Letters in Applied Microbiology 2004, **38,** 499–504 doi:10.1111/j.1472-765X.2004.01525.x

Thermal and chemical resistance of *Lactobacillus casei* and *Lactobacillus paracasei* bacteriophages

M.L. Capra, A. Quiberoni and J.A. Reinheimer

Programa de Lactología Industrial, Facultad de Ingeniería Química, (Universidad Nacional del Litoral), Santiago del Estero, Santa Fe, Argentina

2003/0415: received 19 May 2003, revised 30 December 2003 and accepted 15 March 2004

ABSTRACT

M.L. CAPRA, A. QUIBERONI AND J.A. REINHEIMER. 2004.

Aims: The survival of two collection *Lactobacillus casei* and *L. paracasei* bacteriophages when subjected to thermal and chemical treatments was investigated.

Methods and Results: Thermal resistance was evaluated by heating phage suspensions at 63, 72 and 90°C in three different media [Tris-magnesium gelatin (TMG) buffer: 10 mmol l^{-1} Tris-Cl, 10 mmol l^{-1} $MgSO_4$ and 0·1% w/v gelatin; Man Rogosa Sharpe (MRS) broth and reconstituted nonfat dry skim milk (RSM)]. A marked heat sensitivity was evident in both phages, as 15 min at 72°C was enough to completely inactivate (6 $\log_{10}$ reduction) them. No clear influence was demonstrated by the suspension media. The phages also showed similar resistance to biocides. Peracetic acid and sodium hypochlorite (800 ppm) were the most effective ones, destroying the phages within 5 min. Concentrations of 75 and 100% ethanol were not suitable to inactivate phage particles even after 45 min. Isopropanol did not show an effect on phage viability.

Conclusions: The data obtained in this work are important to design more effective control procedures in order to inactivate phages in dairy plants and laboratories.

Significance and Impact of the Study: This work will contribute to enhance the background knowledge about phages of probiotic bacteria.

Keywords: biocide, heat, inactivation, phage, survival, viability, viral destruction.

INTRODUCTION

The threat of phage infections is particularly important in dairy production. Phage attacks on lactic acid bacteria (LAB) during cheese and yogurt manufacturing result in an unacceptable low production of lactic acid and flavour compounds, reduced proteolysis and culture lysis. Raw milk is considered to be an important source of phages that propagate at low levels on phage-sensitive nonstarter LAB. Hence, it constitutes the primary entrance-point into the industrial environment for phages (Everson 1991; Josephsen and Neve 1998). Because of the constant risk of economic losses, control of phages is a major area of concern in handling LAB (Josephsen and Neve 1998).

Correspondence to: Andrea Quiberoni, Programa de Lactología Industrial, Facultad de Ingeniería Química, (Universidad Nacional del Litoral), Santiago del Estero 2829-(3000), Santa Fe, Argentina (e-mail: aquibe@fiqus.unl.edu.ar).

Lactobacillus casei, *L. paracasei* and *L. acidophilus* are used worldwide in the manufacture of fermented milk beverages (Jarvis 1989) because of their probiotic properties (Gilliland 1998). In Argentina, they are added to some fermented milks and probiotic cheese (Vinderola *et al.* 2000a,b 2002). It has been reported that *L. casei* and *L. paracasei* phages have caused failures in dairy fermentations (Watanabe *et al.* 1970; Sechaud *et al.* 1988; Forsman *et al.* 1993; Herrero *et al.* 1994). Some characteristics have been thoroughly studied for the phage PL-1, but knowledge regarding its heat or chemical resistance is scarce and scattered (Watanabe *et al.* 1970; Lee *et al.* 1985). Contrarily, much data have been reported on thermal and chemical destruction of *Lactococcus* phages (Hunter and Whitehead 1940; Parker and Elliker 1951; Bennet and Nelson 1954; Wilkowske *et al.* 1954; Daoust *et al.* 1965; Zottola and Marth 1966; Fabrizio *et al.* 1999; Suárez and Reinheimer 2002). Additionally, the

resistance of *L. helveticus* (Quiberoni *et al.* 1999), *Streptococcus thermophilus* (Binetti and Reinheimer 2000) and *L. delbrueckii* (Quiberoni *et al.* 2003) bacteriophages to heat and biocides, was also reported.

The aim of this work was to investigate the thermal and chemical sensitivity of two *L. casei* and *L. paracasei* bacteriophages.

MATERIALS AND METHODS

Bacteria strains, bacteriophages and culture conditions

The host strains *L. paracasei* subsp. *paracasei* ATCC 27092 and *L. casei* subsp. *casei* ATCC 27139, and their specific bacteriophages PL-1 (ATCC 27092-B1) and J-1 (ATCC 27139-B1), respectively, were used in this study. The strains were grown and routinely reactivated overnight (37°C) in Man Rogosa Sharpe (MRS) broth (Britania S.A., Buenos Aires, Argentina). They were maintained as frozen (−80°C) stocks in MRS broth in the presence of 15% v/v glycerol. MRS broth and MRS agar supplemented with 10 mmol l^{-1} $CaCl_2$ (MRS-Ca) were used to replicate and count phage particles, respectively. Phage stocks were prepared as described by Neviani *et al.* (1992) and stored at 4°C (MRS broth) and −80°C (MRS broth added of 15% v/v glycerol). Phage counts, expressed as plaque-forming units (PFU) per millilitre, were performed by the double-layer plaque titration method (Svensson and Christiansson 1991). Incubations were carried out at 34°C.

Thermal treatments

To study the thermal resistance of phages, three temperatures (63, 72 and 90°C) and three suspension media [Tris-magnesium gelatin (TMG) buffer: 10 mmol l^{-1} Tris-Cl, 10 mmol l^{-1} $MgSO_4$ and 0·1% w/v gelatin; MRS broth and reconstituted nonfat dry skim milk (RSM)] were chosen. The temperatures were selected on the basis of the heat treatment conditions used in the dairy industry.

Each phage (*ca* 10^6 PFU ml^{-1}) was mixed with the suspension media and, after being distributed in microfuge tubes (1 ml as final volume), incubated at the temperatures mentioned above. At predetermined intervals, the tubes were removed and cooled in ice water. The surviving bacteriophages were immediately titred by the double-layer agar plate method (Svensson and Christiansson 1991). Three trials of each assay were carried out. The results were expressed as the concentration (PFU ml^{-1}) of active viral particles and plotted against the time. Time (min) to achieve the 99% inactivation (T_{99}) of phages were calculated graphically from the inactivation curves as described by Quiberoni *et al.* (1999, 2003); Binetti and Reinheimer (2000) and Suárez and Reinheimer (2002).

Chemical treatments

The biocides used were commercial sodium hypochlorite (400–800 ppm residual-free chlorine), ethanol (Cicarelli, Buenos Aires, Argentina) (10, 50, 75 and 100% v/v), isopropanol (Cicarelli) (10, 50 and 100% v/v) and peracetic acid (Proxitane 1512; Química General, Santa Fe, Argentina) (0·15% v/v). Sodium hypochlorite was diluted in phosphate buffer (pH 7). Alcohols were diluted in MRS broth, while peracetic acid was diluted in distilled water (with a resulting pH of 3·0). The experiments were carried out at 25°C, except for peracetic acid which was assayed at 40°C (Schröder 1984).

Each phage (*ca* 10^6 PFU ml^{-1}) was mixed with the biocide solution and, after being distributed in Microfuge tubes (1 ml as final volume), incubated at the fixed temperatures according to each chemical agent. To study the influence of pH on the viability of the phages, viral suspensions in MRS broth without the addition of biocides, but with a previous adjustment of pH, were titred. At predetermined intervals, tubes were removed and the surviving bacteriophages were immediately diluted and counted. All assays were performed in triplicate. The results were expressed as the concentration (PFU ml^{-1}) of infectious bacteriophages and plotted against time. Time to achieve the 99% inactivation (T_{99}) of phages was calculated from the inactivation curves.

RESULTS

Thermal treatments

When PL-1 and J-1 phages were heated at 63 and 72°C, fast reductions in the number of infectious phage particles ($T_{99} < 5$ min) were detected in the three media used (Table 1). Thermal inactivation of phages did not follow a first-order kinetics. In general, both phages exhibited similar behaviour with regard to temperature (Fig 1a,b). Within 45 min, phage PL-1 suspensions were completely inactivated (6 $\log_{10}$ reduction) at 63°C in MRS broth and RSM and showed a reduction of 4·6 $\log_{10}$ in TMG buffer (Fig. 1a). From the phage J-1 inactivation curves (Fig. 1b), a fall of 4·7 $\log_{10}$ was obtained after 45 min in RSM, while in TMG buffer and MRS broth, undetectable (<10 PFU ml^{-1}) counts were reached in 30 min (6 $\log_{10}$ reduction). PL-1 and J-1 phages exhibited a noticeable heat sensitivity at 72°C. No infectious bacteriophages were detected after 5 min for both phages in MRS broth and RSM, whereas a slightly higher heat resistance was detected in TMG buffer (Fig. 1).

Table 1 Thermal resistance of *Lactobacillus casei* and *L. paracasei* phages in different suspension media

	T_{99}*								
	MRS broth			RSM			TMG Buffer		
Phage	63°C	72°C	90°C	63°C	72°C	90°C	63°C	72°C	90°C
PL-1	2·4 ± 0·1	<5	<5	2·5 ± 0·3	<5	<5	2·6 ± 0·2	1·4 ± 0·3	<5
J-1	2·5 ± 0·2	<5	<5	3·1 ± 0·2	<5	<5	2·8 ± 0·4	2·1 ± 0·4	<5

*Time (min) to achieve 99% inactivation of phage particles.
MRS, Man Rogosa Sharp; RSM, reconstituted nonfat dry skim milk; TMG buffer, Tris-magnesium gelatin buffer.

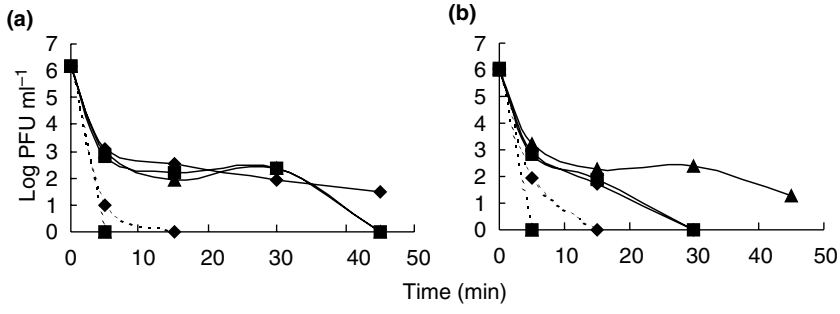

Fig. 1 Thermal destruction kinetics of phages PL-1 (a) and J-1 (b) at 63°C (—) and 72°C (- - -) in MRS broth (■), TMG buffer (◆) and RSM (▲). The values are the mean of three determinations

A treatment of 90°C during 5 min was enough to obtain phage counts <10 PFU ml^{-1} in all suspension media tested (Table 1).

Chemical treatments

A similar behaviour was demonstrated by the two phages when they were treated with biocides.

Sodium hypochlorite at 800 ppm was effective to produce undetectable counts (<10 PFU ml^{-1}) within 5 min. A concentration of 700 ppm was insufficient to achieve a total inactivation of the PL-1 (data not shown) and J-1 (Fig. 2) phages within 45 min (reductions of 4·8 and 5·0 $\log_{10}$ orders, respectively). In this case, the T_{99} was 7 min (Table 2).

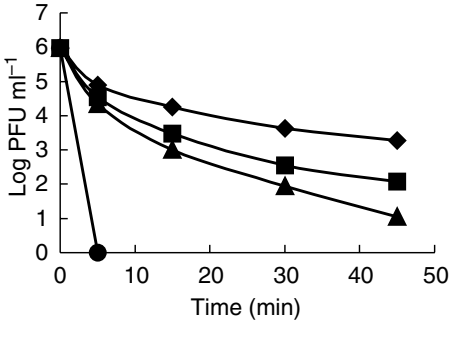

Fig. 2 Destruction kinetics of phage J-1 with 400 ppm (◆), 600 ppm (■), 700 ppm (▲) and 800 ppm (●) of residual-free chlorine. The values are the mean of three determinations

Ethanol at concentrations of 75 and 100% were the most effective solutions to inactivate phages, although they did not achieve a complete loss of viability within 45 min (Fig. 3a). For these concentrations, T_{99} values ranged from 5·5 to 10·3 min (Table 2). Indeed, little or no effects on phage viability were observed when concentrations of 10 and 50% ethanol were used (T_{99} >45 min) (Table 2, Fig. 3a).

Both PL-1 and J-1 phages were scarcely affected by isopropanol (Fig. 3b). For all concentrations assayed, T_{99} values were >45 min (Table 2).

Peracetic acid (0·15%) was the most effective biocidal agent tested, as it produced the fastest inactivation. Neither of the phages were detectable within 5 min of treatment. T_{99} values were <5 min (Table 2).

DISCUSSION

Few phages of *L. casei* and *L. paracasei* are well characterized compared with other LAB phages (Sechaud *et al.* 1988; Jarvis 1989; Forsman *et al.* 1993; Herrero *et al.* 1994; Nakashima *et al.* 1998). Mesophilic species of *Lactobacillus* are used in the manufacture of yakult (a Japanese lactic acid beverage), and meat and vegetable fermentations. The industrial importance of lactobacilli may soon be reinforced by their use as probiotics. In Argentina, some probiotic dairy beverage manufactures include a slow milk fermentation with *L. casei* and

Table 2 Resistance of *Lactobacillus casei* and *L. paracasei* phages to ethanol, isopropanol, sodium hypochlorite and peracetic acid

	T_{99}*											
	Ethanol (% v/v)				Isopropanol (% v/v)			Sodium hypochlorite (ppm)				Peracetic acid (% v/v)
Phage	10	50	75	100	10	50	100	400	600	700	800	0·15
PL-1	>45	>45	9·7 ± 0·2	10·3 ± 0·6	>45	>45	>45	26·8 ± 0·7	14·8 ± 0·4	6·9 ± 0·3	<5	<5
J-1	>45	>45	5·5 ± 0·5	9·0 ± 0·3	>45	>45	>45	20·6 ± 0·5	9·0 ± 0·3	7·0 ± 0·2	<5	<5

*Time (min) to achieve 99% inactivation of phage particles.

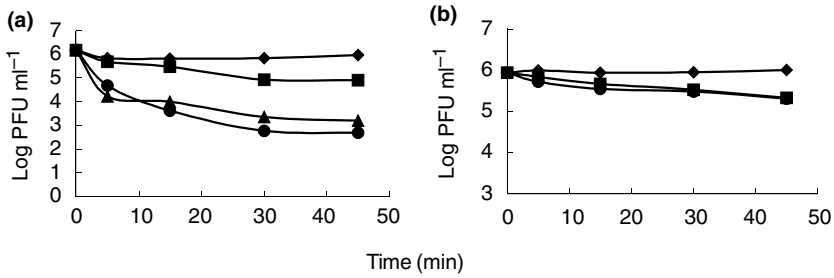

Fig. 3 Destruction kinetics of phage J-1 with 10% (◆), 50% (■), 75% (▲) and 100% (●) (v/v) of ethanol (a) and 10% (◆), 50% (■) and 100% (●) (v/v) of isopropanol (b). The values are the mean of three determinations

L. paracasei strains for several days. The increasing use of valuable lactobacilli as starters will probably lead to bacteriophage infection problems (Alvarez *et al.* 1999). These must be avoided because lactobacilli cells should reach the gut being viable, and colonize it to achieve the desired beneficial probiotic effect (Salminen *et al.* 1998; Lee *et al.* 1999). Consequently, effective control measures are necessary to minimize problems derived from phage attacks. To establish such measures, thermal and chemical resistance must be investigated in advance.

The thermal sensitivity of phages PL-1 and J-1 was not affected by the media for the experiment. Comparing the T_{99} values obtained for the thermal exposures of the phages studied with other LAB phages previously investigated by our group, it was evident that *L. casei* and *L. paracasei* phages were the most sensitive ones. For all the phages studied, including *L. casei* and *L. paracasei* phages, it was demonstrated that usual pasteurization procedures were insufficient to inactivate viral suspensions in milk completely. Except for phage Ib3 (*L. delbrueckii*), a heat treatment at 90°C for 5 min killed high titre suspensions of all phages studied by our group, in all tested media (Quiberoni *et al.* 1999, 2003; Binetti and Reinheimer 2000; Suárez and Reinheimer 2002). Watanabe *et al.* (1970) reported that PL-1 phage was stable below 50°C, but less stable above this point and almost completely inactivated at 60°C in 5 min in TM broth (initial phage titre $1{\cdot}7 \times 10^4$ PFU ml^{-1}).

In this study, it was not possible to demonstrate a significant influence of the suspension media used. A similar evidence was previously obtained for *L. helveticus* (Quiberoni *et al.* 1999) and *S. thermophilus* phages (Binetti and Reinheimer 2000). On the contrary, recent data on thermal inactivation of *L. delbrueckii* phages (Quiberoni *et al.* 2003) demonstrated that MRS broth was the least protective medium and RSM was the most protective one. The same behaviour regarding milk phage suspensions was also reported by Daoust *et al.* (1965) and Fabrizio *et al.* (1999). The latter reported a moderate increase in the heat resistant level for *L. lactis*, when whole milk was used instead of skim milk. For Argentinian *L. lactis* phages (Suárez and Reinheimer 2002), M17 broth was generally less effective in protecting phages against heat exposures than TMG buffer.

The similar T_{99} values obtained for both *L. casei* and *L. paracasei* phages could be associated with the fact that they are serologically related (Sechaud *et al.* 1988). Wilkowske *et al.* (1954) reported that members of bacteriophage groups established by serological typing, appeared to be relatively homogeneous regarding the conditions required for heat inactivation. However, Fabrizio *et al.* (1999) did not find a clear correlation between heat resistance and serological groups. They found that only two phages with an extremely high degree of immunological homology had very similar heat resistance. In addition, Parada (1995) reported that even within the same serological group, significant differences could be observed for inactivation values of phages treated with biocides, although the closest values were obtained for immunologically related phages. This could serve as an explanation in our case, as almost the same

behaviour was also evidenced when the two phages used in the present study were exposed to various chemical agents.

Peracetic acid was found to be the most effective biocide tested, because completely inactivate phage suspensions of high titre in short time. This biocide was similarly effective on the other LAB phages studied (Quiberoni *et al.* 1999, 2003; Binetti and Reinheimer 2000; Suárez and Reinheimer 2002). Maillard *et al.* (1993) found peracetic acid highly effective against F116 [*Pseudomonas aeruginosa* PAO phage], a concentration of 0·1% achieved 99·99% reduction in phage titre after 10 min contact. Peracetic acid altered F116 nucleic acid and its structure. It is not clear whether the biocide inactivates the phage DNA inside the capside or after its release from a fractured protein coat. Biocide treatment of F116 often resulted in a structural alteration of the phage capside (Maillard *et al.* 1996a,b).

In comparison with our previous studies (Quiberoni *et al.* 1999; Binetti and Reinheimer 2000; Suárez and Reinheimer 2002), phages PL-1 and J-1 were more resistant against residual-free chlorine than *L. helveticus*, Argentinian *S. thermophilus* and *L. lactis* phages. An exposure of 5 min to 800 ppm residual-free chlorine was necessary to totally inactivate suspensions of phages PL-1 and J-1. In contrast, sodium hypochlorite at 100–300 ppm, produced undetectable counts of *L. helveticus* (Quiberoni *et al.* 1999), Argentinian *S. thermophilus* (Binetti and Reinheimer 2000) and *L. lactis* phages (Suárez and Reinheimer 2002). However, phage Ib3 (*L. delbrueckii*) exhibited an extremely high resistance against this chemical agent (Quiberoni *et al.* 2003), requiring 45 min at 1200 ppm to inactivate its suspensions. Maillard *et al.* (1998) found that this biocide caused the aggregation to tail proteins of F116 phage. In addition, sodium hypochlorite caused a high percentage of structural alteration to the bacteriophage head, possibly releasing the phage nucleic acid into the surrounding medium.

Alcohols did not seem to be very efficient as viricidal agents for the *L. casei* and *L. paracasei* phages studied. Concentrations of 75 and 100% ethanol reduced phage suspensions titres only partially, while 10 and 50% ethanol showed little effectiveness for inactivating viral particles. Similar results were obtained for some *L. delbrueckii* phages (Quiberoni *et al.* 2003). The other LAB phages studied in our group (Quiberoni *et al.* 1999; Binetti and Reinheimer 2000; Suárez and Reinheimer 2002) were more sensitive to the treatment with this biocide. Lee *et al.* (1985) reported that concentrations higher than 30% ethanol were required to affect plaque formation, when phosphate buffer suspensions (*ca* 10^2 PFU ml^{-1}) of phage PL-1 were treated at 20°C during 20 h.

Watanabe *et al.* (1970) studied the effect of ethanol 30% and sodium hypochlorite (0·1–1 ppm residual-free chlorine) on PL-1 phage suspensions (titre 10^4 PFU ml^{-1}) at 37°C for 60 min and found that ethanol and sodium hypochlorite (1 ppm) were viricidal to free phage particles, while 89% survival was obtained when the phage suspension was treated with 0·1 ppm residual-free chlorine.

Isopropanol was not an effective biocide against the two phages studied in our work, as it did not affect their viability. The same behaviour was reported by Quiberoni *et al.* (2003) for *L. delbrueckii* phages, whereas a weak viricidal effect was exhibited for *L. helveticus* (Quiberoni *et al.* 1999) and *S. thermophilus* (Binetti and Reinheimer 2000) bacteriophages.

The results of this work give useful information to design heat and chemical treatments to be applied in laboratories and industrial plants which handle *L. casei* and *L. paracasei* strains and phages.

ACKNOWLEDGEMENTS

This work was supported by the Universidad Nacional del Litoral (Santa Fe, Argentina) (Programación CAI+D 2002, Proyecto no. 22-155) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, Argentina) – (Proyecto PICT no. 09-08200).

REFERENCES

Alvarez, M.A., Rodríguez, A. and Suárez, J.E. (1999) Stable expression of the *Lactobacillus casei* bacteriophage A2 repressor blocks phage propagation during milk fermentation. *Journal of Applied Microbiology* **86**, 812–816.

Bennet, F.W. and Nelson, F.E. (1954) Action of certain viricidal agents on lactic *Streptococcus* bacteriophage in liquids. *Journal of Dairy Science* **36**, 847–856.

Binetti, A.G. and 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.

Daoust, D.R., El-Bisi, H.M. and Litsky, W. (1965) Thermal destruction kinetics of a lactic streptococcal bacteriophage. *Applied Microbiology* **13**, 478–485.

Everson, T.C. (1991) Control of phages in the dairy plant. *FIL-IDF Bulletin* **263**, 4–11.

Fabrizio, S.V., Ledford, R.A. and Parada J.L. (1999) Heat resistance of *Lactococcus lactis* bacteriophages. *Microbiologie-Aliments-Nutrition* **17**, 191–198.

Forsman, P., Tanskanen, J. and Alatossava, T. (1993) Structural similarity and genetic homology between *Lactobacillus casei* bacteriophages isolated in Japan and in Finland. *Bioscience-Biotechnology-Biochemistry* **57**, 2043–2048.

Gilliland, S.E. (1998) Fermented milks and probiotics. In *Applied Dairy Microbiology*, chap. 8, ed. Marth, E.H. and Steele, J.L. pp. 195–212. New York: Marcel Dekker, Inc.

Herrero, M., de los Reyes-Gavilán, C.G., Caso, J.L. and Suárez, J.E. (1994) Characterization of ϕ393-A2, a bacteriophage that infects *Lactobacillus casei*. *Microbiology* **140**, 2585–2590.

Hunter, G.J.E. and Whitehead, H.R. (1940) The action of chemical disinfectants on bacteriophages for the lactic streptococci. *Journal of Dairy Research* **11**, 62–66.

Jarvis, A.W. (1989) Bacteriophages of lactic acid bacteria. *Journal of Dairy Science* **72**, 3406–3428.

Josephsen, J. and Neve, H. (1998) Bacteriophages and lactic acid bacteria. In *Lactic Acid Bacteria. Microbiology and Functional Aspects*, chap. 14. ed. Salminen, S. and von Wright, A. pp. 385–436. New York: Marcel Dekker, Inc.

Lee, A., Eschenbruch, R. and Waller, J. (1985) Effect of phenolic compounds, ethyl alcohol, and sodium metabisulphite on the lytic activity of phage PL-1 on a *Lactobacillus casei* S strain. *Canadian Journal of Microbiology* **31**, 873–875.

Lee, Y.K., Nomoto, K., Salminen, S. and Gorbach, S.L. (1999) Introduction. In *Handbook of Probiotics*, chap. 1. pp. 1–22. New York: John Wiley & Sons, Inc.

Maillard, J.Y., Beggs, T.S., Day, M.J., Hudson, R.A. and Russell, A.D. (1993) Effect of biocides on *Pseudomonas aeruginosa* phage F116. *Letters in Applied Microbiology* **17**, 167–170.

Maillard, J.Y., Beggs, T.S., Day, M.J., Hudson, R.A. and Russell, A.D. (1996a) The effect of biocides on proteins of *Pseudomonas aeruginosa* PAO bacteriophage F116. *Journal of Applied Bacteriology* **80**, 291–295.

Maillard, J.Y., Beggs, T.S., Day, M.J., Hudson, R.A. and Russell, A.D. (1996b) Damage to *Pseudomonas aeruginosa* PAO1 bacteriophage F116 DNA by biocides. *Journal of Applied Bacteriology* **80**, 540–544.

Maillard, J.Y., Hann, A.C., Baubet, V. and Perrin, R. (1998) Efficacy and mechanisms of action of sodium hypochlorite on *Pseudomonas aeruginosa* PAO1 phage F116. *Journal of Applied Microbiology* **85**, 925–932.

Nakashima, Y., Hasuwa, H., Kakita Y., Murata, K., Kuroiwa, A., Miake, F. and Watanabe, K. (1998) A temperate phage with cohesive ends induced by mitomicyn C treatment of *Lactobacillus casei*. *Archives of Virology* **143**, 1621–1626.

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

Parada, J.L. (1995) Fagos de bacterias lácticas y estrategias para su control. *Tecnologiá Láctea Latinoamericana* **2**, 38–43.

Parker, R.B. and Elliker, P.R. (1951) Destruction of lactic acid *Streptococcus* bacteriophage by hypochlorite and quaternary ammonium compounds. *Journal of Milk and Food Technology* **44**, 52–54.

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

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

Salminen, S., Deighton, M.A., Benno, Y. and Gorbach, S.L. (1998) Lactic acid bacteria in health and disease. In *Lactic Acid Bacteria. Microbiology and Functional Aspects*, chap. 7. ed. Salminen, S. and vonWright, A. pp. 211–253. New York: Marcel Dekker, Inc.

Schröder, W. (1984) Peracetic acid. Disinfectant for the food stuff industry. *Brauwelt International* **1**, 115–120.

Sechaud, L., Cluzel, P.J., Rousseau, M., Baumgartner, A. and Accolas, J.P. (1988) Bacteriophages of lactobacilli. *Biochimie* **70**, 401–410.

Suárez, V.B. and 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. and Christiansson, A. (1991) Methods for phage monitoring. *FIL-IDF Bulletin* **263**, 29–39.

Vinderola, C.G., Bailo, N. and Reinheimer, J.A. (2000a) Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage. *Food Research International* **33**, 97–102.

Vinderola, C.G., Prosello, W., Ghiberto, D. and Reinheimer, J.A. (2000b) Viability of probiotic (*Bifidobacterium*, *Lactobacillus acidophilus* and *Lactobacillus casei*) and nonprobiotic microflora in Argentinian Fresco Cheese. *Journal of Dairy Science* **83**, 1905–1911.

Vinderola, C.G., Mocchiutti, P. and Reinheimer, J.A. (2002) Interactions among lactic acid starters and probiotic bacteria used for fermented dairy products. *American Dairy Science Association* **85**, 721–729.

Watanabe, K., Takesue, S., Jin-Nai, K. and Yoshikawa, T. (1970) Bacteriophage active against the lactic acid beverage-producing bacterium *Lactobacillus casei*. *Applied Microbiology* **20**, 409–415.

Wilkowske, H.H., Nelson, F.E. and Parmelee, C.E. (1954) Heat inactivation of bacteriophage strains active against lactic streptococci. *Applied Microbiology* **2**, 250–253.

Zottola, E.A. and Marth, E.H. (1966) Thermal inactivation of bacteriophages active against lactic streptococci. *Journal of Dairy Science* **49**, 1338–1342.