Korean Journal of Microbiology (2017) Vol. 53, No. 3, pp. 216-218
DOI https://doi.org/10.7845/kjm.2017.7045

pISSN 0440-2413
eISSN 2383-9902

Note (Genome Announcement)

Complete genome sequence of *Bacillus thuringiensis* C25, a potential biocontrol agent for sclerotia-forming fungal phytopathogens

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생물학적방제 효과가 뛰어난 *Bacillus thuringiensis* C25 균주의 유전체 분석

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(Received July 24, 2017; Revised July 25, 2017; Accepted July 25, 2017)

We here provide the complete genome sequence of *Bacillus thuringiensis* C25, the strain showing antagonistic effects on fungal phytopathogens. The genome comprised of 5,308,062 bp with 35.32% G+C content of a circular chromosome and a plasmid containing 308,946 bp with 32.23% G+C content. The chromosome and plasmid genome included 5,683 protein coding DNA sequences, 107 tRNA and 42 rRNA genes.

Keywords: *Bacillus thuringiensis* C25, cell wall degradation enzyme, genome sequencing, Pac-Bio RSII

Biocontrol agents (BCAs) to protect crop plants from various (a)biotic challenges are nowadays getting attention in agricultural industry. The efficacy and durability of BCAs, especially microbial-driven agents, were reported to be comparable to chemical pesticide (Bardin *et al.*, 2015). Among them, *Bacillus thuringiensis* (i.e., Bt) have been successfully industrialized as biopesticide thanks to the insecticidal crystal excretes (Bravo *et al.*, 2011). Interestingly, recent studies reported that *B. thuringiensis* could be also used to antifungal BCA. *B. thuringiensis* C25, a newly identified strain (Shrestha *et al.*, 2015; Sultana and Kim, 2016), exhibited antagonistic effects against *Sclerotinia minor* and *Ciboria shiraiana*. Noticeably, C25 caused the cell lysis of those fungal pathogens, which was correlated with strong activities of fungal cell wall degrading enzymes (CWDEs) including β-1,3-glucanase, β-glucosidase and chitinase. Field application of the C25 strain resulted in effectively suppression of sclerotial pathogens and popcorn mulberry disease (Sultana and Kim, 2016). To date, even though many *B. thuringiensis* strains have been studied for their properties as insecticidal agents; however, the basis of the newly reported antifungal activities is totally unknown. We here sequenced the complete genome and bioinformatics analysis to investigate the genetic characteristics of *B. thuringiensis* strain C25 as a valuable antifungal BCA.

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Genomic DNA of the *B. thuringiensis* strain C25 (KACC90242P) was extracted from an overnight culture in LB broth using the MagAttract® High-Molecular-Weight Genomic DNA Kit (QIAGEN). A total of 5 μg of genomic DNA was used as input into library preparation, and then constructed into a 20-kb SMRTbell™ template library. A PacBio RSII sequencing platform (Pacific Biosciences) was employed for whole genome sequencing using P6-C4 chemistry in one single molecule real time (SMRT) cell (Pacific Biosciences) with MagBead OneCellPerWell v1 Protocol. A total of 133,941 sequence reads with 1,100,203,668 base pairs after sub-reads filtering were generated and assembled using PacBio Hierarchical Genome Assembly Process (HGAP, Version 2.3), including consensus polishing with Quiver (Chin *et al.*, 2013). The C25 strain genome is composed of 5,308,062 bp of a circular chromosome and 308,946 bp of a circular plasmid with GC contents of 35.32% and 32.23%, respectively (Table 1). A total of 5,683 protein coding genes, 42 rRNA (14 5S, 14 16S, 14 23S) and 107 tRNA were predicted from the chromosome and plasmid by Glimmer v3.02 analysis (Table 1, Delcher *et al.*, 2007). These ORFs were searched using BLAST alignment against the NCBI Non-redundant protein database (NR) for all species (Conesa *et al*., 2005). 5,116 genes were assigned to have functions, but 567 genes are remained as hypothetical or uncharacterized genes.

Functional annotation of the genome revealed the presence of various types of CWDE related genes encoding 41 genes of protease, 13 genes of lipase, 2 genes of N-acetylglucosaminidase (NAGase), 2 genes of β-1,3-glucanase, 3 genes of glucosidase, 3 genes of chitinase, and 2 genes of cell wall hydrolase. Recent studies reported that CWDEs and their activities of *Bacillus* spp. were critical to contribute their antifungal activity (Alamri *et al.*, 2012). In addition, enhanced activity of chitinase of *Bacillus licheniformis* resulted in greatly increased its antifungal effects (Hong *et al.*, 2016). These suggest that identification and functional analysis of CDWEs of BCAs would be important for developing key components in biocontrol field. Taken together, the complete genome of the strain *B. thuringiensis* C25 will contribute to understanding of biological roles of CWDEs and other factors for its antifungal activity. The *Bacillus thuringiensis* C25 (KACC90242P) strain is available at the Korean Agricultural Culture Collection (KACC).

Table 1. General genomic features of the *Bacillus thuringiensis* C25

Genomic features	Chromosome (CP022345)	Plasmid (CP022346)
Contig	1	1
Genome size (bp)	5,308,062	308,946
GC content (%)	35.32	32.23
Protein-coding gene (No.)	5,350	333
tRNAs	106	1
rRNAs (5S, 16S, 23S)	42 (14, 14, 14)	0

Nucleotide sequence accession numbers

The GenBank accession number for the complete genome sequence of *Bacillus thuringiensis* C25 has been deposited at GenBank under the accession CP022345 (Chromosome) and 022346 (Plasmid).

적 요

생물학적방제 효과가 뛰어난 *Bacillus thuringiensis* C25 균주의 유전체 분석을 수행하였다. 본 균주는 5,308,062 bp, G+C 비율 35.32%의 염색체와 308,946 bp, 32.23% G+C 함량이 포함된 plasmid를 지닌 것으로 확인되었다. 염색체와 plasmid DNA에 예측된 유전자의 총 수는 5,683개의 단백질 코딩유전자와 107개 tRNA 그리고 42개의 rRNA였다.

Acknowledgements

This work was carried out with the support of the "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ011917)," Rural Development Administration, Republic of Korea.

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