The Tape Measure Protein Is Involved in the Heat Stability of *Lactococcus lactis* Phages

Hany Geagea,^{a,b} Simon J. Labrie,^{c*} Muriel Subirade,^{a,b} Sylvain Moineau^{c,d}

^aInstitute of Nutrition and Functional Foods (INAF), Université Laval, Québec, Canada

^bDepartment of Food Science, Université Laval, Québec, Canada

^cDépartement de biochimie, de microbiologie et de bio-informatique, Faculté des sciences et de génie, Université Laval, Québec, Canada

^dFélix d'Hérelle Reference Center for Bacterial Viruses, Faculté de médecine dentaire, Université Laval, Québec, Canada

ABSTRACT Virulent lactococcal phages are still a major risk for milk fermentation processes as they may lead to slowdowns and low-quality fermented dairy products, particularly cheeses. Some of the phage control strategies used by the industry rely on heat treatments. Recently, a few *Lactococcus lactis* phages were found to be highly thermo-resistant. To identify the genetic determinant(s) responsible for the thermal resistance of lactococcal phages, we used the virulent phage CB14 (of the *Lactococcus lactis* 936 [now *Sk1virus*] phage group) to select for phage mutants with increased heat stability. By treating phage CB14 to successive low and high temperatures, we were able to select two CB14 derivatives with increased heat stability. Sequencing of their genome revealed the same nucleotide sequences as the wild-type phage CB14, except for a same-sized deletion (120 bp) in the gene coding for the tape measure protein (TMP) of each phage mutant, but at a different position. The TMP protein sequences of these mutant phages were compared with their homologues in other wild-type *L. lactis* phages with a wide diversity in heat stability. Comparative analysis showed that the same nucleotide deletion appears to have also occurred in the gene coding for the TMP of highly thermo-resistant lactococcal phages P1532 and P680. We propose that the TMP is, in part, responsible for the heat stability of the highly predominant lactococcal phages of the *Sk1virus* group.

IMPORTANCE Virulent lactococcal phages still represent a major risk for milk fermentation as they may lead to slowdowns and low-quality fermented dairy products. Heat treatment is one of the most commonly used methods to control these virulent phages in cheese by-products. Recently, a few *Lactococcus lactis* phages, members of the *Sk1virus* group, have emerged with high thermal stability. To our knowledge, the genetic determinant(s) responsible for this thermal resistance in lactococcal phages is unknown. A better understanding of the thermal stability of these emerging virulent lactococcal phages is needed to improve industrial control strategies. In this work, we report the identification of a phage structural protein that is involved in the heat stability of a virulent *Sk1virus* phage. Identifying such a genetic determinant for heat stability is a first step in understanding the emergence of this group of thermostable phages.

KEYWORDS lactococcal phages, *Sk1virus* group, heat stability, genetic determinant, TMP, bacteriophages, cheese, *Lactococcus*, milk, phages, tape measure protein, whey proteins, lactic acid bacteria

Virulent phages infecting lactic acid bacteria (LAB) still represent a major risk to the dairy industry, particularly during the large-scale production of cheeses. The phage risk is even higher if milk is supplemented with particulated whey protein concentrates

Received 20 September 2017 Accepted 10 November 2017

Accepted manuscript posted online 17 November 2017

Citation Geagea H, Labrie SJ, Subirade M, Moineau S. 2018. The tape measure protein is involved in the heat stability of *Lactococcus lactis* phages. Appl Environ Microbiol 84:e02082-17. <https://doi.org/10.1128/AEM.02082-17>.

Editor Edward G. Dudley, The Pennsylvania State University

Copyright © 2018 American Society for Microbiology. All Rights Reserved.

Address correspondence to Sylvain Moineau, Sylvain.Moineau@bcm.ulaval.ca.

* Present address: Simon J. Labrie, SyntBioLab Inc., Lévis, Québec, Canada.

(WPC), an industrial practice used to increase cheese yield. Indeed, WPC often contain virulent phages from previous milk fermentations (1).

To manufacture cheeses or other fermented dairy products, LAB starter cultures are inoculated into heat-treated milk to control the fermentation process and to ensure high-quality foods (2). *Lactococcus lactis* is the most important LAB used by the cheese industry, particularly for the production of cheddar (3). The use of several strains in rotation is generally applied to reduce the risk associated with phage infections. In the nonsterile environment of pasteurized milk, *L. lactis* strains come into contact with virulent phages that are ubiquitous in milk (4) and in dairy environs (5). By infecting phage-sensitive bacterial strains, the vial population can increase rapidly, due to their relatively short latent period and large burst size (6, 7). Therefore, contamination of cheese plants with virulent phages can lead to milk fermentation slowdowns, resulting in low-quality cheeses (2) as well as phage-containing by-products such as whey.

All currently known *L. lactis* phages belong to the *Caudovirales* order (double-stranded DNA genome, tailed phages), and most of them belong to the *Siphoviridae* family, which are characterized by long noncontractile tails (8). Phages of the *Sk1virus* group (previously the *Lactococcus lactis* 936 phage group) are by far the most frequently isolated phages in the cheese industry (9, 10) and therefore pose the greatest threat. In addition to the use of phage-unrelated strains, various other practical strategies have been devised to control these virulent phages.

Heat treatment is one of the most commonly used methods of inactivating phages in milk and milk by-products (whey, WPC, etc.) (11–14). Usually, thermal treatments lead to morphological changes on heat-sensitive phage particles, as observed by transmission electron microscopy (15). These changes include phage DNA release from viral capsids, phage breakdown into head and tail structures, and aggregation of phage tails. Although these heat treatments are generally effective against most LAB phages, they can also impair the functional properties and the nutritional values of dairy products (16).

Numerous studies have examined the susceptibility of *L. lactis* phages to heat treatment using well-defined temperature/time combinations. These studies demonstrated that several phages can withstand pasteurization conditions (9, 17). Furthermore, in the last few years, some virulent members of the *Sk1virus* group have emerged with remarkably high thermal resistance (12, 18). For example, infectious viral particles of lactococcal phage P1532, isolated in Germany, were still detected after they were heated at either 90°C for 20 min or 97°C for 5 min (12). In addition, phage P680 remained infectious after 5 min of exposure to 95°C. To our knowledge, the genetic determinant(s) responsible for this thermal resistance in lactococcal phages is unknown. Understanding the thermal stability of these virulent phages may lead to improved control strategies in the cheese industry.

Here, we report the identification of a phage structural protein that is involved in the heat stability of a virulent *Sk1virus* phage. We combined different approaches such as the selection and characterization of phage mutants with an increased heat resistance as well as comparative genomic analyses to identify a deletion in the gene coding for tape measure protein as responsible for improved heat stability. Similar deletions were observed in other wild-type phages that have been reported to persist after heat treatments.

RESULTS AND DISCUSSION

Search for phages closely related to phage P1532. The first challenge was to select a suitable heat-sensitive phage that could be used as a model for selecting heat-resistant phage mutants. A clustering-based approach was used to identify structural proteins that are conserved among a group of 54 members of the *Sk1virus* group. Of the 18 structural proteins, 15 met the criteria used for clustering analysis (see Materials and Methods). The protein sequences were concatenated and used for phylogenetic analysis. We identified a list of virulent phages closely related to the heat-resistant lactococcal phage P1532 (12, 14, 19). Phylogenetic analysis revealed that

Tree scale: 0.01

FIG 1 Phylogeny of the concatenated structural protein sequences conserved among a set of 54 *Sk1virus* phages. Blue circles indicate branch support values greater than 90%, and their size is proportional to their branch support value.

four phages of the *Sk1virus* group share close relatedness with phage P1532 (Fig. 1), namely, SL4, CB13, CB19, and CB20. Interestingly, these phages infect the same *L. lactis* host that is also infected by phage CB14. We evaluated the heat stability of this set of five phages, which were previously isolated during a study on the evolution of virulent phages in a Canadian cheese factory (20).

Thermal resistance of phages SL4, CB13, CB14, CB19, and CB20. These virulent phages were tested for their thermal stability in LM17 medium (M17 medium supplemented with 0.5% lactose). The heat-resistant and -sensitive lactococcal phages P1532 and p2, respectively, were used as controls for comparison purposes. Figure 2 shows the thermal inactivation of these lytic phages expressed as $\log_{10}$ units reduction after heat treatment at 70, 80, and 90°C for 5 min. At 70°C, all phages remained infectious at high levels. However, inactivation of viral particles was significantly higher for phages CB14 and p2 (reduction of $\approx 1.5 \log_{10}$) than for phages SL4, CB13, CB19, CB20, and P1532 (reduction of $\leq 0.5 \log_{10}$). The increase of heating temperature to 80°C resulted in complete inactivation (below the detection limit of $<10 \text{ PFU} \cdot \text{ml}^{-1}$) of phages CB14 and p2. Under the same conditions, phages SL4, CB13, CB19, CB20, and P1532 showed high thermal stability as evidenced by only a slight loss of infectious particles (reduction of $\leq 2 \log_{10}$). Finally, all phages except for P1532 were completely inactivated after

FIG 2 Reduction ($\log_{10}$ units) of lactococcal phages SL4, CB13, CB14, CB19, CB20, P1532, and p2 after heat treatment at 70, 80, and 90°C for 5 min in LM17 medium. *, above detection limit.

5 min of exposure at 90°C. The heat stability of the P1532 control was noticeably high, as this phage was still detectable at a high concentration (reduction of $\leq 1.2 \log_{10}$) following a 5-min exposure at 90°C in LM17. Taken together, these results showed a noticeable difference in the thermal sensitivity of phage CB14 compared to that of P1532. Phage CB14 was selected for further analyses.

Selection of phage mutants. We designed an assay for the selection/enrichment of phage mutants with higher thermal stability. We conducted a two-step assay using phage CB14. After 5 min of exposure at 100°C (second step), a dozen CB14 plaques were recovered and amplified. Their thermal stability was evaluated through inactivation tests at different temperatures. Among them, two phage samples revealed a slight increase in thermal stability compared to the wild-type CB14. The genomes of both phage preparations were sequenced, and based on the analysis of read alignments, we discovered that one of the two phage mutants contained at least two phages. This phage lysate was subjected to a new set of heat treatments and repurified to obtain a single phage, and its genome was sequenced. The two selected homogeneous phage mutants with increased heat stability were designated CB14a and CB14b.

Thermal inactivation of phages CB14a and CB14b. Both phages were retested for their thermal stability to compare them with the wild-type phage CB14. Heat treatments were conducted in LM17 medium at temperatures ranging from 70 to 80°C for 5 min. Figure 3 shows the thermal inactivation of these phages expressed as $\log_{10}$ units reduction. At 70°C, a reproducible increase in thermal stability was detected for CB14a and CB14b compared to the wild-type phage. The wild-type phage CB14 exhibited a 1.6 $\log_{10}$ reduction in infectious particles while phages CB14a and CB14b lost 0.7 $\log_{10}$ and 0.4 $\log_{10}$, respectively, in their titers. At 75°C, no significant difference in heat stability was detected between the three phages, as they all exhibited a 6 $\log_{10}$ reduction. Finally, a complete loss of infectivity of the three phages was observed following 5 min of exposure at 80°C (below the detection limit of $<10 \text{ PFU} \cdot \text{ml}^{-1}$) (data not shown). These results prompted us to further analyze the genomes of these two phage mutants.

Characterization of CB14a and CB14b genomes. The double-stranded DNA genomes of the three phages (CB14, CB14a, and CB14b) were extracted, sequenced (resequenced in the case of CB14), and analyzed. First, the complete nucleotide sequence of phage CB14 was identical to the one previously reported (20), indicating that this virulent lactococcal phage is stable even after multiple amplifications. This was expected, as the virulent phage CB14 was previously isolated in a cheese factory on two occasions 14 months apart.

Analysis of the genome of the phage mutant CB14a revealed a 120-bp deletion in the gene coding for the tape measure protein (TMP) compared to the genome of the

FIG 3 Reduction ($\log_{10}$ units) of CB14WT, CB14a, and CB14b phages after heat treatment at 70 and 75°C for 5 min in LM17 medium. *, a significant difference ($P < 0.05$) compared to control (CB14WT).

wild-type phage CB14. Interestingly, a same-sized deletion was also detected in the *tmp* of phage CB14b. The two deletions were closely located, within 15 nucleotides (Fig. 4B). No additional mutation was found in a comparison of the whole genomes for both phage mutants with that of CB14. The deletions observed through sequencing and assembly of these genomes were confirmed by PCR amplification directly from the extracted DNA and also from the phage lysate (Fig. 4A). Specifically, an internal section of the *tmp* gene was amplified using primers flanking each deletion, and two PCR amplicons were observed: a 953-bp band corresponding to the wild-type *tmp* was observed with phage CB14, and a band of 833 bp corresponding to the deleted *tmp* was observed with the two phage mutants. Based on the above, the TMP, a structural protein within the phage tail, is responsible for the increased heat stability of phage CB14 mutants.

The gene coding for the TMP is part of the late-expressed transcriptional unit, which also include the genes coding for packaging, morphogenesis, and lysis (20–22). The TMP is a multifunctional structural protein with roles in controlling the tail length during assembly, connecting the capsid to the distal tail regions, and facilitating DNA transit to the cell cytoplasm during infection (23–25). Previous findings showed that deletions in particular domains of the TMP can cause changes in tail assembly, in plaque size, and in phage replication (26–29). Our data showed that the increased heat

FIG 4 (A) Analysis of PCR products after amplification of an internal portion of the *tmp* gene in CB14wt, CB14a, and CB14b phages by electrophoresis in 2% agarose gel. (B) Characterization of the CB14a and CB14b deletions.

FIG 5 Alignment of TMP amino acid sequences from 10 lactococcal *Sk1virus*-type phages is shown.

resistance caused by deletions in that particular region of the TMP of phage mutants was not associated with changes in their infective efficiency (phage titers following amplification were $\approx 10^{10}$ PFU $\cdot$ ml $^{-1}$) or in their plaque size (≈ 2.5 mm).

Comparative analysis of phage genomes of the *Sk1virus* group available in a public database showed that most genetic divergences are usually found within early and middle-expressed genes coding for nonstructural proteins. Although structural proteins are often conserved within this group of lactococcal phages, diversity is still observed among the receptor binding protein (RBP), the neck passage structure (NPS), and the TMP (10, 21, 30–32). To gain additional insights into the genetic diversity within the TMP, a multiple-sequence alignment of the TMP protein carried by 10 lactococcal phages with a wide variability in heat stability (P680, SL4, CB13, CB19, CB20, P1532, p2, CB14wt, CB14a, and CB14b) was performed.

Multiple-sequence alignment of TMP protein. As shown in Fig. 5, the size of the TMP varies from one phage to another, from 916 amino acids for the heat-stable phages P680 and P1532 to 999 amino acids for the heat-sensitive phages p2 and CB14. By comparing the TMP translated sequences of CB14 mutants we noticed that the nucleotide deletions also led to a deletion of 40 amino acids. The deletion in the *tmp* gene of only CB14b also caused a change in two amino acids, those flanking the deletion (data not shown). This difference likely explains the slight variability in the reduction of infective particles between the two phage mutants at 70°C (Fig. 3).

Interestingly, the 120-bp deletions observed in two derivatives of phage CB14 also appeared to have occurred in the *tmp* gene of the highly thermo-resistant *L. lactis* phages P1532 and P680 (Fig. 5), which may be responsible for additional heat stability. Of note, a deletion of three amino acids was observed (starting from residue 521) for six phages except for p2 and CB14. They might be involved in heat stability as well. Another region of interest was between residues 370 and 520 (Fig. 5), which is another diverse region of the TMP in the phages analyzed (see Fig. S1 in the supplemental material). The heat-sensitive phages p2 and CB14 have almost the same amino acid sequence in this region while the other phages, except P680, share a high amino acid identity.

This comparative analysis clearly showed that the deletions of the two selected mutants are in the same position and have their analogues in the TMP of the extremely heat-stable phages P680 and P1532. However, the deletions in phages CB14a and CB14b were not enough to provide the same heat stability found in phages P680 and P1532, indicating that other structural proteins are also involved in this phenotype. In subsequent heat inactivation assays, we could not obtain CB14a and CB14b derivatives

TABLE 1 Phages, host strains, and culture conditions used in this study

Phage(s)	Host strain	Culture conditions	
		Medium	Temperature (°C)
p2	<i>L. lactis</i> subsp. <i>cremoris</i> MG1363	GM17	30
P1532	<i>L. lactis</i> subsp. <i>lactis</i> 7-18	GM17	30
SL4, CB13, CB14, CB19, and CB20	<i>L. lactis</i> subsp. <i>cremoris</i> SMQ-404	LM17	30

that had increased heat stability, likely indicating that we have reached the evolutionary or selection limit of our assay. Another approach would likely be needed to further increase the heat stability of these phages. The very recent availability of a genome-editing tool for these virulent lactococcal phages (33) may allow for the swapping of genes (or a fragment thereof) coding for structural proteins of phages P680 and P1532. However, in the latter case a number of factors will have to be taken into account such as protein-protein interactions.

Nonetheless, a better understanding of these relatively recent emerging heat-stable virulent lactococcal phages is needed to improve industrial control strategies. For example, screening new emerging phages for a deletion in the *tmp* gene may hint at an increased thermo-resistance, and heat treatments may need to be modified in the dairy factory.

Conclusions. In this work, a specific gene involved in increased heat stability of *Sk1virus* was identified. After challenging a heat-sensitive phage to low and high temperatures, two phage mutants with a slightly increased thermal resistance were selected. The sequencing of their genomes revealed a 120-bp deletion in the gene coding for TMP. Comparative analysis of TMP proteins of other lactococcal phages identified the same deletions in the extremely heat-stable phages P680 and P1532. Identifying *tmp* as the genetic determinant for *Sk1virus* heat stability is a first step in understanding the emergence of this group of thermostable phages.

MATERIALS AND METHODS

Selection and comparative genomics of *sk1virus* phages. Amino acid sequences of structural proteins were deduced and extracted from 54 *Sk1virus* phage genomes publicly available at the time of the study. The sequences were extracted using in-house Python scripts and grouped in orthologous clusters using COGsoft 4.2.0 (34) using an E value of $1e-3$ as well as requiring the alignment spanning at least 75% of the orthologous sequences. The amino acid sequences of the structural proteins were concatenated using the same order for each phage. The resulting concatenated protein sequences were aligned with MAFFT v7.130b (35) using the iterative measurement method G-INS-i. The most probable amino acid substitution model was determined using ProtTest (3.2), which was used in PhyML (20110304) to generate the best phylogenetic tree. The branch support values were calculated using the Shimodaira-Hasegawa-like approach.

Bacterial strains, phages, and growth conditions. The virulent lactococcal phages p2, SL4, CB13, CB14, CB19, and CB20 used in this study were obtained from the Félix d'Hérelle Reference Center for Bacterial Viruses (www.phage.ulaval.ca). Phage P1532 was obtained from Horst Neve (Max Rubner-Institut, Kiel, Germany). Phages were amplified on their bacterial hosts (Table 1), which were grown at 30°C in M17 broth (Oxoid, Ltd., Basingstoke, UK) supplemented with 0.5% glucose (GM17) or 0.5% lactose (LM17), until an optical density (at 600 nm) of 0.1 was achieved. Next, 10 mM CaCl_2 was added as well as 10^5 PFU $\cdot$ ml $^{-1}$ of phages. The phage-infected culture was incubated at 30°C until complete bacterial lysis was achieved. The resulting lysate was centrifuged to remove cell debris, and the supernatant was filtered using a 0.45- μ m syringe filter. All filtered lysates were stored at 4°C until use.

Thermal inactivation of lactococcal phages. Phages were separately inoculated in LM17 medium, with an initial titer of 10^8 to 10^9 PFU $\cdot$ ml $^{-1}$. The heat treatments of phage suspensions were conducted in 96-well PCR plates in a C1000 Thermal Cycler equipped with the CFX96 real-time system. One hundred microliters of each sample were pipetted per well of PCR plate. The samples were treated by applying different temperature gradients (70 to 90°C; 70 to 80°C) for 5 min, followed by rapid cooling to 16°C. After heating, the remaining number of infectious phages was determined by plaque assays (36). In brief, unheated and heated samples were serially diluted in phage buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 8 mM MgSO_4), and 100 μ l of an overnight culture of the host strain was added to 3 ml of GM17 or LM17 at 50°C supplemented with 0.75% agar and 10 mM CaCl_2 . The inoculated soft agar was poured over CaCl_2 -GM17/LM17 (1% agar) plates. Then, 10 μ l from various serial dilutions (10^0 to 10^{-7}) was spotted on the top agar. After overnight incubation at 30°C, phage plaques were counted.

Selection of heat-stable phage mutants. The selection of mutants from phage CB14 was performed in two sequential steps. First, CB14 phages were treated at low temperature. In brief, LM17 medium was inoculated with CB14 at a level of 10^9 PFU · ml⁻¹. Phage suspensions were pipetted in PCR plates and heated at 72°C for 5 min, as described above. After cooling, samples were subjected to successive heat treatments (approximately 5) under the same conditions until reaching a minimum concentration of infective phages, which were counted by plaque assay. These remaining phages were amplified directly from the medium and were designated first generation, or CB14 I. In the second step, amplified CB14 I phages were treated at a higher temperature. Briefly, CB14 I phages inoculated in LM17 medium (10^8 PFU · ml⁻¹) were heated at 100°C for 5 min. After the heat treatment, 12 infectious phage plaques were picked using the double-layer agar method as described previously (36). Isolated plaques were picked with a sterile truncated tip and were suspended into labeled Eppendorf tubes containing 500 µl of sterile phage buffer. After diffusion for 30 min at room temperature, 50 µl of each phage suspension was amplified on its host as described previously. The thermal resistance of the newly amplified phages was evaluated and compared to the stability of the wild-type phage CB14 as described above. The genomic DNA of phages that showed an increased thermal resistance compared to that of the wild type was extracted and sent for sequencing.

DNA extraction, genome sequencing, and sequence analysis. Genomic DNA of phages with an increased heat resistance (CB14a and CB14b) and of wild-type phage CB14 was extracted from high-titer lysates (10^9 PFU · ml⁻¹) using the Maxi lambda DNA purification kit (Qiagen, USA) with previously described modifications (37). The sequencing libraries were prepared with the Nextera XT DNA library preparation kit (Illumina) according to the manufacturer's instructions. The library was sequenced using a MiSeq reagent kit v2 (Illumina—500 cycles) on a MiSeq system. The sequences were aligned with the genomic sequence of wild-type phage CB14 (available in GenBank under the accession number [FJ848883](#)) and analyzed using Geneious software version 7.1.9.

PCR and gel electrophoresis analysis. Deletions in gene coding for the TMP were confirmed by PCR amplification and gel electrophoresis. Briefly, an internal part (953 bp, positions 13202 to 14154) of the gene (3,000 bp) was amplified by PCR using the forward (p2.18F, 5'-GGTATTCAAACAGCATGGGC-3') and reverse (SL4.3R, 5'-ACGCTGTTCTACTCAAACC-3') primers flanking the deleted sequence. One hundred picomoles of each oligonucleotide was mixed in *Taq* PCR buffer (Feldan). The PCR protocol consisted of denaturation at 95°C for 5 min (hot start) followed by 35 cycles (45 s at 95°C, 45 s at 58°C, 1 min at 72°C) and a final step of 5 min at 72°C. The resulting PCR products were migrated on a 2% agarose gel in Tris-acetate-EDTA (TAE) buffer (40 mM Tris-acetate, 1 mM EDTA) and visualized by UV photography after staining in ethidium bromide, by using standard protocols (38).

Multiple sequence alignment. The nucleotide sequences of the *tmp* genes of the lactococcal phages P680, SL4, CB13, CB19, CB20, p2, P1532, CB14wt, CB14a, and CB14b were translated into protein sequences using Geneious (version 7.1.9). A multiple alignment of the amino acid sequences was performed with Geneious aligner using default parameters.

Statistical analysis. Phage inactivation tests were independently replicated two times, each consisting of three technical replicates. Statistical analyses were carried out in GraphPad Prism 7 software (GraphPad Software, Inc., La Jolla, CA). The results were expressed as log₁₀ units reduction (log₁₀ N0/N) of phage numbers before and after heat treatment. The data were analyzed under a two-way analysis of variance (ANOVA) followed by a Tukey test to correct the *P* values for the multiple comparisons. Significant differences were reported at an alpha of 5%.

Accession number(s). The genome sequences of phages CB14a and CB14b are available in GenBank under the accession numbers [MG309717](#) and [MG309718](#), respectively.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AEM.02082-17>.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

ACKNOWLEDGMENTS

We are grateful to Denise Tremblay for her help in sequencing the genome of the phage mutants. We thank Alexander Hynes for helpful discussions.

This work was funded by a strategic grant from the Natural Sciences and Engineering Research Council of Canada to M.S. and S.M. S.M. holds a Tier 1 Canada Research Chair in Bacteriophages.

REFERENCES

- Atamer Z, Samtlebe M, Neve H, Heller KJ, Hinrichs J. 2013. Review: elimination of bacteriophages in whey and whey products. *Front Microbiol* 4:191.
- Garneau JE, Moineau S. 2011. Bacteriophages of lactic acid bacteria and their impact on milk fermentations. *Microb Cell Fact* 10:S20. <https://doi.org/10.1186/1475-2859-10-S1-S20>.
- Bissonnette F, Labrie S, Deveau H, Lamoureux M, Moineau S. 2000. Characterization of mesophilic mixed starter cultures used for the manufacture of aged cheddar cheese. *J Dairy Sci* 83:620–627. [https://doi.org/10.3168/jds.S0022-0302\(00\)74921-6](https://doi.org/10.3168/jds.S0022-0302(00)74921-6).
- Moineau S, Lévesque C. 2005. Control of bacteriophages in industrial fermentations, p 285–296. In Kutter E, Sulakvelidze A (ed), *Bacteriophages: biology and applications*. CRC Press, Boca Raton, FL.
- Verreault D, Gendron L, Rousseau GM, Veillette M, Massé D, Lindsley WG,

- Moineau S, Duchaine C. 2011. Detection of airborne lactococcal bacteriophages in cheese manufacturing plants. *Appl Environ Microbiol* 77: 491–497. <https://doi.org/10.1128/AEM.01391-10>.
6. Emond E, Moineau S. 2007. Bacteriophages and food fermentations, p 93–124. In Mc Grath S, van Sinderen D (ed), *Bacteriophage: genetics and molecular biology*. Caister Academic Press, Poole, United Kingdom.
7. Samson JE, Moineau S. 2013. Bacteriophages in food fermentations: new frontiers in a continuous arms race. *Annu Rev Food Sci Technol* 4:347–368. <https://doi.org/10.1146/annurev-food-030212-182541>.
8. Deveau H, Labrie SJ, Chopin M-C, Moineau S. 2006. Biodiversity and classification of lactococcal phages. *Appl Environ Microbiol* 72: 4338–4346. <https://doi.org/10.1128/AEM.02517-05>.
9. Madera C, Monjardin C, Suárez JE. 2004. Milk contamination and resistance to processing conditions determine the fate of *Lactococcus lactis* bacteriophages in dairies. *Appl Environ Microbiol* 70:7365–7371. <https://doi.org/10.1128/AEM.70.12.7365-7371.2004>.
10. Moisan M, Moineau S. 2012. Multilocus sequence typing scheme for the characterization of 936-like phages infecting *Lactococcus lactis*. *Appl Environ Microbiol* 78:4646–4653. <https://doi.org/10.1128/AEM.00931-12>.
11. Atamer Z, Ali Y, Neve H, Heller KJ, Hinrichs J. 2011. Thermal resistance of bacteriophages attacking flavour-producing dairy *Leuconostoc* starter cultures. *Int Dairy J* 21:327–334. <https://doi.org/10.1016/j.idairyj.2010.11.005>.
12. Atamer Z, Dietrich J, Müller-Merbach M, Neve H, Heller KJ, Hinrichs J. 2009. Screening for and characterization of *Lactococcus lactis* bacteriophages with high thermal resistance. *Int Dairy J* 19:228–235. <https://doi.org/10.1016/j.idairyj.2008.10.012>.
13. Atamer Z, Hinrichs J. 2010. Thermal inactivation of the heat-resistant *Lactococcus lactis* bacteriophage P680 in modern cheese processing. *Int Dairy J* 20:163–168. <https://doi.org/10.1016/j.idairyj.2009.09.006>.
14. Geagea H, Goma A, Remondetto G, Moineau S, Subirade M. 2015. Investigation of the protective effect of whey proteins on lactococcal phages during heat treatment at various pH. *Int J Food Microbiol* 210:33–41. <https://doi.org/10.1016/j.jfoodmicro.2015.06.011>.
15. Atamer Z, Dietrich J, Neve H, Heller KJ, Hinrichs J. 2010. Influence of the suspension media on the thermal treatment of mesophilic lactococcal bacteriophages. *Int Dairy J* 20:408–414. <https://doi.org/10.1016/j.idairyj.2009.12.014>.
16. Guglielmotti DM, Mercanti DJ, Reinheimer JA, del L Quiberoni A. 2012. Review: efficiency of physical and chemical treatments on the inactivation of dairy bacteriophages. *Front Microbiol* 2:282. <https://doi.org/10.3389/fmicb.2011.00282>.
17. Mahony J, Murphy J, van Sinderen D. 2012. Lactococcal 936-type phages and dairy fermentation problems: from detection to evolution and prevention. *Front Microbiol* 3:335.
18. Wagner N, Samtlebe M, Franz CM, Neve H, Heller KJ, Hinrichs J, Atamer Z. 2017. Dairy bacteriophages isolated from whey powder: thermal inactivation and kinetic characterisation. *Int Dairy J* 68:95–104. <https://doi.org/10.1016/j.idairyj.2016.12.017>.
19. Geagea H, Goma A, Remondetto G, Moineau S, Subirade M. 2017. Molecular structure of lactoferrin influences the thermal resistance of lactococcal phages. *J Agric Food Chem* 65:2214–2221. <https://doi.org/10.1021/acs.jafc.6b05166>.
20. Rousseau GM, Moineau S. 2009. Evolution of *Lactococcus lactis* phages within a cheese factory. *Appl Environ Microbiol* 75:5336–5344. <https://doi.org/10.1128/AEM.00761-09>.
21. Mahony J, Deveau H, Mc Grath S, Ventura M, Canchaya C, Moineau S, Fitzgerald GF, Van Sinderen D. 2006. Sequence and comparative genomic analysis of lactococcal bacteriophages jf50, 712 and P008: evolutionary insights into the 936 phage species. *FEMS Microbiol Lett* 261:253–261. <https://doi.org/10.1111/j.1574-6968.2006.00372.x>.
22. Murphy J, Bottacini F, Mahony J, Kelleher P, Neve H, Zomer A, Nauta A, van Sinderen D. 2016. Comparative genomics and functional analysis of the 936 group of lactococcal *Siphoviridae* phages. *Sci Rep* 6:21345. <https://doi.org/10.1038/srep21345>.
23. Xu J, Hendrix RW, Duda RL. 2004. Conserved translational frameshift in dsDNA bacteriophage tail assembly genes. *Mol Cell* 16:11–21. <https://doi.org/10.1016/j.molcel.2004.09.006>.
24. Xu J, Hendrix RW, Duda RL. 2014. Chaperone–protein interactions that mediate assembly of the bacteriophage lambda tail to the correct length. *J Mol Biol* 426:1004–1018. <https://doi.org/10.1016/j.jmb.2013.06.040>.
25. Cumby N, Reimer K, Mengin-Lecreux D, Davidson AR, Maxwell KL. 2015. The phage tail tape measure protein, an inner membrane protein and a periplasmic chaperone play connected roles in the genome injection process of *E. coli* phage HK97. *Mol Microbiol* 96:437–447. <https://doi.org/10.1111/mmi.12918>.
26. Katsura I. 1987. Determination of bacteriophage lambda tail length by a protein ruler. *Nature* 327:73–75. <https://doi.org/10.1038/327073a0>.
27. Katsura I, Hendrix RW. 1984. Length determination in bacteriophage lambda tails. *Cell* 39:691–698. [https://doi.org/10.1016/0092-8674\(84\)90476-8](https://doi.org/10.1016/0092-8674(84)90476-8).
28. Mahony J, Alqarni M, Stockdale S, Spinelli S, Feyereisen M, Cambillau C, van Sinderen D. 2016. Functional and structural dissection of the tape measure protein of lactococcal phage TP901-1. *Sci Rep* 6:36667. <https://doi.org/10.1038/srep36667>.
29. Pedersen M, Ostergaard S, Bresciani J, Vogensen FK. 2000. Mutational analysis of two structural genes of the temperate lactococcal bacteriophage TP901-1 involved in tail length determination and baseplate assembly. *Virology* 276:315–328. <https://doi.org/10.1006/viro.2000.0497>.
30. Castro-Nallar E, Chen H, Gladman S, Moore SC, Seemann T, Powell IB, Hillier A, Crandall KA, Chandry PS. 2012. Population genomics and phylogeography of an Australian dairy factory derived lytic bacteriophage. *Genome Biol Evol* 4:382–393. <https://doi.org/10.1093/gbe/evs017>.
31. Sciarra G, Bebeacua C, Bron P, Tremblay D, Ortiz-Lombardia M, Lichière J, van Heel M, Campanacci V, Moineau S, Cambillau C. 2010. Structure of lactococcal phage p2 baseplate and its mechanism of activation. *Proc Natl Acad Sci U S A* 107:6852–6857. <https://doi.org/10.1073/pnas.1000232107>.
32. Tremblay DM, Tegoni M, Spinelli S, Campanacci V, Blangy S, Huyghe C, Desmyter A, Labrie S, Moineau S, Cambillau C. 2006. Receptor-binding protein of *Lactococcus lactis* phages: identification and characterization of the saccharide receptor-binding site. *J Bacteriol* 188:2400–2410. <https://doi.org/10.1128/JB.188.7.2400-2410.2006>.
33. Lemay ML, Tremblay DM, Moineau S. 2017. Genome engineering of virulent lactococcal phages using CRISPR-Cas9. *ACS Synth Biol* 6:1351–1358. <https://doi.org/10.1021/acssynbio.6b00388>.
34. Kristensen DM, Kannan L, Coleman MK, Wolf YI, Sorokin A, Koonin EV, Mushegian A. 2010. A low-polynomial algorithm for assembling clusters of orthologous groups from intergenomic symmetric best matches. *Bioinformatics* 26:1481–1487. <https://doi.org/10.1093/bioinformatics/btq229>.
35. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30:772–780. <https://doi.org/10.1093/molbev/mst010>.
36. Moineau S, Fortier J, Ackermann HW, Pandian S. 1992. Characterization of lactococcal bacteriophages from Quebec cheese plants. *Can J Microbiol* 38:875–882. <https://doi.org/10.1139/m92-143>.
37. Deveau H, Van Calsteren M-R, Moineau S. 2002. Effect of exopolysaccharides on phage-host interactions in *Lactococcus lactis*. *Appl Environ Microbiol* 68:4364–4369. <https://doi.org/10.1128/AEM.68.9.4364-4369.2002>.
38. Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.