



Genome Sequences of Bacteriophages cd2, cd3, and cd4, which Specifically Target *Carnobacterium divergens*

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ABSTRACT Carnobacteria have been implicated in food spoilage, but also in protection against pathogenic bacteria. We report the isolation and complete genome sequences of three bacteriophages (phages cd2, cd3, and cd4) that specifically target *Carnobacterium divergens*. The genome sizes are approximately 57 kbp and have limited homology to known enterococcal and streptococcal phages.

Carnobacterium maltaromaticum and *Carnobacterium divergens* are lactic acid bacteria (LAB) commonly found in foods, particularly dairy, meat, fish, and shrimp (1, 2). Previously, it was thought that high bacterial loads of these organisms resulted in food spoilage (2, 3); however, recent reports suggest that the volatile organic compounds produced by carnobacteria have a negligible impact on food quality (4). Moreover, since carnobacteria produce antimicrobial peptides (bacteriocins) and organic acids, they can act as protective cultures by inhibiting the growth of food spoilage or pathogenic bacteria such as *Listeria monocytogenes* (5, 6). Within the food industry, bacteriophages pose a major threat to LAB that function as starter cultures for fermentation processes or protective cultures; as such, phages targeting LAB have been extensively studied (7–9). However, phages infecting carnobacteria are underrepresented in this field of study, and very few bacteriophages targeting *Carnobacterium* spp. have been reported (10, 11). Here, we report the complete genome sequences of three lytic bacteriophages (cd2, cd3, and cd4) that target various *C. divergens* strains.

Bacteriophages cd2 and cd3 were isolated from minced beef, and cd4 was isolated from ham, all purchased at a grocery store in Edmonton, Alberta, Canada (Table 1). In each case, a 1-g sample of meat was added to 10 ml of brain heart infusion broth (Bacto) and incubated overnight at 25°C. Following centrifugation (9,000 × *g*, 5 min, 4°C), the supernatant was filter-sterilized (0.2- μ m filter) and used to prepare crude phage suspensions (11), using *C. divergens* LV13 (12) as the host strain. Purified suspensions of each bacteriophage were prepared using three consecutive rounds of single-plaque isolation using *C. divergens* LV13 as the host strain. Additionally, *C. divergens* B1 (6) was used to propagate and isolate phages cd2 and cd3.

Phage DNA was isolated using a proteinase K and SDS treatment, followed by phenol-chloroform extraction and ethanol precipitation (13). Libraries were constructed using a Nextera XT DNA library prep kit and sequenced using an Illumina MiSeq PE250 platform. Sequencing reads were trimmed using Trimmomatic v0.39 (14), where reads with an average quality score of a 4-base sliding window of <30 and length of <100 were subsequently removed. Genomes were assembled using SPAdes v3.14.0 (15) with kmers set at 21, 33, 55, 77, 99, and 127 bp. Contigs with a length of <500 bp or coverage of <10 were removed using a Python script (16). The genomes were predicted to be circularly permuted using PhageTerm v1.0.11 (17). Genomes were annotated with PHANOTATE v1.5.0 (18) using the default settings.

Table 1 lists the characteristics of the phage genomes. Using OAT v0.9 (19), the phages were found to have average orthologous nucleotide identities of 98.3 to 99.8% with each

Citation Zhang P, Britton AP, Visser KA, Welke CA, Wassink H, Prins E, Yang X, Martin-Visscher LA. 2021. Genome sequences of bacteriophages cd2, cd3, and cd4, which specifically target *Carnobacterium divergens*. Microbiol Resour Announc 10:e00636-21. <https://doi.org/10.1128/MRA.00636-21>.

Editor Kenneth M. Stedman, Portland State University

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Received 21 June 2021

Accepted 5 August 2021

Published 26 August 2021

TABLE 1 Characteristics of the assembled phage genomes

Isolate	Recovery source	Total no. of forward/reverse reads	Estimated coverage by trimmed reads (×)	Assembled genome size (bp)	GC content (%)	No. of contigs	No. of ORFs ^a	GenBank accession no.	
								SRA	Genome
cd2	Minced beef	94,533	594.7	57,220	39.0	1	111	SRR14848713	MZ398135
cd3	Minced beef	129,946	788.9	57,171	38.9	1	110	SRR14848712	MZ398136
cd4	Ham	97,629	604.2	56,713	38.7	1	109	SRR14848711	MZ399596

^aORFs, open reading frames.

other. A BLASTN (20) search of the genomes against the nucleotide database in NCBI did not find closely related bacteriophages, as the top 10 matched nucleotide sequences displayed limited coverage (<5%) and identity (<75%). Analysis with ViPTree (21) suggests that the three phage strains belong to the *Siphoviridae* family and have limited homology to several enterococcal bacteriophages, including VD13 (22), vB_EfaS_IME198, IME-EF1 (23), SAP6 (24), BC-611 (25), and *Streptococcus* phage SP-QS1.

Data availability. Sequencing data for bacteriophages cd2, cd3, and cd4 are available in GenBank under BioProject number [PRJNA738531](#). The accession numbers for the sequencing reads and genomes are listed in Table 1.

ACKNOWLEDGMENTS

This work was funded by a Natural Sciences and Engineering Research Council (NSERC) Discovery Grant (RGPIN-2014-05457) and the NSERC Undergraduate Student Research Award (USRA) program.

We thank Sophie Dang from the Molecular Biology Service Unit for her assistance with sequencing.

REFERENCES

- Rieder G, Krisch L, Fischer H, Kaufmann M, Maringer A, Wessler S. 2012. *Carnobacterium divergens*: a dominating bacterium of pork meat juice. *FEMS Microbiol Lett* 332:122–130. <https://doi.org/10.1111/j.1574-6968.2012.02584.x>.
- Leisner JJ, Laursen BG, Prévost H, Drider D, Dalgaard P. 2007. *Carnobacterium*: positive and negative effects in the environment and in foods. *FEMS Microbiol Rev* 31:592–613. <https://doi.org/10.1111/j.1574-6976.2007.00080.x>.
- Laursen BG, Leisner JJ, Dalgaard P. 2006. *Carnobacterium* species: effect of metabolic activity and interaction with *Brochothrix thermosphacta* on sensory characteristics of modified atmosphere packed shrimp. *J Agric Food Chem* 54:3604–3611. <https://doi.org/10.1021/jf053017f>.
- Casaburi A, Nasi A, Ferrocino I, Di Monaco R, Mauriello G, Villani F, Ercolini D. 2011. Spoilage-related activity of *Carnobacterium maltaromaticum* strains in air-stored and vacuum-packed meat. *Appl Environ Microbiol* 77:7382–7393. <https://doi.org/10.1128/AEM.05304-11>.
- Mills S, Ross RP, Hill C. 2017. Bacteriocins and bacteriophage; a narrow-minded approach to food and gut microbiology. *FEMS Microbiol Rev* 41:S129–S153. <https://doi.org/10.1093/femsre/fux022>.
- Zhang P, Gänzle M, Yang X. 2019. Complementary antibacterial effects of bacteriocins and organic acids as revealed by comparative analysis of *Carnobacterium* spp. from meat. *Appl Environ Microbiol* 85:e01227–19. <https://doi.org/10.1128/AEM.01227-19>.
- Pujato SA, Quiberoni A, Mercanti DJ. 2019. Bacteriophages on dairy foods. *J Appl Microbiol* 126:14–30. <https://doi.org/10.1111/jam.14062>.
- Mc Grath S, Fitzgerald GF, van Sinderen D. 2007. Bacteriophages in dairy products: pros and cons. *Biotechnol J* 2:450–455. <https://doi.org/10.1002/biot.200600227>.
- Garneau JE, Moineau S. 2011. Bacteriophages of lactic acid bacteria and their impact on milk fermentations. *Microb Cell Fact* 10(Suppl 1):S20. <https://doi.org/10.1186/1475-2859-10-S1-S20>.
- Chibani-Chennoufi S, Dillmann M-L, Marvin-Guy L, Rami-Shojaei S, Brüssow H. 2004. *Lactobacillus plantarum* bacteriophage LP65: a new member of the SPO1-like genus of the family *Myoviridae*. *J Bacteriol* 186:7069–7083. <https://doi.org/10.1128/JB.186.21.7069-7083.2004>.
- Manchester LN. 1997. Characterization of a bacteriophage for *Carnobacterium divergens* NCFB 2763 by host specificity and electron microscopy. *Lett Appl Microbiol* 25:401–404. <https://doi.org/10.1111/j.1472-765x.1997.tb00005.x>.
- Ahn C, Stiles ME. 1990. Plasmid-associated bacteriocin production by a strain of *Carnobacterium piscicola* from meat. *Appl Environ Microbiol* 56:2503–2510. <https://doi.org/10.1128/aem.56.8.2503-2510.1990>.
- Sambrook J, Russell D. 2006. Extraction of bacteriophage λ DNA from large-scale cultures using proteinase K and SDS. *Cold Spring Harb Protoc* 2006:pdb.prot3972. <https://doi.org/10.1101/pdb.prot3972>.
- 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>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Douglass AP, O'Brien CE, Offei B, Coughlan AY, Ortiz-Merino RA, Butler G, Byrne KP, Wolfe KH. 2019. Coverage-versus-length plots, a simple quality control step for *de novo* yeast genome sequence assemblies. *G3*:879–887. <https://doi.org/10.1534/g3.118.200745>.
- Garneau JR, Depardieu F, Fortier L-C, Bikard D, Monot M. 2017. PhageTerm: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data. *Sci Rep* 7:8292. <https://doi.org/10.1038/s41598-017-07910-5>.
- McNair K, Zhou C, Dinsdale EA, Souza B, Edwards RA. 2019. PHANOTATE: a novel approach to gene identification in phage genomes. *Bioinformatics* 35:4537–4542. <https://doi.org/10.1093/bioinformatics/btz265>.
- Lee I, Ouk Kim Y, Park S-C, Chun J. 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 66:1100–1103. <https://doi.org/10.1099/ijsem.0.000760>.
- Chen Y, Ye W, Zhang Y, Xu Y. 2015. High speed BLASTN: an accelerated MegaBLAST search tool. *Nucleic Acids Res* 43:7762–7768. <https://doi.org/10.1093/nar/gkv784>.
- Nishimura Y, Yoshida T, Kuronishi M, Uehara H, Ogata H, Goto S. 2017. ViPTree: the viral proteomic tree server. *Bioinformatics* 33:2379–2380. <https://doi.org/10.1093/bioinformatics/btx157>.

22. 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>.
23. Zhang W, Mi Z, Yin X, Fan H, An X, Zhang Z, Chen J, Tong Y. 2013. Characterization of *Enterococcus faecalis* phage IME-EF1 and its endolysin. *PLoS One* 8:e80435. <https://doi.org/10.1371/journal.pone.0080435>.
24. Lee Y-D, Park J-H. 2012. Complete genome sequence of enterococcal bacteriophage SAP6. *J Virol* 86:5402–5403. <https://doi.org/10.1128/JVI.00321-12>.
25. Horiuchi T, Sakka M, Hayashi A, Shimada T, Kimura T, Sakka K. 2012. Complete genome sequence of bacteriophage BC-611 specifically infecting *Enterococcus faecalis* strain NP-10011. *J Virol* 86:9538–9539. <https://doi.org/10.1128/JVI.01424-12>.