FEMS MICROBIOLOGY LETTERS

GENOME ANNOUNCEMENT

# Complete genome sequence of the bacteriophage YMC/09/04/R1988 MRSA BP: a lytic phage from a methicillin-resistant *Staphylococcus aureus* isolate

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Received 14 July 2014; accepted 12 August 2014. Final version published online 4 September 2014.

DOI: 10.1111/1574-6968.12580

Editor: Mark Enright

**Keywords**
antibiotic resistant bacteria; lytic bacteriophage; alternative therapeutic agent; infection.

## Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing cause of serious infection, both in the community and hospital settings. Despite sophisticated strategies and efforts, the antibiotic options for treating MRSA infection have been narrowed due to the limited number of newly developed antimicrobials. Herein, we analyze the completely sequenced genome of a novel virulent phage YMC/09/04/R1988 MRSA BP as a potential alternative anti-MRSA agent, which lysed clinical isolates from a patient admitted to the hospital due to hip disarticulation. The phage contains a linear double-stranded DNA genome of 44,459 bp in length, with 33.37% GC content, 62 predicted open reading frames (ORFs), and annotated functions of only 23 ORFs that are associated with structural assembly, host lysis, DNA replication, and modification. It showed a broad host range (17 of 30 strains) against MRSA strains in clinical isolates.

Bacteriophages (phages) are specific bacterial viruses that infect and lyse host bacteria. As bacteriophages were discovered and isolated by Felix d'Herelle in the early 20th century, a number of treatments for bacterial infections using bacteriophage therapy have been reported (Wittebole *et al.*, 2014). Bacteriophage therapy was restrictively researched and applied because of the discovery and development of antibiotics. Recently, however, the rise and emergence of multiantibiotic-resistant bacteria has led to an increasing interest in phages as new therapeutic agents (Merril *et al.*, 2003).

*Staphylococcus aureus*, a Gram-positive bacterium, is one of the most important pathogens causing several serious infections (Styers *et al.*, 2006). In particular, since methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in the 1960s, infections with MRSA have been rising globally and are difficult to treat due to acquired resistance to most commonly antimicrobial agents (Grundmann *et al.*, 2006). As a result, bacteriophages such as *S. aureus* phage ϕ812, ϕMR11, and S13 have been newly studied as alternative therapeutic tools to combat pathogens (Pantucek *et al.*, 1998; Matsuzaki *et al.*, 2003; Rahman *et al.*, 2011; Deghorain & Van Melderen, 2012; Takemura-Uchiyama *et al.*, 2014).

In this study, we isolated and characterized a novel lytic bacteriophage, Bϕ-R1988 (YMC/09/04/R1988 MRSA BP), which lyses MRSA strains. To our knowledge, this is the first study of a bacteriophage active against MRSA strains isolated from a hospital patient in Korea, and this bacteriophage has application as a new antibiotic agent.

Bϕ-R1988 was isolated from a sewage sample and purified by the agar double-layer method (Kropinski *et al.*, 2009). A spot test (Kropinski *et al.*, 2009) was used to determine the host range of the Bϕ-R1988. MRSA strains were collected from clinical isolates from a university hospital in South Korea and were used to isolate bacteriophages. The genomic DNA was prepared by the phenol extraction method (Wilcox *et al.*, 1996) and was sequenced using the Illumine Genome Analyzer (272-fold average coverage; Illumina, Inc., San Diego, CA).

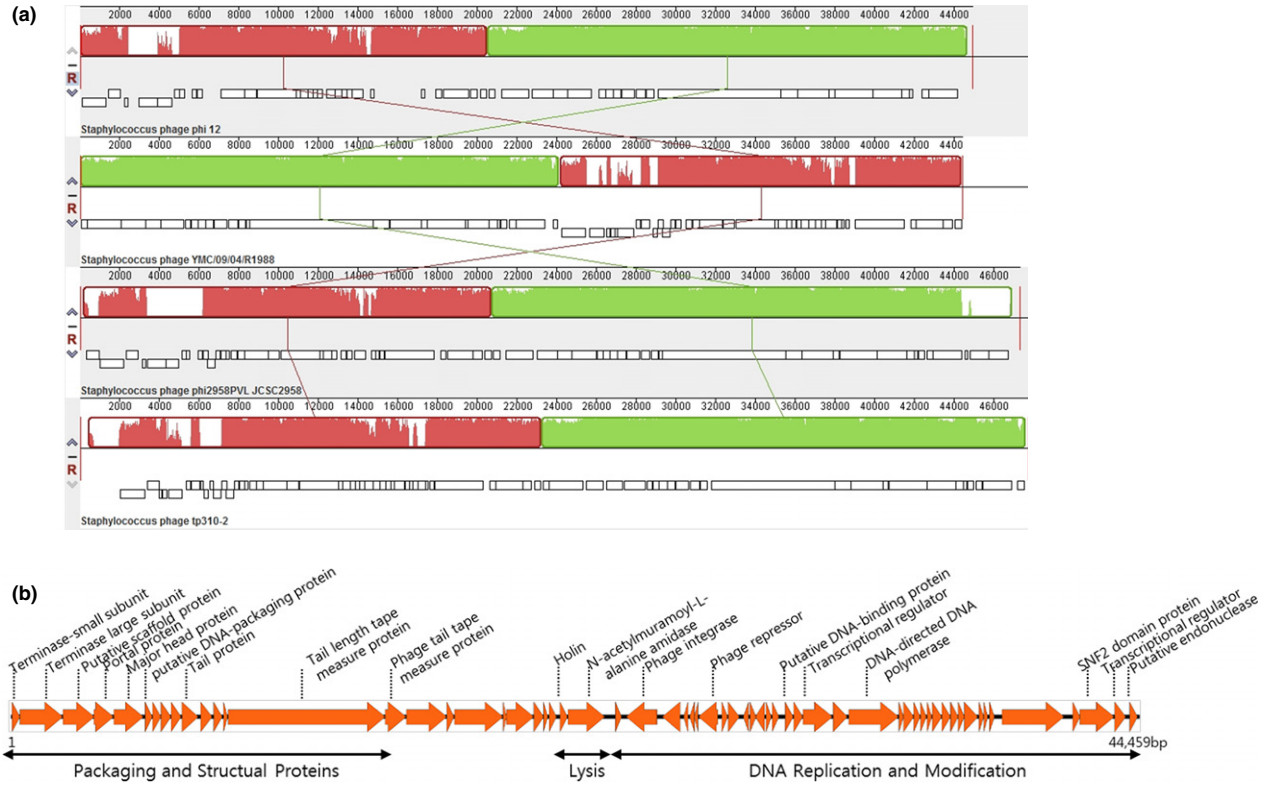


**Fig. 1.** Genome comparison of Bϕ-R1988 and *Staphylococcus* phage phi12, phage phi2958PVL, and phagephi310-1 at the DNA level. This analysis was carried out using MAUVE software 2.3.1 with default parameters. Connection lines indicate similar regions between ORFs of Bϕ-R1988 and three *Staphylococcus* phages. White blocks indicate annotated genes and reverse strands shifted downward (a). The genome map of Bϕ-R1988 indicates a putative function of each ORF with the direction of transcription shown by arrows (b).

The novel MRSA virulent phage, Bϕ-R1988, contained double-stranded DNA with 44,459 bp and a G + C content of 33.37%. Genome analysis of the phage revealed 62 putative open reading frames (ORFs), and tRNA genes were not identified; however, most of the annotated genes (40 of 62 ORFs) were hypothetical proteins (Table S1, Supporting information). According to the morphological characteristics by transmission electronic microscopy (JEOL JEM-1011, Tokyo, Japan), Bϕ-R1988 has a prolate icosahedral head and a long noncontractile tail, and thus, it belongs to the family *Siphoviridae* in the order *Caudovirales*.

Genome alignment was performed using MAUVE software (Darling *et al.*, 2004), and phage Bϕ-R1988 was compared with three other phages (Fig. 1a). The results showed relatively high extensive homology that was located between 20 and 24,049 bp (DNA packaging, structural protein, and lysis regions) and 24,236–44,374 bp (DNA replication, and modification regions) in the Bϕ-R1988 DNA region and between 21,118–44,406 bp and 979–20,694 bp in the phi2958PVL DNA region, respectively. As mentioned above, most of the genome of phage Bϕ-R1988 was arranged in the reverse direction with phage phi2958PVL, phage phi12, and phage tp310-2. However, some ORFs associated with each functional DNA cluster in the genome showed a different direction. Bϕ-R1988 showed 86% similarity to phage phi2958PVL at the DNA level, having a genome size of 47,342 bp (GenBank accession no. AP009363, unclassified phage) and indicated 89% DNA sequence similarities to phage phi 12 (GenBank Accession No. AF424782) and phage tp310-2 (GenBank Accession No. EF462198) belonging to *Siphoviridae*, which have genome sizes of 44,970 and 47,785 bp, respectively (Fig. 1a).

The genome of Bϕ-R1988 is comprised of the following three functional protein clusters: (1) DNA packaging and structural assembly proteins (ORF1, ORF2: small and large subunits of terminase, ORF3: portal protein, ORF5: major head protein, ORF6: DNA packaging protein, ORF10: tail protein, ORF14, ORF15: tail length tape measure protein), (2) host lysis proteins (ORF24: holin, ORF25: endolysin), and (3) DNA replication and modification proteins (ORF27: phage integrase, ORF33: regulatory protein, ORF40: Transcriptional regulator, ORF44: DNA-directed DNA polymerase, ORF61: translational regulator, ORF62: endonuclease; Fig. 1b).

Endolysins are phage-encoded enzymes that lyse target bacteria by specifically destroying them through peptidoglycan digestion (Loessner, 2005). In addition, the holin is a phage-encoded small-membrane protein that forms holes in the cytoplasmic membrane, which allows the endolysin to escape and break the peptidoglycan (Wang *et al.*, 2000).

As ORF24 and ORF25 are lysis modules, ORF24 identified as a holin-exhibited 100% protein sequence identity to the *Staphylococcu*s phage phiSLT (GenBank Accession No. NP_075521.1). The ORF25 identified as a putative *N*-acetylmuramoyl-L-alanine amidase possessing lysis activity of host peptidoglycan showed high protein sequence identity (98%) to sequences from *Staphylococcus* phage tp310-1 (GenBank Accession No. YP_001429893.1). Furthermore, the sequences of ORF5 (phage head protein) and ORF10 (phage tail protein) are highly similar to those of phage phiSLT, both with 100% homology.

In addition, Bϕ-R1988 exhibited a broad lytic spectrum (17 of 30 strains) through host range studies against collected MRSA strains of hospital patients, which were typed using pulsed-field gel electrophoresis (PFGE; data not shown).

In this study, a novel lytic bacteriophage, Bϕ-R1988, which lyses MRSA strains, was isolated and sequenced. This is the first report about a lytic bacteriophage against clinical MRSA strains collected from patients at a hospital in South Korea.

GenBank database Accession Number: KF598856.

## Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2012R1A1A2009064) and by a grant from the Brain Korea 21 PLUS Project for Medical Science, Yonsei University. We are grateful to Ms. Sori Jong for technical assistance.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Data S1.** Material and methods.
**Table S1.** Features of annotated putative ORFs in YMC/09/04/R1988 MRSA BP.