Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Diversity among *Lactobacillus paracasei* phages isolated from a probiotic dairy product plant

M.L. Capra, A.G. Binetti, D.J. Mercanti, A. Quiberoni and J.A. Reinheimer

Instituto de Lactología Industrial (UNL-CONICET), Facultad de Ingeniería Química (Universidad Nacional del Litoral), Santa Fe, Argentina

Keywords
dairy industry, fermentation and processes, *Lactobacillus paracasei,* phage diversity, probiotic bacteria.

Correspondence
Diego Javier Mercanti, Instituto de Lactología Industrial (UNL-CONICET), Facultad de Ingeniería Química (Universidad Nacional del Litoral), Santiago del Estero 2829, 3000 Santa Fe, Argentina. E-mail: diegojav78@gmail.com

2008/2192: received 22 December 2008, revised 19 February 2009 and accepted 2 March 2009

doi:10.1111/j.1365-2672.2009.04313.x

Abstract

Aims: To evaluate the phage diversity in the environment of a dairy industry which manufactures a product fermented with a probiotic strain of *Lactobacillus paracasei*.

Methods and Results: Twenty-two *Lact. paracasei* phages were isolated from an industrial plant that manufactures a probiotic dairy product. Among them, six phages were selected based on restriction profiles, and two phages because of their notable thermal resistance during sample processing. Their morphology, host range, calcium dependency and thermal resistance were investigated. All phages belonged to the *Siphoviridae* family (B1 morphotype), were specific for *Lact. casei* and *paracasei* strains showing identical host spectrum, and only one phage was independent of calcium for completing its lytic cycle. Some of the phages showed an extraordinary thermal resistance and were protected by a commercial medium and milk.

Conclusions: Phage diversity in a probiotic product manufacture was generated to a similar or greater extent than during traditional yogurt or cheese making.

Significance and Impact of the Study: This work emphasizes probiotic phage infections as a new ecological situation beyond yogurt or cheese manufactures, where the balanced coexistence between phages and strains should be directed toward a favourable state, thus achieving a successful fermentation.

Introduction

Bacteriophage attacks against starters of lactic acid bacteria represent one of the major risks of acidifying delays, causing technological difficulties and economic losses (Jarvis 1989; Brüssow *et al.* 1994; Bruttin *et al.* 1997; Moineau and Lévesque 2005). Even though probiotic bacteria are mainly included in fermented dairy products as additives (usually as frozen and/or lyophilized powders) once the main fermentation process is finished, they can also be propagated during this stage, therefore acting as a starter (Watanabe *et al.* 1970; Forsman *et al.* 1993; Saarela *et al.* 2000; Capra *et al.* 2006b). While this function represents an advantage in several aspects, phage infections may occur on these active growing bacteria (Saarela *et al.* 2000). Besides, probiotic strains are unique (probiotic claims are specific of the strain), valuable in time (many well-documented studies are necessary to assure their probiotic properties) and in consequence, expensive cultures. Furthermore, the complete lysis of the strain produces the absence of its specific beneficial properties in the final product (Capra 2007). In consequence, the economic impact of viral infections on probiotic bacteria is even worse than that on lactic acid bacteria.

Two distinct ecological settings can be distinguished in the industrial dairy fermentation environment, which are the yogurt and cheese factories (Brüssow *et al.* 1994; Brüssow and Kutter 2005). Phages are seldom seen in yogurt production while represent a constant problem in cheese plants (Suárez *et al.* 2002; Brüssow and Kutter 2005). Although the dairy industry has accumulated substantial knowledge in the field of phage factory ecology, these data are generally not published. Some authors (Moineau *et al.* 1996; Deveau *et al.* 2006; Szczepańska *et al.* 2007) have investigated lactococcal phage diversity and not many studies have been performed to elucidate

the origin of *Streptococcus thermophilus* phages in dairy environments (Brüssow *et al.* 1994, 1998; Bruttin *et al.* 1997; Brüssow and Desiere 2001), despite these latter lactic acid bacteria are widely used as starters.

The available knowledge about lactobacilli phages is limited and only a few have been studied in detail (Séchaud *et al.* 1988; Moineau and Lévesque 2005). Some of them are lytic *Lactobacillus casei* and *Lact. paracasei* phages isolated from Yakult, a lactic acid beverage fermented with *Lact. casei* (phages PL–1 and J–1; Watanabe *et al.* 1970; Yokokura 1971) and cheeses (phage LC-Nu; Forsman *et al.* 1993), as well as the temperate phages ϕFSW (Shimizu-Kadota and Tsuchida 1984), A2 (Herrero *et al.* 1994), and ϕAT3 (Lo *et al.* 2005).

The expanding use of probiotic strains as starters becomes associated with an increment in the frequency of bacteriophage infections in dairy plants.

In Argentina, the first phage infective for *Lact. paracasei* was isolated in 2003, from a probiotic dairy fermented product (Capra *et al.* 2006b). Since then and up to 2006, the presence of infective phage particles in the same process was almost continuously detected, and allowed the obtainment of 22 independent isolates. The aim of this work was to evaluate the phage diversity in the factory environment of this probiotic dairy product.

Materials and methods

Strains and culture conditions

The commercial strain *Lact. paracasei* A constitutes the starter used in the manufacturing process. It was utilized for the isolation, plaque enumeration and propagation of the phages. To test the host range, 16 strains of *Lact. paracasei* (five collection strains – ATCC 27092, CNRZ 1224, CNRZ 1308, CNRZ 318, CNRZ 1976; a strain isolated from infant faeces and 10 commercial strains), 13 strains of *Lact. casei* (five collection strains – ATCC 27139, ATCC 393, CNRZ 1884, INLAIN 8, INLAIN M; seven strains isolated from cheeses and one commercial strain) and 12 strains of *Lact. rhamnosus* (three commercial strains and nine strains isolated from infant faeces) were used.

Strains were maintained as frozen stocks at −80°C in MRS broth (Britania S.A., Buenos Aires, Argentina) supplemented with 15% v/v of glycerol, and routinely cultured overnight at 37°C in MRS broth.

Samples and their analysis for the presence of phages

A total of 73 samples from the manufacture of an Argentinean probiotic dairy fermented product were investigated from December 2003 to August 2006. Samples were collected from several surfaces, environments and the product at different stages on the manufacture line.

Samples were analysed according to the International Dairy Federation (Neve and Teuber 1991). Firstly, pH values were measured and adjusted, if necessary, to 4·6 with lactic acid in order to precipitate caseins. Then, samples were centrifuged for 15 min at 10 000 ***g*** (8°C) and filtered with Millipore (Billerica, MA, USA) membranes (pore diameter, 0·45 μm). The filtrates with and without thermal treatment (90°C, 15 min) were used to investigate phage presence implementing the turbidity and spot tests, according to Svensson and Christiansson (1991).

Among all the samples that were phage-positive, the following three criteria were applied to select the samples from which phages would be isolated and purified: from samples taken from different infection events on the factory; from samples that were and were not heated during their analysis and from samples of diverse origin (product at different stages of the manufacturing process, plant air, refrigerator ice, swab of surfaces).

Phage enumeration, isolation and purification

For the double-layer plate titration method, 0·1 ml of decimal dilutions of filtrates were added to 0·1 ml of log-phase *Lact. paracasei* A culture, mixed with 1 ml of MRS soft agar (0·6% w/v), and poured onto the surface of MRS-Ca-Gly (10 mmol l^{-1} $CaCl_2$ and 100 mmol l^{-1} glycine) (Lillehaug 1997) agar (1·2% w/v) plates. After incubation at 34°C for 18 h in microaerobic conditions, the lysis plaques were enumerated (Svensson and Christiansson 1991).

To isolate and purify the phages, well-defined single plaques were picked up and placed into 5 ml of MRS broth. Tubes were kept at 4°C for 24 h and were then inoculated with 0·1 ml of an overnight culture of *Lact. paracasei* A. Incubation at 37°C was performed until total lysis of the culture occurred and the lysate was enumerated as described above. These steps were repeated consecutively for three times. The stocks of isolated phages were prepared as described by Neviani *et al.* (1992) in MRS-Ca (10 mmol l^{-1} $CaCl_2$) broth, and stored both at 4°C (MRS broth) and in the INLAIN Collection at −80°C (MRS broth added with 15% v/v of glycerol).

Enzyme restriction analysis of phage genomes

Phage DNAs were obtained and purified according to Binetti *et al.* (2005), but using 10% w/v of polyethylene glycol for viral particle concentrations. Restriction enzymes (*Bgl*II, *Eco*RI, *Eco*RV, *Hin*dIII; GE Healthcare, Buckinghamshire, UK) were used as recommended by the manufacturer. Restricted phage DNAs were

electrophoresed in 0.8% w/v agarose gels in TBE (Tris–Borate–EDTA) buffer and visualized by using standard protocols (Sambrook and Russell 2001). This assay was used to select a low number of phages by choosing those whose profiles were different.

Phages PL–1 and J–1 (host strains *Lact. paracasei* ATCC 27092 and *Lact. casei* ATCC 27139, respectively) were used as reference phages to compare their genomes with those of the phages isolated in this study.

Electron microscopy

Micrographs of phages were obtained by the procedure of Bolondi *et al.* (1995). Phage suspensions were concentrated by centrifugation (1 h, 70 000 ***g***, 5°C) and then stained with uranyl acetate (2% w/v, pH 4·5) or phosphotungstic acid (2% w/v). Electron micrographs were taken with a JEOL 100-C electron microscope operating at 80 kV. Phage morphologies and dimensions (capsid diameter, tail length and width) were recorded.

Host range

Strain cross sensitivity was investigated using the spot and turbidity tests, as described above. These assays were performed for the chosen phages and the strains previously mentioned.

Phage calcium dependency

The influence of calcium on cell lysis was investigated by the turbidity test, incubating infected *Lact. paracasei* A cultures at 37°C in MRS broth with and without $CaCl_2$ (10 mmol l^{-1}), throughout four subcultures.

The effect of calcium ions on phage adsorption was investigated by determining adsorption rates as described by Séchaud *et al.* (1989), modified as follows: exponentially growing (OD_{560}: 0·5) *Lact. paracasei* cultures in MRS broth were centrifuged and the cells suspended (3×10^8–5×10^8 CFU ml^{-1}) in MRS and MRS-Ca broths. Phages were added at a m.o.i. (multiplicity of infection) of 0·01 and the mixtures were incubated at 37°C. After 30 min, aliquots were removed and centrifuged (10 000 ***g***, 5 min) to sediment the phage-adsorbed cells. Then, the titres of nonadsorbed free phages in the supernatant were determined as indicated above, and the results were expressed as percentages of the initial phage counts.

Thermal-inactivation kinetics

A 90°C thermal treatment was applied on suspensions (10^6 PFU ml^{-1}) of the chosen phages. MRS broth, reconstituted skim milk (RSM) and a commercial enriched reconstituted medium (EM) (6% w/v) added with glucose (1% w/v) (pasteurized at 110 for 6 min), were used as suspension media (medium EM has similar features than the ones used for propagating probiotic cultures in this kind of products). At prefixed time intervals, viable phage particles were enumerated.

Results

Phage isolation

From January 2003 to August 2006, 73 samples were analysed: 64·4% (47) of them were taken from the product at different points through the manufacture line, 24·7% (18) were from vessels containing the starter culture, 9·6% (7) from the plant environment and one sample from the manufacture of a similar dairy product using the same strain (Fig. 1a). From each kind of sample, 51% (24), 50% (9) and 43% (3), respectively, were phage-positive (contained phages), while the sample from the related dairy product was phage-positive as well (Fig. 1b).

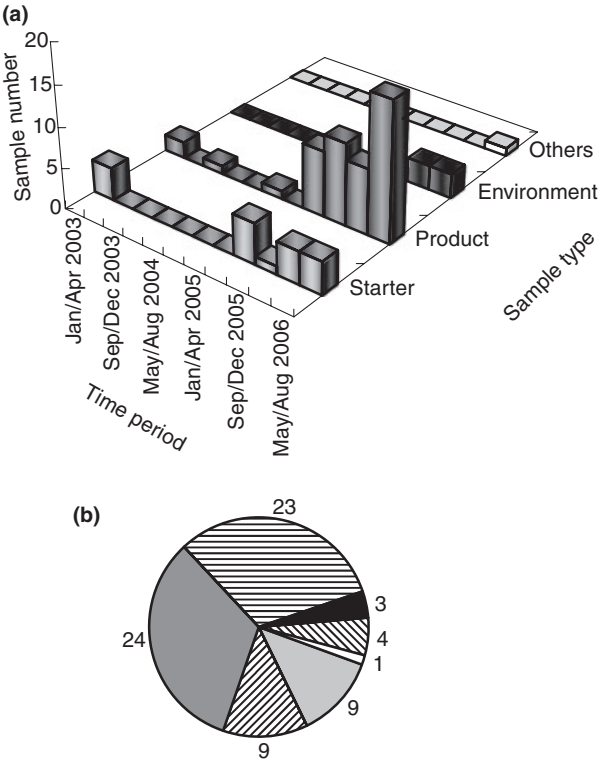

Figure 1 Frequency of phage analyses on samples of diverse origin from a probiotic product factory (a) and the proportion and number of positive and negative results for each kind of sample (b). (▒) starter +; (▨) starter –; (■) product +; (▤) product –; (■) environment +; (▧) environment – and (□) others +.

In view of the criteria previously set in Materials and Methods, from the 37 phage positive samples, a total of 22 isolates infective for *Lact. paracasei* A were selected. The first (MLC-A) and third (MLC-A3) isolates were found at very high concentrations (10^{10} PFU ml^{-1}) and the pH values of the samples were evidently higher (5·12 and 4·86, respectively) than those usually found in the product samples (3·80–4·10). For all the other isolates, titres were lower than 10^5 PFU ml^{-1}, and pH values of product samples practically unchanged in comparison with uncontaminated samples.

Besides, an unusual situation was found since infective phage particles (isolates MLC–A3R, fMLC–A4R, MLC–A7R, MLC–A11R and MLC–A12R) were capable to survive the thermal treatment (90°C–15 min) of the samples, generally performed to inactivate phages.

Enzyme restriction analysis of phage genomes

*Bgl*II gave the best discrimination among the isolates in comparison with *Eco*RI, *Eco*RV and *Hin*dIII (data not shown). Based on the restriction profiles obtained with *Bgl*II, the 22 isolated phages were classified in six groups represented by phages MLC–A, MLC–A2, MLC–A19 (each isolated from intermediate-product samples), MLC–A8, MLC–A17 (both from end-product samples) and MLC–A5 (from the starter culture) (Fig. 2). Besides, all their *Bgl*II profiles were different from the ones obtained for collection phages PL–1 and J–1, though phage MLC–A8 showed a level of similarity higher with J–1 than with all the rest. The genome sizes were estimated for all the restriction enzymes used and average values were calculated: MLC–A, 37 kb (Capra *et al.* 2006b); MLC–A2, 40 kb; MLC–A5, 40 kb; MLC–A8, 43 kb; MLC–A17, 45 kb and MLC–A19, 38 kb.

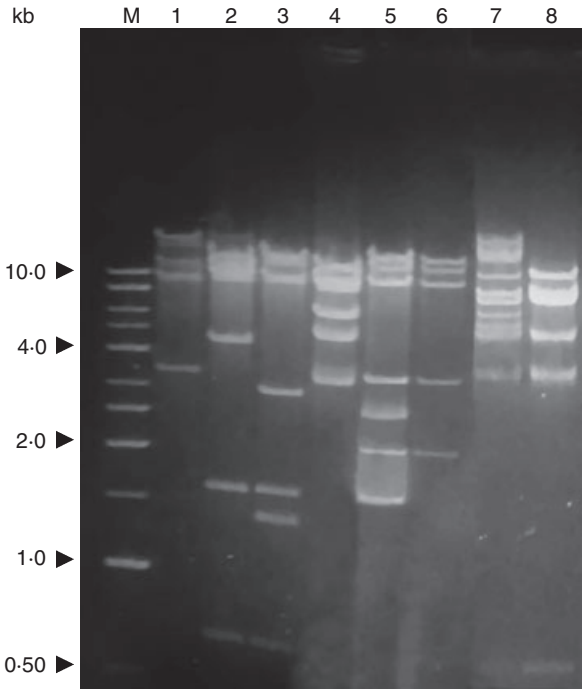

Figure 2 Agarose gel electroforesis of the *Bgl*II generated DNA fragments of *Lactobacillus paracasei* phages. Lane M, 1 kb DNA ladder (GE HealthCare) as molecular marker; lane 1, ϕMLC–A; lane 2, ϕMLC–A5; lane 3, ϕMLC–A2; lane 4, ϕMLC–A8; lane 5, ϕMLC–A17; lane 6, ϕMLC–A19; lane 7, ϕPL–1; lane 8, ϕJ–1.

Besides, two other phages, which had repeated profiles, were also chosen because of their heat resistance during sample analysis; phage MLC–A3R (isolated from an intermediate-product sample) belongs to MLC–A19 group and MLC–A7R (isolated from an end-product sample), to MLC–A group. These eight phages were further characterized in this work.

Phage morphology

All the phages are members of the *Siphoviridae* family and belong to the B1 morphotype (Ackermann 2001; Fig. 3). In general, they have similar dimensions, isometric heads (57–65 nm), and flexible, noncontractile long tails (156–175 nm long; 7–10 nm in diameter). In addition, they present a basal plate, collar and cross-striations along the tail. Their morphologic structures were represented by MLC–A micrograph (previously reported; Capra *et al.* 2006b). Notoriously, phage MLC–A8 showed a particularly long tail of about 300 nm.

Host range

The studied phages showed exactly the same host spectrum and were specific for *Lact. paracasei* and *Lact. casei* strains, being able to infect 43·8% (seven out of 16) and 15·4% (2 out of 13), respectively, of the total strains tested for each species. Collection strains ATCC 27092, ATCC 27139 and ATCC 393 were sensitive for the tested phages. All the *Lact. rhamnosus* strains used in this work were insensitive to the eight selected phages. Thus, this methodology was not useful to differentiate among the isolates.

Phage calcium dependency

MLC–A8 was the only phage able to lyse *Lact. paracasei* A on the first turbidity test subculture either with or without calcium in the broth. On the contrary, the other phages rapidly lysed the sensitive culture in the presence of the cation, but could not do it in its absence, even after four subcultures.

Phages MLC–A and MLC–A2 were chosen as phages which depend on calcium, and MLC–A8 as an

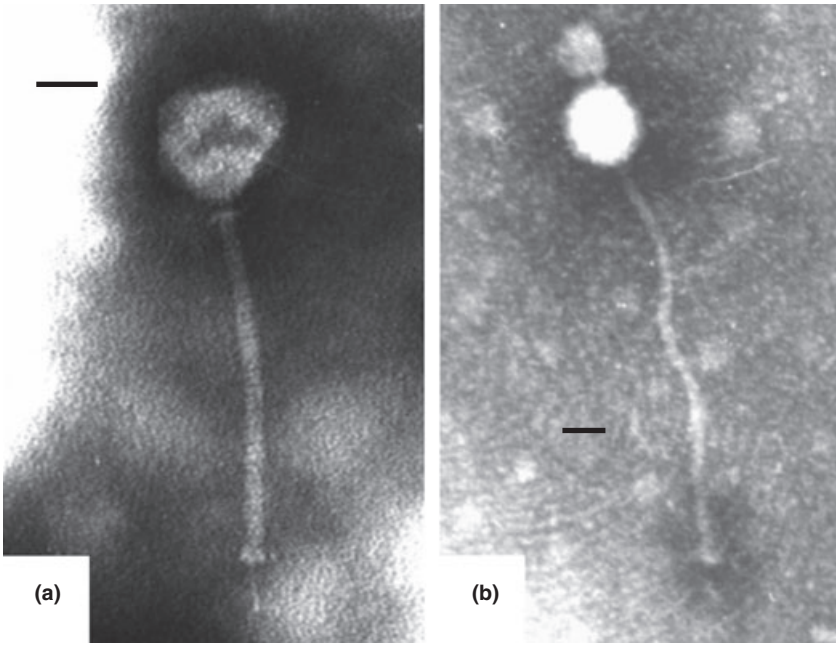

Figure 3 Electron micrograph of *Lactobacillus paracasei* phages MLC–A (a) and MLC–A8 (b) isolated from a probiotic fermented dairy product. Bars represent 25 nm.

independent one, to study the effect of this ion on adsorption. Firstly, MLC–A8 differed in its ability to adsorb on *Lact. paracasei* A in the presence of calcium (at 30 min); whereas 97·0 ± 0·4% of phages MLC–A and MLC–A2 particles adsorbed on the sensitive cells, only 65·8 ± 1·5% of MLC–A8 particles were attached in the same conditions. The second difference was found when the adsorption was performed in the absence of calcium. Phages MLC–A and MLC–A2 showed adsorption rates of 35·0 ± 2·7 and 44·8 ± 2·0%, respectively, while MLC–A8 adsorbed at a higher rate (55·1 ± 1·8%), evidencing once more a singular behaviour.

Thermal inactivation

Phage MLC–A was completely inactivated (at an initial concentration of 10^6 PFU ml^{-1}) after 2 min at 90°C in MRS broth and RSM, though it was slightly protected by EM medium in the same conditions, with a remaining phage count of $1{\cdot}5 \times 10^2$ PFU ml^{-1} (Fig. 4). The other tested phages showed counts from 10 to 10^3 PFU ml^{-1} at 15 min and a protective trend of EM and RSM media with respect to MRS broth. As well, the commercial medium and the milk protected two (MLC–A2 and MLC–A7R) and three (MLC–A2, MLC–A7R and MLC–A19) phages respectively, allowing them to get through the treatment even after 30 min.

Discussion

As previously expressed (Alvarez *et al.* 1999) the expanding use of valuable *Lactobacillus* strains as starters and probiotics will eventually lead to an increase in the frequency of bacteriophage infections in dairy plants. The results obtained in the present study confirm this statement. In our country, the evolution from the end of 2003, when the first Argentinean phage specific for a probiotic strain was isolated, until now, with more than 20 isolations made, highlight the progressive and continuous presence of phages in the industrial environment, especially from the middle of 2004. The substantial increment in the number and diversity of samples, as well as in the frequency of sampling for phage detection, strongly indicates the genuine and rising concern of the industry with respect to this issue. Phages seemed to be widely distributed within the factory; they were present in every place where a sample was collected. Astonishingly, phages MLC–A4R, –A5, –A15 and –A18 were isolated from starter cultures contained in sealed vessels.

Our results showed high similarity among most of the isolates in some phenotypic features such as morphology, host range, thermal resistance and response to calcium, suggesting that they are probably highly related. Although the phage characterization performed in this work showed some phenotypic differences among isolates, the thermal resistance exhibited by them was extraordinary in comparison with either other *Lact. casei/paracasei* phages previously investigated (Capra *et al.* 2004) or phages of lactic acid bacteria in general (Suárez *et al.* 2006). However, Atamer *et al.* (2008) recently suggested that thermoresistant phages are part of the natural phage populations in all types of dairies, being necessary to revise the methodology for bacteriophage detection in suspected industrial samples. This notable thermal resistance is

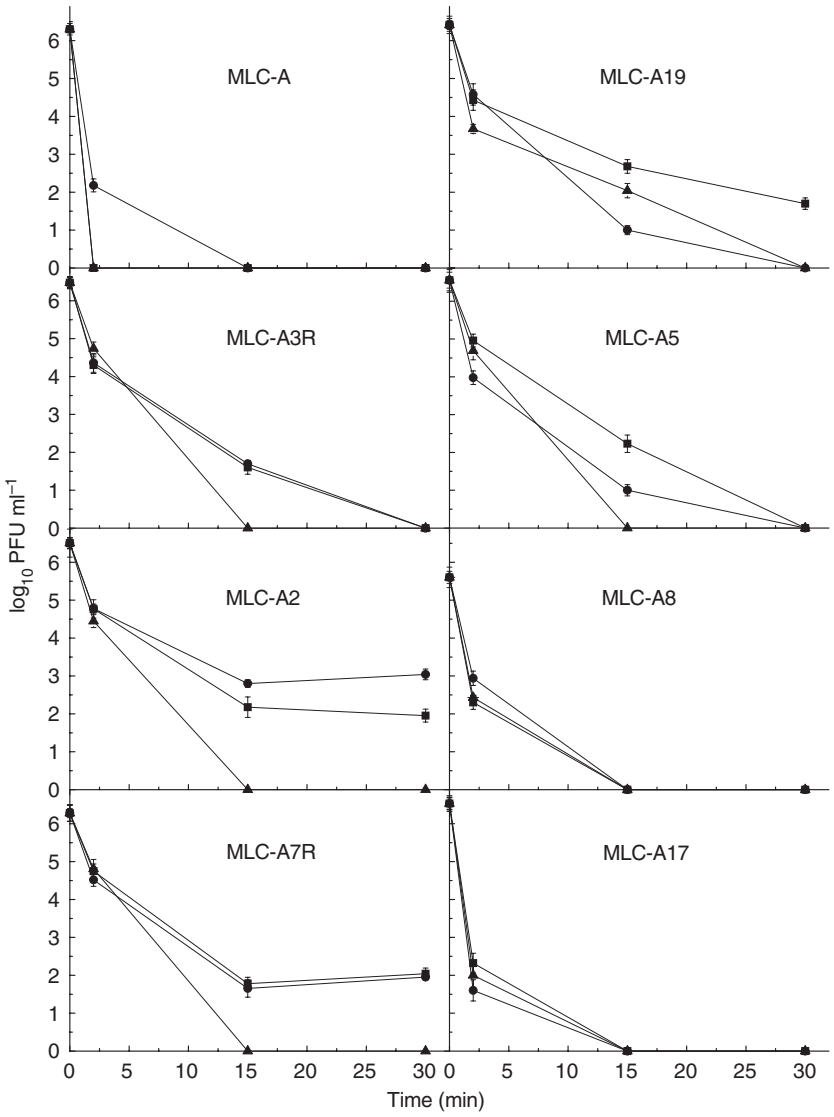

Figure 4 Thermal inactivation kinetics at 90°C of autochthonous phages, isolated from the elaboration of a probiotic fermented dairy product, suspended in RSM (■), EM medium (●) and MRS broth (▲). Values are the mean of three determinations.

particularly important due to the unfeasibility of implementing severe thermal treatments in the dairy industry, because of technological aspects. Among the eight tested phages, MLC-A, MLC–A8 and MLC–A17 showed the highest sensitivity to thermal treatments. Besides, other singularities of MLC–A8 refer to its relatively different restriction profile and the possession of a longer tail. A remarkable feature was its ability to lyse the culture both with and without calcium ions in the broth while all the others could not do it in its absence, even after four subcultures.

Regarding this last characteristic, this phage behaved in the same manner than the collection phages PL-1 and J–1 (Capra *et al.* 2006a), being its restriction profile similar, though not identical, to the one for J–1 phage. Considering all these differences, we might suppose that the relationship between MLC–A8 and the other seven phages is more distant than the latter among themselves. On the other hand, MLC–A8, the other seven tested phages, PL–1 and J–1 (Capra *et al.* 2006a) shared identical host range. This is a surprising characteristic considering results previously obtained by another author. Suárez *et al.* (2002) found 12 different patterns (with no more than four phages with identical host range) when testing 22 streptococcal phages, isolated from yogurt and cheese making processes, on 17 commercial strains. Studying the diversity of virulent phage population in one Argentinean cheese plant, 10 phages were isolated and their sensitivity was checked against seven strains of *Strep. thermophilus* (Quiberoni *et al.* 2006). The authors found seven different patterns, with five phages exhibiting unique profiles, while three and two phages were grouped with the same profiles. Regarding *Lact. delbrueckii* subsp. *bulgaricus*

phages, only three phages were isolated from Argentinean yogurt plants (from 1997 to 2000) and infected only their host strain (Quiberoni *et al.* 2004). Even for lactococcal phages, a different host range was found for 27 isolates tested against 28 strains (Moineau *et al.* 1996).

The dairy industry is dealing with the appearance of a new problem with particular characteristics, and whose resolution shall require complex and different strategies of control with respect to phage attacks against other lactic acid bacteria. Clever tactics to fight against phages of probiotic bacteria are restricted due to the notable resistance of many of these phages to severe thermal treatments, the inappropriateness of strain rotation for probiotic bacteria, and the complexity of the isolation of bacteriophage insensitive mutant strains from a practical point of view (Capra 2007). In consequence, the approach of treatments with disinfectants is becoming important. Future research is necessary to find effective strategies to cope with this new kind of infections. Perhaps, probiotic phage infections could represent a third kind of ecological situation in dairy industry, besides those of yogurt and cheese dairy factories.

This work is the first one evidencing that phage diversity in a probiotic product manufacture can be generated to a similar or even greater extent than during yogurt or cheese production. The conclusion is remarkable because only one strain is used to obtain the fermented probiotic product, the fermentation is performed in closed reactors and both raw materials and plant environment are practically sterile. Consequently, almost no phages or a low diversity of them are expected. Moreover, the noteworthy thermal resistance of some of the isolated phages contributes to aggravate the setting.

Hitherto, phages were known for playing a detrimental role in fermentative process. As most of the strategies implemented for lactic acid bacteria are useless for probiotic cultures, and due to the peculiarity of some phage-probiotic bacteria interactions, it is probably necessary to contemplate the problem from other point of view. Perhaps, it could help us to understand the rules that regulate such behaviours and will reveal the mean in which those phages and strains coexist in certain ecosystems, with the aim of bending the balance toward a favourable state to achieve a successful fermentation.

Acknowledgements

This research was supported by the Consejo Nacional de investigaciones Científicas y Técnicas de Argentina (CONICET; Project PIP 5321), the Agencia Nacional de Promoción Científica y Tecnológica de Argentina (ANPCyT; Proyecto PICT No. 09-08200) and the Universidad Nacional del Litoral (C.A.I.+D. no. 37-200).

References

Ackermann, H.W. (2001) Frequency of morphological phage descriptions in the year 2000. Brief Review. *Arch Virol* **146**, 843–857.

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. *J Appl Microbiol* **86**, 812–816.

Atamer, Z., Dietrich, J., Müller-Merbach, M., Neve, H., Heller, K.J. and Hinrichs, J. (2008) Screening for and characterization of *Lactococcus lactis* bacteriophages with high thermal resistance. *Int Dairy J* **19**, 228–235.

Binetti, A.G., del Río, B., Martín, M.C. and Álvarez, M.A. (2005) Detection and characterization of *Streptococcus thermophilus* bacteriophages based on the antireceptor gene sequence. *Appl Environ Microbiol* **71**, 6096–6103.

Bolondi, A., Gaggino, O. and Monesiglio, J. (1995) *Microscopía electrónica: técnicas generales*. Unidad de Microscopía Electrónica y Bioquímica Aplicada del Centro de Investigación en Ciencias Veterinarias. INTA–Castelar, Argentina.

Brüssow, H. and Desiere, F. (2001) Comparative phage genomics and the evolution of *Siphoviridae*: insight for dairy plants. *Mol Microbiol* **39**, 213–222.

Brüssow, H. and Kutter, E. (2005) Phage ecology. In *Bacteriophages: Biology and Applications* ed. Kutler, E. and Sulakvelidze, A. pp. 129–163. Boca Ratón: CRC Press.

Brüssow, H., Frémont, M., Bruttin, A., Sidoti, J., Constable, A. and Fryder, V. (1994) Detection and classification of *Streptococcus thermophilus* bacteriophages isolated from industrial milk fermentation. *Appl Environ Microb* **60**, 4537–4543.

Brüssow, H., Bruttin, A., Desiere, F., Lucchini, S. and Foley, S. (1998) Molecular ecology and evolution of *Streptococcus thermophilus* bacteriophages – a review. *Virus Genes* **16**, 95–109.

Bruttin, A., Desiere, F., D'Amico, N., Guérin, J-P., Sidoti, J., Huni, B., Luccini, S. and Brüssow, H. (1997) Molecular ecology of *Streptococcus thermophilus* bacteriophage infections in a cheese factory. *Appl Environ Microb* **63**, 3144–3150.

Capra, M.L. (2007). *Thesis: Bacteriofagos de Lactobacillus casei/paracasei*. Caracterización y estudio de la fagorresistencia. Doctor en Ciencias Biológicas. Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.

Capra, M.L., Quiberoni, A. and Reinheimer, J.A. (2004) Thermal and chemical resistance of *Lactobacillus casei* and *Lactobacillus paracasei* bacteriophages. *Lett Appl Microbiol* **38**, 499–504.

Capra, M.L., Quiberoni, A. and Reinheimer, J.A. (2006a) Phages of *Lactobacillus casei/paracasei*: Response to environmental factors and interaction with collection and commercial strains. *J Appl Microbiol* **100**, 334–342.

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

Deveau, H., Labrie, S.J., Chopin, M.-C. and Moineau, S. (2006) Biodiversity and classification of Lactococcal phages. *Appl Environ Microb* **72**, 4338–4346.

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

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.

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

Lillehaug, D. (1997) An improved plaque assay for poor plaque-producing temperate lactococcal bacteriophages. *J Appl Microbiol* **83**, 85–90.

Lo, T.C., Shih, T.C., Lin, C.F., Chen, H.W. and Lin, T.H. (2005) Complete genomic sequence of the temperate bacteriophage ϕAT3 isolated from *Lactobacillus casei* ATCC 393. *Virol* **339**, 42–55.

Moineau, S. and Lévesque, C. (2005) Control of bacteriophages in industrial fermentations. In *Bacteriophages: Biology and Applications* ed. Kutler, E. and Sulakvelidze, A. pp. 285–296. Boca Ratón: CRC Press.

Moineau, S., Borkaev, M., Holler, B.J., Walker, S.A., Kondo, J.K., Vedamuthu, E.R. and Vandenbergh, P.A. (1996) Isolation and characterization of Lactococcal bacteriophages from cultured buttermilk plants in the United States. *J Dairy Sci* **79**, 2104–2111.

Neve, H. and Teuber, M. (1991) Basic microbiology and molecular biology of bacteriophages of lactic acid bacteria in dairies. *Bulletin FIL-IDF* **263**, 3–15.

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

Quiberoni, A., Guglielmotti, D., Binetti, A. and Reinheimer, J. (2004) Characterization of three *Lactobacillus delbrueckii* subsp. *bulgaricus* phages and the physicochemical analysis of phage adsorption. *J Appl Microbiol* **96**, 340–351.

Quiberoni, A., Tremblay, D., Ackermann, H.-W., Moineau, S. and Reinheimer, J.A. (2006) Diversity of *Streptococcus thermophilus* phages in a large-production cheese factory in Argentina. *J Dairy Sci* **89**, 3791–3799.

Saarela, M., Mogensen, G., Fondén, R., Mättö, J. and Mattila–Sandholm, T. (2000) Probiotic bacteria: safety, functional and technological properties. *J Biotechnol* **84**, 197–251.

Sambrook, J. and Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edn. New York: Laboratory Press.

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

Séchaud, L., Callegari, M.L., Rousseau, M., Muller, M.C. and Accolas, J.P. (1989) Relationship between temperate bacteriophage 0241 and virulent bacteriophage 832-B1 of *Lactobacillus helveticus*. *Neth Milk Dairy J* **43**, 261–277.

Shimizu-Kadota, M. and Tsuchida, N. (1984) Physical mapping of the virion and the prophage DNAs of a temperate *Lactobacillus* phage ϕFSW. *J Gen Microbiol* **130**, 423–430.

Suárez, V.B., Quiberoni, A., Binetti, A.G. and Reinheimer, J.A. (2002) Thermophilic lactic acid bacteria phages isolated from Argentinian dairy industries. *J Food Prot* **65**, 1597–1604.

Suárez, V., Binetti, A., Quiberoni, A., Guglielmotti, D. and Capra, M.L. (2006) Infecciones fágicas. Tratamientos térmicos y químicos para la inactivación de fagos. In *Avances en microbiología, bioquímica y tecnología de quesos* ed. Reinheimer, J. and Zalazar, C. pp. 101–115. Santa Fe: Ediciones UNL.

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

Szczepańska, A.K., Hejnowicz, M.S., Kołakowski, P. and Bardowski, J. (2007) Biodiversity of *Lactococcus lactis* bacteriophages in Polish dairy environment. *Acta Biochim Pol* **54**, 151–158.

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

Yokokura, T. (1971) Phage receptor material in *Lactobacillus casei* cell wall. I. Effect of L-rhamnose on phage adsorption to the cell wall. *Jpn J Microbiol* **15**, 457–463.