

Draft Genome Sequence of *Brevibacillus brevis* Strain X23, a Biocontrol Agent against Bacterial Wilt

Wu Chen,^a Yunsheng Wang,^b Dingjun Li,^{b,c} Lin Li,^a Qiming Xiao,^b and Qingming Zhou^a

College of Agronomy, Hunan Agricultural University, Changsha, China^a; College of Bio-safety Science and Technology, Hunan Agricultural University, Changsha, China^b; and Hunan Radio and TV University, Changsha, China^c

***Brevibacillus brevis* X23 is an appropriate biocontrol agent against bacterial wilt caused by *Ralstonia solanacearum*. We report herein the draft genome sequence (6,566,879 bp) and a circular plasmid (6,600 bp) of *B. brevis* X23, data which may be helpful for mining the antagonistic activity against *R. solanacearum*.**

Brevibacillus brevis, also known as *Bacillus brevis*, is a Gram-positive and spore-forming bacterium. It can secrete large amounts of secondary metabolites, which are important for controlling pathogens. Among them, tyrocidine, gramicidin (4, 5, 6), graptisin (12), and edeine (1) have been successively isolated and identified. Only recently, the genome of one *B. brevis* strain, NBRC 100599, is available in the GenBank database (<http://www.ncbi.nlm.nih.gov/nuccore/226309587?report=GenBank>).

Ralstonia solanacearum, a Gram-negative bacterium, causes wilt disease, with a dramatic yield loss for many economically important crops, including tomato, potato, tobacco, and banana (3). We have isolated *B. brevis* X23 from the rhizosphere soil of tobacco (*Nicotiana tabacum*) (7). It can secrete nonribosome peptides, which were shown to effectively inhibit the growth of *R. solanacearum* by disturbing cell membrane integrity (W. Chen, D. Li, and Q. Zhou, unpublished data). Therefore, *B. brevis* X23 is a good biocontrol agent against *R. solanacearum*.

Here, we report the draft genome sequence and a circular plasmid of X23, determined by Illumina Solexa sequencing technology. A total of 1,344 Mb of clean reads from two different insert size libraries (500 bp and 5 kb) was generated using the Illumina HiSeq 2000 paired-end strategy. The genome was assembled with SOAPdenovo (8), and we corrected the assembly with iterative correction of reference nucleotides (iCORN) (10). The assembly genome of X23 consists of 16 scaffold sequences, which contained 28 contigs with a length of approximately 6.58 Mb, and the contig N_{50} was 425 kb.

Putative protein-coding and tRNA genes were predicted by Glimmer3.0 (2) and tRNAscan-SE (9), respectively. The functional annotation was based on BLASTp with Swiss-Prot, KEGG, and NR databases. In all, 6,489 protein-coding genes and 59 tRNA genes were predicted in the X23 genome. Among the 6,489 coding sequences, 4,245 proteins were able to be assigned to COG families. TBLASTn with the genome sequences of NBRC 100599 (NC_012491) showed that 5,822 genes had significant hits with an E value of less than $1e-5$. There were 636 hypothetical proteins (9.8%) that have no match to any known proteins in the databases. In a comparison to the genome of NBRC 100599, X23 has an extra 6.6-kb plasmid with 100% identity to the *Bacillus subtilis* IAM1168 plasmid pLS30 (11). This plasmid may possibly be transmitted from other species by horizontal gene transfer. The GC content of the chromosome is 47.8%, while that of the plasmid is 38.7%.

The genome sequence of X23 is supposed to present more genetic information for synthesis and secretion of antagonistic substances. *In*

silico analysis of the *B. brevis* X23 genome identified 6 giant gene clusters related to nonribosomal peptide synthetases and polyketide synthetases but did not hit any homologous genes that code known antimicrobial peptides. Hence, these 6 gene clusters are likely crucial elements for its antimicrobial activity in *B. brevis* X23. In addition, some interesting genes/gene clusters related to antimicrobial and antibiotic metabolism, such as genes encoding the ABC-type antimicrobial peptide transporter and phosphotransferase, were also observed in the X23 genome sequence.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number [AKYF00000000](https://www.ncbi.nlm.nih.gov/nuccore/AKYF00000000). The version described in this paper is the first version, [AKYF00000000](https://www.ncbi.nlm.nih.gov/nuccore/AKYF00000000).

ACKNOWLEDGMENTS

We acknowledge the Beijing Genomics Institute for genome sequencing in Shenzhen.

This work was supported by grants from the Chinese National Programs for High Technology Research and Development (2009AA10Z403), the National Natural Science Foundation of China (grant no. 31101482), and the Science and Technology Foundation of Hunan Tobacco Corporation (11-13Aa12).

REFERENCES

- Czajgucki Z, et al. 2006. Structure activity relationship studies on the antimicrobial activity of novel edeine A and D analogues. *J. Pept. Sci.* 12:653–662.
- Delcher AL, et al. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23:673–679.
- Elphinstone JG. 2005. The current bacterial wilt situation: a global overview, p 9–28. *In* Allen C, Prior P, Hayward C (ed), *Bacterial wilt disease and the *Ralstonia solanacearum* species complex*. APS Press, St. Paul, MN.
- Hotchkiss RD. 1941. The chemical nature of gramicidin and tyrocidine. *J. Biol. Chem.* 141:171–185.
- Hotchkiss RD, Dubos RJ. 1940. Chemical properties of bactericidal substances isolated from cultures of a soil *Bacillus*. *J. Biol. Chem.* 132:793–794.
- Hotchkiss RD, Dubos RJ. 1941. The isolation of bactericidal substances from cultures of *Bacillus brevis*. *J. Biol. Chem.* 141:155–162.

Received 26 July 2012 Accepted 24 September 2012

Address correspondence to Qingming Zhou, zqm0618@yahoo.com.cn.

W.C. and Y.W. contributed equally to this paper.

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

doi:10.1128/JB.01312-12

7. Li DJ, et al. 1997. A preliminary study of the control of bacterial wilt of tobacco with the inhibiting bacteria in Hunan. *J. Hunan Agric. Univ.* 23(3):256–260.
8. Li R, et al. 2009. SOAP2: an improved ultrafast tool for short read alignment. *Bioinformatics* 25(15):1966–1967.
9. 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.
10. Otto TD, et al. 2010. Iterative correction of reference nucleotides (iCORN) using second generation sequencing technology. *Bioinformatics* 26:1704–1707.
11. Sakaya N, et al. 2006. Experimental basis for a stable plasmid, pLS30, to shuttle between *Bacillus subtilis* species by conjugational transfer. *J. Biochem.* 139(3):557–561.
12. Tamaki M, et al. 1983. Synthetic studies on graptisin, II. *J. Antibiot. (Tokyo)* 36:751–752.