



## Draft Genome Sequence of *Bacillus subtilis* Ia1a, a New Strain for Poly- $\gamma$ -Glutamic Acid and Exopolysaccharide Production

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We report here the 4.092-Mb high-quality draft genome assembly of a newly isolated poly- $\gamma$ -glutamic acid–producing strain, *Bacillus subtilis* Ia1a. The genome sequence is considered a critical tool to facilitate the engineering of improved production strains. Exopolysaccharides and many industrially important enzymes can be produced by this new strain utilizing different carbon sources.

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The endospore-forming, rod-shaped aerobic species *Bacillus* subtilis and its relatives are well-characterized Gram-positive bacteria (1). Several *B. subtilis* strains have been isolated from soil and water sources, showing great ability to adapt to a wide spectrum of environments (2). Important industrial enzymes (protease, amylase, etc.) (3) and valuable metabolites (poly- $\gamma$ -glutamic acid [ $\gamma$ PGA], exopolysaccharides [EPSs], and antibiotics) are known to be produced by different strains of this species (4, 5).

Strain Ia1a was isolated from an Egyptian soil sample and is able to produce EPSs utilizing different carbon substrates such as glycerol, glucose, sucrose, molasses, and starch. In the presence of glutamic acid, high-volumetric production of large-molecularsize  $\gamma$ PGA can be achieved by the strain after a relatively short cultivation time (unpublished data). Phylogenetic analysis of the 16S rRNA genes was used to identify strain Ia1a as a new strain of the species *B. subtilis*. Since the release of the first complete genome sequence of *B. subtilis* (6), comparative genomics of assorted *B. subtilis* strains has revealed several interesting metabolic pathways—such as those involved in sporulation and biofilm formation—that confer to *B. subtilis* a remarkable adaptation capability and that contribute to predominance in diverse environments (7).

For isolation of genomic DNA, a single colony of strain Ia1a grown on a medium E plate (8) was used to inoculate 50 mL of the same medium for shake-flask cultivation. The culture was grown at 37°C for 24 h, and harvested cells were then washed with distilled water and used for genomic DNA extraction and purification with a PureLink genomic DNA minikit (Invitrogen). The genomic DNA library was prepared using a Nextera DNA sample preparation kit (Illumina) following the manufacturer's user guide. Subsequently, genomic DNA was subjected to simultaneous fragmentation and addition of adapter sequences. These adapters were utilized during a limited-cycle (five cycles) PCR, in which unique indices were added to the sample. Following library

preparation, the average library size (750 bp) was determined using an Agilent 2100 bioanalyzer (Agilent Technologies). The library was diluted (to 12 pM), and paired-end reads were obtained using the MiSeq system for 600 cycles (Illumina).

The genome of strain Ia1a was assembled *de novo* by MR DNA (Shallowater, TX, USA) using NGen (DNASTAR, Inc.). Draft genome annotation was performed with the RAST server (9). The genome of strain Ia1a comprises 4,092,291 bp, possesses a GC content of 43.8%, and contains 4,201 coding sequences. The closest neighbor strains were *B. subtilis* 168, SC-8, and QB928, respectively. The complete genome sequence of strain Ia1a should facilitate metabolic understanding and subsequent engineering for designing improved strains for  $\gamma$ PGA and EPS production. Moreover, it will enrich the available *B. subtilis* genome library and contribute to a better understanding of these industrially important organisms.

Accession number(s). The annotated genome sequence was deposited in DDBJ/EMBL/GenBank under accession number FMSN00000000. The version described in this paper is the first version, FMSN01000000.

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## REFERENCES

- Wipat A, Harwood CR. 1999. The *Bacillus subtilis* genome sequence: the molecular blueprint of a soil bacterium. FEMS Microbiol Ecol 28:1–9. http://dx.doi.org/10.1111/j.1574-6941.1999.tb00555.x.
- 2. Earl AM, Losick R, Kolter R. 2008. Ecology and genomics of Bacillus

*subtilis*. Trends Microbiol 16:269–275. http://dx.doi.org/10.1016/ j.tim.2008.03.004.

- Van Dijl JM, Hecker M. 2013. Bacillus subtilis: from soil bacterium to super-secreting cell factory. Microb Cell Fact 12:3. http://dx.doi.org/ 10.1186/1475-2859-12-3.
- 4. Schallmey M, Singh A, Ward OP. 2004. Developments in the use of *Bacillus* species for industrial production. Can J Microbiol 50:1–17. http://dx.doi.org/10.1139/w03-076.
- Marvasi M, Visscher PT, Casillas Martinez L. 2010. Exopolymeric substances (EPS) from *Bacillus subtilis*: polymers and genes encoding their synthesis. FEMS Microbiol Lett 313:1–9. http://dx.doi.org/10.1111/j.1574 -6968.2010.02085.x.
- 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, Codani JJ, Connerton IF, Danchin A. 1997. The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. Nature **390**:249–256. http://dx.doi.org/10.1038/36786.

- Vlamakis H, Chai Y, Beauregard P, Losick R, Kolter R. 2013. Sticking together: building a biofilm the *Bacillus subtilis* way. Nat Rev Microbiol 11:157–168. http://dx.doi.org/10.1038/nrmicro2960.
- Leonard CG, Housewright RD, Thorne CB. 1958. Effects of some metallic ions on glutamyl polypeptide synthesis by *Bacillus subtilis*. J Bacteriol 76: 499–503.
- 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. http://dx.doi.org/10.1186/1471-2164-9-75.