JOURNAL OF BACTERIOLOGY, Aug. 2010, p. 4074–4075 Vol. 192, No. 15
0021-9193/10/$12.00 doi:10.1128/JB.00562-10

GENOME ANNOUNCEMENTS

Complete Genome Sequence of *Bacillus thuringiensis* Mutant Strain BMB171▽

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Received 17 May 2010/Accepted 20 May 2010

***Bacillus thuringiensis* has been widely used as a biopesticide for a long time. Here we report the finished and annotated genome sequence of *B. thuringiensis* mutant strain BMB171, an acrystalliferous mutant strain with a high transformation frequency obtained and stocked in our laboratory.**

Bacillus thuringiensis is an insect pathogen which is widely used as a biopesticide due to its various endogenous crystal proteins and spores (12). To improve the virulence and practical effectiveness of *B. thuringiensis*, genetic transformation of different genes with beneficial traits is a fundamental procedure. Simultaneously, genetic transformation can facilitate functional genomic research. However, wild-type strains are not suitable to be used as recipient strains because of low transformation efficiency. This obstacle is mainly caused by the thick cell wall layer of *B. thuringiensis* together with multiple plasmids inside the cell, which harbor genes encoding insecticidal crystal proteins. We used the method of elevating the growth temperature and adding 0.05% sodium dodecyl sulfate to treat several parental strains and finally obtained mutant strain BMB171, with no resident plasmid, from wild-type crystalliferous strain YBT-1463 (9). The electrotransformation frequency of mutant BMB171 could reach up to 10^7 transformants/μg DNA after optimization of the electrotransformation parameters (7), which was 4.8×10^4-fold higher than that of the parental strain (8). Moreover, mutant strain BMB171 exhibited the same characteristics as YBT-1463, such as metabolic abilities and growth properties, as well as sensitivity to 10 antibiotics (8). Of course, BMB171 could produce parasporal crystals with characteristic geometric shapes through the expression of relevant *cry* genes carried by plasmids (7). Thus, *B. thuringiensis* mutant strain BMB171 has become a major recipient strain and is widely used for insecticidal crystal protein-encoding gene expression (14, 15), cell surface display (10, 13), gene function and regulation researches (2, 5), etc.

The *B. thuringiensis* mutant strain BMB171 genome was sequenced by using a massive parallel pyrosequencing technology (454 GS-FLX). A total of 448,963 high-quality reads with an average read length of 391 bp were produced, providing about 32-fold coverage of the genome. Assembly was performed using the Newbler software of the 454 suite package (454 Life Sciences), which resulted in 193 large (defined as >500 bp) contigs. The relationship of contigs was determined by multiplex PCR, and gaps were filled through sequencing of PCR products by primer walking or shotgun sequencing with an ABI 3730 sequencer. The Phred/Phrap/Consed software package (3) was used for final sequence assembly and quality assessment. Protein-coding genes were predicted by combining the results of Glimmer 3.02 (1) and ZCURVE (4), followed by manual inspection. Both tRNA and rRNA genes were identified by tRNAscan-SE (11) and RNAmmer (6), respectively. Functional annotation was performed by searching against a protein database of the microbial genome developed in house.

The 5.64-Mb genome of *B. thuringiensis* mutant strain BMB171 contains two replicons: a circular chromosome (5.33 Mb) encoding 5,088 open reading frames (ORFs) and a circular plasmid (0.31 Mb), which is named pBMB171, encoding 276 predicted ORFs. The G+C content of the chromosome is 35.3%, while that of the plasmid is 33.3%. The mutant strain BMB171 genome encodes 104 tRNAs and 14 rRNA operons. A previous study indicated that BMB171 is a plasmid-free mutant (9); however, our sequencing results demonstrated that a large plasmid still remains. The reason why the plasmid was not detected previously might be its large size and low copy number. We did not find any crystal protein genes in either chromosome or plasmid sequences, which was consistent with previous observations (9).

In summary, the complete *B. thuringiensis* mutant strain BMB171 genome provides a better-defined genetic back-

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▽ Published ahead of print on 4 June 2010.

ground for gene expression and regulation studies, especially crystal protein production and metabolic network construction.

Nucleotide sequence accession numbers. The sequence and annotation data for *B. thuringiensis* mutant strain BMB171 have been deposited in GenBank under accession numbers CP001903 (chromosome) and CP001904 (plasmid).

This research was supported by the Chinese National Natural Science Funds (grants 30930004, 30770022, and 30900016) and by the National High Technology Research and Development Program of China (863 Program, grant 2008AA02Z112).

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