Note (Genome Announcement)

Complete genome sequence of a novel bacteriophage SPG24 isolated from Cheonggukjang

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청국장에서 분리된 신생 박테리오파지 SPG24의 전체 염기 서열

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A *Bacillus subtilis* strain, designated G24, was isolated from grape during a study on the fermentation of Cheongkukjang, Korean traditional fast-fermented bean paste. Also, a newly isolated bacteriophage SPG24, which was found to inhibit the fermentation process, uses *B. subtilis* G24 as a host. The complete genome sequence of the bacteriophage SPG24 was 152,060 bp in length, with a G+C content of 42.2%. This sequence included 232 ORF; 58 forward ORFs and 174 reverse ORFs.

Keywords: *Bacillus subtilis*, bacteriophage SPG24, Cheongkukjang

Bacillus species are endospore-forming bacteria that can survive as aerobic or facultative anaerobic Gram-positive bacteria (Ackermann, 1974). This genus is commonly found in nature, in soil, water, and other environmental media, and the major representative species are *Bacillus cereus* and *Bacillus subtilis* (Suttle, 2007; Hatful, 2008). Park (2006) isolated a *B.*

subtilis strain, designated G24, from grape during a study on the fermentation of Cheongkukjang, Korean traditional fast-fermented bean paste, and its nutritional improvement after using *B. subtilis*. Also, a newly isolated bacteriophage, SPG24, which was found to inhibit the fermentation process, uses *B. subtilis* G24 as a host. In the present study, the bacteriophage was isolated, and DNA was extracted and then sequenced by ChunLab Ltd. Co., using a GS-FLX Titanium Junior (Roche) system. Although this bacteriophage had no reference genome sequence, we were able to obtain a single long nucleotide sequence without any gaps by taking advantage of the self-assembling process of the phage (Lee *et al*., 2012; Pires *et al.*, 2014). Sequencing and aligning the high number of copies of the phage, we created a consensus sequence and then configured this sequence as the reference genome sequence. To find open reading frames (ORFs) in the sequence, three different programs, NCBI ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), Glimmer (http://www.ncbi.nlm.nih.gov/genomes/MICROBES/glimmer_3.cgi), and GeneMarkS (http://exon.gatech.edu/genemarks.cgi), were used. Also, the program ARAGORN (http://130.235.46.10/ARAGORN/) was used to

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Table 1. Genome features of the bacteriophage SPG24

Genome features	Value
Genome size (bp)	152,060
No. of contigs	1
G + C content (%)	42.2
tRNA genes	5
No. of ORFs	232
No. of ORFs with predicted function	104

determine the location of tRNA genes. To investigate the function(s) of the protein products of each ORF, the Basic Local Alignment Search Tool (BLAST), provided by NCBI (http://www.ncbi.nlm.nih.gov/blast/), was used. tBLASTx, which uses both a translated nucleotide query sequence and translations of all of the nucleotide sequences in the database, was used to analyze the ORF-encoding sequence. In addition, the programs EBI UniProt (http://www.uniprot.org/) and pfam (http://pfam.sanger.ac.uk/search) were used to compare the results in order to improve accuracy in the analysis. The genome sequence of SPG24 was 152,060 bp in length, with a G+C content of 42.2%. This sequence included 58 forward ORFs and 174 reverse ORFs. Of the estimated 232 proteins encoded in the viral genome, 104 included some annotation on function while 128 were proteins without any identified function (putative proteins). In addition, five tRNA genes, encoding tyrosine, aspartic acid, arginine, asparagine, and serine, were found in the genome.

Nucleotide sequence accession number

The complete genome sequence of the bacteriophage SPG24 was deposited in GenBank under the accession number AB930182. The bacteriophage SPG24 is available at the Bacteriophage Bank of Korea (accession number, BP-15001).

적 요

*Bacillus subtilis*를 이용하여 청국장 발효 연구를 진행하던 중 발효능이 뛰어난 *B. subtilis* G24라는 균주를 새로 분리하였고, 이를 숙주로 이용하는 bacteriophage SPG24를 새로 분리하였다. 본 연구에서는 bacteriophage SPG24의 유전체 서열을 해독하였으며 전체길이 152,060 bp, G+C 함량 42.2%, 232개 ORF의 bacteriophage SPG24는 *B. subtilis* G24를 숙주로 이용함으로써 청국장 발효를 저해하는 것으로 확인되었다.

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