LWT - Food Science and Technology 67 (2016) 50–54

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Resistance of foodborne pathogen coliphages to additives applied in food manufacture

David Tomat [a, *], Claudia Balagué [a], Cecilia Casabonne [a], Roxana Verdini [b, c], Andrea Quiberoni [d]

[a] Área de Bacteriología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK, Rosario, Argentina
[b] Área de Bromatología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK, Rosario, Argentina
[c] Instituto de Química Rosario (UNR-CONICET), Suipacha 570, 2000, Rosario, Santa Fe, Argentina
[d] Instituto de Lactología Industrial (UNL - CONICET), Facultad de Ingeniería Química, Santiago del Estero 2829, 3000, Santa Fe, Argentina

ARTICLE INFO

Article history:
Received 4 August 2015
Received in revised form
5 November 2015
Accepted 17 November 2015
Available online 23 November 2015

Keywords:
Bacteriophage
Phage viability
Food additive
Escherichia coli

ABSTRACT

In order to evaluate whether coliphages can be used in combination with food additives, six lytic phages against pathogenic *Escherichia coli* strains were tested for their resistance to additives commonly used in the dairy and meat industries. All the phages evaluated were completely inactivated after a 1-min incubation at 25 °C when exposed to acetic and lactic acids at 4% v/v without pH adjustment, whereas phage viability remained unchanged when pH was adjusted to 5.0 (acetic) and 4.5 (lactic). Likewise, the six phages proved to be highly resistant to both acetate and lactate (4%; sodium salts) after a 24-h incubation. When phage viability was evaluated at 25 °C in Tris-Magnesium-Gelatin buffer supplemented with nitrite (0.015% w/v), phage titers were never below 7–8 $\log_{10}$ PFU ml^{-1} for all the phages tested. Regarding the influence of additives added to dairy products on phage viability, each phage (10^{7-8} PFU ml^{-1}) challenged with nisin (0.25 mg ml^{-1}) remained viable after a 24 h-incubation. In addition, phage viability was either slightly affected or not affected at all when phages were exposed to chymosin. These results proved that phages can be used against pathogenic *E. coli* strains along with other additives as an additional hurdle in order to improve food safety.

1. Introduction

Foodborne diseases caused by pathogenic *Escherichia coli* (*E. coli*) strains are a serious and growing problem. This pathogen has been responsible for hemolytic uremic syndrome cases since 1980 (Karmali et al., 1985). Foodborne bacteria can contaminate food products at any point along the chain production - during slaughtering, milking, storage or packaging (García, Martínez, Obeso, & Rodríguez, 2008). Therefore, several food additives such as weak acids (Ouattara, Simard, Holley, Piette, & Bégin, 1997), nitrite (Honikel, 2008), and nisin (Gharsallaoui, Joly, Oulahal, & Degraeve, 2015) are used at different stages of production in order to ensure food quality and safety. Regarding the maximum concentration allowed in foodstuff, most of these additives are strictly regulated (FDA, 2000; CAA, 2010) as they may be toxic, *e.g.* nitrite (Honikel, 2008), cause alteration of the organoleptic characteristics of food, *e.g.* weak acids at higher concentrations (Kotula & Thelappurate, 1994) or by the activity needed to achieve a high quality product, *e.g.* chymosin (Vallejo, Ageitos, Poza, & Villa, 2012). Although these additives are widely used and accepted, and numerous publications have documented the effectiveness of food preservatives against *E. coli* (Yoder et al., 2012) and other pathogens (Glass et al., 2002; Michaelsen, Sebranek, & Dickson, 2006), novel strategies, such as the use of phages, are needed to fulfill consumer demands for food with lower amounts of chemical compounds. Furthermore, additives are less specific than phages, affecting both foodborne pathogens as well as the normal microflora of food due to their nonspecific mechanisms of action (Kin et al., 2011).

The application of bacteriophages in food safety has been extensively documented against pathogenic *E. coli* strains as well as other foodborne pathogens such as *Salmonella*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Enterobacter sakazakii*, and *Staphylococcus aureus* (Tomat, Mercanti, Balagué, & Quiberoni, 2013a).

* Corresponding author. Área de Bacteriología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (U.N.R), Suipacha 531, S2002LRK, Rosario, Santa Fe, Argentina. Tel.: +54 341 4804592 int. 206.
E-mail address: dtomat@fbioyf.unr.edu.ar (D. Tomat).

http://dx.doi.org/10.1016/j.lwt.2015.11.031
0023-6438/

However, the action of food additives on phage viability was not assessed in most of the studies carried out *in situ*, namely in the food matrix.

There are studies analyzing the effect of acetic acid on phage expression (Wallin-Carlquist et al., 2010), and the effect of lactic acid on phage viability (García, Madera, Martinez, & Rodriguez, 2007), as well as other works where phages are characterized by their acid resistance (Coffey et al., 2011). However, articles reporting the effect of food additives on the infectivity of coliphages are scarce. Several authors have studied different combination of antimicrobials such as bacteriocins and phages (Leverentz et al., 2003; Ly-Chatain, Moussaoui, Vera, Rigobello, & Demarigny, 2013), bacteriocins and endolysins (Schmelcher, Powell, Becker, Camp, & Donovan, 2012), and bacteriocins and essential oils (Bajpai, Yoon, Bhardwaj, & Kang, 2014). However, most of these studies were focused on phages of LAB (Ly-Chatain et al., 2013), *L. monocytogenes* (Leverentz et al., 2003) and *S. aureus* (Martínez, Obeso, Rodríguez, & García, 2008). Regarding studies on phages affecting pathogenic *E. coli* strains, Ly-Chatain et al. (2013) were the only authors who analyzed the antiviral activity of several cationic compounds, specifically nisin, against the bacteriophage MS2, a phage infecting *E. coli* strains, and found a weak antiviral effect (1 $\log_{10}$ reduction after 10 min) only at the highest concentration of nisin (100,000 IU) tested.

In previous studies, phages have proved to be efficient biocontrol agents of pathogenic *E. coli* strains (Tomat, Migliore, Aquili, Quiberoni, & Balagué, 2013b; Tomat, Mercanti, Balagué, & Quiberoni, 2013c; Tomat, Quiberoni, Mercanti, & Balagué, 2014) and to be highly resistant to thermal and physicochemical treatments (Tomat, Balagué, Casabonne, Verdini, & Quiberoni, 2015). Studies on the interaction (*e.g.* challenges) of coliphages with food additives, such as weak acids and their sodic salts, nitrite, and chymosin, have not yet been carried out. The aim of this study was to evaluate the influence of additives added to meat and dairy products on phage viability in order to determine whether they can be used simultaneously as a hurdle technology in the biocontrol of pathogenic *E. coli* strains.

2. Materials and methods

2.1. Bacterial strains and phages

E. coli DH5α was used as the sensitive host strain to propagate all the bacteriophages used in this study. DH5α was maintained as frozen (−80 °C) stock in Hershey broth (8 g l^{-1} Bacto nutrient broth, 5 g l^{-1} Bacto peptone, 5 g l^{-1} NaCl and 1 g l^{-1} glucose) (Difco, Detroit, Michigan, USA) (Cicarelli, San Lorenzo, Santa Fe, Argentina) supplemented with 15% (v/v) glycerol and routinely reactivated overnight at 37 °C in Hershey broth.

Bacteriophages DT1, DT2, DT3, DT4, DT5 and DT6 were isolated from stool samples of patients with diarrhea treated at the Centenary Hospital, Rosario (Tomat et al., 2013c). High-titre phage suspensions were prepared as previously described (Tomat et al., 2013c). Namely, Hershey-Mg broth was inoculated (1%, v/v) with an overnight culture of DH5α, aliquots (100 μl) of phage stocks were added, incubated (37 °C) with shaking until complete lysis. Next, chloroform was added (0.1 ml) and cultures centrifuged at 4000 g for 10 min. Phage stocks were stored at 4 °C and enumerated (plaque-forming units per millilitre; PFU ml^{-1}) by the double-layer plaque technique. Briefly, aliquots of 100 μl of phage stocks were mixed with 100 μl of recipient strain culture (OD_{600} = 1.0), then added with three ml of Hershey-Mg soft agar (Hershey-Mg with 0.7% agar, w/v) at 45 °C. The mixture was poured into plates with Hershey-Mg agar (1.4%, w/v) and incubated overnight at 37 °C (Jamalludeen et al., 2007).

2.2. Viability studies - additives applied in meat products -

2.2.1. Influence of acetic and lactic acid

Phages ($10^7 - 10^8$ PFU ml^{-1}) were suspended in Tris-magnesium gelatin (TMG) buffer (10 mM Tris-Cl, 10 mM $MgSO_4$ and 0.1% (w/v) gelatin) supplemented with acetic (pH 2.72) and lactic (pH 2.28) acid at 4% v/v without pH adjustment. In addition, further assays were carried out with acetic (pH 5.0) and lactic (pH 4.5) acid at 4% v/v with pH adjusted to the same values which result from treating meat with each acid in *in-vitro* preliminary studies.

After each incubation time, namely without (1 and 5 min) and with (1, 8 and 24 h) pH adjustment, at 25 °C, phage suspensions were enumerated by the double-layer plate titration method (Jamalludeen et al., 2007). Assays were carried out in triplicate.

2.2.2. Influence of acetate and lactate (sodium salts)

The influence of acetate and lactate on phage ($10^7 - 10^{-8}$ PFU ml^{-1}) viability was investigated by incubation at 25 °C in TMG buffer supplemented with sodium acetate (4% w/v) or sodium lactate (4% v/v) with the pH adjusted to 5.7, which represents the natural pH of meat. After incubation for 1, 8 and 24 h, phage viability was determined as described above (Jamalludeen et al., 2007). Assays were carried out in triplicate.

2.2.3. Influence of nitrite

The influence of nitrite (sodium salt) on phage ($10^7 - 10^{-8}$ PFU ml^{-1}) viability was investigated by incubation at 25 °C in TMG buffer supplemented with nitrite (0.015% w/v; maximum concentration allowed) (CAA, 2005). After incubation for 1, 8 and 24 h, phage viability was determined as described above (Jamalludeen et al., 2007). Assays were carried out in triplicate.

2.3. Viability studies - additives applied in dairy products -

2.3.1. Influence of nisin

The influence of nisin on phage ($10^7 - 10^8$ PFU ml^{-1}) viability was investigated by incubation at 25 °C in TMG buffer supplemented with nisin (Nisaplin, nisin 2.5% w/w, 1 million IU g^{-1}) at 0.25 mg ml^{-1} (maximum concentration allowed; FDA, 2001). After incubation for 1, 8 and 24 h, phage particles were enumerated as described above (Jamalludeen et al., 2007) and the counts were compared to those at control (TMG) conditions. Assays were carried out in triplicate.

2.3.2. Influence of chymosin

Phages ($10^7 - 10^8$ PFU ml^{-1}) were suspended in TMG buffer supplemented with chymosin (Maxiren 150, 100% chymosin, rennet strength 150,000 IMCU ml^{-1}) at 8.0 mg ml^{-1}. The suspension was incubated at 25 °C. After incubation for 1, 8 and 24 h, phage particles were enumerated as described above (Jamalludeen et al., 2007) and the counts were compared to those at control (TMG) conditions. Assays were carried out in triplicate.

2.4. Statistical analysis

Means (three determinations) were compared using the one-way ANOVA procedure followed by Duncan's multiple range tests at $p < 0.05$.

3. Results and discussion

3.1. Influence of additives applied in meat products on phage viability

Food additives such as weak acids are widely used in the meat

industry with the aim of lowering surface bacterial load prior to packaging (Cherrington, Hinton, Mead, & Chopra, 1991; Ouattara et al., 1997; Ariyapitipun, Mustapha, & Clarke, 1999; Acuff, 2005). Specifically, acetic and lactic acids are regularly used to wash the meat surface while their sodic salts, acetate and lactate, are incorporated into a mass of ground meat in sausage production (FDA, 2000). Thus, the viability of each phage must be evaluated in order to determine whether these acids, or their salts, present in the meat matrix are an obstacle for the use of the phages under study as biocontrol tools.

First, when the six coliphages were tested *in vitro* for their resistance against acetic and lactic acids at 4% v/v without pH adjustment (2.72 and 2.28, respectively), all the phages evaluated were completely inactivated after a 1-min incubation at 25 °C, indicating a high sensitivity to low pH values. When viability was tested at acidic pH values, similar results were obtained for these coliphages (Tomat et al., 2015) as well as for others such as O157:H7 (Coffey et al., 2011), STEC (Dini & De Urraza, 2010), lactic acid bacteria (LAB) (Mercanti, Guglielmotti, Patrignani, Reinheimer, & Quiberoni, 2012) and *Mycobacterium* (Endersen et al., 2013) phages. Regarding treatments with organic weak acids, it is well-known that both acetic and lactic acid possess a biocide effect, *e.g.* they permeabilize gram-negative bacteria by disrupting the outer membrane (Alakomi, Skytta, Saarela, Mattila-Sandholm, Latva-Kala, & Helander, 2000). However, endurance of coliphages other than those analyzed in the present work remains to be assessed.

Next, the action of these acids on phage viability was also evaluated at 25 °C with pH adjustment. Namely, pieces of meat were treated with each acid and pH was determined after a 1-h incubation in order to determine the pH value reached in the food matrix. Unlike inactivation observed without pH adjustment, when phages were exposed to acetic and lactic acid, both at 4% v/v, with pH adjusted to 5.0 and 4.5, respectively, phage viability remained unchanged and no significant difference after 8 h was observed, although there was a sligh but significant reduction in most cases after a 24-h incubation (Table 1). Accordingly, acetic acid at pH 5.5 induced a *S. aureus* prophage and its subsequent replication, indicating that the phage remained viable (Wallin-Carlquist et al., 2010). On the other hand, the production of lactic acid by starter cultures and the concomitant reduction of pH negatively affected the viability of *S. aureus* phages since their titre declined by approx. 2 $\log_{10}$ PFU when the pH dropped from 6.19 to 5.38 (Garcia et al., 2007). It is noteworthy that, though phage inactivation depends on pH, concentration, and identity of the acidifying agent used (Alvarado & McKee, 2010), only the change in the pH value (pH adjusted) produced a significant impact on phage viability.

Acetate and lactate (sodium salts) are commonly used to replace their acids in order to prevent acidification leading to protein denaturation and color changes in meat products (Smulders & Greer, 1998; Kotula & Thelappurate, 1994; Lin & Chuang, 2001). At the concentration assayed in this study, neither acetate nor lactate were shown to change the pH value of meat (data not shown), namely ca. 5.7, as was also found by other authors at 0.25% (acetate) and 4.8% (lactate) (Alvarado & McKee, 2010). The effect of acetate and lactate on phage viability is shown in Fig. 1. The six phages (DT1 to DT6) showed high resistance to both acetate (Fig. 1A) and lactate (Fig. 1B) after a 24-h incubation since phage counts were never below 10^7 PFU ml^{-1} and no significant reductions were observed. These results proved the high resistance of the phages evaluated, which remain active and are useful biocontrol tools during the manufacture and after the packaging of meat products. Acetate and lactate are normally used to eliminate foodborne pathogens such as *L. monocytogenes* (Alvarado & McKee, 2010) and both acids are detrimental to the growth of *E. coli* (Kim et al., 2015); yet, no scientific data on the interaction between weak acids and coliphages has been found.

Food-grade biocides such as nitrite are widely used in the food industry, specifically in the manufacture of cold cuts and sausages, both as meat preservatives and color fixers (Sindelar & Milkowski, 2012). Regarding their influence on phages, the results of the present work showed that the presence of nitrite (0.015% w/v) had no significant effect after a 24-h incubation for all the phages evaluated, suggesting high resistance against this food additive (Fig. 2). Spores of *Clostridium botulinum* are the main reason for using nitrite; yet, it also has a bacteriostatic activity against other pathogenic strains such as *E. coli* (Gutiérrez-Cortés & Suarez-Mahecha, 2012). It is noteworthy that no assays on the viability of *E. coli* phages when challenged with nitrite have been carried out in order to determine whether their simultaneous use in a particular food matrix is possible.

3.2. Influence of additives applied in dairy products on phage viability

Several food additives are artificially added to dairy products (O'Sullivan, Ross, & Hill, 2002; EFSA., 2006), and although several of them - such as nisin and chymosin - are not expected to affect the viability of phages, their effect must be evaluated. Bacteriocins, produced by LAB strains, have been extensively studied for many years (O'Sullivan et al., 2002; Joerger, 2003). Specifically, nisin has the generally recognized as safe (GRAS) status granted by the Food and Drug Administration (FDA, 2001; 21 CFR 184.1538). Although there is abundant scientific literature regarding the use of bacteriocins in combination with phages (Leverentz et al., 2003; Martínez et al., 2008; Nascimento, Guerreiro-Pereira, Costa, Sao Jose, & Santos, 2008), each specific bacteriocin-phage system must be individually evaluated in order to determine whether they can be used together in a treatment.

Table 1
Phage viability in TMG buffer at 25 °C supplemented with acetic and lactic acid.

Phage	* Phage particles ($\times 10^7$) (PFU ml^{-1}) [α]					
	Acetic acid at 4% (pH 5.0)			Lactic acid at 4% (pH 4.5)		
	1 h	8 h	24 h	1 h	8 h	24 h
DT1	[a]1.74 ± 0.37	[a]1.39 ± 0.41	[b]0.86 ± 0.13	[a]1.97 ± 0.11	[a]1.89 ± 0.23	[b]0.83 ± 0.08
DT2	[a]4.41 ± 1.31	[a]4.02 ± 1.06	[b]3.16 ± 0.10	[a]4.18 ± 0.26	[a]4.18 ± 0.04	[b]3.28 ± 0.76
DT3	[a]6.46 ± 0.74	[a]6.17 ± 0.95	[a]5.78 ± 0.46	[a]7.36 ± 0.40	[a]6.72 ± 0.16	[b]4.33 ± 0.43
DT4	[a]7.59 ± 2.27	[a]7.18 ± 1.86	[a]5.58 ± 0.32	[a]7.94 ± 0.62	[a]7.46 ± 0.02	[b]4.72 ± 1.08
DT5	[a]6.18 ± 0.74	[a]5.56 ± 0.92	[b]4.19 ± 0.17	[a]5.89 ± 0.05	[a]4.72 ± 0.96	[b]3.21 ± 0.17
DT6	[a]2.87 ± 0.05	[a]2.70 ± 0.55	[b]1.69 ± 0.34	[a]3.18 ± 0.10	[a]2.95 ± 0.11	[b]1.80 ± 0.29

* Initial inoculum range ($\times 10^7$): DT1 = 1.81–2.12; DT2 = 4.08–4.36; DT3 = 6.21–6.84; DT4 = 6.71–7.08; DT5 = 5.12–5.14; DT6 = 2.46–3.01. [α] Mean value of three determinations ± standard deviation. Different letters indicate significant differences.

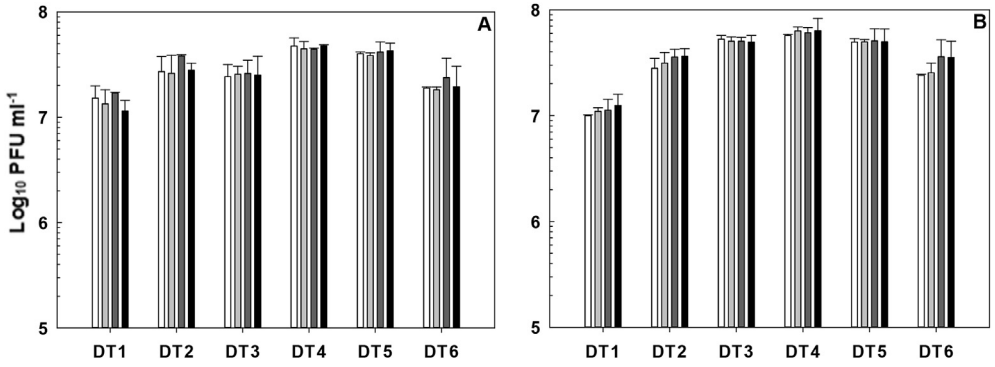

Fig. 1. Phage viability at 25 °C in Tris-magnesium gelatin (TMG) buffer supplemented with acetate (A) and lactate (B) (sodium salts; 4% w/v) at the beginning (□) and after 1 (▒), 8 (▒) and 24 h (■) of incubation. Error bars represent the standard deviation of three determinations ($p < 0.05$).

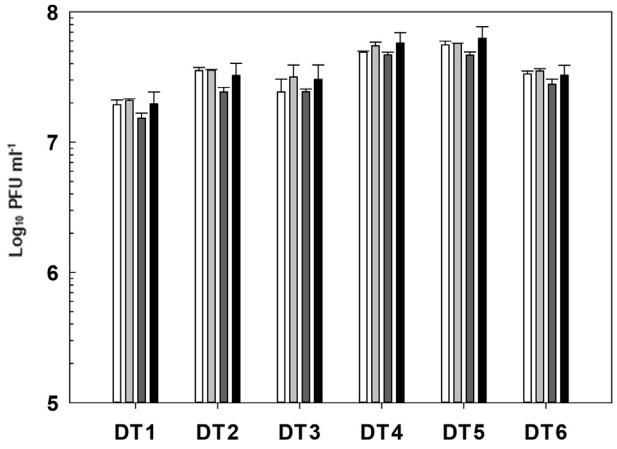

Fig. 2. Phage viability at 25 °C in TMG buffer supplemented with nitrite (0.015% w/v) at the beginning (□) and after 1 (▒), 8 (▒) and 24 h (■) of incubation. Error bars represent the standard deviation of three determinations ($p < 0.05$).

Our results indicated that phage viability was unaffected at 25 °C for all nisin/phage systems evaluated (Fig. 3A). Specifically, each phage - DT1 to DT6 - (~10^{7-8} PFU ml^{-1}) was challenged with nisin at 0.25 mg ml^{-1} and phage viability remained at ~ 7–8 log_{10} PFU ml^{-1}, showing no significant reductions after a 24-h incubation. Accordingly, Ly-Chatain et al. (2013) found similar results for the coliphage MS2 and the *Lactococcus lactis* phage c2, when challenged against nisin at 100,000 UI. In addition, Leverentz et al. (2003) observed that nisin and phage complemented each other for the control of *L. monocytogenes*, showing a synergistic effect, rather than mutually interfering or being inactivated, as it was found for other combinations of antimicrobials such as endolysins and bacteriocins (Becker, Foster-Frey, & Donovan, 2008; Schmelcher et al., 2012) and nisin and essential oils (Bajpai et al., 2014).

Regarding the influence of chymosin, a widely used additive in the dairy industry (Vallejo et al., 2012), on phage viability, the six phages (~10^{7-8} PFU ml^{-1}) evaluated were slightly affected or insensitive - not significantly reduced - after a 24-h incubation, even at concentrations 10-fold higher (8 mg ml^{-1}) than those commonly used, although the phage count was always higher than 7 log_{10} PFU ml^{-1} (Fig. 3B). Previous studies related to the interaction between dairy additives and phages had shown that the viability of LAB phages (Keogh & Pettingill, 1983; Emond & Moineau, 2007) and the phages evaluated in the present work (*e.g.* Ca2+) (Tomat et al., 2015) were not affected. However, the chymosin-coliphage systems had never been evaluated. As for other phages, no viability data against chymosin were found. Results of the present work showed that phage viability, in the presence of routinely used additives such as nisin and chymosin, was unaffected for all the phages tested.

4. Conclusion

Our study showed that the six bacteriophages, lytic against pathogenic *E. coli* strains, resisted well when they were challenged with different additives used in the food industry. These results imply that the coliphages evaluated can be used simultaneously with the additives assayed as an additional hurdle in order to maximize food safety, although further experiments should be conducted in real food environments.

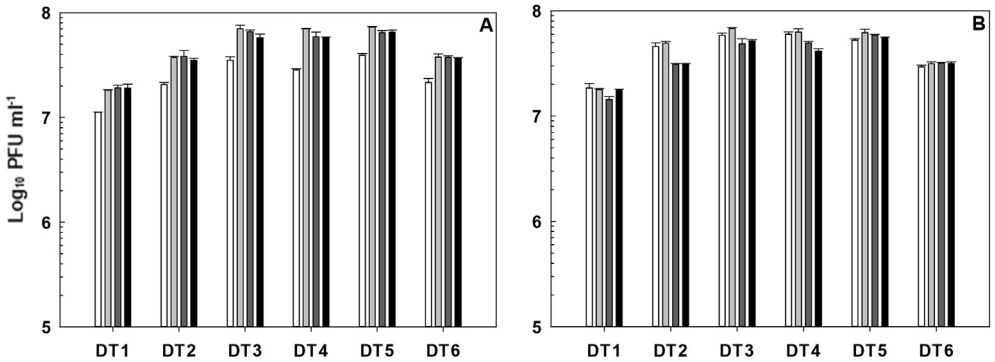

Fig. 3. Phage viability at 25 °C in TMG buffer supplemented with nisin (A) (0.25 mg ml^{-1}) and chymosin (B) (8.0 mg ml^{-1}) at the beginning (□) and after 1 (▒), 8 (▒) and 24 h (■) of incubation. Error bars represent the standard deviation of three determinations ($p < 0.05$).

It is noteworthy that the viability of coliphages had not been previously challenged with most of the food additives tested, especially with those additives used in meat products.

Acknowledgments

This research was supported by the Agencia Nacional de Promoción Científica y Tecnológica (Project FONARSEC – FITS Health 001 – 2012–2015) of Argentina. We would like to thank Maria Robson, Mariana de Sanctis, Geraldine Raimundo, Carolina Perret and Romina Ricardo for their assistance in the language correction.

References

Acuff, G. R. (2005). Chemical decontamination strategies for meat. In J. N. Sofos (Ed.), *Improving the safety of fresh meat* (pp. 351–363). New York: Woodhead Publishing Limited. CRC Press.

Alvarado, C., & McKee, S. (2010). Control de *Listeria monocitogenes* marinando con Ácidos Orgánicos. *Mundo Lácteo y Cárnico*, 12–14.

Ariyapitipun, T., Mustapha, A., & Clarke, A. D. (1999). Microbial shelf life determination of vaccum-packaged fresh beef treated with polilactic acid, lactic acid and nisin solutions. *Journal of Food Protection, 62*(8), 913–920.

Bajpai, V., Yoon, J., Bhardwaj, M., & Kang, S. (2014). Anti-listerial synergism of leaf essential oil of *Metasequoia glyptostroboides* with nisin in whole, low and skim milks. *Asian Pacific Journal of Tropical Medicine*, 602–608.

Becker, S., Foster-Frey, J., & Donovan, D. (2008). The phage K lytic enzyme LysK and lysostaphin act synergistically to kill MRSA. *FEMS Microbiology Letters, 287*, 185–191.

Cherrington, C. A., Hinton, M., Mead, G. C., & Chopra, I. (1991). Organic acids: chemistry, antibacterial activity and practical applications. *Advances in Microbial Physiology, 32*, 87–108.

Código Alimentario Argentino (CAA). (2005). *Alimentos cárneos y afines*. Capítulo VI http://www.msal.gov.ar/argentina-saludable/pdf/CAPITULO_VI.pdf.

Código Alimentario Argentino (CAA). (2010). *Aditivos alimentarios*. Capítulo XVIII http://www.anmat.gov.ar/alimentos/codigoa/capitulo_xviii.pdf.

Coffey, B., Rivas, L., Duffy, G., Coffey, A., Ross, R. P., & McAuliffe, O. (2011). Assessment of *Escherichia coli* O157:H7-specific bacteriophages e11/2 and e4/1c in model broth and hide environments. *International Journal of Food Microbiology, 147*, 188–194.

Dini, C., & De Urraza, P. J. (2010). Isolation and selection of coliphages as potential biocontrol agents of enterohemorrhagic and Shiga toxin-producing *E. coli* (EHEC and STEC) in cattle. *Journal of Applied Microbiology, 109*, 873–887.

EFSA.. (2006). The use of nisin (E 234) as a food additive. *The EFSA Journal, 314*, 1–16.

Emond, E., & Moineau, S. (2007). Bacteriophages and food fermentations. In S. McGrath, & D. van Sinderen (Eds.), *Bacteriophage: Genetics and molecular biology* (pp. 93–124). Horizon Scientific Press/Caister Academic Press.

Endersen, L., Coffey, A., Neve, H., McAuliffe, O., Paul Ross, R., & O'Mahony, J. (2013). Isolation and characterization of six novel mycobacteriophages and investigation of their antimicrobial potential in milk. *International Dairy Journal, 28*(1), 8–14.

Food and Drug Administration, U.S. (2000). Food additives for use in meat and poultry products: sodium diacetate, sodium acetate, sodium lactate, and potassium lactate. *Federal Register, 65*(13), 3121–3123.

Food and Drug Administration, U.S. (2001). *Agency response letter GRAS notice N°*. Department of Health and Human Services. GRN 000065, April 20 2001.

García, P., Madera, C., Martinez, B., & Rodriguez, A. (2007). Biocontrol of *Staphylococcus aureus* in curd manufacturing processes using bacteriophages. *International Dairy Journal, 17*, 1232–1239.

García, P., Martínez, B., Obeso, J. M., & Rodríguez, A. (2008). Bacteriophages and their application in food safety. *Letters in Applied Microbiology, 47*, 479–485.

Gharsallaoui, A., Joly, C., Oulahal, N., & Degraeve, P. (2015). Nisin as a food preservative: part 2: antimicrobial polymer materials containing nisin. *Critical Reviews in Food Science and Nutrition*. http://dx.doi.org/10.1080/10408398.2013.763766.

Glass, K. A., Granberg, D. A., Smith, A. L., McNamara, A. M., Hardin, M., & Mattias, J. (2002). Inhibition of *Listeria monocytogenes* by sodium diacetate and sodium lactate on wieners and cooked bratwurst. *Journal of Food Protection, 65*, 116–123.

Gutiérrez-Cortés, C., & Suarez-Mahecha, H. (2012). Efecto conservante de propóleos en chorizos. *Vitae, 19*(1). S1559–S161.

Honikel, K. O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science, 78*(1–2), 68–76.

Jamalludeen, N., Jonson, R. P., Friendship, R., Kropinski, A. M., Lingohr, E. J., & Gyles, C. L. (2007). Isolation and characterization of nine bacteriophages that lyse O149 enterotoxigenic *Escherichia coli*. *Veterinary Microbiology, 124*, 47–57.

Joerger, R. D. (2003). Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. *Poultry Science, 82*, 640–647.

Karmali, M. A., Petric, M., Lim, C., Fleming, P. C., Arbus, G. S., & Lior, H. (1985). The association between idiopathic haemolytic uraemic syndrome and infection by verotoxin producing *E. coli*. *Journal of Infectious Diseases, 151*, 775–782.

Keogh, B., & Pettingill, G. (1983). Adsorption of bacteriophage eb7 on *Streptococcus cremoris* eb7. *Applied and Environmental Microbiology, 45*(6), 1946–1948.

Kim, T., Jung, H., Kim, S., Zhang, L., Li, J., Sigdel, S., et al. (2015). Reduction of acetate and lactate contributed to enhancement of a recombinant protein production in *E. coli* BL21. *Journal of Microbiology and Biotechnology, 25*(7), 1093–1100.

Kin, S., Schilling, M. W., Smith, B. S., Silva, J. L., Kim, T., Pham, A. J., et al. (2011). Potassium acetate and potassium lactate enhance the microbiological and physical properties of marinated catfish fillets. *Journal of Food Science, 76*(4), S242–S250.

Kotula, K. L., & Thelappurate, R. (1994). Microbiological and sensory attributes of retail cuts of beef treated with acetic and lactic acid solutions. *Journal of Food Protection, 57*, 665–670.

Leverentz, B., Conway, W., Camp, M., Janisiewicz, W., Abuladze, T., Yang, M., et al. (2003). Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. *Applied and Environmental Microbiology, 69*(8), 4519–4526.

Lin, K. W., & Chuang, C. H. (2001). Effectiveness of dipping with phosphate, lactate and acetic acid solutions on the quality and shelf-life of pork loin chop. *Journal of Food Science, 66*(3), 494–499.

Ly-Chatain, M., Moussaoui, S., Vera, A., Rigobello, V., & Demarigny, Y. (2013). Antiviral effect of cationic compounds on bacteriophages. *Frontiers in Microbiology, 4*(46), 1–6.

Martínez, B., Obeso, J. M., Rodríguez, A., & García, P. (2008). Nisin-bacteriophage crossresistance in *Staphylococcus aureus*. *International Journal of Food Microbiology, 122*(3), 253–258.

Mercanti, D. J., Guglielmotti, D. M., Patrignani, F., Reinheimer, J. A., & Quiberoni, A. (2012). Resistance of two temperate *Lactobacillus paracasei* bacteriophages to high pressure homogenization, thermal treatments and chemical biocides of industrial application. *Food Microbiology, 29*, 99–104.

Michaelsen, A. R., Sebranek, J. G., & Dickson, J. S. (2006). Effects of microbial inhibitors and modified atmosphere packaging on growth of *Listeria monocytogenes* and *Salmonella enterica* typhimurium and on quality attributes of injected pork chops and sliced cured ham. *Journal of Food Protection, 69*(11), 2671–2680.

Nascimento, J., Guerreiro-Pereira, M., Costa, S., Sao Jose, C., & Santos, M. (2008). Nisin-triggered activity of Lys44, the secreted endolysin from *Oenococcus oeni* phage fOg44. *Journal of Bacteriology, 190*(1), 457–461.

Ouattara, B., Simard, R., Holley, R., Piette, G., & Bégin, A. (1997). Inhibitory effect of organic acids upon meat spoilage bacteria. *Journal of Food Protection, 60*(3), 246–253.

O'Sullivan, L., Ross, R. P., & Hill, C. (2002). Potential of bacteriocin producing lactic acid bacteria for improvements in food safety and quality. *Biochimie, 84*, 593–604.

Schmelcher, M., Powell, A., Becker, S., Camp, M., & Donovan, D. (2012). Chimeric phage lysins act synergistically with lysostaphin to kill mastitis-causing *Staphylococcus aureus* in murine mammary glands. *Applied and Environmental Microbiology, 78*(7), 2297–2305.

Sindelar, J. J., & Milkowski, A. L. (2012). Human safety controversies surrounding nitrate and nitrite in the diet. *Nitric Oxide, 26*(4), 259–266.

Smulders, F. J., & Greer, G. G. (1998). Integrating microbial decontamination with organic acid in HACCP programmes from muscle foods: prospects and controversies. *Journal of Food Microbiology, 44*, 149–169.

Tomat, D., Balagué, C., Casabonne, C., Verdini, R., & Quiberoni, A. (2015). Resistance of foodborne pathogen coliphages to thermal and physicochemical treatments applied in food manufacture. *Innovative Food Science and Emerging Technologies, 30*, 184–191.

Tomat, D., Mercanti, D., Balagué, C., & Quiberoni, A. (2013a). Bacteriophages as biocontrol tools of pathogens in meat and dairy products. In Clark Denton, & Richard J. Crosby (Eds.), *Bacteriophages: Biology, applications and role in health and disease* (pp. 73–91). Hauppauge, NY, USA: Nova Science Publishers.

Tomat, D., Mercanti, D., Balagué, C., & Quiberoni, A. (2013c). Phage biocontrol of enteropathogenic and Shiga toxin-producing *Escherichia coli* during milk fermentation. *Letters in Applied Microbiology, 57*, 3–10.

Tomat, D., Migliore, L., Aquili, V., Quiberoni, A., & Balagué, C. (2013b). Phage biocontrol of enteropathogenic and Shiga toxin-producing *Escherichia coli* in meat products. *Frontiers in Cellular and Infection Microbiology, 3*(20), 1–10.

Tomat, D., Quiberoni, A., Mercanti, D., & Balagué, C. (2014). Hard surfaces decontamination of enteropathogenic and Shiga toxin-producing *Escherichia coli* using bacteriophages. *Food Research International, 57*, 123–129.

Vallejo, J. A., Ageitos, J. M., Poza, M., & Villa, T. G. (2012). Short communication: a comparative analysis of recombinant chymosins. *Journal of Dairy Science, 95*(2), 609–613.

Wallin-Carlquist, N., Cao, R., Marta, D., Sant'Ana da Silva, A., Schelin, J., & Radstrom, P. (2010). Acetic acid increases the phage-encoded enterotoxin A expression in Staphylococcus aureus. *BMC Microbiology, 10*, 147.

Yoder, S. F., Henning, W. R., Mills, E. W., Doores, S., Ostiguy, N., & Cutter, C. N. (2012). Investigation of chemical rinses suitable for very small meat plants to reduce pathogens on beef surfaces. *Journal of Food Protection, 75*(1), 14–21.