Original Paper

Intervirology

Intervirology 2015;58:49–56
DOI: 10.1159/000369207

Received: March 20, 2014
Accepted after revision: October 18, 2014
Published online: January 15, 2015

Characterization of Two Temperate *Lactobacillus paracasei* Bacteriophages: Morphology, Kinetics and Adsorption

Diego J. Mercanti[a] Hans-Wolfgang Ackermann[b] Andrea Quiberoni[a]

[a]Instituto de Lactología Industrial, Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina; [b]Département de Microbiologie, Infectiologie et Immunologie, Faculté de Médecine, Université Laval, Québec, Qué, Canada

Key Words

Lactobacillus · Bacteriophages · Phage adsorption · Phage kinetics · Lactic acid bacteria

Abstract

Background/Aims: Adsorption and kinetic parameters, latent period, burst size and burst time, are characteristics of phage/host systems and can be affected by several environmental factors. As only few studies have focused on temperate dairy phages, we characterized these parameters on temperate *Lactobacillus paracasei* phages Φ i*Lp*84 and Φ i*Lp*1308, infective for probiotic strains. ***Methods:*** Phages were characterized by transmission electron microscopy and genomic DNA restriction. Adsorption under different environmental conditions, phage kinetics and efficiency of plating (EOP) were determined using the double-layer titration method. ***Results:*** Phages Φ i*Lp*84 and Φ i*Lp*1308 belong to the *Siphoviridae* family and have genome sizes of 38 and 34 kbp, respectively. Adsorption was affected by calcium concentration, pH, temperature and host viability, and reached a limit at very high multiplicity of infection. Latency, burst time and burst size were of 85 min, 131 min and 46 for Φ i*Lp*84, and 51 min, 92 min and 28 for Φ i*Lp*1308, respectively, at 37°C. A clear influence of temperature on phage kinetics was observed. Regarding EOP, Φ i*Lp*84 produced plaques on only 1 out of 8 strains tested. ***Conclusion:*** Noticeable differences in adsorption, kinetics and EOP were found for two morphologically identical temperate *L. paracasei* phages of similar origin.

Introduction

Adsorption is the first step in phage infection. It is a strain-specific event, involving host recognition of the bacterial surface (reversible binding) followed by irreversible fixation of another tail protein to a secondary receptor molecule [1]. Both adsorption velocity and efficiency are characteristics of every individual phage/host system, but can be considerably affected by environmental factors, as well as by the host's physiological state [2]. The environmental factors include pH, temperature and presence or absence of divalent ions, whereas the influence of the physiological state may correspond to changes in the levels of some cell surface molecules that are crucial for phage recognition [3]. In the infection process of a lytic phage, the time needed to complete one cycle (burst time) and the number of virions released by the infected cell (burst size) are characteristics of every phage/host system studied [1].

The adsorption was studied in many phages of lactic acid bacteria (LAB), including *Streptococcus thermophi-*

0300–5526/15/0581–0049$39.50/0

E-Mail karger@karger.com
www.karger.com/int

Diego J. Mercanti
Santiago del Estero 2829
3000 Santa Fe (Argentina)
E-Mail diegojav78@gmail.com

lus [4], *Lactococcus lactis* [5–9] and several species of *Lactobacillus* such as *L. casei* and *L. paracasei* [10–12], *L. fermentum* [13], *L. helveticus* [14], *L. delbrueckii* [15–18] and *L. plantarum* [19]. Most adsorption studies focused on lytic phages, while only a few concerned temperate phages of *L. plantarum*, *L. delbrueckii* and *L. helveticus* [15, 18, 20].

Although LAB phages actually occur as free virions in dairy factories, lysogenic strains represent a good source of new phages. Under favourable conditions, a prophage can be induced from the genome of a given strain and infect related bacteria, or even the same lysogenic strain that produced the phage [10]. In a dairy plant, this might lead to large economic losses [21, 22]. Lysogeny is very widespread in the genus *Lactobacillus* [23–25]. Presently, most probiotic strains used in the dairy industry belong to the *L. casei* group [25]. As any probiotic strain has unique traits, it is hard to replace it by another strain with the same characteristics. Consequently, phage infections have worse consequences for probiotics than for LAB used for acidification only, for which relatively easy industrial anti-phage strategies exist, such as strain rotation [10].

In a previous study, we confirmed the high incidence of lysogeny in the *L. casei* group [25]. In the present work, we phenotypically characterized two phages induced by mitomycin C and able to multiply on related *L. paracasei* strains. We particularly focused on the influence of diverse physicochemical factors on phage adsorption to indicator strains, as well as the efficiency of this process on related bacteria. The effect of lowering temperature on phage multiplication parameters such as burst size, burst time and latent time was also recorded. Our results illustrate the dynamics of temperate phage infection of potentially probiotic bacteria and thus help improving current strategies of phage control in the dairy industry.

Materials and Methods

Bacterial Strains, Phages and Culture Conditions

Bacteriophages Φ i*Lp*84 and Φ i*Lp*1308 were induced by mitomycin C (Sigma-Aldrich Chemical Co., St. Louis, Mo., USA) from the strains *L. paracasei* 84 (isolated from dairy products) and *L. paracasei* CNRZ1308 (CNRZ collection) [25]. Both phages were propagated on their respective indicator strains: Φ i*Lp*84 on *L. paracasei* INL3 (INLAIN collection strain) and Φ i*Lp*1308 on *L. paracasei* A14 (commercial strain). Phages were stocked at –80°C in De Man, Rogosa and Sharpe (MRS) broth (Biokar, Beauvais, France) with 15% (v/v) glycerol (Cicarelli, San Lorenzo, Argentina), but a single high-titred 100-ml suspension of each one, prepared as described by Neviani et al. [26], was stored at 4°C and used for all the assays. Phages were in all cases counted by the double-layer plate titration method, as described by Svensson and Christiansson [27], and using glycine for a better visualization of lysis plaques [28]. Incubation was carried out at 34°C for 18 h under microaerophilic conditions; phage titres (plaque forming units; pfu ml^{-1}) were determined by counting lysis plaques.

In addition to the two above-mentioned indicator strains, seven additional *L. paracasei* strains were used for some tests, namely A, Dn, Hn, Yk, A13 (commercial strains), ATCC 27092 and ATCC 27139 (ATCC collection strains). These seven strains were selected because they showed a diverse degree of sensitivity to bacteriophages Φ i*Lp*84 and Φ i*Lp*1308 in a previous work [25]. Strains were stocked at –80°C in MRS broth (Biokar) supplemented with 15% (v/v) glycerol (Cicarelli) and periodically cultured overnight at 37°C in MRS broth.

Transmission Electron Microscopy

Several micrographs of each phage were obtained using exactly the same method and equipment as described by Capra et al. [12].

Phage DNA Extraction and Restriction Analysis

Phage DNA extraction started from 50 ml of high-titred lysates in MRS with 10 mM $CaCl_2$ (MRS-Ca) broth. Lysates were centrifuged (5,000 *g*, 10 min, 4°C), filtered with Millipore (Sao Paulo, Brazil) membranes (pore diameter 0.45 μm), and phages were precipitated by addition of 0.5 M NaCl (Cicarelli) and 10% (w/v) polyethylene glycol 8000 (USB, Cleveland, Ohio, USA), followed by incubation at 4°C for 18 h. After a new centrifuging step (10,000 *g*, 1 h, 4°C), concentrated phages were resuspended in 1 ml SM buffer [29] and DNA was extracted as described by Binetti et al. [30]. Restriction analysis was carried out with *Bam*HI, *Bgl*II and *Eco*RV enzymes according to the manufacturer's recommendations (Genbiotech, Buenos Aires, Argentina), and restriction fragments were resolved by 0.8% (w/v) agarose gel electrophoresis in 1× TAE buffer, using a 1 kbp DNA ladder (GE Healthcare Life Sciences, Bucks, UK) as molecular weight marker and GelRed™ (Biotium, Inc., Hayward, Calif., USA) as nucleic acid binding dye.

Factors Influencing Adsorption

Influence of calcium on adsorption of Φ i*Lp*1308 and Φ i*Lp*84 was evaluated in both MRS and MRS-Ca as described by Capra et al. [12] and starting from a multiplicity of infection (MOI) between 5×10^{-3} and 1×10^{-2}. Sampling was carried out at 2, 5, 15, 30, 45 and 60 min of incubation.

The influence of pH and temperature were also assessed according to Capra et al. [12]. In the first case, MRS-Ca broth was adjusted to pH 4, 5, 6, 7, 8 and 9. The influence of temperature was determined in aliquots of infected cells incubated in MRS-Ca at 0, 10, 20, 30, 37 (control) or 45°C. In both experiments, samples were taken after 30 min (for Φ i*Lp*1308) or 45 min (for Φ i*Lp*84) of incubation, i.e. the minimum time required to reach a plateau in the number of phages adsorbed in MRS-Ca broth at pH 7 (see Results).

To evaluate the influence of cell viability, adsorption kinetics of phages Φ i*Lp*1308 and Φ i*Lp*84 at 37°C in MRS-Ca broth were determined both on cells in the mid-exponential growth phase (control) and on one aliquot of the same cells heated at 100°C for 2 min in a boiling water bath [19]. The thermal inactivation of the cells was verified by plate counts in MRS agar. The effect of chloramphenicol (Sigma, St. Louis, Mo., USA), a protein synthesis inhibitor, on phage adsorption was also recorded. Among four concen-

trations tested (10, 20, 50 and 100 μg/ml), 50 μg/ml was the minimum required to completely stop cell growth of both bacterial strains (as followed by OD_{560} measurement) after 75 min of incubation at 37°C in MRS-Ca broth (data not shown). As the bacteriostatic effect after chloramphenicol removal lasted for at least the duration of the assays, cells were centrifuged (10,000 *g* for 5 min) and suspended in MRS-Ca broth free of chloramphenicol to conduct adsorption studies.

Finally, the influence of MOI on adsorption of Φ i*Lp*1308 and Φ i*Lp*84 was determined in cultures infected at different initial MOI, at 37°C in MRS-Ca broth.

For all the factors studied, adsorption rates were always calculated as the ratio of initial phages, measured in the corresponding dilution of the original phage suspension, and non-adsorbed phages, measured in the supernatants after centrifugation [12]. Reduction in the number of phages due to adsorption was plotted against time (for the assessment of the influence of calcium and cell viability), pH, temperature or MOI.

One-Step Growth Curve

Both Φ i*Lp*84 on *L. paracasei* INL3 and Φ i*Lp*1308 on *L. paracasei* A14 were subjected to one-step growth assays, which were carried out as described by Briggiler et al. [19], but using a MOI of about 4×10^{-4}. An aliquot (1 ml) of the culture was centrifuged (10,000 *g*, 5 min, 4°C), suspended in 9 ml of MRS-Ca broth (first decimal dilution), and subsequent decimal dilutions were made and incubated at 30 or 37°C. At defined time points, 100 μl of each dilution were collected for phage enumeration [12].

Efficiency of Plating

The efficiency of plating (EOP), defined as the ratio between pfu ml^{-1} for a given phage on a sensitive strain and pfu ml^{-1} on its indicator strain, was investigated for all strains that were sensitive to phages Φ i*Lp*84 or Φ i*Lp*1308 in MRS-Ca broth.

Statistical Analysis

Experiments were carried out in triplicate and data analysed using the Statgraphics™ Plus software (v 3.0, Statistical Graphics Corp.). For adsorption studies, one-way ANOVA and Duncan's multiple range tests at $p < 0.05$ were applied. For the determination of latent period, burst time and burst size on the one-step growth curves, DMFit web edition was used (available online at: http://modelling.combase.cc/DMFit.aspx); data were fitted using the complete model of Baranyi and Roberts [31].

Results and Discussion

Phage Morphology and Restriction Analysis

Both Φ i*Lp*84 and Φ i*Lp*1308 have identical dimensions as measured on 10 phage particles each: icosahedral heads of 61 ± 3 nm in diameter and long non-contractile tails of 184 ± 4 nm × 8 ± 1 nm. Hence, they belong to the morphotype B1 of the *Siphoviridae* family (online suppl. fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000369207) [32]. They also show cross-striations with a periodicity of approximately 4 nm along the tail and a tiny basal plate (about 13 × 10 nm). As expected, *Bgl*II profiles of both phages matched those previously obtained from the supernatants of induction during their isolation (before propagation on sensitive strains) (data not shown) [25]. Genome sizes, roughly estimated from restriction fragments (online suppl. fig. S2) in 38 ± 2 kbp for Φ i*Lp*84 and 34 ± 4 kbp for Φ i*Lp*1308, were similar to those of most LAB phages.

Study of Factors Influencing Adsorption

Influence of Calcium

Phage Φ i*Lp*84 adsorbed slightly faster than Φ i*Lp*1308 during the first 2 min at 37°C in MRS-Ca broth. Differences in adsorption were more evident after 5 min, and large after 30 min (fig. 1a). The amount of Φ i*Lp*1308 virions adsorbed was approximately constant after 30 min, but Φ i*Lp*84 particles continued to adsorb for up to 45 min; these times were therefore selected to assess the effect of the rest of the parameters. Adsorption was verified (though at a lesser extent) in MRS broth with no calcium added. Adsorption rates reported for *L. paracasei* phages C_L1 and C_L2 were intermediate between those of Φ i*Lp*84 and Φ i*Lp*1308 in MRS-Ca broth but similar to Φ i*Lp*1308 in the absence of calcium [10]. Lysis was not detected without calcium (data not shown), suggesting that this ion is essential at some point after adsorption for both phages to complete the infective cycle. A similar influence of calcium on adsorption and lysis in MRS broth was reported for phages MLC-A [12], C_L1 and C_L2 [10], but, on the contrary, both processes showed to be independent of calcium for other *L. casei* and *L. paracasei* phages [11, 33].

Influence of pH

Throughout the entire pH range tested, adsorption remained higher for Φ i*Lp*84 than for Φ i*Lp*1308 (fig. 1b). Most reports indicate that adsorption of LAB phages is stable between pH 5 and 7 and decreases more or less markedly at more extreme pHs; in the present work, adsorption was stable between pH 5 and 8. At pH 9, adsorption is in general clearly affected, as is the case of *L. paracasei* phage MLC-A [12]. Phages in our study showed a moderate decrease of adsorption at pH 9 (slightly higher for Φ i*Lp*1308), but phages PL-1 and J-1 adsorbed efficiently even at pH 10 [11]. On the other hand, adsorption of phages Φ i*Lp*84 and Φ i*Lp*1308 was strongly inhibited at pH 4, more than for most LAB phages. It is important to note that both phages retain full viability at pH 4 and 9 (MRS-Ca broth, 37°C, 30 min) [34], so, the observed titre decreases were only due to a decrease in adsorption.

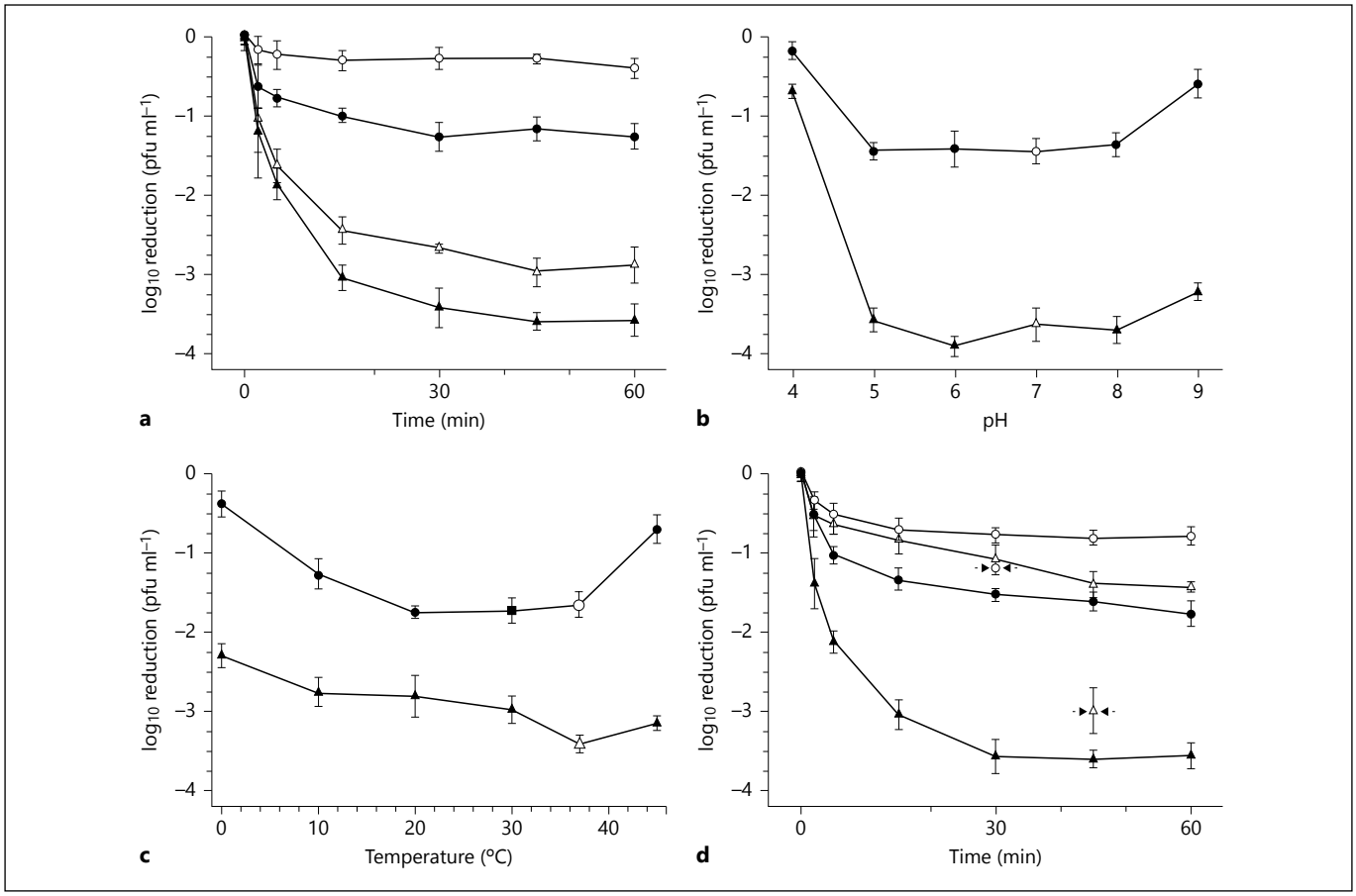

Fig. 1. Influence of different parameters on the adsorption kinetics of phages Φ i*Lp*84 on *L. paracasei* INL3 (▲, Δ) and Φ i*Lp*1308 on *L. paracasei* A14 (●, ○). **a** Influence of calcium at 37°C in MRS (empty symbols) or MRS-Ca (with $CaCl_2$ 10 mM; filled symbols) broth. **b** Influence of pH at 37°C; empty symbols indicate control samples at neutral pH. **c** Influence of temperature; empty symbols indicate control samples at 37°C. **d** Adsorption on control host cells (filled symbols), heat-killed cells (2 min at 100°C; empty symbols) or chloramphenicol-treated cells (50 μg/ml, 75 min at 37°C; symbols indicated by arrows) in MRS-Ca broth at 37°C. **b**, **c** Incubation times were 45 min for Φ i*Lp*84 and 30 min for Φ i*Lp*1308 in MRS-Ca. Experiments were always carried out in triplicate.

Influence of Temperature

For both Φ i*Lp*84 and Φ i*Lp*1308, the efficiency of adsorption was minimal at 0°C and increased with the temperature up to 37°C. Adsorption in other *L. casei* group phages previously studied was also highest at 37°C [11, 12]. For Φ i*Lp*84, adsorption was less dependent on changes of temperature, and even at 0°C it was much more efficient than for Φ i*Lp*1308 at 37°C. For Φ i*Lp*1308, adsorption was similar to that observed in *L. paracasei* phage MLC-A on *L. paracasei* A [12] and phage J-1 on *L. paracasei* ATCC 27139, although for the latter, adsorption fluctuated when it was tested on other strains and was already affected at 10°C [11] (fig. 1c). In contrast, Φ PL-1 was reported to adsorb equally well at 0 and 37°C, either to *L. paracasei* ATCC 27092 [33] or to *L. casei* ATCC 393 [11]. On the other hand, a temperature higher than the optimum (45°C) had little effect on the adsorption of Φ i*Lp*84, but it greatly affected that of Φ i*Lp*1308 (fig. 1c).

Influence of Cell Viability

Adsorption was reduced by thermal inactivation of host cells, considerably for Φ i*Lp*84 (at least 2 log) and moderately for Φ i*Lp*1308 (fig. 1d). Although adsorption rates were still higher for Φ i*Lp*84 than for Φ i*Lp*1308 on heat-killed cells, differences were not significant up to 15 min and slight thereafter. Phage Φ PL-1 (on the strain *L. paracasei* ATCC 27092) [35] adsorbed to heat-inactivated bacteria, indicating that phage receptors are thermostable and that adsorption does not depend on the physiological state of the cells. On the other hand, adsorp-

tion to non-viable cells was moderately or strongly inhibited in phages PL-1 (on *L. paracasei* A), J-1 [11] and MLC-A [12], as well as in phages Φ i*Lp*84 and Φ i*Lp*1308 in this study. These results suggest that adsorption strongly depends on the physiological state of the culture and/or that phage receptors are thermo-sensitive and suffered conformational changes during heating that made them no longer able to adsorb phage particles. On non-growing chloramphenicol-treated cells, adsorption levels of both phages were somewhat lower than on control cells, but still higher than those obtained on heat-killed cells (fig. 1d). These results indicate that the physiological state actually contributes to the efficiency of phage adsorption, especially for phage Φ i*Lp*1308. This factor was, however, less important than changes due to heat treatment of host cells: modifications in the conformation of phage receptors or the dropping in the energy levels (ATP content) of killed cells. To discriminate between the latter, further studies employing drugs which decrease without heating the intracellular concentration of ATP (NaF, NaN_3 or arsenate) would be necessary.

Influence of MOI

Adsorption of LAB phages is usually studied at low MOI (<0.02) to avoid variances due to the influence of the relative amounts of phage particles and host cells present. In the present work, adsorption remained more or less constant for both phages at MOI <0.1. At higher MOI values, the percentage of adsorption was sharply reduced and almost blocked (online suppl. fig. S3). Although the intrinsic error of the double-layer plate titration rapidly increases with MOI, an inhibition in the adsorption at MOI of 1 (Φ i*Lp*84) and 5 (Φ i*Lp*1308) was verified. Capra et al. [11] studied adsorption on *L. paracasei* phages at MOI of up to 1.5, where a decrease in the adsorption is reasonably expected, likely due either to a low number of available sites for phage attachment on the cell wall or to another sort of steric obstruction. Therefore, more attention should be paid to this parameter when evaluating adsorption. To our knowledge, this has not been reported in phages of *Lactobacillus*. In addition, it would be interesting to carry out complementary studies by transmission electron microscopy.

One-Step Growth

Figure 2 shows the one-step growth curves and growth parameters estimated for Φ i*Lp*84 and Φ i*Lp*1308 at 37 and 30°C. At both temperatures, the complete infective cycle of Φ i*Lp*1308 was shorter (lower latent and burst time) and the burst size of Φ i*Lp*84 was higher. A great variation in latent time (5–140 min) and burst time (120–240 min) has been reported among phages of *L. casei* and *L. paracasei* [10–12, 36]. At 37°C, Φ i*Lp*84 lysed the bacterial host slowly, similar to temperate phage Φ 393-A2 [36]. It is worth pointing out that growth parameters were in most studies visually deduced from drawn curves, but we have observed that it underestimates latent and burst times in relation to parameters calculated with DMFit (unpublished results), so the latter was used in our work. The influence of external variables on LAB phages multiplication has received little attention, even if changes in temperature and pH are key stress factors in dairy fermentation. Therefore, we decided to carry out one-step growth curves at 30°C. This sub-optimal temperature had the same effect on both phages, but it was more evident for Φ i*Lp*84 than for Φ i*Lp*1308: infection cycles were slower (burst time 73 and 25% higher, respectively); however, burst sizes were higher (57 and 27%, respectively). Also Müller-Merbach et al. [8] found, for lactococcal phage P008, longer infection cycles at a low temperature but, in contrast, these authors observed larger burst sizes at a higher temperature. Wang et al. [37] observed that the better the physiological condition of host cells, the shorter the latency period; in our study, it corresponds to results obtained at optimal growth temperature. Abedon [38] stated that for two identical phages only differing in lysis time, the one with the longer latent period will have a larger burst size. He postulated eclipse periods of the same length and constant rates of progeny production, so a longer progeny-producing period would produce more phage particles at the time of the burst, in accordance with the results presented here. Future investigations should also consider the influence of factors such as pH and cell density on phage population growth.

Efficiency of Plating

Several *L. casei* and *L. paracasei* phages previously characterized in our laboratory and different at the molecular level showed highly conserved host ranges [10–12, 22]. This makes this test less powerful in discriminating between phages of other LAB species. Table 1 shows EOP values for both phages on nine strains shown to be sensitive to one or another phage in MRS-Ca broth [25]. EOP values for Φ i*Lp*1308 were of 0.2 for *L. paracasei* INL3 and approximately 1 for the rest of the strains. On the other hand, Φ i*Lp*84 did not produce plaques on some strains sensitive to this phage in liquid media [25]. In that case, however, lysis was sometimes partial or achieved after two subcultures, and uneven results were obtained after repeating the test (results not shown).

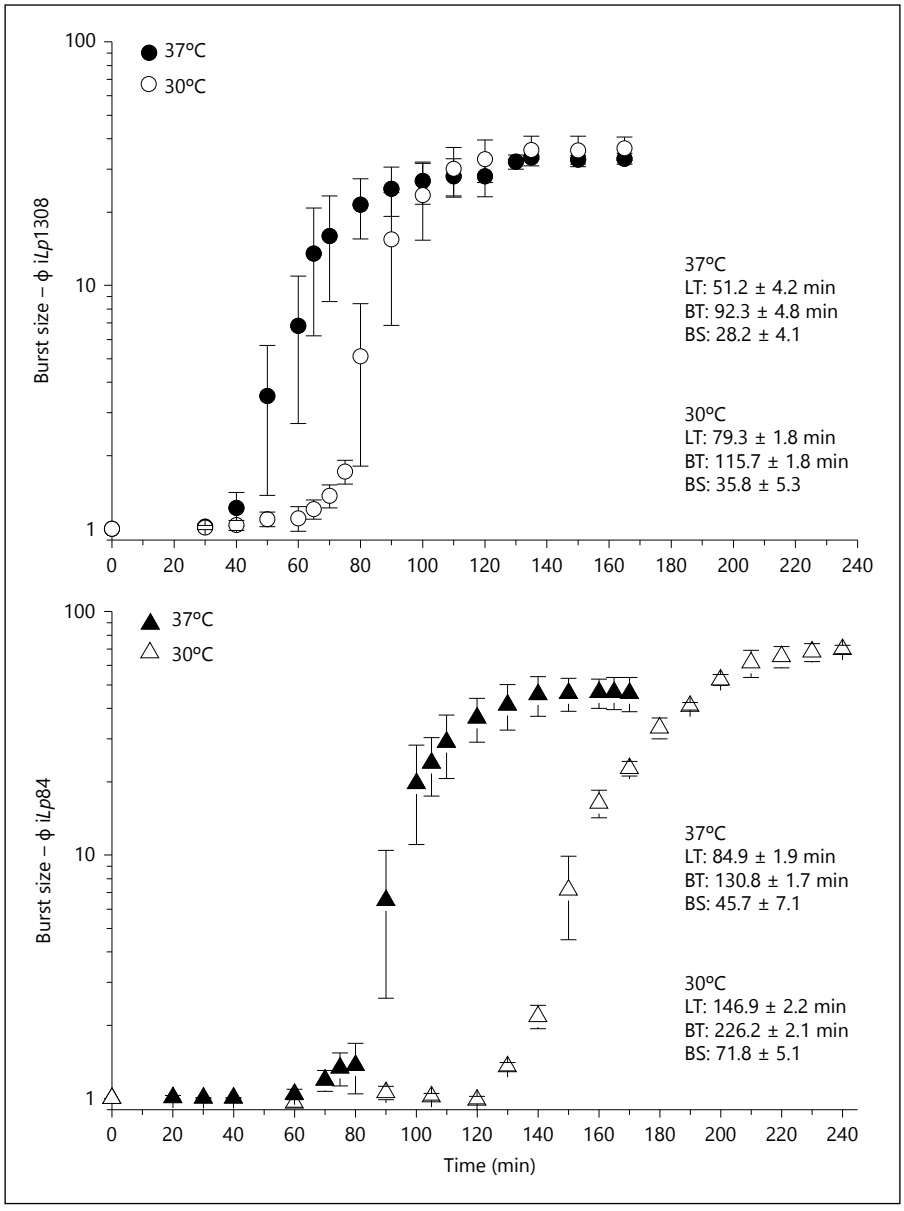

Fig. 2. One-step growth curves in MRS-Ca broth for phages Φ i*Lp*84 on *L. paracasei* INL3 and Φ i*Lp*1308 on *L. paracasei* A14. Experiments were carried out in triplicate. The three parameters were calculated with DMFit web edition using the complete model of Baranyi and Roberts [31] for data fitting. LT = Latent period; BT = burst time; BS = burst size.

Table 1. EOP of phages Φ i*Lp*84 and Φ i*Lp*1308, defined as the ratio between phage counts on different *L. paracasei* strains and phage counts on the respective indicator strain

Strain	Phage	
	Φ i*Lp*84	Φ i*Lp*1308
L. paracasei A	n.d.[a]	1.0
L. paracasei Dn	n.d.[a]	0.9
L. paracasei Hn	n.d.[a]	0.8
L. paracasei Yk	n.d.[a]	1.0
L. paracasei A13	n.d.[a]	1.0
L. paracasei A14	n.d.[a]	[b]
L. paracasei ATCC 27092	0.6	1.7
L. paracasei ATCC 27139	n.d.[a]	1.1
L. paracasei INL3	[b]	0.2

n.d. = Not detected. [a] EOP in these cases were $<1.0 \times 10^{-8}$ considering both the detection limit (10 pfu ml^{-1}) and the titre of Φ i*Lp*84 on *L. paracasei* INL3 (9.9×10^{8} pfu ml^{-1}). [b] Indicator strains for each phage (EOP = 1).

Conclusion

Clear differences in adsorption and multiplication kinetics were found for morphologically identical temperate *L. paracasei* phages Φ i*Lp*1308 and Φ i*Lp*84. While both phages were induced with mitomycin C from *L. paracasei* strains, they showed different EOP. Particularly, the host range of Φ i*Lp*84 was narrower than that of most *L. casei* group phages studied until now. Taking this into consideration, and in order to attain a complete phage characterization, it may be interesting to analyse the genomic sequence and organization of both phages while paying special attention to their temperate nature.

Acknowledgements

This work was supported by the the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, Project PICT 2010-0138; Argentina), the Universidad Nacional del Litoral (UNL) (Project CAI+D 2009 No. 57-275; Santa Fe, Argentina) and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Project PIP No. 112-200801-01206; Argentina).

Disclosure Statement

The authors declare that they have no conflicts of interest.

References

1 Guglielmotti DM, Mercanti DJ, Briggiler Marcó M: Infective cycle of dairy bacteriophages; in Quiberoni A, Reinheimer JA (eds): Bacteriophages in Dairy Processing. Hauppauge, Nova Science Publishers, 2012, pp 99–122.

2 Guttman B, Raya R, Kutter E: Basic phage biology; in Kutter E, Sulakvelidze A (eds): Bacteriophages: Biology and Applications. Boca Raton, CRC Press, 2005, pp 29–66.

3 Kutter E, Goldman E: Introduction to bacteriophages; in Goldman E, Green HL (eds): Practical Handbook of Microbiology. Boca Raton, CRC Press, 2008, pp 685–707.

4 Binetti AG, Quiberoni A, Reinheimer J: Phage adsorption to *Streptococcus thermophilus*: influence of environmental factors and characterization of cell receptors. Food Res Int 2002; 35:73–83.

5 Sijtsma L, Sterkenburg A, Wouters JT: Properties of the cell walls of *Lactococcus lactis* subsp. *cremoris* sk110 and sk112 and their relation to bacteriophage resistance. Appl Environ Microbiol 1988;54:2808–2811.

6 Valyasevi R, Sandine WE, Geller BL: The bacteriophage kh receptor of *Lactococcus lactis* subsp. *cremoris* kh is the rhamnose of the extracellular wall polysaccharide. Appl Environ Microbiol 1990;56:1882–1889.

7 Monteville MR, Ardestani B, Geller BL: Lactococcal bacteriophages require a host cell wall carbohydrate and a plasma membrane protein for adsorption and ejection of DNA. Appl Environ Microbiol 1994;60:3204–3211.

8 Müller-Merbach M, Kohler K, Hinrichs J: Environmental factors for phage-induced fermentation problems: replication and adsorption of the *Lactococcus lactis* phage P008 as influenced by temperature and pH. Food Microbiol 2007;24:695–702.

9 Suarez V, Moineau S, Reinheimer J, Quiberoni A: Argentinean *Lactococcus lactis* bacteriophages: genetic characterization and adsorption studies. J Appl Microbiol 2008;104: 371–379.

10 Capra ML, Mercanti DJ, Reinheimer JA, Quiberoni AL: Characterisation of three temperate phages released from the same *Lactobacillus paracasei* commercial strain. Int J Dairy Technol 2010;63:396–405.

11 Capra ML, Quiberoni A, Reinheimer J: Phages of *Lactobacillus casei*/*paracasei*: response to environmental factors and interaction with collection and commercial strains. J Appl Microbiol 2006;100:334–342.

12 Capra ML, Quiberoni A, Ackermann HW, Moineau S, Reinheimer JA: Characterization of a new virulent phage (MLC-A) of *Lactobacillus paracasei*. J Dairy Sci 2006;89:2414–2423.

13 Foschino R, Perrone F, Galli A: Characterization of two virulent *Lactobacillus fermentum* bacteriophages isolated from sourdough. J Appl Bacteriol 1995;79:677–683.

14 Quiberoni A, Reinheimer J: Physicochemical characterization of phage adsorption to *Lactobacillus helveticus* ATCC 15807 cells. J Appl Microbiol 1998;85:762–768.

15 Caso JL, de Los Reyes Gavilan C, Herrero M, Montilla A, Rodriguez A, Suarez JE: Isolation and characterization of temperate and virulent bacteriophages of *Lactobacillus plantarum*. J Dairy Sci 1995;78:741–750.

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

17 Wang S, Kong J, Gao C, Guo T, Liu X: Isolation and characterization of a novel virulent phage (phiLdb) of *Lactobacillus delbrueckii*. Int J Food Microbiol 2010;137:22–27.

18 Trucco V, Reinheimer J, Quiberoni A, Suarez VB: Adsorption of temperate phages of *Lactobacillus delbrueckii* strains and phage resistance linked to their cell diversity. J Appl Microbiol 2011;10:935–942.

19 Briggiler Marcó M, Reinheimer JA, Quiberoni A: Phage adsorption to *Lactobacillus plantarum*: influence of physiological and environmental factors. Int J Food Microbiol 2010;138:270–275.

20 Séchaud L, Callegari ML, Rousseau M, Muller MC, Accolas JP: Relationship between temperate bacteriophage 0241 and virulent bacteriophage 832-b1 of *Lactobacillus helveticus*. Neth Milk Dairy J 1989;43:261–277.

21 Bruttin A, Desiere F, d'Amico N, Guerin JP, Sidoti J, Huni B, Lucchini S, Brussow H: Molecular ecology of *Streptococcus thermophilus* bacteriophage infections in a cheese factory. Appl Environ Microbiol 1997;63:3144–3150.

22 Capra ML, Binetti AG, Mercanti DJ, Quiberoni A, Reinheimer JA: Diversity among *Lactobacillus paracasei* phages isolated from a probiotic dairy product plant. J Appl Microbiol 2009;107:1350–1357.

23 Ventura M, Canchaya C, Bernini V, Altermann E, Barrangou R, McGrath S, Claesson MJ, Li Y, Leahy S, Walker CD, Zink R, Neviani E, Steele J, Broadbent J, Klaenhammer TR, Fitzgerald GF, O'Toole PW, van Sinderen D: Comparative genomics and transcriptional analysis of prophages identified in the genomes of *Lactobacillus gasseri*, *Lactobacillus salivarius*, and *Lactobacillus casei*. Appl Environ Microbiol 2006;72:3130–3146.

24 Suarez V, Zago M, Quiberoni A, Carminati D, Giraffa G, Reinheimer J: Lysogeny in *Lactobacillus delbrueckii* strains and characterization of two new temperate prolate-headed bacteriophages. J Appl Microbiol 2008;105:1402–1411.

25 Mercanti DJ, Carminati D, Reinheimer JA, Quiberoni A: Widely distributed lysogeny in probiotic lactobacilli represents a potentially high risk for the fermentative dairy industry. Int J Food Microbiol 2011;144:503–510.

26 Neviani E, Carminati D, Giraffa G: Selection of some bacteriophage and lysozyme-resistant variants of *Lactobacillus helveticus* CNRZ 892. J Dairy Sci 1992;75:905–913.

27 Svensson U, Christiansson A: Methods for phage monitoring. FIL-IDF Bull 1991;263: 29–39.
28 Lillehaug D: An improved plaque assay for poor plaque-producing temperate lactococcal bacteriophages. J Appl Microbiol 1997;83:85–90.
29 Sambrook JJ, Russell DDW: Molecular Cloning: A Laboratory Manual. Cold Spring, Harbor Laboratory Press, vol 2, 2001.
30 Binetti A, Del Rio B, Martin M, Alvarez M: Detection and characterization of *Streptococcus thermophilus* bacteriophages by use of the antireceptor gene sequence. Appl Environ Microbiol 2005;71:6096–6103.
31 Baranyi J, Roberts TA: A dynamic approach to predicting bacterial growth in food. Int J Food Microbiol 1994;23:277–294.
32 Ackermann HW: Frequency of morphological phage descriptions in the year 2000. Brief review. Arch Virol 2001;146:843–857.
33 Watanabe K, Takesue S: The requirement for calcium in infection with *Lactobacillus* phage. J Gen Virol 1972;17:19–30.
34 Mercanti DJ, Guglielmotti DM, Patrignani F, Reinheimer JA, Quiberoni A: Resistance of two temperate *Lactobacillus paracasei* bacteriophages to high pressure homogenization, thermal treatments and chemical biocides of industrial application. Food Microbiol 2012; 29:99–104.
35 Watanabe K, Shirabe M, Fukuzaki T, Kakita Y, Nakashima Y, Miake F: Electron microscope studies on the host cell energy requirement for injection of PL-1 phage DNA into *Lactobacillus casei*. Curr Microbiol 1993;26: 293–298.
36 Herrero M, de los Reyes-Gavilán CG, Caso JL, Suárez JE: Characterization of ø393-A2, a bacteriophage that infects *Lactobacillus casei*. Microbiology 1994;140:2585–2590.
37 Wang I-N, Dykhuizen D, Slobodkin L: The evolution of phage lysis timing. Evol Ecol 1996;10:545–558.
38 Abedon S: Selection for bacteriophage latent period length by bacterial density: a theoretical examination. Microb Ecol 1989;18:79–88.