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PII: S0168-1605(08)00126-8
DOI: doi: 10.1016/j.ijfoodmicro.2008.03.006
Reference: FOOD 4319

To appear in: *International Journal of Food Microbiology*

Received date: 9 January 2008
Revised date: 12 March 2008
Accepted date: 14 March 2008

Please cite this article as: Binetti, Ana G., Capra, M. Luján, Álvarez, Miguel A., Reinheimer, Jorge A., PCR method for detection and identification of *Lactobacillus casei* / *paracasei* bacteriophages in dairy products, *International Journal of Food Microbiology* (2008), doi: 10.1016/j.ijfoodmicro.2008.03.006

PCR Method for Detection and Identification of *Lactobacillus casei* / *paracasei* Bacteriophages in Dairy Products

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Running title: Detection of *L. casei* / *paracasei* phages.

Keywords: bacteriophages, *Lactobacillus casei*/ *paracasei*, probiotic lactic acid bacteria, PCR detection

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ABSTRACT

Bacteriophage infections of starter lactic acid bacteria (LAB) pose a serious risk to the dairy industry. Nowadays, the expanding use of valuable *Lactobacillus* strains as probiotic starters determines an increase in the frequency of specific bacteriophage infections in dairy plants.

This work describes a simple and rapid Polymerase Chain Reaction (PCR) method that detects and identifies bacteriophages infecting *Lactobacillus casei* / *paracasei*¸ the main bacterial species used as probiotic. Based on a highly conserved region of the NTP-binding genes belonging to the replication module of *L. casei* phages ϕA2 and ϕAT3 (the only two whose genomes are completely sequenced), a pair of primers was designed to generate a specific fragment. Furthermore, this PCR detection method proved to be a useful tool for monitoring and identifying *L. casei* / *paracasei* phages in industrial samples since specific PCR signals were obtained from phage contaminated milk (detection limit: 10^4 PFU/mL milk) and other commercial samples (fermented milks and cheese whey) that include *L. casei* / *paracasei* as probiotic starter (detection limit: 10^6 PFU/mL fermented milk). Since this method can detect the above phages in industrial samples and can be easily incorporated into dairy industry routines, it might be readily used to earmark contaminated milk for use in processes that do not involve susceptible starter organisms, or processes which involve phage-deactivating conditions.

INTRODUCTION

Bacteriophage infections of lactic acid bacteria (LAB) pose a serious risk to the industrial production of dairy foodstuffs. Dairy fermentations are susceptible to phage infection since the starting material (mainly, raw milk) is not sterile (Moineau, 1999) and, in general, pasteurization processes are not adequate to deactivate viral particles (Binetti & Reinheimer, 2000; Capra, Quiberoni & Reinheimer, 2004). In addition, the continued use of the same starter cultures provides a constant host for phage proliferation (Coveney, Fitzgerald, & Daly, 1994; Neve, Berger & Heller, 1995) which, consequently, can lead to slow lactic acid production or even the complete failure of fermentation, with the substantial damages associated (Josephsen & Neve, 1998). These losses are particularly severe when highly specialized strains, which are a valuable product of scientific discovery, become susceptible to phage attack. In this case, the costs of strain development will not be recovered if the expected lifetime of a very specialized strain is diminished by the appearance of lytic phages capable of infecting it.

Some species of *Lactobacillus* are used worldwide as industrial starters for the manufacture of fermented milks and cheeses and particularly, specific strains of *Lactobacillus casei* / *paracasei* with probiotic characteristics are also added in functional foods and health products (Lee, Nomoto, Salminen & Gorbach, 1999; Tynnkynen, Satokary, Saarela, Mattila-Sandholm & Saxelin, 1999). The available knowledge of lactobacilli phages is limited when compared with that of lactococci and streptococci bacteriophages (Séchaud, Cluzel, Rousseau, Baumgartner & Accolas, 1988; Moineau & Lévesque, 2005). Only a small number of them have been studied in detail, particularly, lytic phages *L. casei* phage J1, *L. paracasei* phage PL-1, both isolated from Yakult (Hino & Ikebe, 1965; Watanabe, Takesue, Jin-Nai & Yoshikawa, 1970, respectively), as well as *L.*

casei temperate phages from different origins: ϕFSW (Shimizu-Kadota & Tsuchida, 1984), ϕA2 (Herrero, de los Reyes-Gavilán, Caso & Suárez, 1994), phage PL-2 (Nakashima et al., 1998) and ϕAT3 (Lo, Shih, Lin, Chen & Lin, 2005). Among them, only the genome sequences of ϕA2 and ϕAT3 are available in databases. Nowadays, the expanding use of valuable *Lactobacillus* strains as probiotic starters will eventually lead to an increase in the frequency of specific bacteriophage infections in dairy plants (Álvarez, Rodríguez & Suárez, 1999; Capra, 2007). Over the last years, and following this global tendency, probiotic bacteria (predominantly *L. casei / paracasei*) are used in Argentinean fermented dairy products (Vinderola, Prosello, Ghiberto & Reinheimer, 2000). As an immediate consequence, the first phage (ϕMLC-A) isolated in South America from probiotic dairy milk of a commercial strain of *L. paracasei* was recently described (Capra, Quiberoni, Ackermann, Moineau & Reinheimer, 2006).

Usually, milk products are examined for phages using standard microbiological methods. However, these assays are time-consuming (a critical variable in industrial processes) and rapid and sensitive methods are therefore required to detect and identify phages at all stages of milk product manufacture. Polymerase Chain Reaction (PCR) methods are fast and reliable and, considering the importance of phage monitoring in the dairy industry, it has already been successfully used to detect bacteriophages that infect various LAB including *Lactococcus lactis* (Labrie & Moineau, 2000; Dupont, Vogensen, & Josephsen, 2005), *Streptococcus thermophilus* (Brüssow, Fremont, Bruttin, Sidoti, Constable & Fryder, 1994; Binetti, del Río, Martin & Álvarez, 2005), *Lactobacillus delbrueckii* subsp. *lactis* (Zago, De Lorentiis, Carminati, Comaschi & Giraffa, 2006) and, simultaneously, phages of three bacterial species of industrial relevance as *L. lactis*, *S.*

thermophilus and *L. delbrueckii* (del Río, Binetti, Martín, Fernández, Magadán & Álvarez, 2007).

In spite of all these data, there are no previous reports on the PCR detection of phages that infect *L. casei* / *paracasei*. Therefore, bearing in mind the expanding incidence of *L. casei* / *paracasei* virus in dairy fermentations, the aim of this work was to design a PCR method to detect these bacteriophages, based on a highly conserved region of the replication module of *L. casei* phages ϕA2 and ϕAT3. The developed system performed from a minimally treated phage suspension and any procedure is required to enrich the samples. Simply a minimal sample treatment prevents the contribution to PCR signal from prophages, frequently integrating the chromosome of *L. casei / paracasei* strains.

MATERIAL AND METHODS

Bacterial strains and bacteriophages. Table 1 shows the *L. casei / paracasei* strains and bacteriophages used in this study. Only 7 phages out of 21 isolated since 2003 to 2006 from industrial samples belonging to the INLAIN (Instituto de Lactología Industrial, Santa Fe, Argentina) Collection were chosen, based on their diverse origins and the differences detected among them by restriction patterns with different enzymes (*Bgl*II, *Eco*RI, *Eco*RV and *Hin*dIII). Phages from ATCC (American Type Culture Collection) were used as references (Figure 1 shows the *Bgl*II restriction patterns of all different phages). Bacteriophages specific to *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *lactis*, *L. lactis* and *L. helveticus* were exclusively used to check the specificity of the primers proposed for the PCR detection method. Moreover, 3 lysogenic *L. casei* strains from ATCC were included, since they harbor different prophages. Host and lysogenic

strains were conserved as frozen stocks at -80 ºC in MRS (*Lactobacillus*) or Elliker (*S. thermophilus* and *Lactococcus*) broth (Britania, Buenos Aires, Argentina) supplemented with 15% glycerol, and routinely cultured overnight at 37 ºC for *Lactobacillus*, at 42 ºC for *S. thermophilus* and at 30 ºC for *Lc. lactis*. Phage stocks were prepared as described by Neviani, Carminati and Giraffa (1992) in broth supplemented with 10 mM $CaCl_2$ (MRS- or Elliker-Ca), and stored at 4 ºC and at -80 ºC (with 15% glycerol). Phage enumerations (PFU/mL) of *L. casei* / *paracasei* bacteriophages were performed by the double-layer plate titration method (Svensson & Christiansson, 1991), using MRS-Ca agar supplemented with 100 mM glycine (Lillehaug, 1997). Plates were incubated at 34 ºC under microaerophilic conditions to improve the formation of lysis plaques (Capra et al., 2006).

Phage multiplication and concentration. Overnight *L. casei* / *paracasei* host bacterial cultures were inoculated (1%) into 100 mL of MRS-Ca broth, infected with phage suspensions at a multiplicity of infection from 0.1 to 1 and incubated at 37 ºC until complete lysis occurred. The suspensions were centrifuged (10,000 *g*, 15 min, 4 ºC), filtered (0.45 μm pore size, Millipore, Billerica, MA, USA) to eliminate bacterial debris, and treated with 1 μg μL^{-1} RNase A (usb, Cleveland, Ohio, USA) and 1 μg/μL DNase I (usb) for 1 h at 37 ºC. Phage particles were then precipitated with 10% PEG 8000 (usb) and 0.5 M NaCl for 24 h at 4 ºC and centrifuged (17,000 *g*, 60 min, 4 ºC). Pellets were resuspended in 2 mL of SM buffer (0.1 M NaCl; 0.01 M $MgSO_4.7H_2O$; 0.05 M Tris-Cl, pH 7.5; Sambrook & Russell, 2001) and stored at 4 ºC as a concentrated suspension.

DNA isolation. Phage DNA was obtained from 1 mL of concentrated suspension of phage particles treated with 100 μL of SDS mix solution (0.25 M EDTA; 0.5 M Tris-HCl, pH 9.0; 2.5% SDS) for 30 min at 65 ºC. Then, 125 μL of 8 M potassium acetate were added

and incubated for 30 min on ice before centrifugation (16,100 *g*, 10 min, 4 ºC). Three phenol-chloroform extractions were performed on the resulting lysates in the presence of 0.3 M sodium acetate followed by an isopropanol (4 ºC) precipitation. The DNA pellet was washed in 70% ethanol, then dried, resuspended in 10 mM Tris-HCl, pH 8.0 and stored at -20 ºC. Total DNA from *L. casei* / *paracasei* strains was obtained from 10 mL overnight cultures in MRS following the method described by Foley, Lucchini, Zwahlen and Brüssow (1998) with modifications: cells were washed before centrifugation with TSE buffer (6.7% sucrose; 50 mM Tris-HCl; 1 mM EDTA, pH 8.0); the pellets were resuspended in 1 mL TSE buffer containing 30 mg/mL lysozyme and incubated on ice for 1 h. SDS was added (125 μL of 10% stock solution, usb) with proteinase K (20 μL of 20 mg/mL stock, usb) and the lysates were incubated at 65 ºC for 30 min. RNase A (50 mg/mL, usb) was subsequently added, and incubation was continued at 37 ºC for an additional 30 min. Three phenol-chloroform extractions were performed, followed by an isopropanol precipitation. The DNA pellet obtained was washed in 70% ethanol and finally resuspended in 10 mM Tris-HCl, pH 8.0 and stored at -20 ºC.

Restriction analysis. Purified viral DNA was digested with restriction endonucleases *Bgl*II, *Eco*RI, *Eco*RV and *Hin*dIII (GE Healthcare, Buckinghamshire, United Kingdom) according to the manufacturer's instructions. Restricted phage DNA was electrophoresed in a 0.8% agarose gel in TBE (Tris-Borate-EDTA) buffer and visualized by using standard protocols (Sambrook & Russell, 2001).

Treatment of the samples. Samples constituted by whole phage particles in suspension (in MRS broth and industrial samples –fermented milks and cheese whey– containing *L. casei* / *paracasei* as probiotic starter) were filtered (0.45 μm pore size,

Millipore) to eliminate bacterial debris. In case of industrial samples–fermented milks and cheese whey–, a centrifugation step (21000 *g*, 15 min, 4 ºC) was included before filtration. Then, all the samples were treated with 1 μg/μL DNase I (usb) for 1 h at 37 ºC to remove free DNA from uncoated phages and prophages (from potential *L. casei / paracasei* lysogenic strains). Then, to inactivate this enzyme and allow lysis of phage particles, 0.625 mM EDTA was added, followed by a heat treatment (10 min at 100 ºC). When pH of samples was lower than 4.0, a variable volume of 2 M Tris-HCl buffer (pH 9.0) was used to reach this pH value. In case of cheese whey, 20 mM sodium citrate pH 7.0 was added to stabilize pH (Dupont et al., 2005) and a heat treatment (10 min at 94 ºC) was applied to precipitate whey proteins.

PCR conditions. PCR reactions were performed using PuReTaq Ready-To-Go™ PCR Beads (GE Healthcare) with each primer (Sigma-Genosys, The Woodlands, TX, USA) at a concentration of 400 nM in 25 μL of final volume. One microliter of the template was added, consisting of either phage DNA [10 ng diluted in 25 μL of ultraPure water (Gibco™, Invitrogen, Grand Island, NY, USA)] or phage suspensions. In the latter case, 0.5 U of pyrophosphatase (usb) was added in the reaction mix (del Río et al., 2007).

Amplifications were performed in a GeneAmp PCR System (Applied Biosystems, Foster City, CA, USA) under the following conditions: 3 min at 94 ºC, 35 cycles of 45 sec at 94 ºC, 30 sec at 57 ºC and 1 min at 72 ºC, and a final step of 7 min at 72 ºC. A negative control (no phages) was included in all experiments and DNA from *L. casei* ATCC 393 (harboring ϕAT3 as prophage) was used as positive control. PCR products were visualized on 1.5% agarose gels in TBE buffer (Sambrook & Russell, 2001). In order to determine the detection limit of the PCR method, sterile skim milk and industrial fermented milks were

contaminated with serial dilutions (from 10^3 to 10^8 PFU/mL) of treated phage suspensions and 1 µL sample was used directly in the PCR reaction. With the purpose of analyzing the possible inhibitory effect of the sample, positive controls of viral DNA suspended both in TE buffer and in the sample were included. The lowest visible concentration in agarose gels was taken as the detection limit. All experiments were performed by triplicate.

Nucleotide sequence analysis. PCR amplicons obtained with primers NTP-Lc3/4 were purified using MicroSpin Columns (GE Healthcare) and their nucleotide sequences were determined by primer extension at the DNA Sequencing Service of Macrogen Inc. (Seoul, Korea). Sequence data were assembled and compared using a sequence analysis software package available from the EMBL Spanish node (CNB, CSIC, Spain). Alignment was performed using the ClustalW algorithm (Thompson, Higgins &. Gibson, 1994).

RESULTS

Selection of bacteriophages. 7 out of 21 *L. paracasei* bacteriophages were selected among all of those isolated from a fermented milk process (during the period 2003-2006), belonging to INLAIN Collection. They were arranged into 7 groups according to the origin of the samples and their restriction analysis with different enzymes (*Bgl*II, *Eco*RI, *Eco*RV and *Hin*dIII). Additionally, 2 *L. casei* and *L. paracasei* phages from ATCC showed particular restriction patterns (Figure 1 shows the *Bgl*II restriction patterns of all different phages). Moreover, 3 lysogenic *L. casei* strains from ATCC were included, since they harbor different prophages. Therefore, 12 particular *L. casei* / *paracasei* phages and prophages were considered for the detection method design and validation.

Primer design and validation of the PCR method. Primer pairs selection was made by comparing and aligning NTP-binding protein genes from ϕA2 (*orf*31) and ϕAT3

(*orf*28) genomes (GenBank accession numbers AJ251789 and AY605066, respectively). This region was chosen due to the high similarity demonstrated between both genomes (Lo et al., 2005). In this alignment, *orf*29 sequence of *L. rhamnosus* phage Lc-Nu (GenBank accession number AY131267) was also included, since high nucleotide conservation (> 92% similarity) over this gene was shared with *L. casei* phages ϕA2 and ϕAT3 (Tuohimaa, Riipinen, Brandt & Alatossava, 2006). A pair of primers (named NTP-Lc3 and NTP-Lc4, Table 1) was designed to amplify a DNA fragment of 589 bp, and used to verify the conservation of NTP-binding protein gene from ϕAT3 (present as prophage in *L. casei* ATCC 393) and homologous genes in all analyzed phages and lysogenic strains. A positive PCR signal was obtained with these primers for all viral and bacterial assayed DNA (data not shown). The sequences of the purified PCR products were compared and aligned with those of *orf*31, *orf*28 and *orf*29 from bacteriophages ϕA2, ϕAT3 and ϕLc-Nu, respectively. All amplicons showed nucleotide sequence similarities higher than 90% (Figure 2), indicating that the region (belonging to NTP-binding protein and homologous genes) chosen as target was conserved among a considerable number (n=12) of *L. casei / paracasei* phages and prophages from different origins. Based on these results, another pair of oligonucleotides, NTP-Lc1 and NTP-Lc2 (Table 1) was designed based on highly conserved regions of amplicons obtained from PCR reactions, which amplify a 356 bp internal region for all DNA samples (data not shown). They were also selected taking into account the fact that nucleotides of 3´end of both primers were not conserved in *orf29* sequence of ϕLc-Nu, the only *L. rhamnosus* phage with known genome sequence. Additionally, oligonucleotides were assayed against different phage types infecting other LAB (*S. thermophilus*, *L. delbrueckii*, *L. helveticus* and the main phage types of *L. lactis*)

and were found to be specific for the target species (data not shown). The sequences of the four primers (NTP-Lc1, NTP-Lc2, NTP-Lc3 and NTP-Lc4) were checked in the NCBI database using the nucleotide-nucleotide homology search BLAST for short, nearly exact matches (www.ncbi.nlm.nhi.gov/blast) to ensure that no matches with other genes were present (Altschul et al., 1997).

PCR detection in milk and fermented industrial samples. Determination of the detection limit. The PCR amplification with primers NTP-Lc1 and NTP-Lc2 was successfully tested for sterile skim milk, treated commercial fermented milks and probiotic cheese whey artificially contaminated with 10^8 PFU/mL for all distinct *L. casei* / *paracasei* phages (Figure 3 shows the amplification products obtained from ϕC in all the tested samples). For all phages (n=12), positive PCR signals of the expected size were observed. No interference due to the sample nature was observed in PCR reactions when milk and fermented industrial samples were used as template. In order to determine the sensitivity of the PCR assay, serial dilutions of all phages in sterile skim milk and in treated fermented milk were assayed. All tested phages were detected with a limit of 10^4 PFU/mL in milk and 10^6 PFU/mL in fermented milk (Figures 4A and B show the amplification products for milk and treated milk fermented samples, respectively, artificially contaminated with decreasing concentrations of phages ϕA and ϕB).

DISCUSSION

Taking into account the nucleotide sequence conservation reported for the NTP-binding protein genes of *L. casei* phages with known complete sequences (Lo et al., 2005) and the high probability that this genome region could be specific for *L. casei* / *paracasei* bacteriophages, a pair of preliminary primers (NTP-Lc3 and NTP-Lc4) was designed based

on the genome sequence of the corresponding genes (*orf*31 and *orf*28 from *L. casei* bacteriophages ϕA2 and ϕAT3, respectively). The nucleotide sequence analysis of PCR products obtained from 12 *L. casei* / *paracasei* phages and prophages from diverse origins (phages isolated from Argentinean fermented dairy milks and phages or lysogenic strains belonging to international collections) confirmed this hypothesis, extending the validity of the detection method. Therefore, a new pair of oligonucleotides (NTP-Lc1 and NTP-Lc2) was designed on an internal and even more conserved DNA region. These primers, due to their verified specificity – since they gave negative PCR signals from DNA of the other dairy phages of industrial relevance (*S. thermophilus*, *L. delbrueckii*, *L. helveticus* and *L. lactis* bacteriophages) – were used for the development of a PCR detection method of *L. casei* / *paracasei* bacteriophages, the first PCR assay described for these phages. Furthermore, this system is a useful tool for monitoring *L. casei* / *paracasei* phages in industrial samples since specific PCR signals were obtained for all phages in milk (detection limit: 10^4 PFU/mL milk) and other commercial samples (fermented milks and cheese whey) that include *L. casei* / *paracasei* as probiotic starter (detection limit: 10^6 PFU/mL fermented milk). Similar detection systems for LAB bacteriophages were previously reported (Brüssow et al., 1994; Labrie & Moineau, 2000; Binetti et al., 2005; Dupont et al., 2005; Zago et al., 2006; del Río et al., 2007) but none of them refers to phages of *L. casei* / *paracasei*. When reactions involved direct PCR amplification from phage suspensions, the detection limits were comparable to those determined in the present work. The designed system could be very promising for routine applications since it can be performed from a minimally treated phage suspension (basically by filtering, eliminating free DNA and boiling the sample) and neither phage particle concentration (purification by

precipitation, CsCl gradient, etc.), DNA extraction, nor any other procedure is usually required to enrich the samples (Brüssow et al., 1994; Labrie & Moineau, 2000; Zago et al., 2006). DNase I treatment implies extra hands-on time for the assay, but it allows to assume that PCR signals resulted exclusively from virions and not from prophages integrating the host chromosome or from uncoated viral DNA. Even though it is presumed that the commercial strains are tested for lysogeny, in case of spontaneous induction of prophages from lysogenic strains, this molecular method would be detect a contribution of temperate free phages that is impossible to detect by classical methods. This fact would represent an advantage since when a viral additional population is present in a sample, adds to the diversity and represents a potential source of infective bacteriophages that could attack the starter.

It is important to highlight that the proposed PCR method could be successfully used to guarantee minimum quality in routine controls. Even though the phage concentration from which fermentation delays are detected depends on the virulence of infective phages, in most cases, concentrations below 10^5 PFU per mL of milk are not considered a threat to fermentation processes (Svensson & Christiansson, 1991; Suárez, Quiberoni, Binetti & Reinheimer, 2002).

Phage analysis is particularly important in the dairy industry and, while traditional assays are time-consuming and mostly rely on the availability of single indicator strains, the PCR assay described in this paper can detect bacteriophages in dairy samples in just 5-6 h. The correct and rapid identification of bacteriophages potentially able to attack starter cultures allows for prompt decisions with regard to the destination of contaminated milk. Such milk might be earmarked for use in processes in which phages are deactivated, that do not require starters, or that employ starter bacteria insensitive to the detected phage. For

example, it could be derived to UHT treatments or be used as pasteurized milk for drinking or for yoghurt or cheese fermentations in which *L. casei* / *paracasei* strains are not required. In case of phage detection in different steps of the manufacture process or in the final product, plant disinfection should be recommended even when fermentation failures were not detected, to avoid subsequent economic losses. Moreover, as primers were found to be specific for the target phages, the proposed method shows that not only a presumptive detection of phage is possible but also a rapid identification of *L. casei* / *paracasei* bacteriophages can be performed. It is also important to remark that the size of amplified DNA fragment by these primers was chosen considering the possibility to utilize them with others by multiplex PCR (Brüssow et al., 1994; Labrie & Moineau, 2000; Binetti et al., 2005; Zago et al., 2006; del Río et al., 2007). Thus, the obtained PCR products can be easily separated by agarose gel electrophoresis and simultaneously, they would allow detecting and identifying different dairy phages, based on their amplicon sizes.

The dairy industry needs reliable methods for rapid phage monitoring. Consequently, future works should evaluate whether *L. casei* / *paracasei* phages may be detected by means of a PCR (or multiplex-PCR) diagnostic kit based on the use of the method described in combination with previously described dairy phages PCR detection systems.

AKNOWLEDGMENTS

We are grateful to Andrea Quiberoni (INLAIN, FIQ-UNL, CONICET) for her support at the initial stage of this work. This research was supported by PIP - CONICET Nº 532 (Consejo Nacional de Investigaciones Científicas y Tecnológicas), C.A.I.+D. Nº 37-200 (Universidad Nacional del Litoral) and PICT ANCyT Nº 20358 (Agencia Nacional de Promoción Científica y Tecnológica) projects.

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FIGURE LEGENDS

FIGURE 1 - Agarose gel electrophoresis of the *BglII*-generated fragments of the indicated *L. casei* / *paracasei* bacteriophages. Lane M, 1 Kb marker (GE HealthCare); lane 1, ϕMLC-A; lane 2, ϕA; lane 3, ϕB; lane 4, ϕC; lane 5, ϕE; lane 6, ϕF; lane 7, phage PL-1; lane 8, phage J1.

FIGURE 2 - DNA sequence alignment of genomic fragments from the NTP-binding protein gene of *L. casei* / *paracasei* bacteriophages obtained with primers NTP-Lc3 and NTP-Lc4. Numbers represent the nucleotide position from the start of the amplified region. The relevant primers are indicated.

FIGURE 3 – Amplification products (356 bp) of the highly conserved DNA fragments from the NTP-binding protein gene of *L. paracasei* bacteriophage ϕC (10^8 PFU/mL) obtained with primers NTP-Lc1 and NTP-Lc2, from different suspension media. Lane M, 100 bp Ladder (GE HealthCare); lane 1, MRS; lane 2, milk; lane 3, yoghurt; lane 4, probiotic fermented milk; lane 5, probiotic cheese whey; lane 6, phage DNA (positive control); lane 7, negative control.

FIGURE 4 – Amplification products (356 bp) of the highly conserved DNA fragments from the NTP-binding protein gene of *L. casei* / *paracasei* bacteriophages obtained with primers NTP-Lc1 and NTP-Lc2, from sterile skim milk (A) and treated fermented milk (B) samples artificially infected with bacteriophage ϕC, 10^8 PFU/mL in decreasing

concentrations. Lane M, 100 bp Ladder (GE HealthCare); lane 1, ϕA, of MRS; lanes 2 -7, ϕA: 10^8- 10^3 PFU/mL of sample; lane 8, ϕB, 10^9 PFU/mL of MRS; lanes 9 -14, ϕB: 10^8-10^3 PFU/mL of sample; lane 15, ϕMLC-A DNA diluted in TE buffer (PCR positive control); lane 16, ϕMLC-A DNA diluted (1:100) in sample (sample PCR positive control); lane 17, negative control.

TABLE 1 - Bacterial strains, phages and primers used in this study

Strain, phage or primer	Relevant characteristics	Reference and/or source [a]
Strains		
L. casei ATCC 393	Lysogenic strain harboring ϕAT3.	ATCC
L. casei ATCC 27139	Lysogenic strain harboring ϕFSW. Sensitive to phage J1.	ATCC
L. paracasei ATCC 27092	Lysogenic strain harboring prophage PL-2. Sensitive to phage PL-1.	ATCC
L. paracasei A	Commercial strain. Sensitive to ϕMLC-A, ϕA, ϕB, ϕC and ϕD.	INLAIN Collection; Capra et al., 2006.
S. thermophilus M10-C	Commercial strain. Sensitive to ϕCP.	INLAIN Collection; Binetti et al., 2005.
L. delbrueckii subsp. *bulgaricus* YDSV	Commercial strain. Sensitive to ϕBYM.	INLAIN Collection; del Río et al., 2007.
L. delbrueckii subsp. *lactis* 204	Lysogenic commercial strain harboring ϕCb1.	INLAIN Collection.
L. lactis F4-2	Sensitive to ϕP335.	del Río et al., 2007.
L. lactis MG1614	Sensitive to ϕc2.	del Río et al., 2007.
L. lactis IL1403	Sensitive to ϕbIL170.	del Río et al., 2007.
L. helveticus ATCC15807	Sensitive to ϕATCC 15807-B1.	ATCC
Phages		

J1[b]	*L. casei* phage.	ATCC; Hino & Ikebe, 1965.
PL-1	*L. paracasei* phage.	ATCC; Watanabe et al., 1970.
ϕMLC-A	*L. paracasei* phage isolated from Argentinean probiotic fermented milk.	INLAIN Collection; Capra et al., 2006.
ϕA ϕB ϕC ϕD ϕE ϕF	*L. paracasei* phages isolated from Argentinean probiotic fermented milk.	INLAIN Collection; this work.
ϕCP[b]	*S. thermophilus* phage isolated from Argentinean cheese plant.	INLAIN Collection; Binetti et al., 2005.
ϕBYM[b]	*L. delbrueckii* subsp. *bulgaricus* phage isolated from an Argentinean probiotic yoghurt plant.	INLAIN Collection; del Río et al., 2007.
ϕCb1[b]	*L. delbrueckii* subsp. *lactis* phage induced from a commercial strain.	INLAIN Collection.
ϕP335[b]	*L. lactis* phage belonging to P335 phage species.	del Río et al., 2007.
ϕc2[b]	*L. lactis* phage representative to c2 phage species.	del Río et al., 2007.
ϕbIL170[b]	*L. lactis* phage belonging to 936 phage species.	del Río et al., 2007.
ϕATCC 15807-B1[b]	*L. helveticus* phage.	ATCC

Primers	*Sequence (5´→3´)*	
NTP-Lc1	GATCTGGATGACAGTTCAAAAGTGCTATCC	This work.
NTP-Lc2	GCTGAATGATTGTCCAGTTTCGCTTGTAAC	This work.
NTP-Lc3	GCATCTGCAATTGATCGAACAAAGAACTGGC	This work.
NTP-Lc4	GCATCTGAACCTGCAAGGATAACTCCTCGG	This work.

[a] ATCC: American Type Culture Collection (Manassas, VA, USA).

[b] Phages used to verify the specificity of the PCR method.

FIGURE 1 –

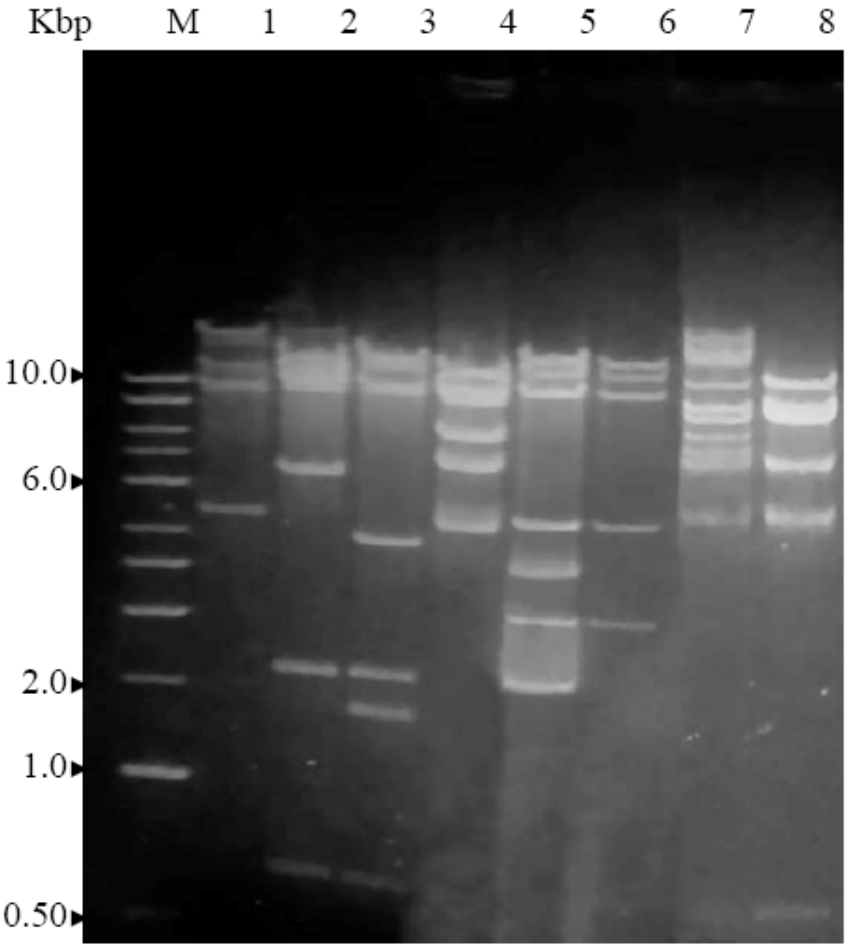

FIGURE 2 –

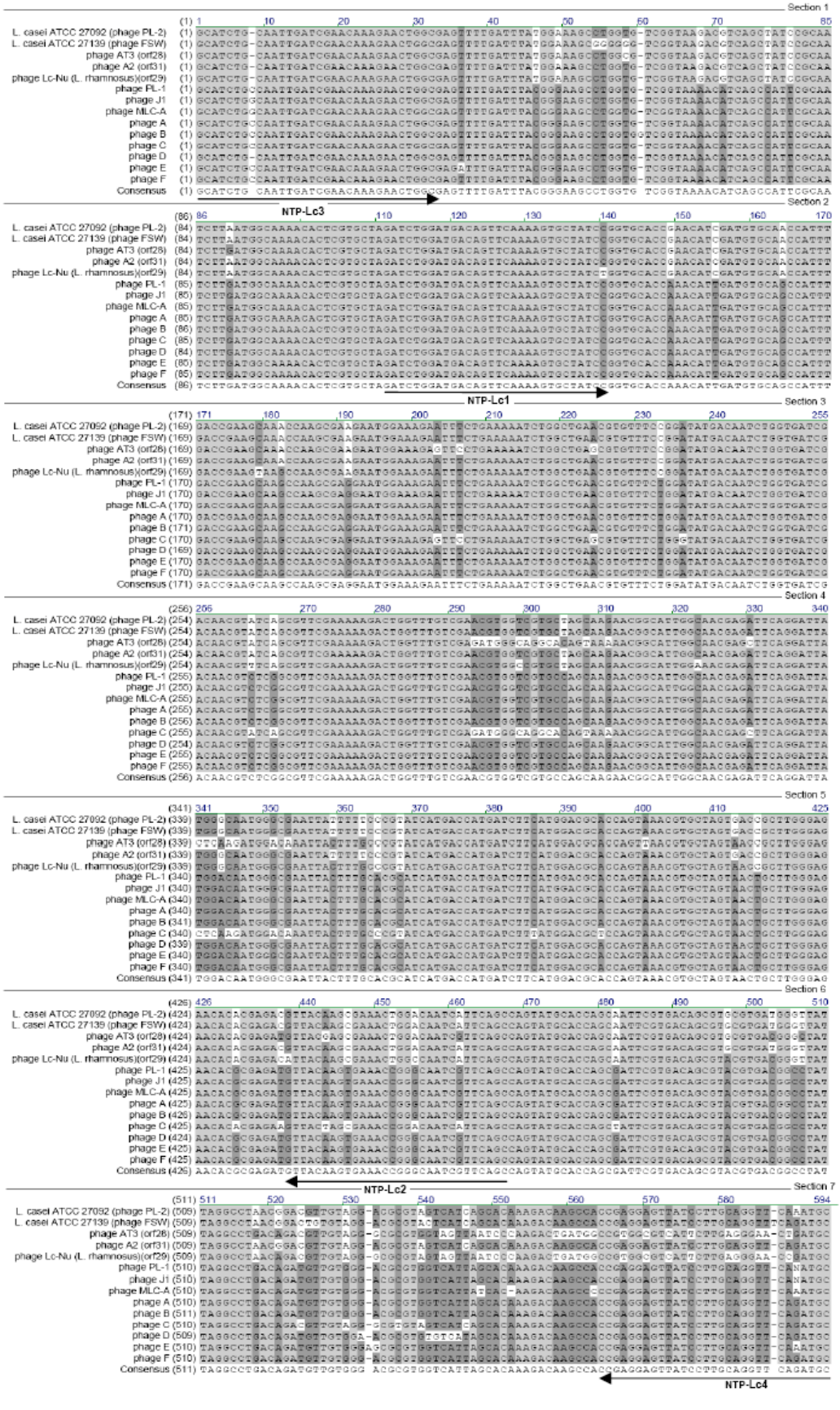

FIGURE 3 –

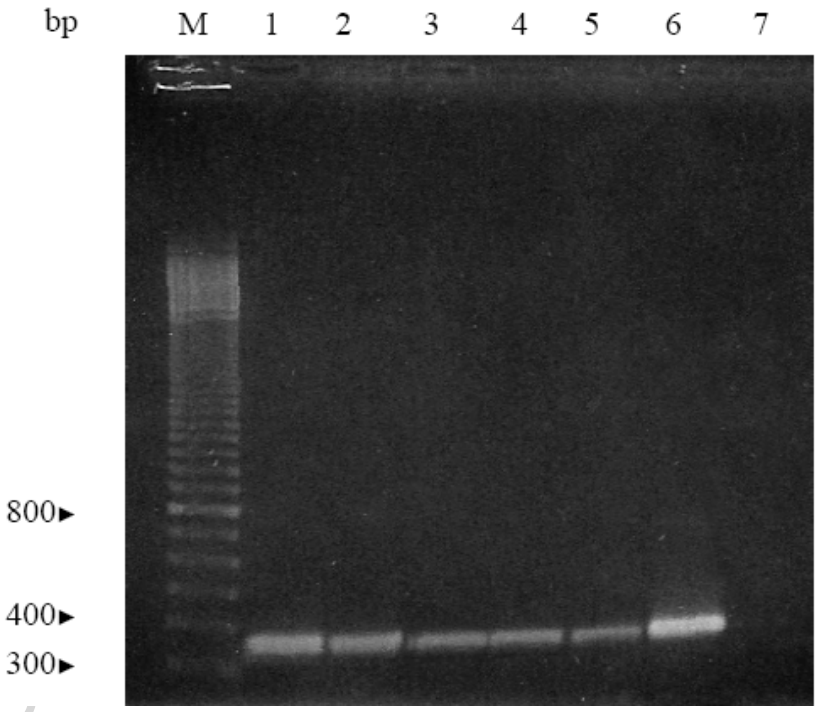

FIGURE 4 –