

VIRUSES



Whole-Genome Sequence of the Novel *Enterobacter* Bacteriophage Arya with an Integrase Pseudogene, Isolated from the Gut of the Formosan Subterranean Termite

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ABSTRACT We isolated and sequenced the novel *Enterobacter* bacteriophage Arya from termite gut. The genome showed synteny to lytic bacteriophage genomes; however, the genome encodes a truncated, putatively nonfunctional integrase pseudogene. Lysogeny-related genes were previously observed in certain lytic phages, but their role and evolution remain unclear.

Previous studies suggest that the termite gut contains a population of unexplored novel bacteriophages (1, 2). In this study, we isolated a novel bacteriophage, Arya, infecting *Enterobacter* sp. CT7 (GenBank accession no. KT204538), which is the third bacteriophage isolated from the termite gut and the second to infect *Enterobacter* sp. CT7.

Bacteriophage Arya was isolated from the gut of the Formosan subterranean termite, Coptotermes formosanus. Phage isolation, DNA extraction, DNA sequencing and annotation, and electron microscopy were carried out as described previously (1). Bacteriophage Arya produced small but clear plaques on Enterobacter sp. CT7. DNA sequencing produced a linear contig with terminal redundancy. PCR and restriction digestion confirmed the DNA to be circularly permuted. Arya has a genome length of 41,918 bp, with a G+C content of 54%. The genome has a total of 64 predicted protein-coding sequences and an arginine tRNA gene. Of the 64 genes, 55 produced a match in the NCBI GenBank protein nr database. Based on similarity to sequences in the database, 22 proteins were assigned a putative function. The overall genome architecture is conserved, with a packaging module, a structural module, a DNA replication/ metabolism module, and a lysis cassette. The genome of phage Arya showed a high level of synteny to Escherichia phage vB EcoM ECO1230-10 (3), Escherichia phage vB_EcoM-ep3 (4), and Pseudomonas phage PPpW-3 (5). The Escherichia bacteriophages vB_EcoM_ECO1230-10 and vB_EcoM-ep3 are lytic, and no integrase or other lysogeny genes are present in their genomes (3, 4). Despite being lytic, phage PPpW-3 harbors a predicted integrase pseudogene. The Arya genome also contains a predicted integrase pseudogene. It encodes a 43-amino-acid product which is small compared to products of other integrases and is most likely nonfunctional (6). Both the PPpW-3 and Arya genomes contain an arginine tRNA gene located next to the integrase pseudogene. Also, similar to phage PPpW-3, Arya did not show the presence of other essential lysogeny genes (repressor and antirepressor genes). The presence of an integrase pseudogene in phage PPpW-3 is hypothesized to be the result of a horizontal gene transfer event (3). Electron microscopy and VIRFAM (7) analysis confirmed phage Arya to be a member of the Myoviridae family.

In another study, we isolated bacteriophage Tyrion, which also infects *Enterobacter* sp. CT7 (2). Phage Tyrion is lysogenic and is predicted to alter the host cell receptors to provide superinfection immunity against the host. Isolation and sequencing of bacte-

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* Present address: Chinmay Vijay Tikhe, W. Harry Feinstone Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA. riophage Arya provides us with a model system for studying superinfection immunity and the dynamics among a bacterial host, a lytic phage, and a lysogenic phage.

Accession number(s). The complete annotated sequence of the *Enterobacter* phage Arya genome can be accessed under the GenBank accession no. KX231828.

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REFERENCES

- Tikhe CV, Martin TM, Gissendanner CR, Husseneder C. 2015. Complete genome sequence of *Citrobacter* phage CVT22 isolated from the gut of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Genome Announc 3(4):e00408-15. https://doi.org/10.1128/genomeA .00408-15.
- Tikhe CV, Martin TM, Gissendanner CR, Husseneder C. 2018. Wholegenome sequence of the novel temperate *Enterobacter* bacteriophage Tyrion, isolated from the gut of the Formosan subterranean termite. Genome Announc 6(1): e00839-17. https://doi.org/10.1128/genomeA.00839-17.
- Santos TM, Bicalho RC. 2011. Complete genome sequence of vB_EcoM_ ECO1230-10: a coliphage with therapeutic potential for bovine metritis. Vet Microbiol 148:267–275. https://doi.org/10.1016/j.vetmic.2010.08.034.
- 4. Lv M, Wang S, Yan G, Sun C, Feng X, Gu J, Han W, Lei L. 2015. Genome

sequencing and analysis of an *Escherichia coli* phage vB_EcoM-ep3 with a novel lysin, Lysep3. Virus Genes 50:487–497. https://doi.org/10.1007/s11262-015-1195-8.

- Park SC, Shimamura I, Fukunaga M, Mori KI, Nakai T. 2000. Isolation of bacteriophages specific to a fish pathogen, *Pseudomonas plecoglossicida*, as a candidate for disease control. Appl Environ Microbiol 66:1416–1422. https://doi.org/10.1128/AEM.66.4.1416-1422.2000.
- Groth AC, Calos MP. 2004. Phage integrases: biology and applications. J Mol Biol 335:667–678. https://doi.org/10.1016/j.jmb.2003.09.082.
- Lopes A, Tavares P, Petit MA, Guérois R, Zinn-Justin S. 2014. Automated classification of tailed bacteriophages according to their neck organization. BMC Genomics 15:1027. https://doi.org/10.1186/1471-2164 -15-1027.