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Short communication

# Extreme thermal resistance of phages isolated from dairy samples: Updating traditional phage detection methodologies

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ABSTRACT

Starter culture failures due to bacteriophage attacks reduce product quality and may cause financial losses. It was the aim of this work to revise and update the Standard IDF microbiological methodology for phage detection which includes a mandatory control, treated for 15 min at 90 °C for discrimination of phage- and non-phage inhibitors. Phages specific for *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus casei* and *Lactobacillus paracasei* were investigated to establish a reliable heat-treatment for the processing of industrial dairy samples suspected of containing phages. A prolonged heating-step of 45 min at 90 °C is proposed.

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## 1. Introduction

Bacteriophage infections of starter cultures still represents a nuisance in the dairy industry, since slow or even completely failed fermentation batches may result in poor product quality and financial losses (Deveau, Labrie, Chopin, & Moineau, 2006). As phages are ubiquitous in dairy environments, they cannot be eradicated completely, but efforts must be undertaken to control phage proliferation efficiently (Moineau & Lévesque, 2005). It is well known that the majority of dairy phages are resistant to standard pasteurisation procedures (Binetti & Reinheimer, 2000; Capra, Quiberoni, Ackermann, Moineau, & Reinheimer, 2006; Capra, Quiberoni, & Reinheimer, 2004; Chopin, 1980; Madera, Monjardín, & Suárez, 2004; Quiberoni, Suárez, & Reinheimer, 1999; Suárez & Reinheimer, 2002). Furthermore, Quiberoni, Guglielmotti, and Reinheimer (2003) reported that *Lactobacillus delbrueckii* phage $Ib_3$ required a 15-min treatment at 90 °C for complete inactivation. Later, other phages infecting different lactic acid species from various industrial environments were reported to exhibit extraordinary thermal resistances (Atamer, Ali, Neve, Heller, & Hinrichs, 2011; Atamer, Dietrich, Neve, Heller, & Hinrichs, 2010; Atamer et al., 2009; Atamer & Hinrichs, 2010; Atamer, Neve, & Heller, 2012; Buzrul, Öztürk, Alpasa, & Akcelik, 2007; Capra, Binetti, Mercanti, Quiberoni, & Reinheimer, 2009). Phage populations in dairy factories are in a dynamic state and are continuously subjected to natural selection (Madera et al., 2004). Furthermore, reliable long-term phage control measures in dairies are hindered by the high biodiversity and rapid evolution of these phages (Deveau et al., 2006).

Phage monitoring is critical in dairies to assess their contamination and to allow for rapid response to new phage infections. A number of methods are available for the detection of phages and include not only direct tests (i.e., microbiological, immunological, molecular and electron microscopic methods) but also indirect tests (i.e., activity test, impedimetric methods, flow cytometry-based methods) (Binetti, 2012; Zago et al., 2012). Standard microbiological phage detection methods allow for the documentation and enumeration of active phage particles. Since they are suitable for the discrimination between phage and non-phage inhibitors, they are still in use despite of their disadvantages (i.e., long time requirement, availability of a phage-sensitive indicator strain).

International standard tests for phage monitoring (International Dairy Federation, IDF Bulletin 263 1991) are conducted in standard glass test tubes and include internal controls, which are heat-treated for 15 min at 90 °C. This time–temperature combination was thought to inactivate all phage particles presumably present within the sample but with no effect on other possible inhibitors (i.e., residues of antibiotics or – to a lesser extent – disinfectants). However, since heat-resistant phages have emerged for both mesophilic and thermophilic lactic acid bacteria, these internal

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controls no longer guarantee a valid discrimination between phage-derived and non-phage-derived inhibitors. It was the aim of this study to revise and update the standard protocol for phage detection in dairy samples.

## 2. Materials and methods

### 2.1. Bacteria, bacteriophages and culture conditions

Phages, host strains and culture conditions used in this study are listed in Table 1. Strains were maintained as frozen stocks at −80 °C in the corresponding medium supplemented with 15% (v/v) glycerol, and routinely reactivated overnight at the appropriate temperature and in the growth media indicated in Table 1. Phage stocks were prepared as described by Neviani, Carminatti, and Giraffa (1992). *Lactobacillus* phages were propagated in de Man, Rogosa and Sharp broth (MRS, Biokar, Beauvais, France; de Man, Rogosa, & Sharpe, 1960) at 37 °C; *Streptococcus thermophilus* phages were propagated in Elliker (Biokar, Beauvais, France; Elliker, Anderson, & Hannesson, 1956) broth at 42 °C. M17 broth supplemented with 0.5% lactose (LM17) was used for preparing stocks of *Lactococcus lactis* phages at 30 °C and was prepared from the original compounds (Becton, Dickinson, Sparks, USA) according to Terzaghi and Sandine (1975). All three growth media were supplemented with 10 mM $CaCl_2$. All phages were stored at 4 °C in the corresponding media and at −80 °C in the same broths supplemented with 15%, v/v, glycerol.

Phage counts, expressed as plaque-forming units per millilitre (pfu $mL^{-1}$), were performed by the double-layer plaque titration method (Svensson & Christiansson, 1991) and were incubated in microaerophilic conditions (*S. thermophilus* and lactobacilli phages; Capra et al., 2006) or aerobically (*L. lactis* phages) at temperatures indicated in Table 1. For the *Lactobacillus casei/paracasei* and for the *S. thermophilus* phages, the detection limit for this methodology was 10 pfu $mL^{-1}$. Since residual titres of heat-sensitive lactococcal reference phage P008 had already fallen below that detection limit during the heating-up times both in the glass and in metal tubes, 4 × 0.25 mL of each undiluted sample were also included for counting of lactococcal residual phages.

### 2.2. Preparation of test phage suspensions

Phage stocks in broth were used to prepare test suspensions in 10% (w/v) reconstituted skim milk (Difco, Sparks, MD, USA) and were diluted to final concentration of approximately $10^7$ pfu $mL^{-1}$. Phages J-1, 031-D and P008 (Table 1) were used as heat-sensitive control phages for *Lb. paracasei*, (Capra et al., 2006), *S. thermophilus* (Binetti & Reinheimer, 2000) and *L. lactis* phages (Müller-Merbach, Neve, & Hinrichs, 2005), respectively.

### 2.3. Thermal treatments

For all samples, 2 mL of skim milk with phages were subjected to heat treatments in thin-walled (0.5 mm thickness) borosilicate standard glass test tubes (length: 160 mm, inner diameter 16 mm; Schott Fiolax, Mainz, Germany). Additionally, all lactococcal phages were tested in 1.5 mL milk in (self-made) screw-capped stainless steel tubes (length, 50 mm; inner diameter, 6.2 mm; wall thickness,

**Table 1**
Phages, host strains and culture conditions used in this study.

| Phage | Host strain[a] | Source | Reference | Culture conditions | Phage titration conditions[b] |
|---|---|---|---|---|---|
| J-1 | *Lactobacillus casei* subsp. *casei* ATCC 27139 | Yakult elaboration | Hino and Ikebe (1965) | MRS, 37 °C | MRS-Ca-Gly, 34 °C |
| MLC-A3R | *Lactobacillus paracasei* A | Probiotic fermented milk | Capra et al. (2009) | MRS, 37 °C | MRS-Ca-Gly, 34 °C |
| $C_L2$ | *Lactobacillus paracasei* A | Noninfected lysed-cultures of commercial *Lb. paracasei* A | Capra, Mercanti, Reinheimer, and Quiberoni (2010) | MRS, 37 °C | MRS-Ca-Gly, 34 °C |
| 031-D | *Streptococcus thermophilus* ST 12-5 | Cuartirolo cheese whey | Binetti and Reinheimer (2000) | Elliker, 42 °C | M17-Ca-Gly, 37 °C |
| CGL-3 | *Streptococcus thermophilus* SC-36 | Cheese whey | This study | Elliker, 42 °C | M17-Ca-Gly, 37 °C |
| CGL-4 | *Streptococcus thermophilus* SC-36 | Cheese whey | This study | Elliker, 42 °C | M17-Ca-Gly, 37 °C |
| WPC-1 | *Streptococcus thermophilus* WPC-1 | Whey protein concentrate | This study | Elliker, 42 °C | M17-Gly, 37 °C |
| P1532 | *Lactococcus lactis* subsp. *lactis* 7-18 | Sour cream | Atamer et al. (2009) | LM17, 30 °C | LM17-Ca, 30 °C |
| P680 | *Lactococcus lactis* subsp. *lactis* F7/2 | Quarg sample | Atamer et al. (2009) | LM17, 30 °C | LM17-Ca, 30 °C |
| P008 | *Lactococcus lactis* subsp. *lactis* F7/2 | Cheese whey | Braun, Hertwig, Neve, Geis, and Teuber (1989) | LM17, 30 °C | LM17-Ca, 30 °C |

[a] ATCC: American Type Culture Collection, Manassas, VA, USA.
[b] MRS-Ca-Gly: MRS agar containing 10 mM $CaCl_2$ and 100 mM glycine; M17-Ca-Gly: M17 agar containing 10 mM $CaCl_2$ and 100 mM glycine; LM17-Gly: LM17 agar containing 100 mM glycine; LM17-Ca: LM17 agar containing 10 mM $CaCl_2$.

2 mm: i.e., no head-space after filling) as described previously (Atamer et al., 2009). All samples were heated in a thermostatically controlled water bath at 90 °C for 0–45 min. The temperature increase was documented in parallel in a glass tube with a calibrated thermometer. As soon as 90 °C was reached (i.e., after 2–3.5 min), samples were withdrawn in 5 min intervals and cooled rapidly in ice-water. For the stainless-steel test tubes, a 1 min heating time was chosen as reported before (Atamer et al., 2009). When all heat treatment experiments were completed, phage titres (initial input phage titres, phage titres immediately after heating-up time and residual titres after all sequential 5 min heating treatments) were calculated. All trials were done in triplicate and mean values and standard deviations are indicated.

*2.4. Electron microscopy*

Transmission electron microscopy of *L. lactis* phages was done with phages concentrated and purified by CsCl gradient centrifugation, as conducted previously (Vegge et al., 2005). The other phages were analysed directly from high-titre raw lysates in complex growth media. Negative staining of all phages was with 2% uranyl acetate and transmission electron microscopy (Tecnai 10 FEI Company, Eindhoven, The Netherlands) was performed as reported previously (Mc Grath et al., 2006).

## 3. Results and discussion

The International Dairy Federation (IDF Bulletin 263, 1991) has published a standard test for phage monitoring. This IDF assay has to be conducted in standard glass test tubes and includes a mandatory internal control, which is heat-treated for 15 min at 90 °C. To evaluate a revised thermal phage inactivation step, we performed a series of heat treatments on heat-resistant phages infecting both mesophilic (*L. lactis*) and thermophilic (*Lb. casei*, *Lb. paracasei*, *S. thermophilus*) lactic acid bacteria (Table 1, Fig. 1). For the lactococcal phages, heat treatment of milk samples was not only carried out in thin-walled borosilicate glass tubes but also – for comparison – in screw-capped stainless steel tubes as described earlier (Atamer et al., 2009). In these metal tubes, the heating-up phase was shorter (1 min; Atamer et al., 2009) than measured in the thin-walled glass tubes (2–3.5 min).

Heat inactivation experiments in glass tubes clearly showed that heat-sensitive control phages *S. thermophilus* phage 031-D and *Lb. casei* phage J-1 were inactivated to titres below the detection limit (<10 pfu mL$^{-1}$) within a short 5–10 min incubation at 90 °C (Fig. 2). Titres for the heat-sensitive *L. lactis* phage P008 had already fallen below the detection limit during the heating-up times both in the glass and in metal tubes (Fig. 3). Previously, it was reported that the titre of phage P008 rapidly decreased by 6 log cycles during a 1-min heating-up phase at 80 °C (Müller-Merbach et al., 2005). All other phages tested were capable of overcoming this thermal 15-min/90 °C hurdle. At the 15-min/90 °C threshold, residual titres of $10^2$–$10^4$ pfu mL$^{-1}$ were still detected (Fig. 2). Similar numbers of residual phages were also determined for the heat-resistant lactococcal phage P680 (Fig. 3). The most heat-resistant lactococcal phage P1532 revealed a minor 2-log reduction of phage titre after the first 15 min of treatment (Fig. 3).

To our knowledge, phage P1532 is currently the most heat-stable dairy phage known and can be used as a worst-case model phage for thermal inactivation experiments. It is obvious that the heat-resistant phenotype is not correlating with the growth preference of the bacterial host cultures. The *S. thermophilus* phage CGL-4 represents the most heat-stable phage for the thermophilic group of dairy phages (Fig. 2), but its inactivation curve is still below the curve for lactococcal phage P1532.

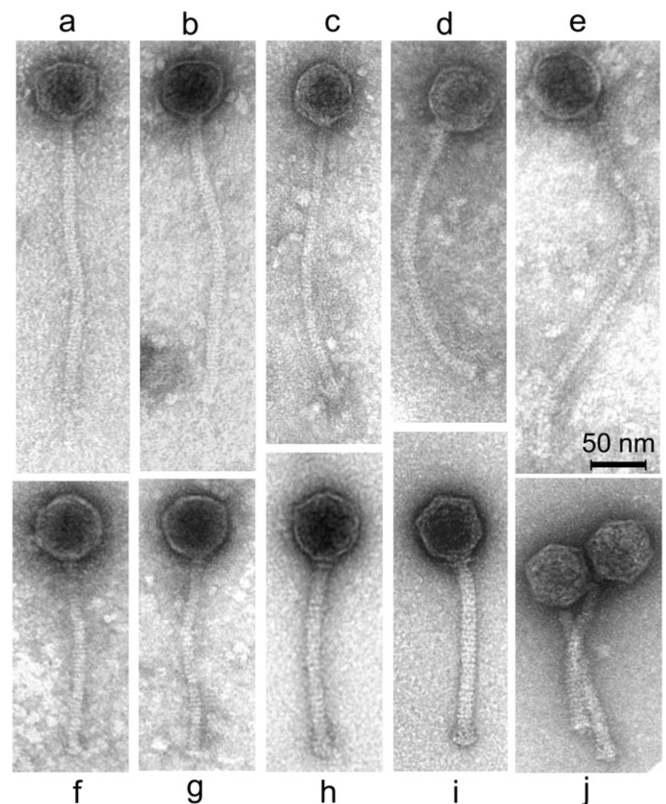


**Fig. 1.** Transmission electron micrographs of *S. thermophilus* bacteriophages CGL-3 (a), CGL-4 (b), WPC-1 (c), 031-D (d), of *Lb. casei/paracasei* phages J-1 (e), $C_L2$ (f), MLC-A3R (g), and of *L. lactis* phages P008 (h), P680 (i), P1532 (j).

All ten phages in our study are members of the small isometric-headed *Siphoviridae* (head diameter: approx. 55 nm) with long non-contractible tails of different lengths (270-nm tails: *Lb. casei* phage J-1, 240-nm tails: *S. thermophilus* phages CGL-3, CGL-4, WPC-1 and 031-D, 160-nm tails: *Lb. paracasei* phages $C_L2$ and

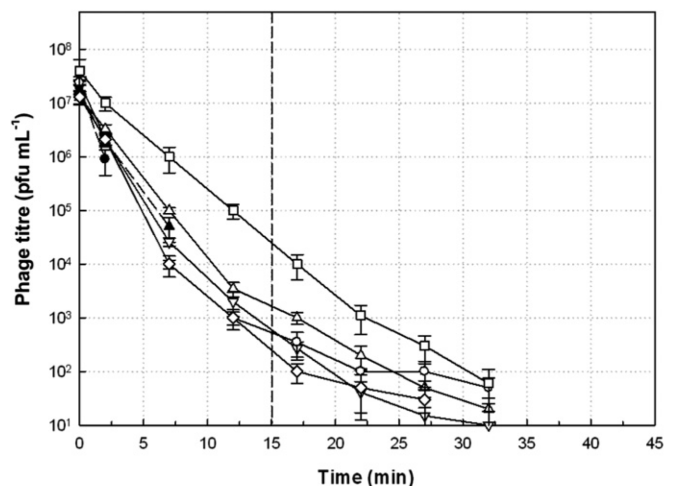


**Fig. 2.** Thermal inactivation of phages in thin-wall glass tubes at 90 °C in 2 mL of reconstituted skim milk. Streptococcal phages: 031-D (●), CGL-3 (○), CGL-4 (□) and WPC-1 (◇). Lactobacilli phages: J-1 (▲), MLC-A3R (△) and $C_L2$ (▽). Error bars represent the standard deviation of triplicate determinations. The dashed vertical line illustrates the 15-min boundary of the 90 °C incubation step originally considered as secure in phage detection methodologies for complete thermal inactivation of dairy phages in milk in glass test tubes (details see text and Svensson & Christiansson, 1991).

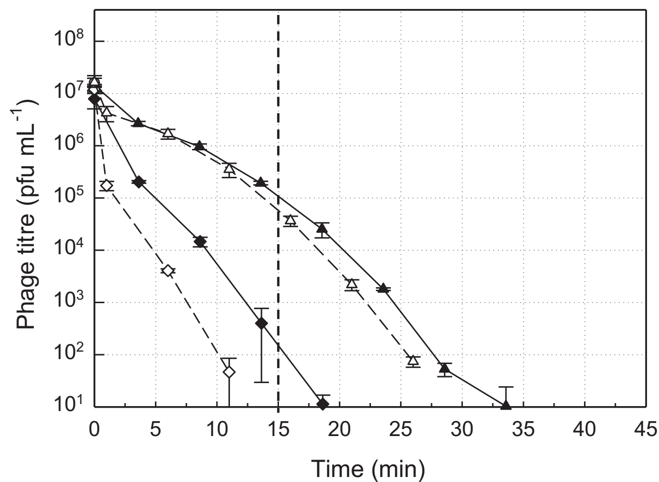


**Fig. 3.** Thermal inactivation of lactococcal phages at 90 °C in 2 mL of reconstituted skim milk in thin-wall glass tubes and in 1.5 mL of reconstituted skim milk in stainless steel tubes. Phages: P008 (glass tube: ●; stainless steel tube: ○), P680 (glass tube: ◆; stainless-steel tube: ◇) and P1532 (glass tube: ▲; stainless steel tube: △). Error bars represent the standard deviation of triplicate determinations. Residual titre for phage P008 was omitted because it was below the detection limit immediately after the heating-up time of 1 min in the metal tube or of 3.5 min in the glass tubes. The dashed vertical line illustrates the 15-min boundary of the 90 °C incubation step originally considered as secure in phage detection methodologies for complete thermal inactivation of dairy phages in milk in glass test tubes (details see text and Svensson & Christiansson, 1991).

MLC-A3R, *L. lactis* phages P008, P680 and P1532) (Fig. 1). Our data show that heat stability does not correlate with this morphological characteristic. For the *L. lactis* phages P008 and P680, DNA release and disintegration of capsids from tails have been shown earlier to occur as the first critical morphological changes during thermal destruction (Atamer et al., 2010). These thermal effects have also been confirmed recently by electron microscopy for a prolate-headed lactococcal phage (Marvig et al., 2011).

For all phages tested, no residual phage-derived plaques were detected after 40 min (Figs. 2 and 3). This thermal hurdle therefore assures reliable inactivation of all 10 heat-stable dairy phages tested by 7 log units. Since phage titres higher than $10^7$ pfu $mL^{-1}$ may be present in dairy samples and since phages may develop even higher degrees of thermal stability, we recommend adding an extra 5 min “auxiliary” time. Therefore we propose to prolong the 90 °C heat treatment in control assays significantly from 15 min to 45 min.

For lactococcal phages, heat inactivation usually performed in glass tubes was compared for the first time with data obtained in parallel in stainless-steel test tubes (Fig. 3). The systems differ in terms of sample volumes and head space (1.5 mL – no head space versus 2 mL – large head space) and heat conductivity (metal versus glass). Longer heating times to reach 90 °C were measured in the glass tube (2–3.5 min) compared with metal (1 min). For lactococcal phage P680, the inactivation curves in the glass and the metal test tubes showed a more pronounced difference (Fig. 3) than the curves for lactococcal phage P1532. This indicates this latter phage is a suitable worst-case model phage for different heat-treatment systems.

In suggesting a revised heating-step of 90 °C for 45 min (total time including heating up-period), we are aware of the possibility that non-phage inhibitors (e.g., antibiotics) might also be effected and degraded by a prolonged heat treatment. The majority of antibiotics used for dairy cattle belong to β-lactam antibiotics (i.e., penicillins and cephalosporins) as reported recently for The Netherlands (Anonymous, 2011). It has been shown that β-lactam antibiotics (in particular penicillins) loose only moderate activity after prolonged heating (up to 3 h) at temperatures up to 100 °C (Roca, Villegas, Kortabitarte, Althaus, & Molina, 2011), while thermal degradation was much greater at harsher thermal conditions used for milk sterilisation (i.e., 120 °C, 20 min) (Zorraquino, Roca, Fernandez, Molina, & Althaus, 2008). Similar results have also been reported for macrolide and lincosamide antibiotics (Zorraquino, Althaus, Roca, & Molina, 2011), for aminoglycosides (Zorraquino, Althaus, Roca, & Molina, 2009) and for quinolones (Roca, Castillos, Marti, Althaus, & Molina, 2010).

Hence, in an updated phage detection protocol with a 45 min treatment at 90 °C, the chance of a false positive result, in the absence of any phage particles in the sample (i.e., absence of starter culture inhibition in a heat-treated milk sample containing antibiotic residues) is low, but possible depending on the initial and residual concentrations of corresponding antibiotic traces.

## 4. Conclusions

Thermo-resistant phages infecting different lactic acid bacterial species have been documented in various dairy environments. Phages specific for *L. lactis*, *S. thermophilus*, *Lb. casei* and *Lb. paracasei* were investigated to establish a novel heat-treatment for the processing of the industrial dairy samples suspected of containing phages. In this regard it is proposed the Standard IDF microbiological methodology be revised to a longer heating-step (45 min at 90 °C). This prolonged 90 °C thermal treatment should be validated again in due time, since phage genomic plasticity and phage adaptation ability might lead to the origin of new phage populations with a higher thermal stability.

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## References


Anonymous. (2011). *Veterinary antibiotic usage in the Netherlands in 2010*. www.maran.wur.nl.

Atamer, Z., Ali, Y., Neve, H., Heller, K. J., & Hinrichs, J. (2011). Thermal resistance of bacteriophages attacking flavour-producing dairy *Leuconostoc* starter cultures. *International Dairy Journal, 21*, 327–334.

Atamer, Z., Dietrich, J., Müller-Merbach, M., Neve, H., Heller, K. J., & Hinrichs, J. (2009). Screening for and characterization of *Lactococcus lactis* bacteriophages with high thermal resistance. *International Dairy Journal, 19*, 228–235.

Atamer, Z., Dietrich, J., Neve, H., Heller, K. J., & Hinrichs, J. (2010). Influence of the suspension media on the thermal treatment of mesophilic lactococcal bacteriophages. *International Dairy Journal, 20*, 408–414.

Atamer, Z., & Hinrichs, J. (2010). Thermal inactivation of the heat-resistant *Lactococcus lactis* bacteriophage P680 in modern cheese processing. *International Dairy Journal, 20*, 163–168.

Atamer, Z., Neve, H., & Heller, K. J. (2012). Thermal resistance of bacteriophages in the dairy industry. In A. Quiberoni, & J. A. Reinheimer (Eds.), *Bacteriophages in dairy processing* (pp. 195–214). New York, NY, USA: Nova Science Publishers.

Binetti, A. G. (2012). Detection and quantification of dairy bacteriophages. In A. Quiberoni, & J. A. Reinheimer (Eds.), *Bacteriophages in dairy processing* (pp. 239–252). New York, NY, USA: Nova Science Publishers.

Binetti, A. G., & Reinheimer, J. A. (2000). Thermal and chemical inactivation of indigenous *Streptococcus thermophilus* bacteriophages isolated from Argentinian dairy plants. *Journal of Food Protection, 63*, 509–515.

Braun, V., Hertwig, S., Neve, H., Geis, A., & Teuber, M. (1989). Taxonomic differentiation of bacteriophages of *Lactococcus lactis* by electron microscopy, DNA–DNA hybridization, and protein profiles. *Journal of General Microbiology, 135*, 2551–2560.

Buzrul, S., Öztürk, P., Alpasa, H., & Akcelik, M. (2007). Thermal and chemical inactivation of lactococcal bacteriophages. *LWT Food Science and Technology, 40*, 1671–1677.

Capra, M. L., Binetti, A. G., Mercanti, D. J., Quiberoni, A., & Reinheimer, J. A. (2009). Diversity among *Lactobacillus paracasei* phages isolated from a probiotic dairy product plant. *Journal of Applied Microbiology, 107*, 1350–1357.

Capra, M. L., Mercanti, D. J., Reinheimer, J. A., & Quiberoni, A. L. (2010). Characterisation of three temperate phages released from the same *Lactobacillus paracasei* commercial strain. *International Journal of Dairy Technology, 63*, 396–405.

Capra, M. L., Quiberoni, A. L., Ackermann, H. W., Moineau, S., & Reinheimer, J. A. (2006). Characterization of a new virulent phage (MLC-A) of *Lactobacillus paracasei*. *Journal of Dairy Science, 89*, 2414–2423.

Capra, M. L., Quiberoni, A., & Reinheimer, J. A. (2004). Thermal and chemical resistance of *Lactobacillus casei* and *Lactobacillus paracasei* bacteriophages. *Letters in Applied Microbiology, 38*, 499–504.

Chopin, M. C. (1980). Resistance of 17 mesophilic lactic *Streptococcus* bacteriophages to pasteurization and spray-drying. *Journal of Dairy Research, 47*, 131–139.

de Man, J. D., Rogosa, M., & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology, 23*, 130–135.

Deveau, H., Labrie, S. J., Chopin, M. C., & Moineau, S. (2006). Biodiversity and classification of lactococcal phages. *Applied and Environmental Microbiology, 72*, 4338–4346.

Elliker, P. R., Anderson, A. W., & Hannesson, G. (1956). An agar culture medium for lactic acid streptococci and lactobacilli. *Journal of Dairy Science, 39*, 1611–1612.

Hino, M., & Ikebe, N. (1965). Studies on the lactic acid bacteria employed for beverage production II. Isolation and some properties of a bacteriophage infected during the fermentation of lactic acid beverage. *Journal of the Agricultural Chemical Society of Japan, 39*, 472–476.

Madera, C., Monjardín, C., & Suárez, J. E. (2004). Milk contamination and resistance to processing conditions determine the fate of *Lactococcus lactis* bacteriophages in dairies. *Applied and Environmental Microbiology, 70*, 7365–7371.

Marvig, C. L., Aideh, B., Neve, H., Heller, K. J., Knoechel, S., & Vogensen, F. K. (2011). Heat tolerance of dairy lactococcal c2 phages. *International Dairy Journal, 21*, 556–560.

Mc Grath, S., Neve, H., Seegers, J. F. M. L., Eijlander, R., Vegge, C. S., Brøndsted, L., et al. (2006). Anatomy of a lactococcal phage tail. *Journal of Bacteriology, 188*, 3972–3982.

Moineau, S., & Lévesque, C. (2005). Control of bacteriophages in industrial fermentation. In E. Kutter, & A. Sulakvelidze (Eds.), *Bacteriophages: Biology and applications* (pp. 286–296). Boca Raton, FL, USA: CRC Press.

Müller-Merbach, M., Neve, H., & Hinrichs, J. (2005). Kinetics of the thermal inactivation of the *Lactococcus lactis* bacteriophage P008. *Journal of Dairy Research, 72*, 281–286.

Neviani, E. N., Carminatti, D., & Giraffa, G. (1992). Selection of some bacteriophage- and lysozyme-resistant variants of *Lactobacillus helveticus* CNRZ 892. *Journal of Dairy Science, 75*, 905–913.

Quiberoni, A., Guglielmotti, D. M., & Reinheimer, J. A. (2003). Inactivation of *Lactobacillus delbrueckii* bacteriophages by heat and biocides. *International Journal of Food Microbiology, 84*, 51–62.

Quiberoni, A., Suárez, V. B., & Reinheimer, J. A. (1999). Inactivation of *Lactobacillus helveticus* bacteriophages by thermal and chemical treatments. *Journal of Food Protection, 62*, 894–898.

Roca, M., Castillos, M., Marti, P., Althaus, R. L., & Molina, M. P. (2010). Effect of heating on the stability of quinolones in milk. *Journal of Agricultural and Food Chemistry, 58*, 5427–5431.

Roca, M., Villegas, L., Kortabitarte, M. L., Althaus, R. L., & Molina, M. P. (2011). Effect of heat treatments on stability of β-lactams in milk. *Journal of Dairy Science, 94*, 1155–1164.

Suárez, V. B., & Reinheimer, J. A. (2002). Effectiveness of thermal treatments and biocides in the inactivation of Argentinian *Lactococcus lactis* phages. *Journal of Food Protection, 65*, 1756–1759.

Svensson, U., & Christiansson, A. (1991). Methods for phage monitoring. *Bulletin of the International Dairy Federation (FIL-IDF), 263*, 29–39.

Terzaghi, B. E., & Sandine, W. E. (1975). Improved medium for lactic streptococcic and their bacteriophages. *Applied Microbiology, 29*, 807–813.

Vegge, C. S., Brøndsted, L., Neve, H., Mc Grath, S., van Sinderen, D., & Vogensen, F. K. (2005). Structural characterization and assembly of the distal tail structure of the temperate lactococcal bacteriophage TP901-1. *Journal of Bacteriology, 187*, 4187–4197.

Zago, M., Scaltriti, E., Fornasari, M. E., Rivetti, C., Grolli, S., Giraffa, G., et al. (2012). Epifluorescence and atomic force microscopy: two innovative applications for studying phage–host interactions in *Lactobacillus helveticus*. *Journal of Microbiological Methods, 88*, 41–46.

Zorraquino, M. A., Althaus, R. L., Roca, M., & Molina, M. P. (2009). Effect of heat treatments on aminoglycosides in milk. *Journal of Food Protection, 72*, 1338–1341.

Zorraquino, M. A., Althaus, R. L., Roca, M., & Molina, M. P. (2011). Heat treatment effects on the antimicrobial activity of macrolide and lincosamide antibiotics in milk. *Journal of Food Protection, 74*, 311–315.

Zorraquino, M. A., Roca, M., Fernandez, N., Molina, M. P., & Althaus, R. (2008). Heat inactivation of β-lactam antibiotics in milk. *Journal of Food Protection, 71*, 1193–1198.