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Genome Sequences of *Listeria* Phages Induced from Lysogenic Isolates of *Listeria monocytogenes* from Seafood and a Seafood Processing Environment in Thailand

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ABSTRACT We report here the complete genome sequences of three *Listeria* phages (PSU-VKH-LP019, PSU-VKH-LP040, and PSU-VKH-LP041), which were newly induced from lysogenic isolates of *Listeria monocytogenes* from seafood and a seafood processing environment in Thailand. The three phages show circularly permuted double-stranded DNA genomes with sizes of 38.6, 39.6, and 48.3 kb.

Prophage diversity is of interest since prophages are commonly present in the genomes of *Listeria monocytogenes* strains (1, 2). They play an important role in the evolution (3), survival, and persistence (4, 5) of *L. monocytogenes*. We have an ongoing project for screening lysogenic isolates of *Listeria* from various sources, including seafood and a seafood processing environment. Of these lysogenic isolates, an induced form of prophage(s) from selected isolates of *L. monocytogenes* was examined to understand the prophage diversity. We report here the complete genome sequences of three induced phages, PSU-VKH-LP019, PSU-VKH-LP040, and PSU-VKH-LP041 (hereafter referred to as LP019, LP040, and LP041, respectively).

Phage DNA was extracted by the phenol-chloroform method, as previously described (6). Fragmentation of DNA was performed, and high-quality sequencing libraries were sequenced using the Illumina HiSeq 2500 platform with 100-bp paired-end reads at Macrogen, Inc. (Seoul, South Korea). Then, low-quality reads were filtered by Trimmomatic (7). SOAP*denovo*2 was utilized for *de novo* assembly (8) before a prediction of open reading frames (ORFs) using Glimmer 2 (9) was made. An automatic genome annotation was performed by RAST (10) and Phaster (11) and then verified by BLAST (12), InterPro (http://www.ebi.ac.uk/interpro) (13), and Artemis (14). tRNA was detected using the tRNAscan-SE search server (15).

Sequencing of the induced phages by the Illumina HiSeq 2500 platform yielded 7 to 11 million reads, with an average sequencing coverage of 15,000×. *De novo* assembly resulted in a single contig for each phage, suggesting a complete genome. These genomes were circularly permuted terminally redundant double-stranded DNA genomes. The genome sizes of these phages ranged from 38 to 48 kb, which is consistent with the size range of previously reported temperate *Listeria* phages (16–19). A lysogeny module, including integrase and transcriptional regulator/repressor genes, was observed, thus confirming the temperate characteristic of these sequenced phages. No tRNAs were found in the genomes of these phages.

Phage LP019 was induced from a lysogenic *L. monocytogenes* isolate, PSU-KV-134LM, obtained from a seafood product (fish stick) using *L. monocytogenes* FSL J1-208 as a propagating host (20). This phage is 38,601 bp in length, with a GC content of

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35.7%. A total of 66 ORFs were detected, of which 28 ORFs were assigned functions. Genome comparison by BLASTN of phage LP019 with the NCBI database showed 93% similarity with 62% sequence coverage to *Listeria* phage vB_LmoS_188, which was previously isolated from wild mushroom (21).

Two phages, LP040 and LP041, were induced from lysogenic *L. monocytogenes* isolate PSU-KV-036LM (from a seafood processing environment) using F2365 and FSL F2-695 as propagating hosts, respectively (H. T. K. Vu, S. Benjakula, and K. Vongkamjan, submitted for publication). Phage LP040 presents a genome size of 39,585 bp, with a GC content of 37.1%, whereas phage LP041 is 48,286 bp in length, with a GC content of 35.8%. For phage LP040, a total of 67 ORFs were detected, of which 32 ORFs were assigned functions. For phage LP041, a total of 81 ORFs were detected, but only 24 were assigned functions. Nucleotide sequence comparison by BLASTN revealed that LP040 showed a 91% similarity with 77% sequence coverage to *Listeria* phage vB_LmoS_293, isolated from mushroom compost (20). The genome of phage LP041 showed 96% similarity with 82% sequence coverage to phage B054 (16) previously induced from *Listeria innocua* WSLC 2054 (21).

Accession number(s). The genome sequences of these three induced *Listeria* phages, LP019, LP040, and LP041, have been deposited in GenBank under the accession no. MH341451, MH341452, and MH341453, respectively.

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REFERENCES

1. Kuenne C, Billion A, Mraheil MA, Strittmatter A, Daniel R, Goesmann A, Barbuddhe S, Hain T, Chakraborty T. 2013. Reassessment of the *Listeria monocytogenes* pan-genome reveals dynamic integration hotspots and mobile genetic elements as major components of the accessory genome. BMC Genomics 14:47. https://doi.org/10.1186/1471-2164-14-47.
2. Nelson KE, Fouts DE, Mongodin EF, Ravel J, DeBoy RT, Kolonay JF, Rasko DA, Angiuoli SV, Gill SR, Paulsen IT, Peterson J, White O, Nelson WC, Nierman W, Beanan MJ, Brinkac LM, Daugherty SC, Dodson RJ, Durkin AS, Madupu R, Haft DH, Selengut J, Van Aken S, Khouri H, Fedorova N, Forberger H, Tran B, Kathariou S, Wonderling LD, Uhlich GA, Bayles DO, Luchansky JB, Fraser CM. 2004. Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. Nucleic Acids Res 32:2386–2395. https://doi.org/10.1093/nar/gkh562.
3. den Bakker HC, Desjardins CA, Griggs AD, Peters JE, Zeng Q, Young SK, Kodira CD, Yandava C, Hepburn TA, Haas BJ, Birren BW, Wiedmann M. 2013. Evolutionary dynamics of the accessory genome of *Listeria monocytogenes*. PLoS One 8:e67511. https://doi.org/10.1371/journal.pone.0067511.
4. Orsi RH, Borowsky ML, Lauer P, Young SK, Nusbaum C, Galagan JE, Birren BW, Ivy RA, Sun Q, Graves LM, Swaminathan B, Wiedmann M. 2008. Short-term genome evolution of *Listeria monocytogenes* in a non-controlled environment. BMC Genomics 9:539. https://doi.org/10.1186/1471-2164-9-539.
5. Ferreira V, Barbosa J, Stasiewicz M, Vongkamjan K, Moreno Switt A, Hogg T, Gibbs P, Teixeira P, Wiedmann M. 2011. Diverse geno- and phenotypes of persistent *Listeria monocytogenes* isolates from fermented meat sausage production facilities in Portugal. Appl Environ Microbiol 77:2701–2715. https://doi.org/10.1128/AEM.02553-10.
6. Vongkamjan K, Moreno Switt A, den Bakker HC, Fortes ED, Wiedmann M. 2012. Silage collected from dairy farms harbors an abundance of listeriaphages with considerable host range and genome size diversity. Appl Environ Microbiol 78:8666–8675. https://doi.org/10.1128/AEM.01859-12.
7. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
8. Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, Tang J, Wu G, Zhang H, Shi Y, Liu Y, Yu C, Wang B, Lu Y, Han C, Cheung DW, Yiu S-M, Peng S, Xiaoqian Z, Liu G, Liao X, Li Y, Yang H, Wang J, Lam T-W, Wang J. 2012. SOAPdenovo2: an empirically improved memory-efficient short-read *de novo* assembler. Gigascience 1:1–6. https://doi.org/10.1186/2047-217X-1-18.
9. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with Glimmer. Nucleic Acids Res 27:4636–4641. https://doi.org/10.1093/nar/27.23.4636.
10. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
11. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387.
12. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.
13. Quevillon E, Silventoinen V, Pillai S, Harte N, Mulder N, Apweiler R, Lopez R. 2005. InterProScan: protein domains identifier. Nucleic Acids Res 33:W116–W120. https://doi.org/10.1093/nar/gki442.
14. Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream M-A, Barrell B. 2000. Artemis: sequence visualization and annotation. Bioinformatics 16:944–945. https://doi.org/10.1093/bioinformatics/16.10.944.
15. Lowe TM, Chan PP. 2016. tRNAscan-SE on-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res 44:W54–W57. https://doi.org/10.1093/nar/gkw413.
16. Dorscht J, Klumpp J, Bielmann R, Schmelcher M, Born Y, Zimmer M, Calendar R, Loessner MJ. 2009. Comparative genome analysis of *Listeria* bacteriophages reveals extensive mosaicism, programmed translational frameshifting, and a novel prophage insertion site. J Bacteriol 191:7206–7215. https://doi.org/10.1128/JB.01041-09.
17. Zimmer M, Sattelberger E, Inman RB, Calendar R, Loessner MJ. 2003.

Genome and proteome of *Listeria monocytogenes* phage PSA: an unusual case for programmed + 1 translational frameshifting in structural protein synthesis. Mol Microbiol 50:303–317. https://doi.org/10.1046/j.1365-2958.2003.03684.x.
18. Denes T, Vongkamjan K, Ackermann H-W, Moreno Switt AI, Wiedmann M, den Bakker HC. 2014. Comparative genomic and morphological analyses of *Listeria* phages isolated from farm environments. Appl Environ Microbiol 80:4616–4625. https://doi.org/10.1128/AEM.00720-14.
19. Loessner MJ, Inman RB, Lauer P, Calendar R. 2000. Complete nucleotide sequence, molecular analysis and genome structure of bacteriophage A118 of *Listeria monocytogenes*: implications for phage evolution. Mol Microbiol 35:324–340. https://doi.org/10.1046/j.1365-2958.2000.01720.x.
20. Casey A, Jordan K, Coffey A, McAuliffe O. 2015. Complete genome sequences of vB_LmoS_188 and vB_LmoS_293, two bacteriophages with specificity for *Listeria monocytogenes* strains of serotypes 4b and 4e. Genome Announc 3:e00040-15. https://doi.org/10.1128/genomeA.00040-15.
21. Loessner MJ, Busse M. 1990. Bacteriophage typing of *Listeria* species. Appl Environ Microbiol 56:1912–1918.