

Complete Genome Sequence of *Bacillus subtilis* Strain ATCC 6051a, a Potential Host for High-Level Secretion of Industrial Enzymes

Haeyoung Jeong,^{a,b} Young Mi Sim,^c Seung-Hwan Park,^{a,b} Soo-Keun Choi^{a,b}

Super-Bacteria Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Republic of Korea^a; Biosystems and Bioengineering Program, University of Science and Technology (UST), Daejeon, Republic of Korea^b; Korean Bioinformation Center (KOBIC), Daejeon, Republic of Korea^c

***Bacillus subtilis* ATCC 6051a (=KCTC 1028), which is less domesticated than strain 168, is widely used for the secretory expression of industrial enzymes. Herein, we present the complete genome sequence of the *Bacillus subtilis* strain ATCC 6051a.**

Received 20 April 2015 Accepted 27 April 2015 Published 21 May 2015

Citation Jeong H, Sim YM, Park S-H, Choi S-K. 2015. Complete genome sequence of *Bacillus subtilis* strain ATCC 6051a, a potential host for high-level secretion of industrial enzymes. *Genome Announc* 3(3):e00532-15. doi:10.1128/genomeA.00532-15.

Copyright © 2015 Jeong et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Haeyoung Jeong, hyjeong@kribb.re.kr, or Soo-Keun Choi, sookeun@kribb.re.kr.

Bacillus subtilis, the most widely studied Gram-positive bacterium, has long been used for biotechnology applications. Some *Bacillus* species, including *B. subtilis*, are generally regarded as safe (GRAS) organisms, which makes them feasible microorganisms for direct human use. *B. subtilis* is also widely used for the secretory expression of many industrial enzymes and pharmaceutical proteins.

Although *B. subtilis* strain 168 is considered a model system for *B. subtilis* (1), it carries many mutations occurring through domestication, such as mutagenesis from irradiation and selection (2). The complete genome sequence of the wild-type strain ATCC 6051^T was recently determined by a German group (3). In this study, ATCC 6051a (=KCTC 1028, also known as P31K6), a strain that is widely used for the secretory production of industrial enzymes (4–7), was chosen for genome sequencing and analysis. Although the strain name implies the possible derivation of ATCC 6051a from ATCC 6051^T, we could not delineate the origin of ATCC 6051a from the literature, with the exception of the source information from the ATCC website. Differences in antibiotic sensitivity against amoxicillin and nalidixic acid, and fatty acid methyl ester analysis profiles suggests its distinctive feature of ATCC 6051a (http://www.ec.gc.ca/ese-ees/E35B18C1-E940-4204-9500-DAF41BA56F79/DSAR_Micro-organisms_Bacillus_EN.pdf).

The genome sequencing of *B. subtilis* ATCC 6051a was carried out using an Illumina HiSeq 2000 platform. Cells were purchased from the Korean Collection for Type Cultures. Library construction (average insert length of ca. 400 bp) and 101-cycle paired-end sequencing were carried out by the National Instrumentation Center for Environmental Management from Seoul National University (Seoul, Korea). A total of 33,192,942 reads (ca. 3.35 Gb) were quality trimmed, filtered by length, and mapped against the genome sequences of *B. subtilis* strains 168 and ATCC 6051 using the CLC Genomics Workbench version 6.5 (CLC bio). The complete genome sequence of ATCC 6051a was obtained by revising the reference sequences according to the quality-based variants identified by the mapping procedure. Ambiguous bases and larger indels were confirmed through the Sanger sequencing

of the PCR products. The final genome sequence was reconfirmed through a remapping analysis, which did not detect any variants (single nucleotide variants [SNVs], indels, or structural variations) or read breakpoints. The *de novo* assembly of unmapped reads did not suggest the presence of any ATCC 6051a-specific genomic regions. Genome annotation was carried out using the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) service. A complete list of variants among strains 168, ATCC 6051, and ATCC 6051a is available from http://wiki.genoglobe.kr/kribb/ATCC_6051a.

Nucleotide sequence accession number. This complete genome sequence was deposited at DDBJ/EMBL/GenBank under the accession no. CP011115.

ACKNOWLEDGMENTS

We thank Sung Hong Kim for her assistance with the finishing process.

This work was supported by the Military Biodefense Laboratory Program (FDC0901513) and the KRIBB Research Initiative Program, Ministry of Science, ICT and Future Planning, Republic of Korea.

REFERENCES

- Kunst F, Ogasawara N, Moszer I, Albertini AM, Alloni G, Azevedo V, Bertero MG, Bessières P, Bolotin A, Borchert S, Borriss R, Boursier L, Brans A, Braun M, Brignell SC, Bron S, Brouillet S, Bruschi CV, Caldwell B, Capuano V, Carter NM, Choi SK, Cordani JJ, Connerton IF, Cummings NJ, Daniel RA, Denzot F, Devine KM, Dusterhof A, Ehrlich SD, Emmerson PT, Entian KD, Errington J, Fabret C, Ferrari E, Foulger D, Fritz C, Fujita M, Fujita Y, Fuma S, Galizzi A, Galleron N, Ghim SY, Glaser P, Goffeau A, Golightly EJ, Grandi G, Guiseppi G, Guy BJ, Haga K, et al. 1997. The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature* 390:249–256. <http://dx.doi.org/10.1038/36786>.
- Zeigler DR, Prágai Z, Rodriguez S, Chevreux B, Muffler A, Albert T, Bai R, Wyss M, Perkins JB. 2008. The origins of 168, W23, and other *Bacillus subtilis* legacy strains. *J Bacteriol* 190:6983–6995. <http://dx.doi.org/10.1128/JB.00722-08>.
- Kabisch J, Thürmer A, Hübel T, Popper L, Daniel R, Schweder T. 2013. Characterization and optimization of *Bacillus subtilis* ATCC 6051 as an expression host. *J Biotechnol* 163:97–104. <http://dx.doi.org/10.1016/j.jbiotec.2012.06.034>.
- Widner B, Thomas M, Sternberg D, Lammon D, Behr R, Sloma A. 2000. Development of marker-free strains of *Bacillus subtilis* capable of secreting

- high levels of industrial enzymes. *J Ind Microbiol Biotechnol* 25:204–212. <http://dx.doi.org/10.1038/sj.jim.7000051>.
5. Skolpap W, Scharer JM, Douglas PL, Moo-Young M. 2004. Fed-batch optimization of α -amylase and protease-producing *Bacillus subtilis* using Markov chain methods. *Biotechnol Bioeng* 86:706–717. <http://dx.doi.org/10.1002/bit.20079>.
 6. Widner B, Behr R, Von Dollen S, Tang M, Heu T, Sloma A, Sternberg D, Deangelis PL, Weigel PH, Brown S. 2005. Hyaluronic acid production in *Bacillus subtilis*. *Appl Environ Microbiol* 71:3747–3752. <http://dx.doi.org/10.1128/AEM.71.7.3747-3752.2005>.
 7. Shin SK, Kim K-K. 1996. Effect of elevated pressure on α -amylase and protease synthesis by *Bacillus subtilis*. *J Ind Eng Chem* 2:91–96.