *Bacillus pumilus* TUAT1. Microbiol Resour Announc 8:e00076-19. <https://doi.org/10.1128/MRA.00076-19>.

Editor David Rasko, University of Maryland School of Medicine

Copyright © 2019 Okazaki et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Tadashi Yokoyama, tadashiy@cc.tuat.ac.jp.

* Present address: Naoto Sano, Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, Versailles, France; Kazuo Ishii, Biostatistics Center, Kurume University, Fukuoka, Japan; Naoko Ohkama-Ohtsu, Institute of Global Innovation Research, Tokyo University of Agriculture and Technology, Tokyo, Japan.

Received 9 February 2019

Accepted 23 April 2019

Published 23 May 2019

total coding sequences (CDS), 3,732 protein-coding genes, 46 pseudogenes, 81 tRNA genes, 24 rRNA genes, and 5 noncoding RNA genes. The whole-genome structure and 16S rRNA gene sequence of TUAT1 were most similar to those of *B. pumilus* MTCC B6033 (3,763,493 bp, 3,659 genes, 81 tRNAs, 6 rRNA clusters, and 41.4% G+C content), which was originally isolated from soil in India as a biocatalyst for the stereospecific oxidation of β -lactams (9).

TUAT1 has been shown to exert plant growth promotion effects on several plants, but the underlying mechanism has not yet been elucidated. Genome analysis of TUAT1 revealed the presence of several genes that have been reported to be involved in the plant growth promotion effects, including those involved in indole-3-acetic acid synthesis (*ysnE*, locus tag BTUAT1_32950, and *yhcX*, BTUAT1_08910), siderophore biosynthesis protein (BTUAT1_10350), or acetoin metabolism (*acuABC*, locus tags BTUAT1_26940, BTUAT1_26950, and BTUAT1_26960). Interestingly, our preliminary experiments indicated that the spore of TUAT1 exhibited a higher plant growth promotion activity than vegetative cells of TUAT1 (10). Understanding of sporulation and its regulation in TUAT1 would contribute to the development of a better inoculant using TUAT1.

Data availability. The complete genome sequence was deposited in GenBank under the accession number [AP014928](https://doi.org/10.1093/nar/nzab111). The version described in this paper is the first version (AP014928.1). The sequences have been submitted to the Sequence Read Archive under the accession number [PRJNA286715](https://doi.org/10.1093/bioinformatics/btab111).

ACKNOWLEDGMENTS

We thank E. Omori (University of Tokyo) for technical support.

This study was supported by the following funds: (i) MAFF Japan, “Research and development projects for application in promoting new policy of Agriculture Forestry and Fisheries (number 26073C), research and development of new rice cultivation technology using multifunctional biofertilizer in order to increase of grain yield under low fertilizer input condition” (2014 to 2016); (ii) special research fund of MEXT Japan, “Research and development of security and safe crop production to reconstruct agricultural lands in Fukushima prefecture based on novel techniques to remove radioactive compounds using advanced bio-fertilizer and plant protection strategies” (2012 to 2016); and (iii) the Human Resource Development Program in Agricultural Genome Sciences at the Tokyo University of Agriculture and Technology.

REFERENCES

- Aung HP, Djedidi S, Oo AZ, Aye YS, Yokoyama T, Suzuki S, Sekimoto H, Bellingrath-Kimura SD. 2015. Growth and 137Cs uptake of four *Brassica* species influenced by inoculation with a plant growth-promoting rhizobacterium *Bacillus pumilus* in three contaminated farmlands in Fukushima prefecture, Japan. *Sci Tot Environ* 521–522:261–269. <https://doi.org/10.1016/j.scitotenv.2015.03.109>.
- Win KT, Oo AZ, Ohkama-Ohtsu N, Yokoyama T. 2018. *Bacillus pumilus* strain TUAT-1 and nitrogen application in nursery phase promote growth of rice plants under field conditions. *Agronomy* 8:216. <https://doi.org/10.3390/agronomy8100216>.
- Djedidi S, Kojima K, Yamaya H, Ohkama-Ohtsu N, Bellingrath-Kimura SD, Watanabe I, Yokoyama T. 2014. Stable cesium uptake and accumulation capacities of five plant species as influenced by bacterial inoculation and cesium distribution in the soil. *J Plant Res* 127:585–597. <https://doi.org/10.1007/s10265-014-0647-x>.
- Djedidi S, Terasaki A, Aung HP, Kojima K, Yamaya H, Ohkama-Ohtsu N, Bellingrath-Kimura SD, Meunchang P, Yokoyama T. 2015. Evaluation of the possibility to use the plant-microbe interaction to stimulate radioactive 137Cs accumulation by plants in a contaminated farm field in Fukushima, Japan. *J Plant Res* 128:147–159. <https://doi.org/10.1007/s10265-014-0678-3>.
- Djedidi S, Kojima K, Ohkama-Ohtsu N, Bellingrath-Kimura SD, Yokoyama T. 2016. Growth and 137Cs uptake and accumulation among 56 Japanese cultivars of *Brassica rapa*, *Brassica juncea* and *Brassica napus* grown in a contaminated field in Fukushima: effect of inoculation with a *Bacillus pumilus* strain. *J Environ Radioact* 157: 27–37. <https://doi.org/10.1016/j.jenvrad.2016.02.024>.
- Green MR, Sambrook J. 2012. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Wilson K. 1987. Preparation of genomic DNA from bacteria. *Curr Protoc Mol Biol* 2.4.1–2.4.5. <https://doi.org/10.1002/0471142727.mb0204s56>.
- 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>.
- Villanueva J, Switala J, Ivancich A, Loewen PC. 2014. Genome sequence of *Bacillus pumilus* MTCC B6033. *Genome Announc* 2:e00327-14. <https://doi.org/10.1128/genomeA.00327-14>.
- Seerat AY, Ookawa T, Kojima K, Ohkama-Ohtsu N, Maeda M, Djedidi S, Habibi S, Sekimoto H, Abe A, Yokoyama T. 2019. Evaluation of the effects of spores and their heat-treated residues from different *Bacillus* strains on the initial growth of rice plants. *Soil Sci Plant Nutr*. <https://doi.org/10.1080/00380768.2018.1551042>.