



Complete Genome Sequence of *Bacillus subtilis* Strain DKU_NT_03, Isolated from a Traditional Korean Food Using Soybean (Chung-gook-jang) for High-Quality Nattokinase Activity

Hee-Won Jeong,^a Man-Seok Bang,^a Yea-jin Lee,^a Su Ji Lee,^a Sang-Cheol Lee,^a Jang-In Shin,^b  Chung-Hun Oh^{a,b}

^aDepartment of Medical Laser, Graduate School, Dankook University, Cheonan, Choongnam, Republic of Korea

^bDepartment of Oral Physiology, College of Dentistry, Dankook University, Cheonan, Choongnam, Republic of Korea

ABSTRACT We present here the complete genome sequence of *Bacillus subtilis* strain DKU_NT_03 isolated from the traditional Korean food chung-gook-jang, which is made from soybeans. This strain was chosen to identify genetic factors with high-quality nattokinase activity.

Chung-gook-jang is a traditional fermented Korean food that is made from soybeans and thus contains many proteins. The fermentation of the soybeans also produces a viscous, physiologically active substance (1, 2). This substance has been reported to be useful for its antimicrobial, thrombolytic, and immunological activities and, as a result, chung-gook-jang has been attracting attention as a functional food (3–7). This study was conducted to obtain strains with proteolytic capacity and viscosity, which are two of the most important criteria for producing high-quality fermented foods. We found a *Bacillus subtilis* strain, DKU_NT_03, that produces viscous material with high efficiency.

B. subtilis strain DKU_NT_03 was cultivated from traditional Korean food using soybean (chung-gook-jang) and LB agar. Samples were grown and maintained at 37°C in LB broth overnight until they were axenic. DNA was then extracted using the Wizard genomic DNA purification kit (Promega) following the manufacturer's instructions. Whole-genome sequencing was performed on the PacBio RS II sequencing platform (PacBio Biosciences, USA) at Macrogen (Seoul, Republic of Korea) (8).

Sequencing libraries from the genomic DNA extracts were prepared using SMRT Cell 8Pac version 3.0 and the DNA polymerase binding kit P6 and were sequenced using PacBio RS II technology (PacBio Biosciences).

The 113,728 PacBio reads of *B. subtilis* strain DKU_NT_03 were assembled using the Hierarchical Genome Assembly Process version 3.0 (HGAP3) protocol, and the ends of each contig were overlapped to one circular chromosome, which comprised 4,196,031 bp with a GC content of 43.3% and an average sequencing depth of 203× (9–11). The genomes were annotated with Prokka software, and functional categories were predicted with the Rapid Annotations using Subsystems Technology (RAST) version 2.0 server (12, 13). The chromosome contains 4,369 coding sequences, 87 tRNAs, and 30 rRNAs. A plasmid was not found in this strain.

Accession number(s). The complete genome sequence of *B. subtilis* strain DKU_NT_03 was deposited in GenBank under the accession number [CP022891](https://doi.org/10.1128/genomeA.00526-18).

ACKNOWLEDGMENT

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries (IPET) through the High-Value-

Received 11 May 2018 Accepted 13 May 2018 Published 21 June 2018

Citation Jeong H-W, Bang M-S, Lee Y-J, Lee SJ, Lee S-C, Shin J-I, Oh C-H. 2018. Complete genome sequence of *Bacillus subtilis* strain DKU_NT_03, isolated from a traditional Korean food using soybean (chung-gook-jang) for high-quality nattokinase activity. *Genome Announc* 6:e00526-18. <https://doi.org/10.1128/genomeA.00526-18>.

Copyright © 2018 Jeong 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 Jang-In Shin, jishin@dankook.ac.kr, or Chung-Hun Oh, choh@dankook.ac.kr.

H.-W.J. and M.-S.B. contributed equally to this work as co-first authors.

J.-I.S. and C.-H.O. contributed equally to this work.

Added Food Technology Development Program funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA, grant 31606203).

REFERENCES

- Ogawa Y, Hosoyama H, Hamano M, Motai H. 1991. Purification and properties of gamma-glutamyltranspeptidase from *Bacillus subtilis* (natto). *Agric Biol Chem* 55:2971–2977.
- Chettri R, Bhutia MO, Tamang JP. 2016. Poly- γ -glutamic acid (PGA)-producing *Bacillus* species isolated from *Kinema*, Indian fermented soybean food. *Front Microbiol* 7:971. <https://doi.org/10.3389/fmicb.2016.00971>.
- Zheng G, Slavik MF. 1999. Isolation, partial purification and characterization of a bacteriocin produced by a newly isolated *Bacillus subtilis* strain. *Lett Appl Microbiol* 28:363–367. <https://doi.org/10.1046/j.1365-2672.1999.00545.x>.
- Wang Y, Lu WQ, Li DF, Liu XT, Wang HL, Niu S, Piao XS. 2014. Energy and ileal digestible amino acid concentrations for growing pigs and performance of weanling pigs fed fermented or conventional soybean meal. *Asian Australas J Anim Sci* 27:706–716. <https://doi.org/10.5713/ajas.2013.13612>.
- Radnaabazar C, Park CM, Kim JH, Cha J, Song YS. 2011. Fibrinolytic and antiplatelet aggregation properties of a recombinant *Cheonggukjang* kinase. *J Med Food* 14:625–629. <https://doi.org/10.1089/jmf.2010.1233>.
- Omura K, Hitosugi M, Zhu X, Ikeda M, Maeda H, Tokudome S. 2005. A newly derived protein from *Bacillus subtilis natto* with both antithrombotic and fibrinolytic effects. *J Pharmacol Sci* 99:247–251. <https://doi.org/10.1254/jphs.FP0050408>.
- Lee S-J, Rim H-K, Jung J-Y, An H-J, Shin J-S, Cho C-W, Rhee YK, Hong H-D, Lee K-T. 2013. Immunostimulatory activity of polysaccharides from *Cheonggukjang*. *Food Chem Toxicol* 59:476–484. <https://doi.org/10.1016/j.fct.2013.06.045>.
- Tombácz D, Csabai Z, Oláh P, Balázs Z, Likó I, Zsigmond L, Sharon D, Snyder M, Boldogkői Z. 2016. Full-length isoform sequencing reveals novel transcripts and substantial transcriptional overlaps in a herpesvirus. *PLoS One* 11:e0162868. <https://doi.org/10.1371/journal.pone.0162868>.
- Liao YC, Lin S-H, Lin H-H. 2015. Completing bacterial genome assemblies: strategy and performance comparisons. *Sci Rep* 5:8747. <https://doi.org/10.1038/srep08747>.
- 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>.
- Chien J-T, Pakala SB, Geraldo JA, Lapp SA, Humphrey JC, Barnwell JW, Kissinger JC, Galinski MR. 2016. High-quality genome assembly and annotation for *Plasmodium coatneyi*, generated using single-molecule real-time PacBio technology. *Genome Announc* 4(5):e00883-16. <https://doi.org/10.1128/genomeA.00883-16>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.