

Genome Sequence of *Bacillus licheniformis* WX-02

Wuming Yangtse, Yinhua Zhou, Yang Lei, Yimin Qiu, Xuetao Wei, Zhixia Ji, Gaofu Qi, Yangchun Yong, Lingling Chen, and Shouwen Chen

State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, People's Republic of China

***Bacillus licheniformis* is an important bacterium that has been used extensively for large-scale industrial production of exoenzymes and peptide antibiotics. *B. licheniformis* WX-02 produces poly- γ -glutamate increasingly when fermented under stress conditions. Here its genome sequence (4,270,104 bp, with G+C content of 46.06%), which comprises a circular chromosome, is announced.**

Bacillus licheniformis is a Gram-positive bacterium which inhabits soil and readily forms endospores. Agriculturally, it has been used as a probiotic and microbial fertilizer (9); industrially, it is used to produce enzymatic agents (12), peptide antibiotics (7), acetoin (8), and poly- γ -glutamic acid (γ -PGA) (1, 2), etc. *B. licheniformis* WX-02 was isolated from saline soil in Yingcheng City, Hubei Province, China. It has been noted that this strain can produce larger amounts of γ -PGA under stress conditions than under normal ones; the stresses refer to high salt, high temperature, caustic alkali, ultrasonic shock, and so on. The yield of γ -PGA can be raised 5.28 times against that of the control group when the salt concentration reaches 8% (13). In order to explore the mechanism of high production of γ -PGA under adverse circumstances, the genome of *B. licheniformis* WX-02 was sequenced and compared with that of the type strain ATCC 14580 (10, 12). *B. licheniformis* WX-02 has been maintained at the China Center for Type Culture Collection and designated *B. licheniformis* CCTCC M208065.

Whole-genome sequencing was performed using a Solexa genome analyzer (BGI; Shenzhen, China) using a shotgun strategy, which produced paired reads totaling ~555 Mb with about 131-fold coverage of the genome. Genome sequence data were processed and assembled into 45 contigs and 29 scaffolds (5). Gaps between contigs were closed by combinatorial PCR and sequencing amplicons by primer walking. Finally, this assembling process produced 3 large scaffolds in one potential circular chromosome. Open reading frames were identified using Fgenesb (<http://linux1.softberry.com/berry.phtml?topic=fgenesb&group=programs&subgroup=gfindb>), Prodigal (<http://compbio.ornl.gov/prodigal/>), and Glimmer (http://www.ncbi.nlm.nih.gov/genomes/MICROBES/glimmer_3.cgi) and BLAST against the RefSeq database. tRNA and rRNA genes were identified by the tRNAscan and RNAmmer software programs, respectively (4, 6). The genome of strain CCTCC M208065 is 4,270,104 bp long with a GC content of 46.06%, containing 4,320 open reading frames, 72 tRNA genes, and 7 rRNA operons. Although the genome of CCTCC M208065 shares great similarity (94.8%) with that of ATCC 14580, genome comparison indicates that two large (more than 50 kb) fragments are translocated in CCTCC M208065, which extends our understanding of genome evolution. In addition, there is a fragment of about 76 kb in this genome sharing very low homology with the genome of ATCC 14580 and other sequences in GenBank; this fragment encodes the DNA replication initiator protein, conjugation protein, RNA polymerase sigma factors, transcriptional regulators, recombinases, hypothetical proteins, and others.

It has been reported that the gene cluster *comQXPA* is involved in regulation of γ -PGA synthesis (3, 11). The *comP* gene in the ATCC 14580 strain is inactivated by two insertional fragments, while this gene in CCTCC M208065 is complete. The difference in the *comP* gene between these two strains might be associated with their different abilities for γ -PGA production, although much evidence is needed to verify this.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited in DDBJ/EMBL/GenBank under the accession no. AHIF00000000. The version described in this paper is the first version, AHIF01000000.

ACKNOWLEDGMENTS

This study is funded by the National Natural Science Foundation of China (grant no. 30970036 and no. 31170046).

We are grateful to Sun Ming for his kind and generous suggestions.

REFERENCES

- Birrer GA, Cromwick AM, Gross RA. 1994. γ -Poly (glutamic acid) formation by *Bacillus licheniformis* 9945A: physiological and biochemical studies. *Int. J. Biol. Macromol.* 16:265–275.
- Buescher JM, Margaritis A. 2007. Microbial biosynthesis of polyglutamic acid biopolymer and applications in the biopharmaceutical, biomedical and food industries. *Crit. Rev. Biotechnol.* 27:1–19.
- Hoffmann K, et al. 2010. Facilitation of direct conditional knockout of essential genes in *Bacillus licheniformis* DSM13 by comparative genetic analysis and manipulation of genetic competence. *Appl. Environ. Microbiol.* 76:5046–5057.
- Lagesen K, et al. 2007. RNAmmer: consistent annotation of rRNA genes in genomic sequences. *Nucleic Acids Res.* 35:3100–3108.
- Li R, et al. 2009. SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics* 25:1966–1967.
- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25:955–964.
- McInerney MJ, Javaheri M, Nagle DP. 1990. Properties of the biosurfactant produced by *Bacillus licheniformis* strain JF-2. *J. Ind. Microbiol.* 5:95–102.
- Perego P, Converti A, Del Borghi M. 2003. Effects of temperature, inoculum size and starch hydrolyzate concentration on butanediol production by *Bacillus licheniformis*. *Bioresour. Technol.* 89:125–131.

Received 5 April 2012 Accepted 10 April 2012

Address correspondence to Shouwen Chen, chenshouwen@mail.hzau.edu.cn.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JB.00572-12

9. Pötter M, Oppermann-Sanio FB, Steinbüchel A. 2001. Cultivation of bacteria producing polyamino acids with liquid manure as carbon and nitrogen source. *Appl. Environ. Microbiol.* **67**:617–622.
10. Rey MW, et al. 2004. Complete genome sequence of the industrial bacterium *Bacillus licheniformis* and comparisons with closely related *Bacillus* species. *Genome Biol.* **5**:R77. doi:10.1186/gb-2004-5-10-r77.
11. Stanley NR, Lazazzera BA. 2005. Defining the genetic differences between wild and domestic strains of *Bacillus subtilis* that affect poly- γ -DL-glutamic acid production and biofilm formation. *Mol. Microbiol.* **57**:1143–1158.
12. Veith B, et al. 2004. The complete genome sequence of *Bacillus licheniformis* DSM13, an organism with great industrial potential. *J. Mol. Microbiol. Biotechnol.* **7**:204–211.
13. Wei X, Ji Z, Chen S. 2010. Isolation of halotolerant *Bacillus licheniformis* WX-02 and regulatory effects of sodium chloride on yield and molecular sizes of poly- γ -glutamic acid. *Appl. Biochem. Biotechnol.* **160**:1332–1340.