JB Journals.ASM.org

GENOME ANNOUNCEMENT

Complete Genome Sequence of *Bacillus subtilis* Strain QB928, a Strain Widely Used in *B. subtilis* Genetic Studies

Chi-Shing Yu,[a] Kay-Yuen Yim,[a,c] Stephen Kwok-Wing Tsui,[b,c] and Ting-Fung Chan[a,c]

School of Life Sciences,[a] School of Biomedical Sciences,[b] and Hong Kong Bioinformatics Centre,[c] The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

The complete genome sequence of *Bacillus subtilis* strain QB928 was constructed to facilitate studies in the evolution of the genetic code. With a widespread use of the strain in *Bacillus subtilis* genetics studies, its complete genome sequence would facilitate deeper understanding of *Bacillus subtilis* genetics.

Bacillus subtilis is the workhorse of a wide range of industrial processes and the model organism for Gram-positive bacteria (4). The strain QB928 was previously constructed to aid in genetic mapping due to the presence of various markers [*aroI*(*aroK*)*906 purE1 dal*(*alrA*)*1 trpC2*] (3). Since then, it has been widely adopted in a wide range of bacterial studies. According to the Google Scholar search engine, QB928 has been cited by 171 articles, with topics ranging from genetic code modification through sporulation and industrial food production to antibiotic production. Most importantly, QB928 was used to generate a set of mutants leading to codon displacement phenotypes (7, 11); we set out to complete the genome for QB928 to facilitate downstream comparative genomic studies.

High-throughput DNA sequencing of QB928 was done on the Illumina GA IIx 75-bp paired-end platform with an average insert size of 200 bp at BGI-Shenzhen (BGI). Altogether, 4,954,514 reads were achieved, resulting in ~89× coverage of the 4.15-Mbp QB928 genome. Reads were filtered to remove adapter sequences, low-quality bases (Phred score, <10), and singletons. A draft genome was generated by *de novo* assembly using Velvet 1.0.09 (12). Twenty-five scaffolds with an N_{50} value of 488,188 were constructed. Gaps within scaffolds were iteratively closed using a previously described method (10). Gaps between scaffolds and the remaining gaps within scaffolds were closed by Sanger sequencing of the PCR products spanning gap regions.

The size of the QB928 genome is 4,146,839 bp, which is about 69 kbp smaller than the previously published *Bacillus subtilis* strain 168 genome (1). The mean GC content of QB928 is 43.61%. By the use of RATT (8), confidently homologous annotations from *B. subtilis* 168 were transferred to the QB928 genome. The ISGA server (5) was used for novel gene discovery. Altogether, 4,292 genes were annotated, composed of 4,113 coding genes, 30 rRNA genes in 10 rRNA operons, 86 tRNAs, and 63 miscellaneous RNAs (e.g., small RNAs). Whole-genome comparison using progressiveMauve (2) revealed 1,528 variants in QB928 with respect to *B. subtilis* 168. Nonsense mutations W115Stop and E175Stop were found in *aroI* (*aroK*) and *dal* (*alrA*), respectively, which are consistent with its tryptophan and D-alanine auxotrophic phenotype. However, no mutation can be found in *purE*. Instead, we found a missense E195K mutation in *purC*, which suggested that the genotype notation from the Bacillus Genetic Stock Center could be incorrect.

We found two large deleted regions in QB928 with respect to *B. subtilis* 168. The first region spans from positions 529424 to 549937 relative to *B. subtilis* 168, which contain genes found in or associated with transposons, or bacteriophages. The second region spans from positions 2653333 to 2701362 relative to *B. subtilis* 168, which contain the *skin* (*sigK* intervening) element (6) between *spoIVCB* and *spoIIIC*, and is assumed to be a remnant of a phage (9). In previous studies, it was shown that deletion of the *skin* element from *B. subtilis* would not impair growth or sporulation (6).

QB928 is one of the widely used *Bacillus subtilis* strains and is currently available from the Bacillus Genetic Stock Center. Its complete genome sequence should benefit the research community in general.

Nucleotide sequence accession number. The sequence is accessible from GenBank under the accession number CP003783.

ACKNOWLEDGMENTS

This study is supported by a Focused Investment Scheme of the Chinese University of Hong Kong granted to S.K.-W.T. and a General Research Fund (GRF461809) from the Research Grant Committee, Hong Kong SAR Government, granted to T.-F.C.

REFERENCES

1. **Barbe V, et al.** 2009. From a consortium sequence to a unified sequence: the *Bacillus subtilis* 168 reference genome a decade later. Microbiology **155**:1758–1775.
2. **Darling AE, Mau B, Perna NT.** 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One **5**:e11147. doi:10.1371/journal.pone.0011147.
3. **Dedonder RA, et al.** 1977. Construction of a kit of reference strains for rapid genetic mapping in *Bacillus subtilis* 168. Appl. Environ. Microbiol. **33**:989–993.
4. **Harwood CR.** 1992. Bacillus subtilis and its relatives: molecular biological and industrial workhorses. Trends Biotechnol. **10**:247–256.
5. **Hemmerich C, Buechlein A, Podicheti R, Revanna KV, Dong Q.** 2010. An Ergatis-based prokaryotic genome annotation web server. Bioinformatics **26**:1122–1124.
6. **Kunkel B, Losick R, Stragier P.** 1990. The *Bacillus subtilis* gene for the development transcription factor sigma K is generated by excision of a dispensable DNA element containing a sporulation recombinase gene. Genes Dev. **4**:525–535.
7. **Mat WK, Xue H, Wong JT.** 2010. Genetic code mutations: the breaking of a three billion year invariance. PLoS One **5**:e12206. doi:10.1371/journal.pone.0012206.

Received 23 August 2012 **Accepted** 29 August 2012

Address correspondence to Ting-Fung Chan, tf.chan@cuhk.edu.hk.

doi:10.1128/JB.01533-12

8. **Otto TD, Dillon GP, Degrave WS, Berriman M.** 2011. RATT: rapid annotation transfer tool. Nucleic Acids Res. **39**:e57. doi:10.1093/nar/gkq1268.
9. **Takemaru K, Mizuno M, Sato T, Takeuchi M, Kobayashi Y.** 1995. Complete nucleotide sequence of a skin element excised by DNA rearrangement during sporulation in *Bacillus subtilis*. Microbiology **141**:323–327.
10. **Tsai IJ, Otto TD, Berriman M.** 2010. Improving draft assemblies by iterative mapping and assembly of short reads to eliminate gaps. Genome Biol. **11**:R41. doi:10.1186/gb-2010-11-4-r41.
11. **Wong JT.** 1983. Membership mutation of the genetic code: loss of fitness by tryptophan. Proc. Natl. Acad. Sci. U. S. A. **80**:6303–6306.
12. **Zerbino DR, Birney E.** 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. **18**:821–829.