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# Complete Genome Sequence of *Bacillus subtilis* Strain CU1050, Which Is Sensitive to Phage $SP\beta$

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The Gram-positive bacterium *Bacillus subtilis* is used as a model organism to study cellular and molecular processes. Here, we announce the complete genomic sequence of *B. subtilis* strain CU1050, derived from *B. subtilis* strain 168. CU1050 has historically been used to study suppressor mutations and phage biology, especially the lysogenic phage SPβ.

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**B***acillus subtilis* has been used to study many fundamental biological processes, including sporulation, competence development, and horizontal gene transfer (1). The temperate phage SP $\beta$ is found in many common laboratory strains of *B. subtilis*, including strain 168. The study of SP $\beta$  was greatly facilitated by the identification of a sensitive strain, *B. subtilis* CU1050 (originally su + 3) (2–8).

We report here the genome sequence of CU1050. This strain is derived from 168 and was mutagenized using 2-aminopurine to generate a nonsense suppressor (9). The suppressor mutation is a T-to-A transversion that changes the anticodon of the gene encoding tRNA-lys in the *trnS* operon from UUU to UUA (10), causing lysine to be inserted at ochre nonsense mutations (11). The strain also supports efficient plaque formation by certain defective mutants of  $\phi e$  (9), the temperate phage SP $\beta$ , for which CU1050 is null (2), and the temperate phage H2 from *Bacillus amyloliquefaciens* (12).

CU1050 has a circular chromosome of 4,056,281 bp. We prepared genomic DNA by phenol-chloroform extraction with RNase A treatment, followed by ethanol precipitation. We sheared DNA using a Covaris sonicator, recovered 300- to 600-bp fragments using a Beckman Coulter SPRIworks system, and obtained 150-bp paired-end reads using an Illumina MiSeq. A total of 1,488,671 reads assembled to the final genome, giving 55-fold mean coverage. We used de novo assembly with Velvet (13) and whole-genome alignment with Mauve (14) to identify potential large differences between CU1050 and strain 168 (accession no. NC\_000964). We used breseq (15) to identify and correct polymorphisms, using 2 rounds of refinement, and then used Geneious (Biomatters) to compare the reads to the resulting sequence for a further round of refinement. Gene prediction was done using the NCBI Prokaryotic Genome Annotation Pipeline (16).

Compared to strain 168 (17, 18), strain CU1050 is null for the mobile genetic elements SP $\beta$  and ICE*Bs1*, has a 4,123-bp deletion in the region from *yozF* to *yoaU*, a 255-bp deletion of the intergenic region from *ydbT* to *ydcA*, a 45-bp in-frame deletion within *codY*, and other smaller deletions. It also contains approximately

3,950 single-nucleotide polymorphisms (SNPs) and indels, most of which are located in discrete regions, including the *panB* to *hepT* hypervariable region (30 kb) (19) and other smaller regions: *ydbM* to *ydcC* (12 kb), *pbuE* to *rrnE* (11.5 kb), *yqfU* to *sigA* (10 kb), *moaB* to *ackA* (2 kb), and *sacA* to *ywcL* (5 kb). We confirmed that CU1050 carries nonsense mutations in *metA* and *thrC* and the previously identified nonsense suppressor mutation.

Different auxotrophic requirements have been reported for CU1050 (3, 9). We found that CU1050 requires supplementation with methionine, threonine, and leucine, but not adenine, when grown in  $S7_{50}$  defined minimal medium (20). Suppression of the *metA* and *thrC* mutations is evidently not sufficient to support normal growth in this medium lacking methionine and threonine.

**Nucleotide sequence accession number.** The complete genome sequence is available from GenBank under accession no. CP014166. Strain CU1050 is available from the Bacillus Genetic Stock Center.

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#### REFERENCES

- 1. Sonenshein AL, Hoch JA, Losick R. 2002. *Bacillus subtilis* and its closest relatives: from genes to cells. ASM Press, Washington, DC.
- Warner FD, Kitos GA, Romano MP, Hemphill HE. 1977. Characterization of SP beta: a temperate bacteriophage from *Bacillus subtilis* 168M. Can J Microbiol 23:45–51. http://dx.doi.org/10.1139/m77-006.
- 3. Zahler SA, Korman RZ, Rosenthal R, Hemphill HE. 1977. *Bacillus subtilis* bacteriophage SP beta: localization of the prophage attachment site, and specialized transduction. J Bacteriol **129**:556–558.
- 4. Rosenthal R, Toye PA, Korman RZ, Zahler SA. 1979. The prophage of SP beta c2dcitK1, a defective specialized transducing phage of *Bacillus subtilis*. Genetics **92**:721–739.

- 5. Spancake GA, Hemphill HE. 1985. Deletion mutants of *Bacillus subtilis* bacteriophage SP beta. J Virol 55:39–44.
- McLaughlin JR, Wong HC, Ting YE, Van Arsdell JN, Chang S. 1986. Control of lysogeny and immunity of *Bacillus subtilis* temperate bacteriophage SP beta by its *d* gene. J Bacteriol 167:952–959.
- Lazarevic V, Düsterhöft A, Soldo B, Hilbert H, Mauël C, Karamata D. 1999. Nucleotide sequence of the *Bacillus subtilis* temperate bacteriophage SPbetac2. Microbiology 145:1055–1067. http://dx.doi.org/10.1099/ 13500872-145-5-1055.
- Abe K, Kawano Y, Iwamoto K, Arai K, Maruyama Y, Eichenberger P, Sato T. 2014. Developmentally regulated excision of the SPbeta prophage reconstitutes a gene required for spore envelope maturation in *Bacillus* subtilis. PLoS Genet 10:e1004636. http://dx.doi.org/10.1371/ journal.pgen.1004636.
- Georgopoulos CP. 1969. Suppressor system in *Bacillus subtilis* 168. J Bacteriol 97:1397–1402.
- Garrity DB, Zahler SA. 1993. The *Bacillus subtilis* ochre suppressor *sup-3* is located in an operon of seven tRNA genes. J Bacteriol 175:6512–6517.
- 11. Mulbry WW, Ambulos NP, Jr, Lovett PS. 1989. *Bacillus subtilis* mutant allele *sup-3* causes lysine insertion at ochre codons: use of *sup-3* in studies of translational attenuation. J Bacteriol 171:5322–5324.
- Zahler SA, Korman RZ, Thomas C, Fink PS, Weiner MP, Odebralski JM. 1987. H2, a temperate bacteriophage isolated from *Bacillus amyloliq-uefaciens* strain H. J Gen Microbiol 133:2937–2944. http://dx.doi.org/ 10.1099/00221287-133-10-2937.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res 18:821–829. http:// dx.doi.org/10.1101/gr.074492.107.
- 14. Darling AC, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple

alignment of conserved genomic sequence with rearrangements. Genome Res 14:1394–1403. http://dx.doi.org/10.1101/gr.2289704.

- Deatherage DE, Barrick JE. 2014. Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using breseq. Methods Mol Biol 1151:165–188. http://dx.doi.org/10.1007/978-1 -4939-0554-6\_12.
- Tatusova T, Ciufo S, Federhen S, Fedorov B, McVeigh R, O'Neill K, Tolstoy I, Zaslavsky L. 2015. Update on RefSeq microbial genomes resources. Nucleic Acids Res 43:D599–D605. http://dx.doi.org/10.1093/ nar/gku1062.
- 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.
- Barbe V, Cruveiller S, Kunst F, Lenoble P, Meurice G, Sekowska A, Vallenet D, Wang T, Moszer I, Médigue C, Danchin A. 2009. From a consortium sequence to a unified sequence: the *Bacillus subtilis* 168 reference genome a decade later. Microbiology 155:1758–1775. http:// dx.doi.org/10.1099/mic.0.027839-0.
- Smith JL, Goldberg JM, Grossman AD. 2014. Complete genome sequences of *Bacillus subtilis* subsp. *subtilis* laboratory strains JH642 (AG174) and AG1839. Genome Announc 2(4):e00663-14. http:// dx.doi.org/10.1128/genomeA.00663-14.
- Jaacks KJ, Healy J, Losick R, Grossman AD. 1989. Identification and characterization of genes controlled by the sporulation-regulatory gene spo0H in Bacillus subtilis. J Bacteriol 171:4121–4129.