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ORIGINAL ARTICLE

Isolation and preliminary characterization of *Lactobacillus plantarum* bacteriophages from table olive fermentation

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Abstract Nine table olive fermentation brines were analysed to demonstrate the presence of lytic bacteriophages active against *Lactobacillus plantarum* strains. Five brines, out of the nine studied, were added to selected strains of *L. plantarum* as starter cultures, while the other four brines were left to ferment spontaneously. Turbidity tests with different host strains inoculated in growth medium with added filter-sterilized brines demonstrated the presence of phages able to lyse the sensitive strains. Phages were isolated from fermented brines and their presence was confirmed by scanning electron microscopy. A subsequent phage characterization based on host range, restriction analysis and protein profiles was performed. This study reported for the first time the isolation and characterization of *L. plantarum* phages from table olive fermentation. This demonstrated presence of lytic phages active against *L. plantarum* could represent a serious obstacle in the regular table olive fermentation process. For these reasons, the search for phage-resistant strains, to use as starter cultures, could be important to counteract the development of fermentation problems.

Keywords Lactic acid bacteria · *Lactobacillus plantarum* · Phage infection · Table olive fermentation

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Introduction

Lactic acid bacteria (LAB) are among the most important groups of micro-organisms used in food fermentations (Giraffa 2004; Leroy and De Vuyst 2004). LAB contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing large amounts of lactic acid and other growth-inhibiting substances (bacteriocins).

Table olives (*Olea europaea* L.) are fermented products that are largely diffused (Garrido Fernandez et al. 1997). The most important production zones of table olives are located in the Mediterranean area, but they are consumed on a large scale all over the world, and their consumption is expanding, due to the increasing popularity of the Mediterranean diet. Olives are picked at different stages of maturity and, after harvesting, they are processed to eliminate the characteristic bitterness caused by the presence of the oleuropein glucoside and to become suitable for human consumption. To obtain naturally fermented olives (Greek-style), the fruits are directly placed into a solution of 6–8% sodium chloride (brine) in which a fermentation takes place. The hydrolysis of oleuropein is attributed to the enzymatic reactions of the indigenous microorganisms by their β-glucosidase and esterase activity (Ciafardini et al. 1994; Marsilio et al. 1996; Marsilio and Lanza 1998), in particular the spontaneous fermentation of green olives depends on LAB, mostly represented by *Lactobacillus plantarum,* and yeasts that dominate the brines. LAB may also inhibit undesirable microorganisms, increasing the safety of end product (Ruiz-Barba et al. 1994; Brito et al. 2004; Rubia-Soria et al. 2006). In a controlled fermentation, selected bacteria are added as starter cultures (single or mixed starter cultures), mainly to degrade the olive bitter glucoside (oleuropein) but also to produce desirable acids and alcohols, offering organoleptic and nutritional advantages (Leal-Sanchez et al. 2002; Panagou et al. 2003; Marsilio et al.

Table 1 Host range of the nine *L. plantarum* bacteriophages

Strain	Source	Bacteriophage								
		Φ S1 (B1)	Φ S2 (B126)	Φ S3 (B165)	Φ S4 (B142)	Φ S5 (B3)	Φ S6 (B130)	Φ S7 (B138)	Φ S8 (B19)	Φ S9 (B136)
Isolated from olive cultivar:										
B1	unknown	1[a]	1	1	1	1	1	1	1	1
B2	unknown	1	1	1	1	1	1	1	1	1
B3	I77	1	1	1	1	1	1	1	1	1
B7	unknown	1	1	1	1	1	1	1	1	1
B10	Ascolana tenera	1	1	1	1	1	1	1	1	1
B13	Picholine	R[b]	R	R	R	R	R	R	R	R
B15	Picholine	R	R	R	R	R	R	R	R	R
B17	Leccino	R	R	R	R	R	R	R	R	R
B19	S. Agostino	1	1	1	1	1	1	1	1	1
B27	Coratina	R	R	R	R	R	R	R	R	R
B28	Coratina	R	R	R	R	R	R	R	R	R
B39	Cassanese	R	R	R	R	R	R	R	R	R
B44	S. Caterina	R	R	R	R	R	R	R	R	R
B51	Cucco	R	R	R	R	R	R	R	R	R
B53	Cucco	R	R	R	R	R	R	R	R	R
B124	S. Agostino	R	R	R	R	R	R	R	R	R
B126	Nocellara del Belice	1	1	1	1	1	1	1	1	1
B130	Ascolana tenera	1	1	1	1	1	1	1	1	1
B136	Nocellara del Belice	1	1	1	1	1	1	1	1	1
B138	Bella di Cerignola	1	1	1	1	1	1	1	1	1
B142	Intosso	1	1	1	1	1	1	1	1	1
B146	Ascolana tenera	1	1	1	1	1	1	1	1	1
B165	Nocellara etnea	1	1	1	1	1	1	1	1	1
Isolated from cheese:										
Lp751	Pecorino toscano	R	R	R	R	R	R	R	R	R
Lp752	Pecorino toscano	R	R	R	R	R	R	R	R	R
Lp754	Pecorino toscano	R	R	R	R	R	R	R	R	R
Lp755	Pecorino toscano	R	R	R	R	R	R	R	R	R
Lp790	Morlacco cheese	R	R	R	R	R	R	R	R	R
Lp791	Canestrato pugliese	R	R	R	R	R	R	R	R	R
Lp793	Canestrato pugliese	R	R	R	R	R	R	R	R	R
Lp794	Monte veronese	R	R	R	R	R	R	R	R	R
Lp797	Morlacco	R	R	R	R	R	R	R	R	R
Lp799	Monte veronese	R	R	R	R	R	R	R	R	R
Lp800	Morlacco	R	R	R	R	R	R	R	R	R
Lp803	Canestrato pugliese	R	R	R	R	1	1	1	1	1
Lp804	Monte veronese	R	R	R	R	R	R	R	R	R
Lp805	Monte veronese	R	R	R	R	R	R	R	R	R
Lp813	Spressa	R	R	R	R	1	1	1	1	1
Lp814	Canestrato pugliese	1	1	1	1	1	1	1	1	1
Lp14	Argentinean	R	R	R	R	R	R	R	R	R
Lp994	Argentinean	R	R	R	R	R	R	R	R	R
Lp995	Argentinean	1	1	1	1	R	R	R	R	R
Lp996	Argentinean	1	1	1	1	R	R	R	R	R
Lp997	Argentinean	1	1	1	1	R	R	R	R	R
Lp998	Argentinean	1	1	1	1	R	R	R	R	R

Table 1 (continued)

Strain	Source	Bacteriophage								
		Φ S1 (B1)	Φ S2 (B126)	Φ S3 (B165)	Φ S4 (B142)	Φ S5 (B3)	Φ S6 (B130)	Φ S7 (B138)	Φ S8 (B19)	Φ S9 (B136)
Lp999	Argentinean	1	1	1	1	R	R	R	R	R
Lp885	Pannerone	R	R	R	R	R	R	R	R	R
From commercial collections:										
DSM 20174^T	DSM collection	R	R	R	R	R	R	R	R	R
LMG 9211	LMG collection	R	R	R	R	R	R	R	R	R

Strain used to isolate and propagate the bacteriophage in parentheses

[a] *1* Lysis after the first infection with the filter-sterilized brine sample

[b] *R* No lysis after three consecutive subcultures

2005; Servili et al. 2006). The growth of the starter culture could be influenced by physico-chemical factors such as temperature or the presence of phenolic compounds and by microbiological factors such as the presence of bacteriophages (Lanza et al. 2009; Zago et al. 2003). In vegetable fermentations, *L. plantarum* bacteriophages were isolated from cucumber (Lu et al. 2003a) and commercial sauerkraut fermentations (Lu et al. 2003b; Yoon et al. 2002), and were active against fermentation isolates and selected strains, including those used as starter cultures. Bacteriophages that infect *L. plantarum* have also been isolated from meat (Trevors et al. 1983), silage (Caso et al. 1995; Doi et al. 2003), homemade cheese whey (Caso et al. 1995), fermented maize and coffee (Chibanni-Chennoufi et al. 2004) and kefir grains (De Antoni et al. 2010).

The aim of the present work was to search for the presence of *L. plantarum* bacteriophages in table olive fermentations, both spontaneously fermented and inoculated with strains of *L. plantarum* selected for oleuropeinolytic activity as starter culture. This is the first report of isolation and characterization of *L. plantarum* phages from table olive fermentation.

Materials and methods

Bacterial strains, bacteriophages and culture media

Lactobacillus plantarum strains and phages used or isolated in this study are listed in Table 1. Twenty-three strains were isolated from olive cultivars, 24 from cheeses (belonging to CRA-OLI and CRA-FLC collections, respectively), and 2 were reference strains (DSM 20174^T and LMG 9211). Strains and phages were maintained as frozen stocks at −80°C in the presence of 15% (v/v) glycerol as cryoprotective agent. MRS (Oxoid, Milan, Italy) broth and agar, supplemented with 10 mM-$CaCl_2$ when specified (MRS-Ca), were routinely used to grow and plate bacteria or to propagate and count phages at 30°C.

Olive processing and bacteriophage isolation

Fruits from *Olea europaea* of four cultivars were hand-harvested at their mature-green stage of ripening (on mid-October) and processed as "natural green olives" according to the *Trade Standard Applying to Table Olives* (IOOC 2004). Nine fermentation trials were carried out: four olive samples were left to ferment spontaneously in a 7% NaCl solution (w/v), whereas the other five olive samples, after 1 week of brining, were inoculated with selected strains of *Lactobacillus plantarum* (CRA-OLI Collection) (Table 2).

Lactobacillus plantarum phages were searched into brines at the end of fermentation by means of a turbidity test (Zago et al. 2006b). Each strain used as starter (Table 1) was inoculated (2%) in MRS-Ca broth, added with 5% of brines previously centrifuged and filter-sterilized (sterile filter units 0.45-μm pore size; Millipore, Milan, Italy). A control culture without addition of brine filtrate was also prepared. Cultures were incubated at 30°C and checked for turbidity. If no lysis occurred in the tubes added with the brine filtrates, three further subcultures were made to detect possible delayed lysis. If lysis was

Table 2 Fermentation brines used in this study

Brine	Matrix (*Olea europaea* L. cultivar)	Fermentation process
S1	Nocellara Etnea	Inoculated
S2	Itrana	Spontaneous
S3	Ascolana tenera	Spontaneous
S4	Ascolana tenera	Inoculated
S5	Nocellara del Belice	Spontaneous
S6	Nocellara Etnea	Spontaneous
S7	Nocellara Etnea	Inoculated
S8	Nocellara del Belice	Inoculated
S9	Nocellara Etnea	Inoculated

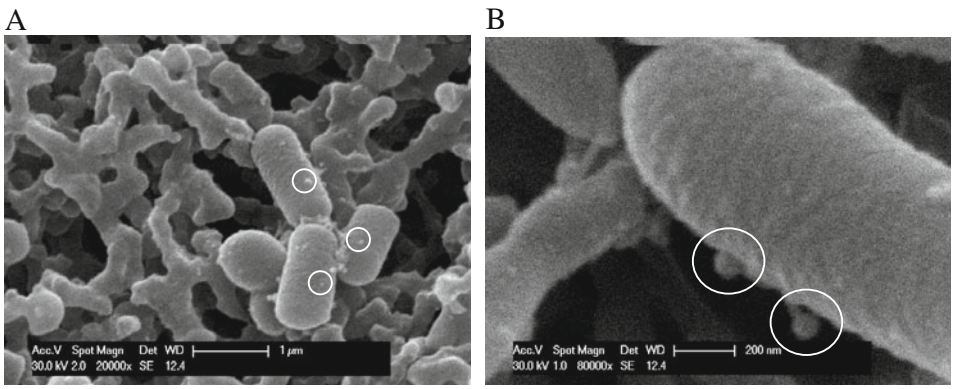

Fig. 1 SEM images of adhesion of phage particles to bacterial surface. Phage particles are indicated by *white circles*. Pictures were taken at ×20,000 (**a**) and ×80,000 (**b**) magnification

observed, the culture was filtered and phage particles isolated and purified according to standard methods. Plaque-forming capability of phages was performed by the double-layer plate titration method (Svensson and Christiansson 1991) using MRS-Ca with addition of 100 mM-glycine (Lillehaug 1997).

Host range determination

Strains and bacteriophages were inoculated in MRS-Ca broth at a multiplicity of infection (m.o.i.) of approx 1. As a control, a tube without the bacteriophage was used. Three subcultures were performed to confirm the phage resistance (no lysis) or sensitivity (lysis) of strains.

Restriction analysis of phage DNA

Phages were propagated on related hosts in 50 ml of MRS-Ca broth. After lysis, the cultures were filtered and treated for 30 min with 1 μg/ml DNase I and 1 μg/ml RNase (Sigma-Aldrich, Milan, Italy). Phage particles were concentrated by centrifugation (10,000*g*, 1 h, 4°C). Phage DNAs were extracted and purified by the methods previously described (Zago et al. 2006a).

Phage DNAs were cleaved with *Hind*III, *Mlu*I, *Pst*I, *Sal*I and *Xba*I restriction enzymes (New England BioLabs, Hertfordshire, UK), according to manufacturer's instructions. Digested DNA fragments were separated on 1.5% (w/v) agarose gel and visualized by GelRed™ solution (Biotium, Hayward, CA, USA) staining. One-kb plus DNA Ladder and λ-*Hind*III DNA ladder (Invitrogen, Milan, Italy) were used as DNA molecular weight marker.

Bacteriophage structural proteins

Concentrated phage particles were used to extract structural phage proteins and analyzed by SDS-PAGE electrophoresis according to De Antoni et al. (2010).

Scanning electron microscopy

The presence of phage particles was detected by Scanning Electron Microscopy (SEM). After 1 h of contact, the infected culture was fixed with 2.5% (v/v) glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.4) and filtered (0.45-μm pore size). The samples were washed with the same buffer, dehydrated in ethanol series (30, 50, 70, 85, 95 and 100% v/v), then transferred to 100% (v/v) acetone before drying with CO_2 at the critical point in a Critical Point Dryer CPD 030 (Balzers Union, Liechtenstein). The dry filters were then mounted on aluminium stubs and coated with gold (20 nm thick) in a Sputter Coater SCD 050 (Balzers Union). Representative specimens were examined with a Philips XL 20 SEM (FEI Europe, Eindhoven, The Netherlands) and then photographed.

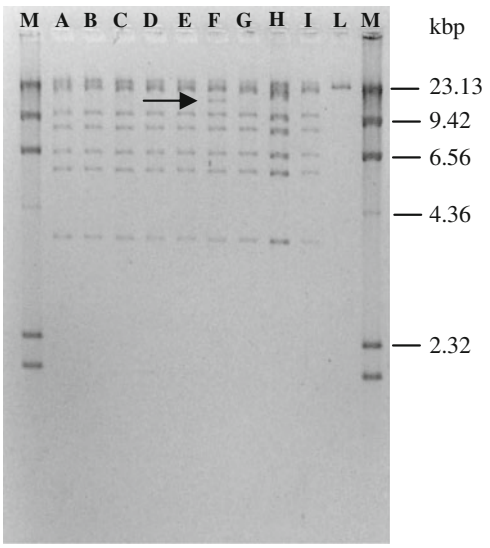

Fig. 2 *Mlu*I digests of *L. plantarum* phage DNAs. *Lanes A* ΦS1, *B* ΦS2, *C* ΦS3, *D* ΦS4, *E* ΦS5, *F* ΦS6, *G* ΦS7, *H* ΦS8, *I* ΦS9, *L* undigested DNA, *M* λ-*Hind*III DNA ladder as molecular marker (Invitrogen). An additional band of ΦS6 is indicated by the *arrow*

Results and discussion

This study demonstrated for the first time the presence of *L. plantarum* phages in the fermentation of table olives. The presence of phages in vegetable fermentations, as well as in other fermented foods, follows the development of the LAB which drive the fermentation process. The continuous multiplication of the most adapted strains involves the selection of host-related phages, which may enter the fermentation process through raw materials, environment, or that originated from lysogenic starter strains (Sanders 1987; Cogan et al. 1991). Each of the nine table olive fermentation brines investigated, both non-inoculated and inoculated with starter strains of *L. plantarum,* resulted in infection by phages causing the lysis of several *L. plantarum* strains used as hosts. The presence of phages was confirmed by the formation of plaque of lysis on agar medium and also visualised by scanning electron microscopy; Fig. 1 shows the adhesion of phage particles to bacterial surface and an initial cell wall disruption. To differentiate the nine isolated phages, the activity spectrum against 23 *L. plantarum* strains isolated from *Olea europaea* cultivars, 24 from cheeses, and 2 reference strains was carried out. The *L. plantarum* phages isolated from the different samples of olive fermentation brines, both inoculated and non-inoculated with starter strains, showed a low level of diversity. In fact, the host range analysis allowed the division of the phages into only two groups according to the lytic pattern observed (Table 1). Both groups included phages isolated from inoculated or uninoculated fermentation brines. The phages belonging to one group (ΦS1–ΦS4) were able to infect 19 strains, while phages of the other group (ΦS5–ΦS9) infected 16 strains. The different host range was determined only by the differences in the lytic pattern on dairy strains. These host range data, with phages that separated only into two groups despite the high number of strains used from various sources, confirmed recent results obtained by De Antoni et al. (2010) on *L. plantarum* phages isolated from fermented milks. These phages also showed a fast-growing capability, demonstrated by the lysis of sensitive host strains during the first infection with the brine samples.

The similarity of phages was confirmed by phage DNA restriction analyses, which showed the same restriction profiles for eight of the nine phages studied, except for ΦS6 which, after *Mlu*I digestion, showed an additional band of about 13.0 kbp (Fig. 2). No phage DNAs were cut by *Pst*I and *Xba*I. The estimated genome sizes were about 36.0±6.8 kbp for eight phages and about 47.0±4.6 kbp for ΦS6 only. They were similar to other known *L. plantarum* phages: ΦJL-1 (36.7 kbp; Lu et al. 2003a), ΦFAGK-1 and ΦFAGK-2 (34.8±3.2 kbp; De Antoni et al. 2010) and ΦLP2 (47 kbp; Caso et al. 1995). Also, structural protein profiles were very similar for all the nine phages, and they were characterized by the presence of three major bands at about 28, 33 and 37 kDa (data not shown). The molecular masses were within the values reported for the other *L. plantarum* phages, in fact, the protein profiles were similar to those obtained by Yoon et al. (2001) for the phage ΦSC921, with two major bands (32 and 34 kDa), and minor bands that ranged between 13 and 112 kDa. On the basis of the characterisation results, the nine phages could be distinguished into three groups, one of which was represented only by the phage ΦS6 isolated from a non-inoculated fermentation brine, while the other two grouped phages were separated by the different host range profiles.

This study is the first report of *L. plantarum* phages from table olive fermentations. This phage infection could adversely affect the fermentation process, mainly in the case of use of starter cultures. These findings imply the need to evaluate the phage impact on these systems, and to confirm the importance of select suitable strains to control table olive fermentations also taking into account their phage sensitivity.

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References

Brito MD, Delgado AM, Catulo L, Quintans F, Perese C (2004) The role of bacteriocin producers in table-olive fermentation. In: 5th International Symposium on Olive Growing, Izmir, Turkey

Caso JL, de los Reyes-Gavilàn CG, Herrero M, Montilla A, Rodriguez A, Suarez JE (1995) Isolation and characterization of temperate and virulent bacteriophage of *Lactobacillus plantarum*. J Dairy Sci 78:741–750

Chibanni-Chennoufi S, Dillmann ML, Marvin-Guy L, Rami-Shojaei S, Brussow H (2004) *Lactobacillus plantarum* bacteriophage LP65: a new member of the SPO1-like genus of the family Myoviridae. J Bacteriol 186:7069–7083

Ciafardini G, Marsilio V, Lanza B, Pozzi N (1994) Hydrolysis of oleuropein by *Lactobacillus plantarum* strains associated with olive fermentation. Appl Environ Microbiol 60:4142–4147

Cogan TM, Peitersen N, Sellars RL (1991) Starter systems. In: Bulletin International Dairy Federation (FIL-IDF), Practical phage control, 263: 16–23

De Antoni G, Zago M, Vasek O, Giraffa G, Carminati D, Briggiler M, Reinheimer J, Suarez V (2010) *Lactobacillus plantarum* bacteriophages isolated from Kefir grains: phenotypic and molecular characterization. J Dairy Res 77:7–12

Doi K, Zhang Y, Nishizaki Y, Umeda A, Ohmomo S, Ogata S (2003) A comparative study and phage typing of silage-making *Lactobacillus* bacteriophages. J Biosci Bioeng 95:518–525

Garrido Fernandez A, Fernandez Diaz MJ, Adams RM (1997) Table olives: production and processing. Chapman & Hall, London

Giraffa G (2004) Studying the dynamics of microbial populations during food fermentation. FEMS Microbiol Rev 28:251–260

IOOC (2004) Trade Standard Applying to Table Olives, COI/OT/NC No 1 December 2004 (Resolution No. RES-2/91-IV/04). International Olive Oil Council, Madrid, Spain

Lanza B, Rossetti L, Zago M, Carminati D, Russi F, Iannucci E, Di Serio MG, Mucciarella MR, Giraffa G (2009) Presenza di fagi nelle salamoie di fermentazione di olive da tavola, In: Proceedings of Convegno Internazionale RIOM, Rende (CS), Italy, 11–12 giugno, pp. 447–449

Leal-Sanchez MV, Ruiz-Barba JL, Sanchez AH, Rejano L, Jimenez-Diaz R, Garrido A (2002) Fermentation profile and optimization of green olive fermentation using *Lactobacillus plantarum* LPCO10 as a starter culture. Food Microbiol 20:421–430

Leroy F, De Vuyst L (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol 15:67–78

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

Lu Z, Breidt F, Fleming HP, Altermann E, Klaenhammer TR (2003a) Isolation and characterization of a *Lactobacillus plantarum* bacteriophage, Φ JL-1, from a cucumber fermentation. Int J Food Microbiol 84:225–235

Lu Z, Breidt F, Plengvidhya V, Fleming HP (2003b) Bacteriophage ecology in commercial sauerkraut fermentations. Appl Environ Microbiol 69:3192–3202

Marsilio V, Lanza B (1998) Characterization of an oleuropein degrading strain of *Lactobacillus plantarum*. Combined effect of compounds present in olive fermenting brines (phenols, glucose and NaCl) on bacterial activity. J Sci Food Agric 76:520–524

Marsilio V, Lanza B, Pozzi N (1996) Progress in table olives debittering: degradation in vitro of oleuropein and its derivatives by *L. plantarum*. J Am Oil Chem Soc 73:593–597

Marsilio V, Seghetti L, Iannucci E, Russi F, Lanza B, Felicioni M (2005) Use of a lactic acid bacteria starter culture during green olive (*Olea europaea* L., cv. Ascolana tenera) processing. J Sci Food Agric 85:1084–1090

Panagou RZ, Tassou CC, Katsaboxakis CZ (2003) Induced lactic acid fermentation of untreated green olives of the Conservolea cultivar by *Lactobacillus pentosus*. J Agric Food Sci 83:667–674

Rubia-Soria A, Abriouel H, Lucas R, Ben Omar N, Martinez-Canamero M, Galvez A (2006) Production of antimicrobial substances by bacteria isolated from fermented table olives. World J Microbiol Biotechnol 22:765–768

Ruiz-Barba JL, Cathcart DP, Warner PJ, Jimenez-Diaz R (1994) Use of *Lactobacillus plantarum* LPCO10, a bacteriocin producer, as a starter culture in Spanish-Style green olive fermentations. Appl Environ Microbiol 60:2059–2064

Sanders ME (1987) Bacteriophages of industrial importance. In: Goyal SM, Gerba CP, Bitton G (eds) Phage ecology. Wiley, New York, pp 211–244

Servili M, Settanni L, Veneziani G, Esposto S, Massitti O, Taticchi A, Urbani S, Montedoro GF, Corsetti A (2006) The use of *Lactobacillus pentosus* 1MO to shorten the debittering process time of black table olives (cv. Itrana and Leccino): a pilot-scale application. J Agric Food Chem 54:3869–3875

Svensson V, Christiansson A (1991) Methods for phage monitoring. In: Bulletin International Dairy Federation (FIL-IDF), Practical phage control, 263: 29–39.

Trevors KE, Holley RA, Kempton AG (1983) Isolation and characterization of a *Lactobacillus plantarum* bacteriophage isolated from a meat starter culture. J Appl Bacteriol 54:281–288

Yoon SS, Kim JW, Breidt F, Fleming HP (2001) Characterization of a lytic *Lactobacillus plantarum* bacteriophage and molecular cloning of a lysin gene in *Escherichia coli*. Int J Food Microbiol 65:63–74

Yoon SS, Barrangou-Poueys R, Breidt F, Klaenhammer TR, Fleming HP (2002) Isolation and characterization of bacteriophages from fermenting sauerkraut. Appl Environ Microbiol 68:973–976

Zago M, Carminati D, Giraffa G (2003) Characterization of Streptococcus thermophilus phages from cheese. Ann Microbiol 53: 171–178

Zago M, De Lorentiis A, Carminati D, Comaschi L, Giraffa G (2006a) Detection and identification of *Lactobacillus delbrueckii* subsp. *lactis* bacteriophages by PCR. J Dairy Res 73:146–153

Zago M, Rossetti L, Fornasari ME, De Lorentiis A, Bonioli A, Perrone A, Giraffa G, Carminati D (2006b) Identificazione e caratterizzazione di batteriofagi di lattobacilli termofili isolati da innesti naturali. Sci Tec Latt Cas 57:525–534