A temperate phage was isolated from emetic *Bacillus cereus* NCTC11143 by mitomycin C and characterized by transmission electron microscopy and DNA and protein analyses. Whole genome sequencing of *Bacillus* phage 11143 was performed by GS-FLX. The phage has a dsDNA genome of 39,077 bp and a 35% G+C content. Bioinformatic analysis of the phage genome revealed 49 putative ORFs involved in replication, morphogenesis, DNA packaging, lysogeny, and host lysis. *Bacillus phage* 11143 could be classified as a member of the Siphoviridae family by morphology and genome structure. Genomic comparisons at the DNA and protein levels revealed homologous genetic modules with patterns and morphogenesis proteins similar to those of other *Bacillus* phages. Thus, *Bacillus* phages might have a mosaic genetic relationship.

**Keywords:** *B. cereus*, temperate phage, DNA sequence, sequence analysis

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# Supplementary data for this paper are available on-line only at http://jmb.or.kr.

*Genome Organization of Temperate Phage 11143 from Emetic Bacillus cereus NCTC11143*

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Laboratories, Detroit, MI, USA) or agar (LBC broth or agar) supplemented with 10 mmol CaCl$_2$ (Sigma-Aldrich, St. Louis, MO, USA) at 37°C overnight in a shaking incubator. Subsequently, 0.1 ml of each bacterial culture was transferred into 50 ml of fresh LBC broth. Induction of temperate phages was performed by addition of mitomycin C (Merck Korea, Seoul, Korea) at a final concentration of 1 µg/ml for about 2 h after inoculation (OD ≈ 0.2–0.3). Induction of temperate phage was confirmed by its growth curve and plaque formation (Fig. 1A). Bacterial cell lysate was centrifuged for 15 min at 10,000 × g and the supernatant was passed through a 0.45 µm syringe filter (Millipore, Billerica, MA, USA) and stored at 4°C and –80°C. Phage concentrations were determined by precipitation with polyethylene glycol 8000 [23]. Phage particles (10$^{10–11}$ PFU/ml) were negatively stained with 2% (w/v) aqueous uranyl acetate (pH 4.5) on a carbon-coated grid and examined by transmission electron microscopy using a JEOL JEM-100S apparatus (Japan Electronics and Optics Laboratory, Tokyo, Japan) at an accelerating voltage of 80 kV. According to morphological analysis, the Bacillus phage 11143 particles had long, non-contractile tails and icosahedral heads belonging to the Siphoviridae family in the order Caudovirales (Fig. 2). Bacteriophages can be tailed, polyhedral, filamentous, or pleomorphic, and most of them contain double-stranded DNA. About 5,568 bacterial viruses have been examined by electron microscopy. At least 5,360 (96.2%) of these are tailed bacteriophages known as Caudovirales [2]. Tailed bacteriophages are considered to be the most diverse and widespread of all viral groups because their properties are highly wide-ranging. Some of these differences include DNA content and composition, host range, physiology, serology, and the nature of constitutive proteins [1].

Phage DNA was isolated from polyethylene glycol-precipitated phage particles by the method of Manfioletti and Schneider [20] with some modification. DNase I (10 µg/ml) and RNase A (20 µg/ml) were added to the phage lysate. After incubation at room temperature for 15 min, 0.5 M EDTA (pH 8) and proteinase K (1 mg/ml) were added, followed by incubation at 65°C for 30 min. After incubation, the nucleic acid was extracted with phenol-chloroform-isoamyl alcohol. The nucleic acid was precipitated with isopropanol and dissolved in sterile distilled water. Phage DNA was stored at –80°C. The DNA was digested with restriction enzymes according to the manufacturer’s recommendations. Additionally, we analyzed purified phage particles for structural protein composition using SDS-PAGE. Protein bands were visualized using Coomassie blue stain. The genomic DNA...
of Bacillus phage 11143 was isolated and digested with restriction enzymes. Various genomic DNA fragmentation patterns and structural proteins were revealed (Fig. 1B).

The genome sequence was determined by ultrahigh-throughput GS-FLX sequencing to 20-fold redundancy on average. The genomic sequences were compared with others in GenBank by the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). Open reading frames (ORFs) were identified with the ORF Finder at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The molecular weight and isoelectric point were calculated with the Compute pl/Mw program (http://www.expasy.ch/tools/pi_tool.html). Rho-independent terminators and tRNA were identified with the Trans Term program (http://nbc11.biologie.uni-kl.de/transterm/) and tRNAscan-SE Search Server (http://lowelab.ucsc.edu/tRNAscan-SE/), respectively. Genomic comparisons at the nucleotide level were made with Mauve software (http://gel.ahabs.wisc.edu/mauve/), using a progressive alignment with the default settings. Comparisons at the proteomic level were made using CoreGenes (http://binf.gmu.edu:8080/CoreGenes3.0). The complete genome sequence of Bacillus phage 11143 was deposited in GenBank under Accession No. GU233956.

Analysis of the Bacillus phage 11143 sequence revealed a dsDNA genome of 39,077 bp. The total G+C content was calculated to be 35%. According to a BlastN search, the sequence shared a very high similarity with a putative prophage region of the genome of B. cereus AH187 (Accession No. CP001177). B. cereus NCTC11143 and AH187 are emetic strains and might have very similar prophage sequences. Forty-nine putative ORFs were identified in the Bacillus phage 11143 genome. Putative functions could be assigned to only 23 of the predicted gene products (46.9%), based on DNA and amino acid sequence similarities. Bacteriophage genomes consist of various structural and functional genes for survival or propagation. Using the tRNAscan-SE program, tRNA genes were not identified. In this study, Bacillus phage 11143 was morphologically most reminiscent of the Siphoviridae family in Caudovirales. Functional ORFs from Bacillus phage 11143 are involved in replication, regulation, DNA packaging, head and tail morphogenesis, lysogeny module, and host lysis (Fig. 3 and Supplementary Material).

Within the DNA packaging modules, the proteins encoded by ORF1 and ORF2 were identified as a small and large subunit of a terminase, respectively. The protein encoded by ORF3 shows similarity to a phage portal protein. Head morphogenesis-related proteins are encoded by ORF4, ORF5, and ORF7. Moreover, tail morphogenesis-associated proteins are encoded by ORF10 and ORF12–15. In the case of ORF15, it is a putative prophage endopeptidase tail conserved domain located in the upstream region. The protein encoded by ORF20 in the lysis region was identified as N-acetylmuramoyl-l-alanine amidase. Endolysins and holins are essential for host cell lysis by bacteriophages, but the latest reports and experiments have revealed that no holin-encoding genes are located upstream of the endolysin genes [5]. The replication regions contained ORF23, ORF26, ORF30, ORF31, ORF36, ORF39, ORF44, and ORF48. In the lysogeny modules, ORF17 and ORF24 were identified as a phage integrase family site-specific recombinase and a site-specific recombinase, respectively. Moreover, ORF45 showed similarity to a DNA integration/recombination/inversion protein.

Whole genome comparisons were made at the DNA level using Mauve and at the protein level using CoreGenes. CoreGenes performs hierarchical and iterative BlastP analyses using one genome as a reference and another as a query [29]. Bacillus phage 11143 and other Bacillus phages belonged to Siphoviridae and showed similar genome size (Bacillus phage 250: GU229986; Bacillus phage Cherry: DQ222851; Bacillus phage IEBH: EU874396; Bacillus phage wbeta: DQ289555). According to a dot-plot analysis with the Blast algorithm, weak sequence similarities (2–6%) were found between Bacillus phage 11143 and other Bacillus phages (Bacillus phage 250, Bacillus phage IEBH, Bacillus phage wbeta), and Bacillus phage 11143 was not similar to Bacillus phage Cherry (data not shown).

Mauve analysis revealed the alignment of five Bacillus phage genomes, explaining mosaicism at the level of the genetic module. Homologous genetic modules are represented by a similar pattern (Fig. 4). CoreGenes analysis showed that the structure-related genes coding for morphogenesis proteins such as tail component protein, tape measure protein, and minor structural protein are similar in Bacillus phage 11143 and other Bacillus phages. Moreover, a replication gene is similar in Bacillus phage 11143 and other Bacillus

![Fig. 3. Schematic representation of the dsDNA genome of Bacillus phage 11143.](Image 50x121 to 546x182)

Forty-nine putative ORFs are represented by arrows, with predicted functions where available. Proposed modules are based on predicted functions. : DNA packaging; : Head assembly; : Tail assembly; : Replication; : Cell lysis; : Lysogeny; : hypothetical protein; : rho-independent terminator.
phages (Fig. 5). Genomes of phages have a mosaic relationship to one another. There is a strong tendency for these mosaics to have genetic functions arranged on their chromosomes in the same order, but when phages are compared for any given function, the genes may or may not be homologous [8]. Furthermore, Casjens [9] recently reported that the various tailed phages do have features in common, and all tailed bacteriophages by definition have tails and all such phages examined utilize a common mechanism of DNA packaging [9]. These results reveal that the genomes of tailed bacteriophages have a mosaic relationship [10]. Lately, bacteriophage mosaic genomes have been described in reviews including experimental reports. For example, phages λ and HK97 have rather similar tail genes but very different head genes [17], and phages λ and N15 have similar virion assembly genes but apparently non-homologous replication genes [22]. Therefore, genomic comparisons at the DNA and protein levels confirmed that Bacillus phages might be genetic mosaics.

In conclusion, Bacillus phage 11143 isolated from a mitomycin C-induced lysate of emetic B. cereus NCTC 11143 has an isometric head, a noncontractile tail, and a double-stranded DNA genome with a length of 39,077 bp and 35% G+C content. Forty-nine putative ORFs are associated with bacteriophage-related genes. According to morphological analysis, Bacillus phage 11143 belongs to the Siphoviridae family. The genomes of Bacillus phages might have a mosaic relationship.

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**Fig. 4.** Genomic alignment of Bacillus phage 11143 and other Bacillus phages using Mauve.
Regions of nucleotide similarity are indicated by the height of the colored bars, and dissimilar regions are in white.

**Fig. 5.** Proteomic comparisons of Bacillus phage 11143 and other Bacillus phages using CoreGenes (GU233956: Bacillus phage 11143; GU229986: Bacillus phage 250; DQ222851: Bacillus phage Cherry; EU874396: Bacillus phage IEBH; DQ289555: Bacillus phage Wbeta).
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