

Complete Genome Sequence of Enterococcal Bacteriophage SAP6

Young-Duck Lee and Jong-Hyun Park

Department of Food Science and Biotechnology, College of Engineering, Gachon University, Sunghnam-Si, Kyunggi-Do, South Korea

***Enterococcus faecalis* is an important bacterium for use as a probiotic and is an opportunistic pathogen in human beings. The antibiotic resistance acquired by *E. faecalis* is restricted to antibiotics used in the clinical setting. While screening for alternative antibiotics for use against multidrug-resistant *E. faecalis*, we isolated a virulent enterococcal bacteriophage, SAP6, belonging to the family *Siphoviridae*. To our knowledge, this study is the first to report the complete genome sequence of bacteriophage SAP6, which might be used as a therapeutic agent in combination with alternative antibiotics for multidrug-resistant *E. faecalis*.**

Enterococcus faecalis is used as a starter for fermented food and probiotics (5). However, it can also cause infectious diseases in human beings and biogenic amine production in foods (3). Moreover, acquired resistance of *E. faecalis* to aminoglycosides, glycopeptides, and other antibiotics is being increasingly reported in isolates, and the therapeutic effect obtained in these multidrug-resistant strains is limited (14). Acquired resistance to various antibiotics is mediated by various mechanisms such as conjugation or gene transfer. Biocontrol involving biological treatment (e.g., use of bacteriophages) has recently been used to minimize the risk of infection with *E. faecalis* (8, 12). Bacteriophages have been intensively studied and used for various practical applications such as phage therapy (13), bacterial pathogen detection (2), food-borne-pathogen biocontrol (6, 7), and bioremediation (16). This study reports the morphogenetic properties and complete genome sequence of the virulent enterococcal bacteriophage SAP6, which was newly isolated from sewage.

The morphological characteristics of bacteriophage SAP6 were examined by transmission electron microscopy. Bacteriophage particles were negatively stained with 2% aqueous uranyl acetate on a carbon-coated grid and examined. The genomic DNA of SAP6 was isolated using the method reported by Manfioletti and Schneider (10). Its genomic sequence was determined using ultra-high-throughput Genome Sequencer FLX (GS-FLX) sequencing. The nucleotide sequences were compared with those of other genes in GenBank by using the BLAST program (15). The open reading frames (ORFs) were identified using the NCBI ORF Finder (15). The molecular weight and isoelectric point were calculated using the ExPASy Compute pI/M_w program (4). The tRNA sequences were analyzed using the tRNAscan-SE program (9). Conserved protein domain analysis was performed using BLASTP and the NCBI CDD (11).

Morphological analysis showed that bacteriophage SAP6 belonged to the family *Siphoviridae* (1). The genomic sequence of SAP6 was composed of 58,619 bp, with a G+C content of 40.00%. The genome showed 44 ORFs, and the tRNA sequences could not be determined. A BLASTN search of the genomic sequences did not indicate any significant similarity between the genomic sequence of SAP6 and those of other previously reported bacteriophages. ORFs from the SAP6 genome were involved in DNA packaging, morphogenesis, replication, DNA manipulation, and host lysis. The morphogenesis modules and DNA packaging modules contained head morphogenesis-related proteins (major head protein, head morphogenesis protein, minor capsid protein, minor structural protein), tail morphogenesis-associated proteins (ma-

ior tail protein, tail fiber protein, major tail protein), a terminase, and a phage portal protein. The genome encoded replication-related proteins and DNA manipulation proteins (DNA helicase, primase, polymerase, replication protein, transcriptional regulator, deaminase, metalloprotease, glycosyltransferase, endo-DNase, endonuclease, methyltransferase, pyrophosphohydrolase). Bacteriophage SAP6 also contained an *N*-acetylmuramoyl-L-alanine amidase for host lysis.

In conclusion, to our knowledge, this study is the first to report the morphology and complete genome sequence of the virulent enterococcal bacteriophage SAP6. Further study of this bacteriophage might enable its use as a therapeutic agent in combination with alternative antibiotics against multidrug-resistant *E. faecalis*.

Nucleotide sequence accession number. The complete genome sequence of bacteriophage SAP6 is available in GenBank under accession number [JF731128](https://www.ncbi.nlm.nih.gov/nuccore/JF731128).

ACKNOWLEDGMENT

This work was supported by a National Research Foundation (NRF) grant funded by the South Korean government (20110012221).

REFERENCES

- Ackermann HW. 2003. Bacteriophage observations and evolution. *Res. Microbiol.* 154:245–251.
- Dinnes J, et al. 2007. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. *Health Technol. Assess.* 11(3):1–196.
- Franz CM, Stiles ME, Schleifer KH, Holzapfel WH. 2003. Enterococci in foods—a conundrum for food safety. *Int. J. Food Microbiol.* 88:105–122.
- Gasteiger E, et al. 2005. Protein identification and analysis tools on the ExPASy server, p 575–576. *In* Walker JM (ed), *The proteomics protocols handbook*. Humana Press, Totowa, NJ.
- Giraffa G. 2003. Functionality of enterococci in dairy products. *Int. J. Food Microbiol.* 88:215–222.
- Greer GG. 2005. Bacteriophage control of foodborne bacteria. *J. Food Prot.* 68:1102–1111.
- Hudson JA, Billington C, Carey-Smith G, Greening G. 2005. Bacteriophages as biocontrol agents in food. *J. Food Prot.* 68:426–437.
- Letkiewicz S, Miedzybrodzki R, Fortuna W, Weber-Dabrowska B, Górski A. 2009. Eradication of *Enterococcus faecalis* by phage therapy in chronic bacterial prostatitis—case report. *Folia Microbiol.* 54:457–461.
- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detec-

Received 7 February 2012 Accepted 9 February 2012

Address correspondence to Jong-Hyun Park, p5062@kyungwon.ac.kr.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JVI.00321-12

- tion of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25: 955–964.
10. Manfioletti G, Schneider C. 1988. A new and fast method for preparing high quality lambda DNA suitable for sequencing. *Nucleic Acids Res.* 16: 2873–2884.
 11. Marchler-Bauer A, et al. 2007. CDD: a conserved domain database for interactive domain family analysis. *Nucleic Acids Res.* 35:D237–D240.
 12. McLean SK, Dunn LA, Palombo EA. 2011. Bacteriophage biocontrol has the potential to reduce enterococci on hospital fabrics, plastic and glass. *World J. Microbiol. Biotechnol.* 27:1713–1717.
 13. Sulakvelidze A, Alavidze Z, Morris JG. 2001. Bacteriophage therapy. *Antimicrob. Agents Chemother.* 45:649–659.
 14. Tendolkar PM, Baghdayan AS, Shankar N. 2003. Pathogenic enterococci new developments in the 21st century. *Cell. Mol. Life Sci.* 60: 2622–2636.
 15. Wheeler DL, et al. 2003. Database resources of the National Center for Biotechnology. *Nucleic Acids Res.* 31:28–33.
 16. Withey S, Cartmell E, Avery LM, Stephenson T. 2005. Bacteriophages—potential for application in wastewater treatment processes. *Sci. Total Environ.* 339:1–18.