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Complete Genome Sequence of *Bacillus thuringiensis* Serovar *coreanensis* ST7 with Toxicity to Human Cancer Cells

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Abstract

Bacillus thuringiensis (Bt) parasporal crystal proteins were well known to be toxic to certain insects and cytocidal activity against various human cancer cells. Bt serovar *coreanensis* ST7, non-pathogenic to insects and non-hemolytic, has an important parasporin, PS4Aa1 (Cry45Aa1), with potential toxicity to human cancer cells. In this study, we reported the feature of complete genome sequence and the cluster of orthologous groups of proteins function classification of ST7. Meanwhile, the evolutionary of ST7 was also studied. The genome data of ST7 will strongly contribute to a better understanding of the genomic diversity and evolution, and enrich the Bt genome database.

Keywords

Bacillus thuringiensis Serovar *Coreanensis* ST7, Gapless Chromosome, Plasmid Sequences, Genomic Feature, Cluster of Orthologous Groups of Proteins

1. Introduction

Bacillus thuringiensis (Bt) is a typical aerobic, Gram-positive bacterium. During sporulation, Bt produces one or more parasporal crystal proteins [1]. Many Bt strains and toxins have been found. Only a few of them have been used to control some pests [2]. And earlier studies have shown that non-insecticidal Bt strains were ubiquitous in natural environments and even more widely distri-

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buted than insecticidal strains [3] [4]. Interestingly, among the non-insecticidal Bt strains, some strains exhibited highly cytotoxic to a wide range of mammalian cells, but were non-hemolytic and non-insecticidal [4] [5]. The Bt strain ST7 was isolated from the soil sample of Glacier virgin forest in Sichuan Basin of China by our lab and saved in Sichuan Agricultural University with non-pathogenic to insects and non-hemolytic. Scanning electron microscope observation showed that the ST7 could produce three types of square, spherical, and bipyramidal crystals (**Figure 1**), and the results of PCR-restriction fragment length polymorphism (PCR-RFLP) showed that ST7 contained five insecticidal crystal protein genes *cry*22-type, *cry*32-type, *cry*45-type, *cry*62-type, and *cry*73-type.

A previous study showed that PS4Aa1 (Cry45Aa1) exhibits specific cytotoxicity against human cancer cells, such as CACO-2, Sawano, and MOLT-4 cells, in particular [6]. The Cry45-type contained in the strain ST7 has 100% amino acid sequence homology with Cry45Aa1 of Bt *serovar* strain A1470 [6], so that the strain ST7 may have toxicity to the same kinds of human cancer cells. In the past research, thousands of Bt strains have been isolated, but only a few of them have high toxicity to human cancer cells, and have been not obtained the complete genome sequence [4] [5]. This is the first complete genome sequence of Bt with potential toxicity to human cancer. The analysis of complete genome sequence ofST7 may help in the development of strategies to cope with cancer cells in the medical field.

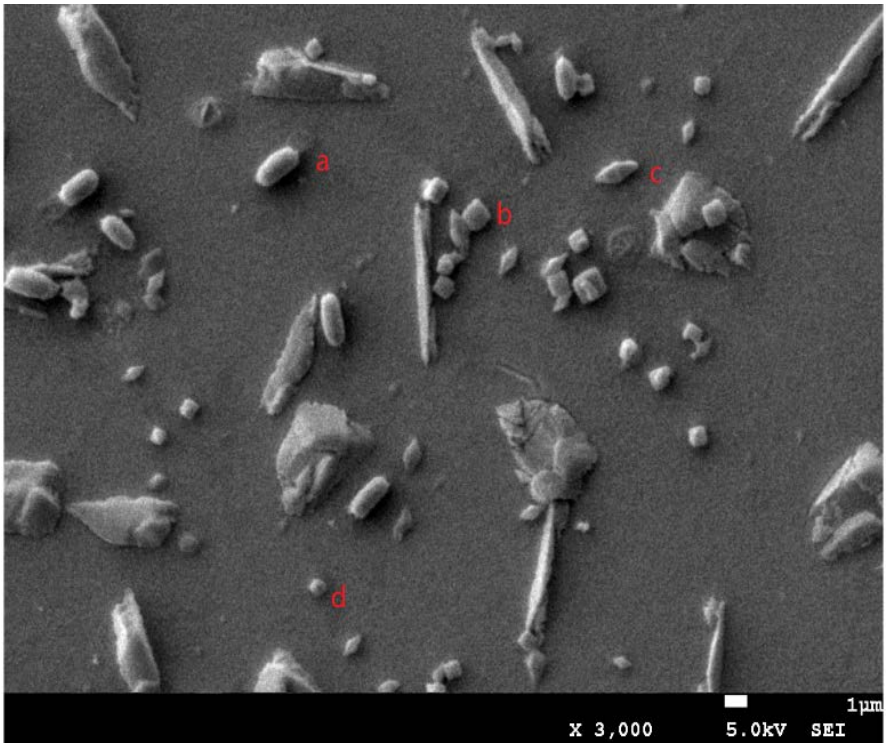

Figure 1. Scanning electron microscopy observation of the spore and crystal mixtures produced by ST7. (a), spore; (b), square crystal; (c), bipyramidal crystal; (d), spherical crystal.

2. Genome Sequencing and Assembly

In this study, Genomic DNA was isolated from ST7 using the QIAGEN Genomic-tip 500/G DNA isolation kit. Whole-genome sequencing of ST7 was performed at the Tianjin Biochip Corporation (Tianjin, China) using the PacBio RS II platform with the Single Molecule Real Time (SMRT), which was claimed to have a further four-fold improvement, with twice the mean read length and twice the throughput of PacBio RS instrument [7]. A SMART bell sequencing library was prepared and sequenced using the Pacific Biosciences RS II instrument DNA Template Prep Kit 2.0 and P6-C4 chemistry [8]. The raw reads were processed into subreads by removing the adaptors and filtered. The filtered date produce 77,741 reads (N_{50} length is 18,452 bp), which were used in the HGAP assembly process to obtain the final genomic sequence [9] [10], tRNA and rRNA genes were identified using tRNAscan-SE 1.21 and RNAmmer 1.2 [11] [12].

3. Genome Properties

The genome of ST7 is shown to contain five replicons with one circular chromosome and four circular plasmids. And submit to GenBank (GenBank accession number: CP016194-CP016198). The circular chromosome (5,665,360 bp) contains 5223 predicted protein coding genes, and the G+C content of the chromosome is 35.31%. Meanwhile, the ST7 complete genome encodes 109 tRNA genes and 42 rRNA operons. Four circular plasmids are named pST7-1 (317,818 bp), pST7-2 (149,450 bp), pST7-3 (92,610 bp), pST7-4 (60,883 bp), and the four circular plasmids contain 452 protein coding genes. The G+C content of the plasmid is 33.321%, 31.52%, 36.12% and 35.71%, respectively (**Table 1**, **Figure 2**).

4. Genome Annotation

ST7 contains most genes that function in replication, recombination and repair nucleotide, transport and metabolism, cell cycle control, and defense mechanisms and so on. 38,484 of the identified genes were classified to functional categories based on clusters of orthologous genes (COG) designation: 3118 genes for translation, ribosomal structure and biogenesis; 2536 genes for transcription; 2656 genes for replication, recombination and repair; 15 genes for chromatin

Table 1. Features of BtST7 genome.

Replicons	Total Length (bp)	%G+C Content	Protein-coding Gene	tRNAs	rRNA	GenBank Accession Nos.
Chromosome	5,665,360	35.31%	5223	109	42	CP016194
pST7-1	317,818	33.32%	219	0	0	CP016195
pST7-2	149,450	31.52%	103	0	0	CP016196
pST7-3	92,610	36.12%	82	0	0	CP016197
pST7-4	60,883	35.71%	48	0	0	CP016198

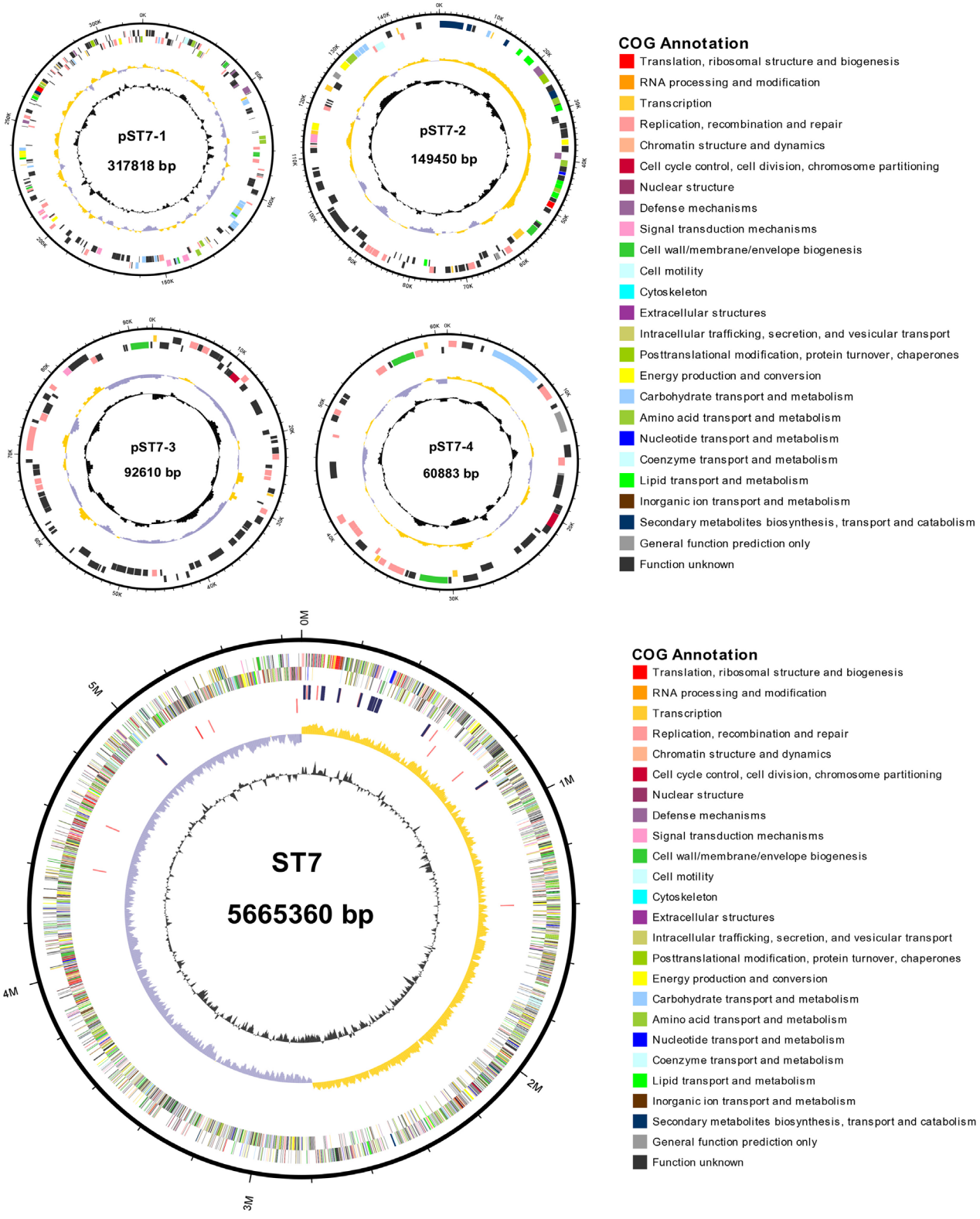

Figure 2. Circular genome map of BtST7.

structure and dynamics; 444 genes for cell cycle control, cell division and chromosome partitioning; 568 genes for defense mechanisms; 1394 genes for signal

transduction mechanisms; 1983 genes for cell wall/membrane/envelope biogenesis; 522 genes for cell motility; 2 genes for extracellular structures; 595 genes for intracellular trafficking, secretion, and vesicular transport; 1180 genes for posttranslational modification, protein turnover and chaperones; 2067 genes for energy production and conversion; 2099 genes for carbohydrate transport and metabolism; 4224 genes for amino acid transport and metabolism; 1469 genes for nucleotide transport and metabolism; 2120 genes for coenzyme transport and metabolism; 1175 genes for lipid transport and metabolism; 2344 genes for inorganic ion transport and metabolism; 626 genes for secondary metabolites biosynthesis, transport and catabolism; 4430 genes for general function prediction. Only the functions of 2917 genes were unknown (**Figure 3**).

The evolutionary of ST7 was presented by the phylogenetic tree, using software MEGA 6.0 by neighbour-joining method based on complete genome sequences of 21 Btstrains and BtKBAB4 strain, which showed that a distinct branch of ST7 with all strains, and BtHD-29 [13], BtYBT-1520 [14], BtHD-73 [15], BtYC-10 [16], and BtHD-1 [17] was identified as the five closest evolutionary relative of ST7 (**Figure 4**).

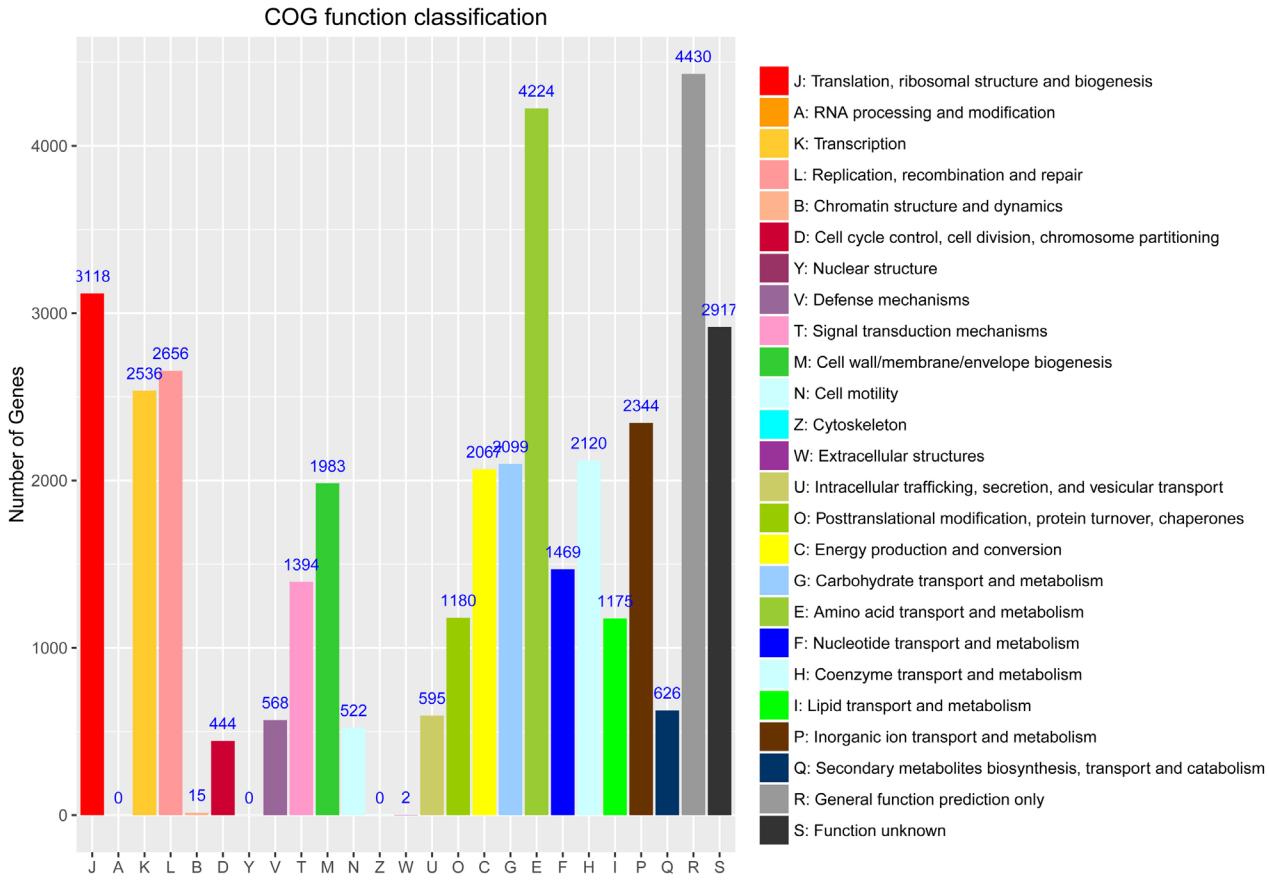

Figure 3. COG (Cluster of Orthologous Groups of proteins) function classification of ST7. The evolutionary of ST7 was presented by the phylogenetic tree, using software MEGA 6.0 by neighbour-joining method based on complete genome sequences of 21 Bt strains and BtKBAB4 strain, which showed that a distinct branch of ST7 with all strains, and BtHD-29 [13], BtYBT-1520 [14], BtHD-73 [15], BtYC-10 [16], and BtHD-1 [17] was identified as the five closest evolutionary relative of ST7 (**Figure 4**).

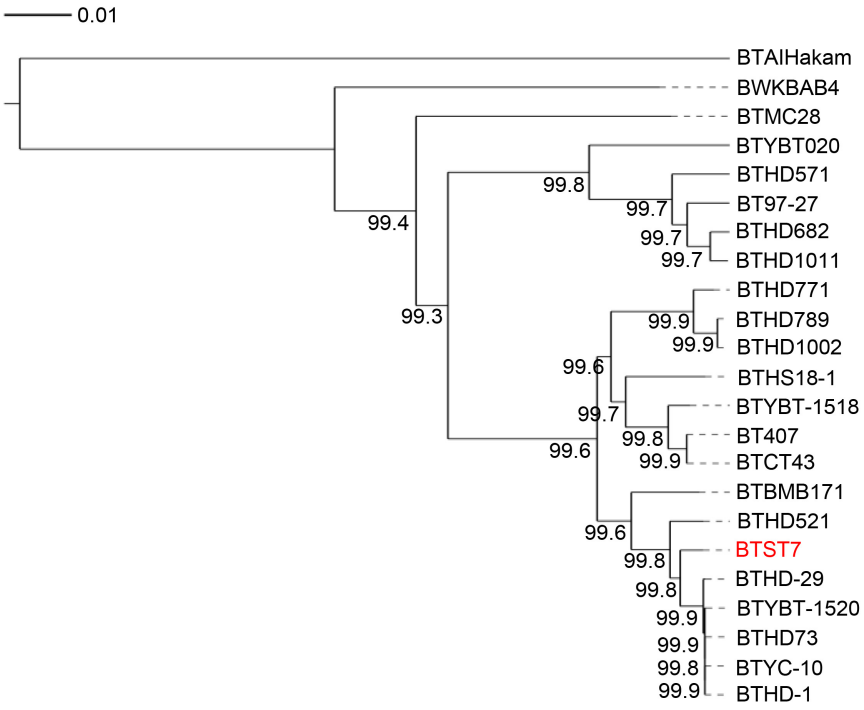

Figure 4. Phylogenetic tree of BtST7.

5. Nucleotide Sequence Accession Numbers

The complete genome sequence of Bt ST7 has been deposited in GenBank. The accession numbers is CP016194 (chromosome), CP016195 (plasmid pST7-1), CP016196 (plasmid pST7-2), CP016197 (plasmid pST7-3), CP016198 (plasmid pST7-4).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Authors' Contributions

The experiments and the writing of the manuscript were performed by Jun Zhu, Yiping Liu, and Jing Zhang. The genome assembling, annotation and analysis and the editing of the manuscript was performed by Rui Liu, XuLiu, Baoli Zhang. The design and support of all the manuscript was performed by Jun Zhu. All authors read and approved the final manuscript.

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