



## Complete Genome Sequence of *Salmonella enterica* Serovar Typhimurium Myophage Mushroom

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*Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) is a leading cause of foodborne illness worldwide. Over the past two decades, strains resistant to antibiotics have begun to emerge, highlighting the need for alternative treatment strategies such as bacteriophage therapy. Here, we present the complete genome of Mushroom, an *S.* Typhimurium myophage.

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**S**almonella enterica serovar Typhimurium (*S*. Typhimurium) is a leading cause of gastroenteritis in humans worldwide. With growing data that shows increasing drug resistance of *S*. Typhimurium, other alternatives, like bacteriophage therapy, are needed (1, 2). Future application of phage therapy in the United States will most certainly require detailed knowledge of the phages utilized (3). Hence, we present the complete genome of *S*. Typhimurium myophage Mushroom. Mushroom is a component of IntestiPhage (developed by the George Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia), a cocktail of 23 phages active against several enterobacteria strains (4).

Bacteriophage Mushroom was isolated from IntestiPhage (Lot #M2-401). DNA was sequenced in an Illumina MiSeq 250-bp paired-end run with a 550-bp insert library at the Genomic Sequencing and Analysis Facility at the University of Texas (Austin, TX). Quality controlled, trimmed reads were assembled to a single contig at 27.3-fold coverage using Velvet version 1.2.10. The contig was confirmed to be complete by PCR using primers that face the upstream and downstream ends of the DNA. Products from the PCR amplification of the junctions of concatemeric molecules were sequenced by Sanger sequencing (Eton Bioscience, San Diego, CA). Genes were predicted using GeneMarkS (5) and corrected using software tools available on the Center for Phage Technology Galaxy instance (https://cpt.tamu.edu/galaxy -public/). Morphology was determined using transmission electron microscopy performed at the Texas A&M University Microscopy and Imaging Center.

Mushroom has an 87,709-bp genome with a G+C content of 39.03% and a coding density of 88.5%. Mushroom shares 48.6% nucleotide sequence identity with *Salmonella* phage Felix O1 (NC\_005282) as determined by Emboss Stretcher (6). It is a member of the "Lytic 15" phage cluster as defined by Grose and Casjens (7). The G+C content was lower compared to the host (52.2%), a common feature of Felix O1-like phages (8, 9). Mushroom encodes twenty-three tRNAs, two of which are pseudo tRNA genes (10, 11). Interestingly, Mushroom does not encode the tmRNA, *ssrA*, encoded by Felix O1 (10). For annotation purposes, the genome has been opened to *rIIa*.

Mushroom encodes core genes representative of Felix O1-like phages involved in biosynthesis, replication, morphogenesis, and lysis (8, 12). Unlike the DNA polymerase of Felix O1, however, which is encoded by a single gene, the DNA polymerase of Mushroom exists as two genes whose products align with the single protein of Felix O1 (8). The region separating the two polymerase genes in Mushroom is 383-bp in length and has no obvious open reading frame corresponding to a homing endonuclease. The anaerobic ribonucleoside diphosphate reductase of Mushroom is disrupted by a free-standing homing endonuclease, as is seen in the ribonucleoside reductase of bacteriophage T4 (13). FelixO1, on the other hand has no reported T4-like intervening homing endonucleases (8). Mushroom encodes a T4-like lysis system with a soluble lysozyme, a class-III holin (1 transmembrane domain; N-in, C-out topology), and imbedded inner and outer membrane spanin proteins (14–16).

**Nucleotide sequence accession number.** The genome sequence of Mushroom was contributed as accession no. KP143762 to GenBank.

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## REFERENCES

- Gomes-Neves E, Antunes P, Manageiro V, Gärtner F, Caniça M, da Costa JM, Peixe L. 2014. Clinically relevant multidrug resistant *Salmonella enterica* in swine and meat handlers at the abattoir. Vet Microbiol 168:229–233. http://dx.doi.org/10.1016/j.vetmic.2013.10.017.
- Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM. 2011. Phage treatment of human infections. Bacteriophage 1:66–85. http://dx.doi.org/10.4161/ bact.1.2.15845.

- 3. Keen EC. 2012. Phage therapy: concept to cure. Front Microbiol 3:238. http://dx.doi.org/10.3389/fmicb.2012.00238.
- Kutter E, De Vos D, Gvasalia G, Alavidze Z, Gogokhia L, Kuhl S, Abedon ST. 2010. Phage therapy in clinical practice: treatment of human infections. Curr Pharm Biotechnol 11:69–86. http://dx.doi.org/10.2174/ 138920110790725401.
- Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a selftraining method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res 29:2607–2618. http://dx.doi.org/10.1093/nar/29.12.2607.
- Myers EW, Miller W. 1988. Optimal alignments in linear space. Comput Appl Biosci 4:11–17. http://dx.doi.org/10.1093/bioinformatics/ 4.1.11.
- Grose JH, Casjens SR. 2014. Understanding the enormous diversity of bacteriophages: the tailed phages that infect the bacterial family Enterobacteriaceae. Virology 468–470C:421–443.
- Whichard JM, Weigt LA, Borris DJ, Li LL, Zhang Q, Kapur V, Pierson FW, Lingohr EJ, She YM, Kropinski AM, Sriranganathan N. 2010. Complete genomic sequence of bacteriophage felix o1. Viruses 2:710–730. http://dx.doi.org/10.3390/v2030710.
- McClelland M, Sanderson KE, Spieth J, Clifton SW, Latreille P, Courtney L, Porwollik S, Ali J, Dante M, Du F, Hou S, Layman D, Leonard S, Nguyen C, Scott K, Holmes A, Grewal N, Mulvaney E, Ryan E, Sun H, Florea L, Miller W, Stoneking T, Nhan M, Waterston R, Wilson RK. 2001. Complete genome sequence of Salmonella en-

terica serovar typhimurium LT2. Nature 413:852-856. http://dx.doi.org/10.1038/35101614.

- Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32:11–16. http://dx.doi.org/10.1093/nar/gkh152.
- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25: 955–964. http://dx.doi.org/10.1093/nar/25.5.0955.
- Lavigne R, Darius P, Summer EJ, Seto D, Mahadevan P, Nilsson AS, Ackermann HW, Kropinski AM. 2009. Classification of Myoviridae bacteriophages using protein sequence similarity. BMC Microbiol 9:224. http://dx.doi.org/10.1186/1471-2180-9-224.
- Friedrich NC, Torrents E, Gibb EA, Sahlin M, Sjöberg BM, Edgell DR. 2007. Insertion of a homing endonuclease creates a genes-in-pieces ribonucleotide reductase that retains function. Proc Natl Acad Sci U S A 104: 6176–6181. http://dx.doi.org/10.1073/pnas.0609915104.
- 14. Moussa SH, Kuznetsov V, Tran TA, Sacchettini JC, Young R. 2012. Protein determinants of phage T4 lysis inhibition. Protein Sci 21:571–582. http://dx.doi.org/10.1002/pro.2042.
- Wang IN, Smith DL, Young R. 2000. Holins: the protein clocks of bacteriophage infections. Annu Rev Microbiol 54:799-825. http:// dx.doi.org/10.1146/annurev.micro.54.1.799.
- Summer EJ, Berry J, Tran TA, Niu L, Struck DK, Young R. 2007. Rz/Rz1 lysis gene equivalents in phages of gram-negative hosts. J Mol Biol 373: 1098–1112. http://dx.doi.org/10.1016/j.jmb.2007.08.045.