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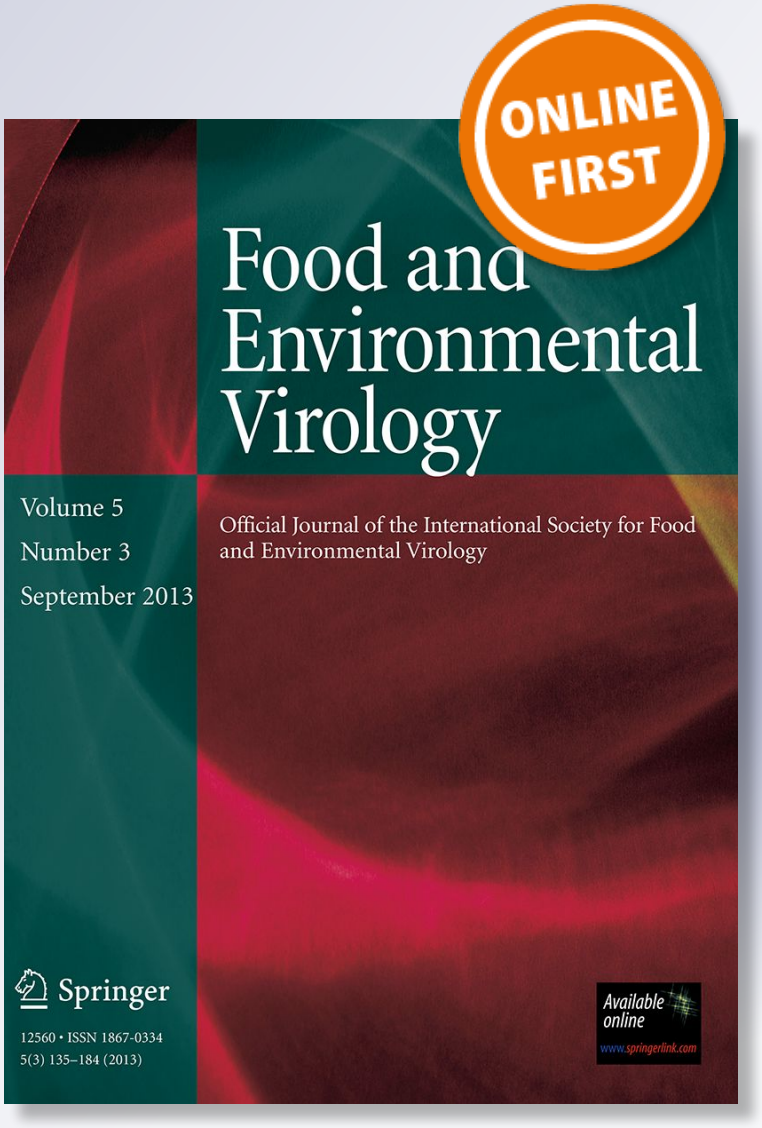

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ORIGINAL PAPER

Changes in Environmental Conditions Modify Infection Kinetics of Dairy Phages

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Abstract Latent period, burst time, and burst size, kinetic parameters of phage infection characteristic of a given phage/host system, have been measured for a wide variety of lactic acid bacteria. However, most studies to date were conducted in optimal growth conditions of host bacteria and did not consider variations due to changes in external factors. In this work, we determined the effect of temperature, pH, and starvation on kinetic parameters of phages infecting *Lactobacillus paracasei*, *Lactobacillus plantarum*, and *Leuconostoc mesenteroides*. For kinetics assessment, one-step growth curves were carried out in MRS broth at optimal conditions (control), lower temperature, pH 6.0 and 5.0 (MRS_6 and MRS_5, respectively), or in medium lacking carbon (MRS_N) or nitrogen (MRS_C) sources. Phage infection was progressively impaired as environmental conditions were modified from optimal. At lower temperature or pH, infection was delayed, as perceived by longer latent and burst times. Burst size, however, was lower, equal or higher than for controls, but this effect was highly dependent on the particular phage–host system studied. Phage infection was strongly inhibited in MRS_C, but only mildly impaired in MRS_N. Nevertheless, growth of all the bacterial strains tested was severely compromised by starvation, without significant differences between MRS_C and MRS_N, indicating that nitrogen compounds are specifically required for a successful phage infection, beyond their influence on bacterial growth.

Keywords Lactic acid bacteria · Phage infection · Burst size · Temperature · pH · Starvation

Introduction

Phage infections on lactic acid bacteria (LAB) are common and can spoil industrial fermentations, thus representing a continuous threat on dairy plants (Capra et al. 2016). The lytic cycle of bacteriophages involves a series of coordinated steps, namely adsorption of the virus to the host cell, injection of viral DNA, synthesis of viral macromolecules, assembling of new phage particles, and their release to the extracellular medium, which for most phages is produced after cell lysis (Guglielmotti et al. 2012). Adsorption, the first step of phage infection, has kinetic parameters characteristic of every phage/host system. The speed and efficiency of this process, as well as the impact of several external factors, have been studied in most LAB species of dairy interest (Binetti et al. 2002; Suarez et al. 2008; Mercanti et al. 2015; Quiberoni and Reinheimer 1998; Trucco et al. 2011; Guglielmotti et al. 2012; Briggiler Marcó et al. 2010; Pujato et al. 2015).

Alternatively, other phage kinetic parameters are also distinctive of individual phage/host systems. Latent period, which extends from phage-bacteria encounter to extracellular release of the first synthesized virions, rise period, during which the entire phage progeny is released, and burst size (the number of virions released per infected cell) have been the most studied parameters of viral infection (Guglielmotti et al. 2012). Burst time is defined as the sum of latent and rise periods. Phage growth kinetics were established for many LAB systems, including phage–host systems of *Lactobacillus casei* group (Mercanti et al. 2015; Capra et al. 2006a, b, 2010; Zhang et al. 2015),

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Lactobacillus plantarum (De Antoni et al. 2010; Briggiler Marcó et al. 2010; Lu et al. 2003; Caso et al. 1995; Nes et al. 1988), *Leuconostoc mesenteroides* subsp. *mesenteroides* (Pujato et al. 2015; Sozzi et al. 1978), and *Leuconostoc oenos* (Arendt et al. 1991), among other LAB species.

Wide variations of phage kinetics were generally reported, even for two or more phages infecting the same bacterial strain. In most cases, assays were conducted in the laboratory in optimal cell growth conditions (temperature, pH and availability of nutrients), which are not those typically found elsewhere, for example, during manufacturing processes in dairy plants (Golec et al. 2014). It was demonstrated that *Escherichia coli* bacteriophage T4 is able to propagate even if conditions for host cells growth are harsh (Golec et al. 2014, 2011), but available analogous data for LAB phages are scarce. Only recently, it was demonstrated that temperature exerts a noticeable effect for two phages infecting *Lactobacillus paracasei* strains (Mercanti et al. 2015) and that *L. plantarum* phage B1 showed reduced propagation when nitrogen sources were removed from the culture (Briggiler Marco et al. 2015). Still, the effect of temperature or starvation on kinetics of phages infecting other LAB species, as well as the impact of low pH, was not reported. Consequently, these studies are interesting, considering that a successful infection generally depends on the presence of host cells in optimal conditions.

The aim of the present work was to assess the impact of changes in temperature, pH, and lack of carbon or nitrogen sources in the medium, on the kinetics parameters latent period, burst time and burst size, for phage/host systems of *L. paracasei*, *L. plantarum*, and *Ln. mesenteroides*. These LAB species were selected considering their significance for the dairy industry. Some *L. paracasei* strains are added as starter or adjunct probiotic cultures for the production of functional dairy products (Saarela et al. 2000). *L. plantarum* has been also marketed as a health-promoting microorganism, which is able to grow in a wide variety of environments, including dairy products, where it can also contribute to flavor development (Briggiler Marcó et al. 2010). Finally, *Ln. mesenteroides* is industrially interesting as the main responsible of the characteristic flavor of butter, sour cream, and some fresh cheeses (Server-Busson et al. 1999). Besides, *Leuconostoc* are heterofermentative bacteria that produce CO_2, which allows curd openness and further colonization by *Penicillium roqueforti* in blue-veined cheeses (Hemme 2012).

Materials and Methods

Bacterial Strains and Bacteriophages

Four phage–host systems were selected for this study: (1) i*Lp*84—*Lactobacillus paracasei* INL3 (Mercanti et al. 2011); (2) i*Lp*1308—*L. paracasei* A14 (Mercanti et al. 2011); (3) ATCC 8014-B1 (referred to as B1 from now on)—*Lactobacillus plantarum* ATCC 8014 (Briggiler Marco et al. 2012); and (4) LDG—*Leuconostoc mesenteroides* R707 (data not published). Phages i*Lp*84 and i*Lp*1308 were obtained after mitomycin C (Sigma-Aldrich., St. Louis, Mo., USA) induction of *L. paracasei* strains 84 and CNRZ 1308, and propagated on *L. paracasei* strains INL3 and A14, respectively (Mercanti et al. 2011).

Culture Conditions

Bacterial strains were grown at their optimal temperature (37 °C for *Lactobacillus*, 30 °C for *Leuconostoc*) in de Man, Rogosa & Sharpe (MRS) broth (Biokar, Beauvais, France), and stored at −80 °C in MRS broth supplemented with 15% (v/v) glycerol at the INLAIN Collection (Santa Fe, Argentina). Phage amplifications were carried out by infecting growing cultures of host bacteria (optical density at 600 nm [DO_{560}] of 0.2) with phage (10^4 PFU/mL), followed by incubation at the optimal temperature in MRS broth supplemented with 10 mM $CaCl_2$ (MRS-Ca). Lysed cultures were then filtered using a 0.45-µm-pore-size filter (Sartorius), to separate phages from bacterial debris; phages in the suspensions were stored at 4 °C (short-term use) or supplemented with 15% (v/v) glycerol and preserved at −80 °C at the INLAIN Collection. Phage stocks were enumerated (PFU/mL) using the double-layer titration method (Svensson and Christiansson 1991), in MRS agar supplemented with 10 mM $CaCl_2$ and 100 mM glycine (MRS-Ca-Gly), the latter added for a better visualization of plaques (Lillehaug 1997).

To standardize, the determination of either bacterial or phage kinetics was conducted using MRS broth prepared in the laboratory, considering the final concentration (w/v) of their components in the commercial broth: glucose (2%), peptone (1%), meat extract (0.8%), sodium acetate (0.5%), yeast extract (0.4%), dipotassium hydrogen phosphate (0.2%), ammonium citrate (0.2%), polysorbate 80 (0.1%), magnesium sulfate (0.02%), and manganese sulfate (0.005%). Culture media lacking carbon (MRS_N) or nitrogen (MRS_C) sources were prepared as described but omitting the addition of either glucose (MRS_N) or peptone, meat extract, and yeast extract (MRS_C). MRS_N is not a media completely devoid of carbon sources, as the mentioned nitrogen sources also contain some carbohydrates. However, this contribution is negligible compared to the concentration of glucose in standard MRS, so the effect of replacing MRS by MRS_N on bacterial growth and phage infection can be primarily attributed to the lack of carbon sources. In acid MRS broth, pH was adjusted with acetic acid prior to sterilization, until reaching pH values of 6.0 (MRS_6) and 5.0 (MRS_5).

Bacterial Growth Kinetics

Strain growth was monitored in the same culture conditions tested for infection kinetics assessment. With this aim, overnight (18 h) cultures of each strain in MRS broth were diluted (2% v/v) in fresh MRS broth, and incubated at the optimal growth temperature of each strain until DO_{560} reached 0.5. Then, cells were harvested by centrifugation (10,000×*g*, 2 min), washed and five-fold diluted in MRS, MRS_C, MRS_N, MRS_6, or MRS_5 broth, each of them supplemented with 10 mM $CaCl_2$. Samples of 200 µL were pipetted in triplicate into wells of sterile covered microplates, and cell growth was monitored for 18 h at 37 °C (*Lactobacillus*) or 30 °C (*Leuconostoc*) through measurement of DO_{560} on a Multiskan™ FC Microplate Photometer (Thermo Scientific). Acquired data were processed with the Thermo Scientific™ SkanIt™ Software.

One-Step Growth Assay

Phage kinetics assessment was carried out by synchronic infection of growing bacteria with the corresponding phage (phage–host systems studied were described above in this section). One-step growth experiments were conducted following the protocol used by Briggiler Marcó et al. (2010) with some modifications. First, bacterial cultures were prepared as described for the determination of bacterial growth kinetics but, in this case, after harvesting and washing, cells were suspended in one-fifth of the initial volume of MRS, MRS_C, MRS_N, MRS_6, or MRS_5 broth, each one supplemented with 10 mM $CaCl_2$. Then, 1 mL of the culture was infected at a multiplicity of infection (MOI) of approximately 0.1 (phages B1, i*Lp*84 and i*Lp*1308) or 0.05 (phage LDG) (time = 0), and incubated at the optimal temperature of the strain (37 or 30 °C) for 10–15 min in a water bath. Cultures were centrifuged (13,000×*g*, 1 min) to remove non-adsorbed phages, suspended and serially diluted (1:10) in the corresponding fresh media, and incubated at the optimal temperature. At specific time points after infection, aliquots of 100 µL of dilutions were taken for phage enumeration.

The influence of low temperature was assessed using the same protocol. In this case, cells were suspended in MRS-Ca broth for phage infection, and incubated at 30 and 25 °C (*Lactobacillus*), or at 25 and 20 °C (*Leuconostoc*), from the time of phage addition and throughout the entire one-step growth assay.

Statistical Analysis

Data from one-step growth curves (PFU/mL vs. time) were fitted by applying the complete model of Baranyi and Roberts (Baranyi and Roberts 1994). Latent period, burst time, and burst size were calculated from the curves using DMFit (web edition; available at: http://browser.combase.cc/DMFit.aspx). Experiments were carried out in triplicates. Data were analyzed by one-way ANOVA using Statgraphics™ Plus software (v 3.0, Statistical Graphics Corp.), followed by Duncan's multiple range test at $P < 0.05$.

Results

Effect of Temperature on Phage Infection

Phages i*Lp*1308, LDG, and B1 showed similar, fast infection curves in optimal conditions (MRS, optimal growth temperature), while lysis was slower for phage i*Lp*84. For all the phages, the use of temperatures lower than the optimal for strain growth conduced to longer latent and burst times (Fig. 1). However, when temperature was moderately reduced (30 °C for *Lactobacillus*, 25 °C for *Leuconostoc*), the increase of the burst time was significant only for *L. paracasei* phages. An additional reduction in temperature (25 °C for *Lactobacillus*, 20 °C for *Leuconostoc*) produced a further delay, which was significant for all the phages, with respect not only to their respective controls but also to the values obtained at intermediate temperatures.

Regarding burst size, the effect of lowering temperature was different for the diverse phage–host systems studied. The value of this parameter did not significantly change at 30 °C (*Lactobacillus*) and 25 °C (*Leuconostoc*), except for the increment showed by *L. paracasei* phage i*Lp*84 (Fig. 1). Nevertheless, burst size was noticeably impaired at 25 °C for the three *Lactobacillus* phages, whereas it slightly increased at 20 °C for *Leuconostoc* phage LDG.

Effect of Acidification on Phage Infection

The effect of lowering pH on phage kinetics was remarkably dissimilar for each phage–host system. Phage i*Lp*1308 kept latent time approximately constant at pH 6 and 5, and burst time was slightly higher only at pH 5. However, infection was noticeably impaired due to the very low burst sizes observed (Fig. 1). Phage i*Lp*84, which is morphologically and genetically related to i*Lp*1308 (Mercanti et al. 2016), had similar variations in infection speed with pH but, unexpectedly, burst size significantly increased to more than two and three times the control value when pH was reduced to 6 and 5, respectively. Latent and burst times for phage B1 were only slightly higher at pH 5, and burst size increased at pH 6 but decreased again at pH 5 to a value still higher than control. This increase in burst size was, however, milder than that observed for phage i*Lp*84.

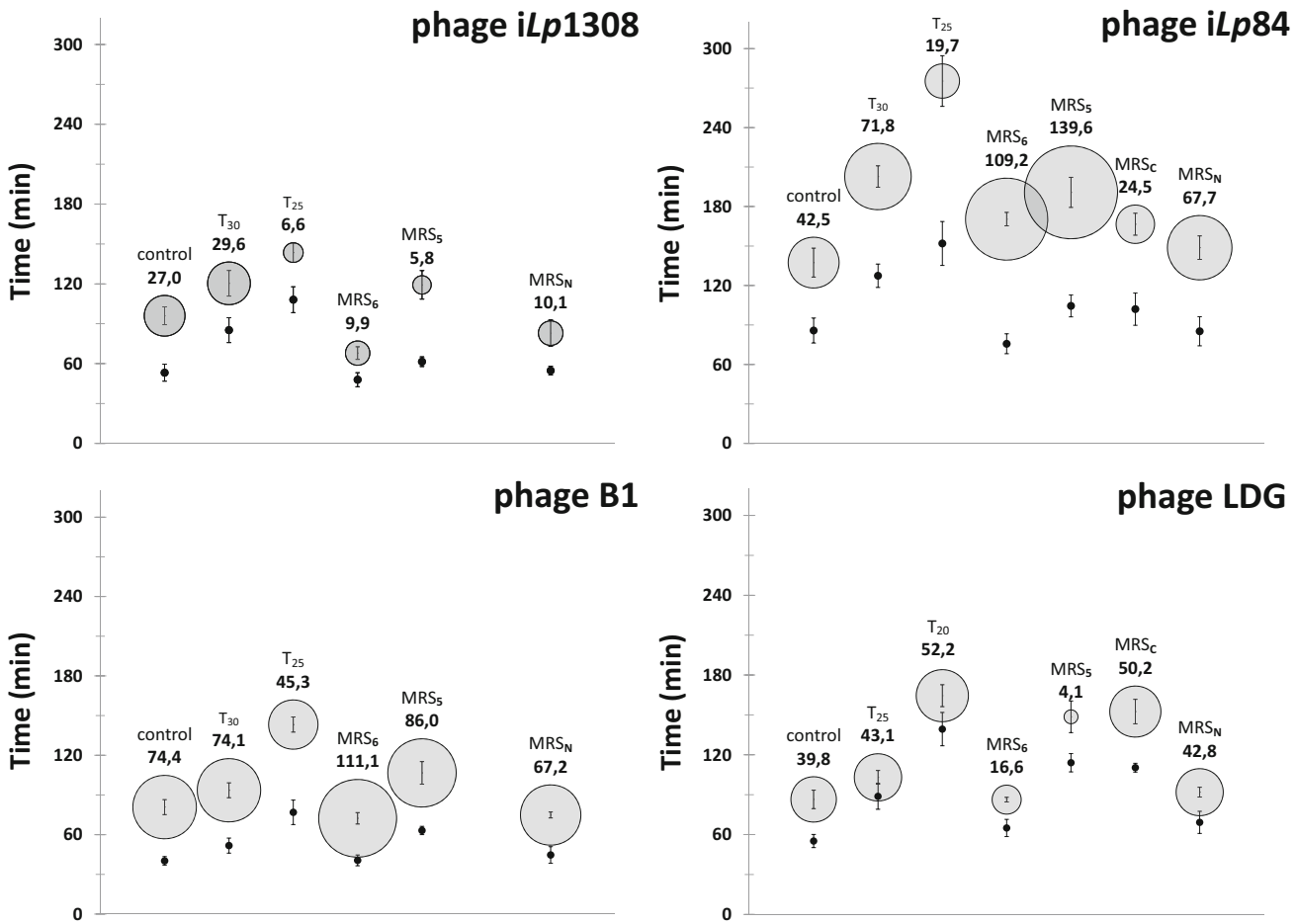

Fig. 1 Kinetic parameters of phage infection. Control (standard MRS broth), acidic (MRS_6 and MRS_5), and starvation (MRS_N and MRS_C) conditions were tested at 37 °C (phages i*Lp*1308, i*Lp*84, and B1) and 30 °C (phage LDG). Lower temperatures are indicated as T_{30} (30 °C), T_{25} (25 °C), and T_{20} (20 °C). Latent time is indicated with black dots. Burst time is indicated with *gray circles* (position in the *y*-axis). Burst size values are indicated above *gray circles*, and are proportional to the area of the *circles*. Lysis was not evidenced for phages i*Lp*1308 and B1 in MRS_C. Values are the mean of three replicates. Standard deviations are shown for latent and burst time

The effect of acidification on phage LDG infection was remarkable at pH 5, where latent time and burst size were about two times and one-tenth of the values obtained at optimal environmental conditions, respectively (Fig. 1). Although severely impaired, lysis of phage LDG was still evident at pH 5.

Bacterial growth was only weakly compromised at pH 6, but it was noticeably slower at pH 5 for all the strains studied. Nonetheless, the inhibition of growth at pH 5 was evidently higher for *Ln. mesenteroides* LDG than for lactobacilli (Fig. 2), which can explain the great influence of reducing pH from 6 to 5 on the lytic cycle of phage LDG.

Effect of Starvation on Phage Infection

MRS broth with 2‰ (w/v) glucose (1/10 that of standard MRS) had virtually no effect on phage lytic kinetics (data not shown). Moreover, the complete elimination of glucose from the culture media (MRS_N) still had no effect on lytic parameters of phages i*Lp*84, B1, and LDG, while for phage i*Lp*1308 only burst size was smaller (Fig. 1). On the other hand, the lack of carbon sources produced a strong inhibition of the growth of all the bacterial strains (Fig. 2), which however did not inhibit lytic cycle, thus indicating that cells exponentially growing in the presence of simple carbohydrates were not mandatory for the success of infection.

Contrarily, the lack of available nitrogen sources (MRS_C) had a drastic effect not only on bacterial growth (Fig. 2), but also on phage infection (Fig. 1). The strongest effect was verified for phages i*Lp*1308 and B1, for which no lysis was inferred from one-step growth curves. To confirm this effect, lysis was also monitored through measurements of DO_{560} in MRS_C in the presence of calcium, and DO_{560} of cultures infected by one or another phage did not decrease after 6 h of incubation at 37 °C

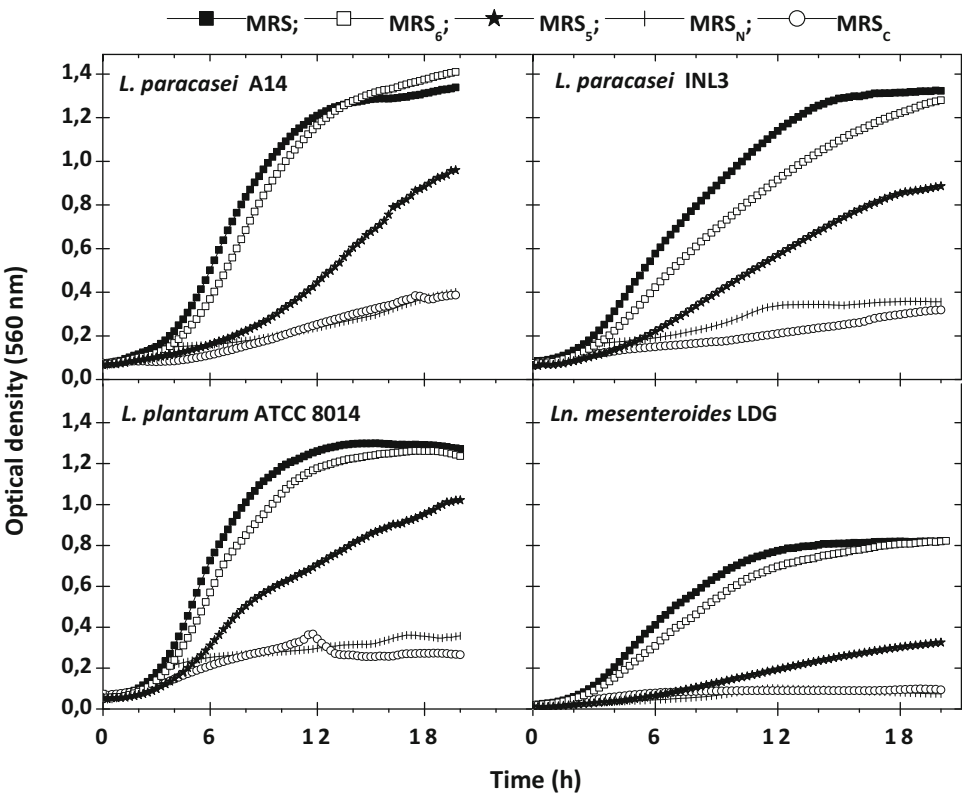

Fig. 2 Growth of bacterial strains in standard MRS broth (control) or under acidic (MRS_6, MRS_5) and starvations (MRS_N, MRS_C) stresses. Values are the mean of three replicates

(data not shown). Phage LDG suffered a significant delay in the infection cycle in MRS_C, but could complete it without alteration in the burst size, unlike the effect observed for this phage at pH 5. Alternatively, phage i*Lp*84 showed only slight alterations in burst time and burst size values. Although statistically significant differences in burst time and burst size were observed, the inhibition of phage infection was not as strong as that produced by low temperature.

Discussion

Efficiency of phage infections has been mostly studied in the presence of all the essential factors required for the growth of the bacterial host. However, these optimal conditions are not found outside the laboratory, for example, in industrial plants (Briggiler Marco et al. 2015). Changes in the environment impact on the metabolism of the host, and consequently the DNA replication, synthesis, and virion assembly machinery would not be fully active. To our knowledge, this is the first study in which temperature, pH, and lack of essential nutrients, key stress factors in dairy fermentations, were systematically evaluated for their effect on kinetics of phages infecting different LAB species.

Phage lysis was impaired by progressive adjustment of ideal environmental conditions. On the whole, infection was deeply compromised by the lack of nitrogen sources, delayed at lower temperature or pH, and slightly or not modified by the lack of fermentable sugars. However, the magnitude and direction of the observed effects greatly differed among the diverse phage–host systems analyzed. While infection speed (latent time, burst time) was reduced in comparison to that observed at optimal conditions, burst size was sometimes lower, equal, or higher. The last case might be explained according to Abedon (1989), who postulated that longer progeny-producing periods would allow a bigger intracellular accumulation of newly assembled virions to be released at the time of burst. Yet, that theoretical postulate assumes that phages have equal eclipse periods and that progeny is produced at a constant speed, and is therefore not applicable to many of our observations. For the cases where lysis was delayed and burst size decreased, altered conditions would be harsh enough to slow down the cellular metabolism in a way that cannot compensate the longer time available for phage synthesis. Antagonistic effects of temperature or host condition on burst size have been reported (Müller-Merbach et al. 2007; Wang et al. 1996; Mercanti et al. 2015). Related *L. paracasei* phages i*Lp*84 and i*Lp*1308 were similarly inhibited by low temperatures, in

concordance with results previously reported by Mercanti et al. (2015). For these phages, acidity exerted a relatively low influence on infection speed, and its effect on burst size was the opposite for both phages; for an unknown mechanism, acidity seems to amplify phage i*Lp*84 progeny. Likewise, the size of phage LDG progeny was reduced by acidity much more strongly than by temperature decrease.

Lack of nitrogen sources in the culture strongly inhibited phage infection, whereas lack of carbon sources did not. However, growth of all the strains was very slow and similar in both types of starvation conditions, indicating that nitrogen compounds are specifically required for a successful phage infection, beyond their simple effect on bacterial growth. Briggiler Marco et al. (2015) tested the effect of nutrient availability on phage B1 propagation, obtaining results similar to those of the present study. Those authors hypothesized that the overproduction of the nucleotide (p)ppGpp, an alarmone that regulates several characteristics of bacterial biology regarding changes in nutrients availability (Potrykus and Cashel 2008), could be responsible for the inhibition of phage replication observed in *L. plantarum* cells, as that effect had been reported before for *E. coli* phages (Nowicki et al. 2013). Nevertheless, replication of phage i*Lp*84 (on *L. paracasei*, another species of the same genus infected by phage B1) in MRS_C was equal or faster than that obtained at lower temperature or pH, while burst size was only slightly affected. On the other hand, *Leuconostoc* phage LDG infected host cells slowly compared to its control, but keeping a normal burst size in MRS_C. Therefore, only some LAB phages would be severely inhibited by the lack of nitrogen sources.

In conclusion, phages infecting different LAB species of significance in the dairy industry showed reduced ability of propagation under non-favorable conditions. However, all phages tested were able to complete the lytic cycle at low temperature, in acidic conditions, and in the absence of a fermentable carbohydrates source, though at lower efficiency. Dairy phages could be induced from lysogenic bacteria and are often present in raw milk, whey and other effluents, or in equipment surfaces. Thus, they cannot be completely eradicated from industrial environment. In this regard, our study points out that phages could replicate well on bacterial cells that remain in a poor metabolic state on pieces of equipment or effluent pipes after industrial fermentations, remarking their ability to persist in dairy plants.

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Compliance with Ethical Standard

Conflict of interest The authors declare that they have no conflict of interest.

References

Abedon, S. (1989). Selection for bacteriophage latent period length by bacterial density: A theoretical examination. *Microbial Ecology, 18*(2), 79–88.

Arendt, E. K., Lonvaud, A., & Hammes, W. P. (1991). Lysogeny in *Leuconostoc oenos*. *Journal of General Microbiology, 137*(9), 2135–2139.

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology, 23*(3–4), 277–294.

Binetti, A. G., Quiberoni, A., & Reinheimer, J. (2002). Phage adsorption to *Streptococcus thermophilus*: Influence of environmental factors and characterization of cell receptors. *Food Research International, 35,* 73–83.

Briggiler Marco, M., Garneau, J. E., Tremblay, D., Quiberoni, A., & Moineau, S. (2012). Characterization of two virulent phages of *Lactobacillus plantarum*. *Applied and Environmental Microbiology, 78*(24), 8719–8734.

Briggiler Marco, M., Reinheimer, J., & Quiberoni, A. (2015). Phage adsorption and lytic propagation in *Lactobacillus plantarum*: Could host cell starvation affect them? *BMC Microbiology, 15,* 273.

Briggiler Marcó, M., Reinheimer, J. A., & Quiberoni, A. (2010). Phage adsorption to *Lactobacillus plantarum*: Influence of physiological and environmental factors. *International Journal of Food Microbiology, 138*(3), 270–275.

Capra, M. L., Guglielmotti, D., Reinheimer, J., & Quiberoni, A. (2016). Bacteriophage: Biological aspects. In *Reference module in food science*. Cambridge: Elsevier.

Capra, M. L., Mercanti, D. J., Reinheimer, J. A., & Quiberoni, A. L. (2010). Characterisation of three temperate phages released from the same *Lactobacillus paracasei* commercial strain. *International Journal of Dairy Technology, 63*(3), 396–405.

Capra, M. L., Quiberoni, A., Ackermann, H. W., Moineau, S., & Reinheimer, J. A. (2006a). Characterization of a new virulent phage (MLC-A) of *Lactobacillus paracasei*. *Journal of Dairy Science, 89*(7), 2414–2423.

Capra, M. L., Quiberoni, A., & Reinheimer, J. (2006b). Phages of *Lactobacillus casei/paracasei*: Response to environmental factors and interaction with collection and commercial strains. *Journal of Applied Microbiology, 100*(2), 334–342.

Caso, J. L., de Los Reyes Gavilan, C., Herrero, M., Montilla, A., Rodriguez, A., & Suarez, J. E. (1995). Isolation and characterization of temperate and virulent bacteriophages of *Lactobacillus plantarum*. *Journal of Dairy Science, 78,* 741–750.

De Antoni, G., Zago, M., Vasek, O., Giraffa, G., Carminati, D., Marco, M. B., et al. (2010). *Lactobacillus plantarum* bacteriophages isolated from Kefir grains: Phenotypic and molecular characterization. *Journal of Dairy Research, 77*(1), 7–12.

Golec, P., Karczewska-Golec, J., Los, M., & Wegrzyn, G. (2014). Bacteriophage T4 can produce progeny virions in extremely slowly growing *Escherichia coli* host: Comparison of a mathematical model with the experimental data. *FEMS Microbiology Letters, 351*(2), 156–161.

Golec, P., Wiczk, A., Los, J., Konopa, G., Wegrzyn, G., & Los, M. (2011). Persistence of bacteriophage T4 in a starved *Escherichia coli* culture: Evidence for the presence of phage subpopulations. *Journal of General Virology, 92,* 997–1003.

Guglielmotti, D. M., Mercanti, D. J., & Briggiler Marcó, M. (2012). Infective cycle of dairy bacteriophages. In A. Quiberoni & J. A. Reinheimer (Eds.), *Bacteriophages in Dairy Processing* (pp. 99–122, Advances in Food Safety and Food Microbiology). Hauppauge, New York, USA: Nova Science Publishers.

Hemme, D. (2012). *Leuconostoc* and its use in dairy technology. In *Handbook of animal-based fermented food and beverage technology* (2nd ed., pp. 73–108). Boca Raton: CRC Press.

Lillehaug, D. (1997). An improved plaque assay for poor plaque-producing temperate lactococcal bacteriophages. *Journal of Applied Microbiology, 83*(1), 85–90.

Lu, Z., Breidt, F., Jr., Fleming, H. P., Altermann, E., & Klaenhammer, T. R. (2003). Isolation and characterization of a *Lactobacillus plantarum* bacteriophage, phiJL-1, from a cucumber fermentation. *International Journal of Food Microbiology, 84*(2), 225–235.

Mercanti, D. J., Ackermann, H. W., & Quiberoni, A. (2015). Characterization of two temperate *Lactobacillus paracasei* bacteriophages: Morphology, kinetics and adsorption. *Intervirology, 58*(1), 49–56.

Mercanti, D. J., Carminati, D., Reinheimer, J. A., & Quiberoni, A. (2011). Widely distributed lysogeny in probiotic lactobacilli represents a potentially high risk for the fermentative dairy industry. *International Journal of Food Microbiology, 144*(3), 503–510.

Mercanti, D. J., Rousseau, G. M., Capra, M. L., Quiberoni, A., Tremblay, D. M., Labrie, S. J., et al. (2016). Genomic diversity of phages infecting probiotic strains of *Lactobacillus paracasei*. *Applied and Environmental Microbiology, 82*(1), 95–105.

Müller-Merbach, M., Kohler, K., & Hinrichs, J. (2007). Environmental factors for phage-induced fermentation problems: Replication and adsorption of the *Lactococcus lactis* phage P008 as influenced by temperature and pH. *Food Microbiology, 24*(7–8), 695–702.

Nes, I. F., Brendehaug, J., & von Husby, K. O. (1988). Characterization of the bacteriophage B2 of *Lactobacillus plantarum* ATCC 8014. *Biochimie, 70*(3), 423–427.

Nowicki, D., Kobiela, W., Węgrzyn, A., Węgrzyn, G., & Szalewska-Pałasz, A. (2013). ppGpp-dependent negative control of DNA replication of Shiga toxin-converting bacteriophages in *Escherichia coli*. *Journal of Bacteriology, 195*(22), 5007–5015.

Potrykus, K., & Cashel, M. (2008). (p)ppGpp: Still magical? *Annual Review of Microbiology, 62,* 35–51.

Pujato, S. A., Mercanti, D. J., Guglielmotti, D. M., Rousseau, G. M., Moineau, S., Reinheimer, J. A., et al. (2015). Phages of dairy *Leuconostoc mesenteroides*: Genomics and factors influencing their adsorption. *International Journal of Food Microbiology, 201,* 58–65.

Quiberoni, A., & Reinheimer, J. (1998). Physicochemical characterization of phage adsorption to *Lactobacillus helveticus* ATCC 15807 cells. *Journal of Applied Microbiology, 85,* 762–768.

Saarela, M., Mogensen, G., Fondén, R., Mättö, J., & Mattila-Sandholm, T. (2000). Probiotic bacteria: Safety, functional and technological properties. *Journal of Biotechnology, 84*(3), 197–215.

Server-Busson, C., Foucaud, C., & Leveau, J.-Y. (1999). Selection of dairy *Leuconostoc* isolates for important technological properties. *Journal of Dairy Research, 66*(2), 245–256.

Sozzi, T., Poulin, J. M., Maret, R., & Pousaz, R. (1978). Isolation of a bacteriophage of *Leuconostoc mesenteroides* from dairy products. *Journal of Applied Bacteriology, 44*(1), 159–161.

Suarez, V., Moineau, S., Reinheimer, J., & Quiberoni, A. (2008). Argentinean *Lactococcus lactis* bacteriophages: Genetic characterization and adsorption studies. *Journal of Applied Microbiology, 104*(2), 371–379.

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

Trucco, V., Reinheimer, J., Quiberoni, A., & Suarez, V. B. (2011). Adsorption of temperate phages of *Lactobacillus delbrueckii* strains and phage resistance linked to their cell diversity. *Journal of Applied Microbiology, 10*(10), 1365–2672.

Wang, I.-N., Dykhuizen, D., & Slobodkin, L. (1996). The evolution of phage lysis timing. *Evolutionary Ecology, 10*(5), 545–558.

Zhang, X., Lan, Y., Jiao, W., Li, Y., Tang, L., Jiang, Y., et al. (2015). Isolation and characterization of a novel virulent phage of *Lactobacillus casei* ATCC 393. *Food and Environmental Virology, 7*(4), 333–341.