

Complete Genome Sequences of Broad-Host-Range *Pseudomonas aeruginosa* Bacteriophages Φ R18 and Φ S12-1

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***Pseudomonas aeruginosa* is an important cause of racehorse keratitis. Bacteriophage therapy has the potential to aid in the prevention and treatment of diseases caused by *P. aeruginosa*. We present here the complete genome sequences of two phages, Φ R18 and Φ S12-1, which exhibit infectivity for a broad range of *P. aeruginosa* isolates.**

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Pseudomonas aeruginosa is a major causative bacterium of ulcerative keratitis in racehorses (1). This pathogen secretes various proteases, including elastase, which is known to degrade corneal collagen. Therefore, bacterial keratitis caused by *P. aeruginosa* tends to be aggravated (2). Moreover, *P. aeruginosa* and other pathogens are attaining antibiotic resistance throughout the world (<http://www.who.int/mediacentre/news/releases/2014/amr-report/en/>). To solve the antibiotic resistance problem, phage therapy has attracted the interest of many researchers (3, 4).

Phages Φ R18 (belonging to *Podoviridae*) and Φ S12-1 (belonging to *Myoviridae*), both of which were isolated from sewage treatment plants at Hokkaido in Japan, were purified from their lysate with CsCl density gradient ultracentrifugation, and their DNA were extracted. We submitted the corresponding DNAs to Hokkaido System Science Co., Ltd., for whole-genome sequencing. The samples were sequenced as paired-end reads on the Illumina HiSeq 2500 (Illumina, Inc., San Diego, CA). A total of 1,129,756 and 1,116,928 reads, each with mean lengths of 100 bp, were obtained from Φ R18 and Φ S12-1, respectively. The obtained reads were assembled *de novo* using Velvet (version 1.2.8) (5). Sequences were annotated using the Rapid Annotation using Subsystem Technology (RAST) server (6).

Overwrapped bases were recognized in both sequences. Therefore, we found two DNAs to be circular. The genomic double-stranded DNA of Φ R18 consists of 63,560 bp, with a G+C content of 60.35%. The genome was scanned for coding DNA sequences (CDSs); a total of 86 CDSs were detected. The Φ R18 genome is 97% identical to that of the *P. aeruginosa* phage KPP25 (GenBank accession no. AB910393) (7). Φ R18 has two unique sequences of about 150 bp and 400 bp in length, including CDSs of unknown functional proteins. The Φ S12-1 phage has a genome of 66,257 bp, with 55.58% G+C content; 94 CDSs were detected. The Φ S12-1 genome is 97% identical to that of *P. aeruginosa* phage vB_PaeM_PA01_Ab27 (GenBank accession no. LN610579) (8). Φ S12-1 has about 3,000 bp of unique sequence, including CDSs

for the putative tail protein, putative internal protein, and 4 hypothetical proteins (function is unknown). The endolysins of both the Φ R18 and Φ S12-1 phages have two lysozyme-like domains. Neither of these phages have indicators of lysogenicity or toxic proteins, as detected by the LLNL Virulence Database (9).

In a fundamental aspect, the relationships between genome information and host range variation of these phages might provide insights into mechanisms of host specificity. In addition, information on endolysins, the agents for potential alternatives to antibiotics, is shown in this study and will be very useful for the development of endolysin therapy.

Nucleotide sequence accession numbers. The complete genome sequences of Φ R18 and Φ S12-1 have been deposited in the DNA Data Bank of Japan (DDBJ) under accession numbers [LC102729](https://www.ncbi.nlm.nih.gov/nuccore/LC102729) and [LC102730](https://www.ncbi.nlm.nih.gov/nuccore/LC102730), respectively.

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REFERENCES

- Wada S, Hobo S, Niwa H. 2010. Ulcerative keratitis in thoroughbred racehorses in Japan from 1997 to 2008. *Vet Ophthalmol* 13:99–105. <http://dx.doi.org/10.1111/j.1463-5224.2010.00767.x>.
- Fleiszig SM, Evans DJ. 2002. The pathogenesis of bacterial keratitis: studies with *Pseudomonas aeruginosa*. *Clin Exp Optom* 85:271–278. <http://dx.doi.org/10.1111/j.1444-0938.2002.tb03082.x>.
- D'Herelle F. 1931. Bacteriophage as a treatment in acute medical and surgical infections. *Bull NY Acad Med* 7:329–348.

4. Dublanchet A, Fruciano E. 2008. A short history of phage therapy. *Med Mal Infect* 38:415–420. <http://dx.doi.org/10.1016/j.medmal.2008.06.016>.
5. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
6. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res* 42:D206–D214. <http://dx.doi.org/10.1093/nar/gkt1226>.
7. Miyata R, Yamaguchi K, Uchiyama J, Shigehisa R, Takemura-Uchiyama I, Kato S, Ujihara T, Sakaguchi Y, Daibata M, Matsuzaki S. 2014. Characterization of a novel *Pseudomonas aeruginosa* bacteriophage, KPP25, of the family *Podoviridae*. *Virus Res* 189:43–46. <http://dx.doi.org/10.1016/j.virusres.2014.04.019>.
8. Eshoh C, Latino L, Midoux C, Blouin Y, Loukou G, Nguetta SP, Lathro S, Cablanmian A, Kouassi AK, Vergnaud G, Pourcel C. 2015. Investigation of a large collection of *Pseudomonas aeruginosa* bacteriophages collected from a single environmental source in Abidjan, Côte d'Ivoire. *PLoS One* 10:e0130548. <http://dx.doi.org/10.1371/journal.pone.0130548>.
9. Zhou CE, Smith J, Lam M, Zemla A, Dyer MD, Slezak T. 2007. MvirDB—a microbial database of protein toxins, virulence factors and antibiotic resistance genes for bio-defence applications. *Nucleic Acids Res* 35:D391–D394. <http://dx.doi.org/10.1093/nar/gkl791>.