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ORIGINAL ARTICLE

"Memorized" modifications on *Listeria monocytogenes*' membrane lipids and fatty acid profile after its survival on soft white feta-type cheese

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Abstract The ability of *Listeria monocytogenes* to adapt to low temperature, low pH, and osmotic stress, to withstand cleaning and disinfection agents, to induce stress-related cross-protection responses, and to form biofilms, enables it to prevail and survive most of the common safety measures employed in industrial-scale food processing to ensure food safety. The objective of this study was to determine the changes that occur in the lipid composition of the membrane of *L. monocytogenes* under the conditions of the manufacture and storage of a laboratory-prepared soft cheese (feta type). The results show that *L. monocytogenes* cells present in the raw milk may survive the cheese manufacturing process as well as the storage period and that the history of the inoculated cells has an effect on their behavior during storage of the cheese. The bacterium seems to follow different pathways and adopt different strategies to preserve the optimal fluidity and functions of the membrane depending on the length of storage time and the history of the inoculated cells. In this context, cells derived from pre-inoculated cells grown in optimal or acidic conditions showed an increase in the number of straight chain fatty acids (SCFA) relative to branched ones (BCFA), without altering the ratio of BCFA C15:0 to BCFA C17:0 and the ratio of iso to anteiso FA. Alternatively, those cells derived from pre-inoculated cells grown in presence of benzalkonium chloride followed a different pathway. These cells achieved a balance between optimal fluidity and solid phase membrane not only by changes in SCFA content, but also by changes in the ratios of BCFA C15:0 to C17:0 and of iso to anteiso fatty acids. This latter approach to maintain membrane fluidity seems to be paired with the different survival trends of the bacterium under the conditions of this study.

Keywords Fatty acid · Feta · Cheese · Lipids · Membrane

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Introduction

Listeria monocytogenes is prevalent in a variety of foods and ready-to-eat products where it has the potential to become a serious public health problem. Dairy products are commonly ready-to-eat products, and the evaluation of their ability to support growth or survival of this pathogen is crucial for risk assessment (Kozak et al. 1996). Previous studies have demonstrated the behavior of *L. monocytogenes* in soft cheese (Papageorgiou et al. 1996, 1997), semi-hard cheese (Papageorgiou and Marth 1989), and hard ripened cheese (Theodoridis et al. 2006), reporting that most cases of contamination occurred in the final product. Nevertheless, contamination of a product in a dairy/milk processing plants can occur at various stages (i.e. storage, production, ripening) and have different sources (i.e., unpasteurized milk; final products; contact with humans, unclean surfaces, insects). *L. monocytogenes* cells from these different sources have a different physiology and probably a different capacity to overcome the hurdles posed to them throughout the manufacture and ripening of dairy products as well as by their own physicochemical properties (Kousta et al. 2010).

The ability of *L. monocytogenes* to adapt to low temperature, low pH, and osmotic stress (Tasara and Stephan 2006; Gandhi and Chikindas 2007), to withstand cleaning and disinfection agents (Lopes 1986; Lunden et al. 2003; Bisbiroulas et al. 2011), and to induce stress-related cross-protection

responses (Beales 2004), combined with its ability to form biofilms (Carpentier and Chassaing 2004; Pereira da Silva and Pereira De Martinis 2013), enable it to prevail and survive most of the commonly used safety measures used in industrial-scale food processing to ensure food safety. Among the strategies undertaken by *L. monocytogenes* to adapt to environmental changes is modification of the membrane lipids; such modifications are essential to maintain sufficient fluidity compatible with the integrity, and functionality of the membrane (Diakogiannis et al. 2013). The effects of reduced temperature on the fatty acid (FA) composition of *L. monocytogenes*' membrane has been studied extensively. The principal change in the FA composition of this bacterium's membrane with a drop in temperature to below the optimum value is an increase in the number of anteiso-C15:0 FA at the expense of anteiso-C17:0 FA. The membrane of *L. monocytogenes* is characterized by an unusual proportion of branched chain FA (BCFA) (>85 %), primarily anteiso-C15:0 and anteiso- C17:0 (Mastronicolis et al. (1996a, b). It has been proposed that the membrane FA composition of *L. monocytogenes* cells, especially the content of anteiso-15:0 FA, may play an important role in its ability to grow at low temperatures (Annous et al. 1997). Cells grown at lower temperatures contain significantly fewer anteiso-17:0 FA than those grown at higher temperatures (Mastronicolis et al. 1998; Chihib et al. 2003). Similarly, the membrane lipid composition can be determined by the pH of the growth medium. The acid adaptation response will lead to alterations in FA composition, mainly in the neutral lipids (NL) class of adapted cultures (Mastronicolis et al. 2010). Changes in the FA profile of newly synthesized NL were also observed in cold (5 °C) adapted *L. monocytogenes* (Mastronicolis et al. 2006).

Bacterial cells have the capacity to remember past environments, which accelerates their physiological adaptation. Memory in bacteria has been studied in the context of epigenetic switches, which can maintain stable phenotypic states over hundreds of generations (Casadesús and D'Ari 2002). Growth conditions are known to alter several bacterial responses, implying that memory may be more than an incidental factor in modulating the regulatory mechanisms which control acclimation of the cells or the status of the metabolic stores (Wolf et al. 2008). Lambert and Kussell (2014) reported two distinct memory mechanisms in the *lac* operon of *Escherichia coli*, namely, phenotypic and response memory, each of which is beneficial over different time scales.

The aims of our study were to determine (1) whether cell history plays a role in pathogen survival and (2) whether this role can be mirrored (memorized) in the membrane lipid composition of *L. monocytogenes* following its survival of the manufacturing process and storage period in a laboratory-prepared, feta-type soft white cheese. Our ultimate goal was therefore to gain a deeper understanding of the adaptive mechanisms adopted by *L. monocytogenes* under food processing conditions.

Material and methods

Culture preparation for inoculation of milk

Listeria monocytogenes DP-L1044 (D. Portnoy, University of Pennsylvania) is the strain used in this study. It is a avirulent mutant of parent strain L1044S (serotype 1/2a, resistant to 1 mg ml^{-1} streptomycin) (Portnoy et al. 1988; Camilli et al. 1991). The strain was first inoculated in brain-heart infusion broth (BHI; BD, Sparks, Maryland) at 30 °C for 20 h, following which three 10-mL aliquots were removed. The first 10-mL aliquot was inoculated into 1 L of BHI broth, which was then incubated at 30 °C (Control, pCO) until the early stationary phase (OD_{600} = 0.8; 8 h). The second 10-mL aliquot was inoculated into 1 L BHI that had been adjusted to an initial pH of 5.5 with L-lactic acid (Fluka, Sigma-Aldrich, Allentown, PA) (culture pLA). The third 10-mL aliquot was used to inoculate 1 L of BHI supplemented with 2.5 mg benzalkonium chloride (Alfa Aesar, Karlsruhe, Germany) and incubated at 30 °C, pH 7.65 until the early stationary phase (culture pBC), as described in previous studies (Mastronicolis et al. 2010; Bisbiroulas et al. 2011).

Aliquots (1 mL) of *L. monocytogenes* cultures were serially decimally diluted in sterile water to attain the desired inoculum level. Inocula (1 mL) of cultures CO, LA, and BC were added to different test samples of milk to yield a concentration of 5 $\log_{10}$ CFU ml^{-1} milk.

Cheese-making procedure

The white feta-type cheese was prepared in the laboratory based on traditional manufacturing process of feta cheese. Two types of milk (Department of Animal Breeding and Husbandry, Agricultural University of Athens) were used in a mixture of 70 % ewe's milk and 30 % goat's milk. Portions (200 mL) of the mixture were thermized at 62 °C for 10 min and cooled at 32 °C. Each portion was inoculated with appropriate culture of *L. monocytogenes*, and 0.1 g rennet was added. The samples were allowed to clot at 36 °C for 20 min, following which the curd was allowed to drain for 24 h at room temperature. The samples were stored in 6 % sterile salt brine (sterile stomacher bag) in the refrigerator (5 °C) for up to 2 months.

Enumeration of microorganisms

The bacterium was enumerated at 1, 30 and 60 days after the cheese preparation (draining of curd). For enumeration, 25 g were taken from each sample and homogenized with 225 mL sterile Frazer broth (Oxoid Ltd., Basingstoke, Hampshire, UK; code: CM0895) in stomacher bags (stomacher 400; Light Interscience, Rockland, MA). Appropriate serial decimal dilutions were made in Frazer broth. *L. monocytogenes*

was counted by surface-plating 0.1 mL of the appropriate dilutions on Oxoid Chromogenic Listeria Agar (OCLA) plates, a selective medium (modification of ALOA; Ottaviani et al. 1997) utilized recently in routine food analysis for the identification and differentiation of *L. monocytogenes* from food samples (Oxoid Ltd; code: PO5165A). The petri dishes were incubated at 36.6 °C for 24 h. This method has been validated by the Association Française de Normalisation and been shown to give equivalent results to ISO 11290 1:1997 (Oxoid Folio No. 1059, available at: http://www.oxoid.com).

Culture preparation for analysis of membrane lipids

For each set of samples (CO, LA, BC) at every enumeration time-point (1, 30, 60 days), we scraped off cells growing on the surface of the selected medium in the petri dishes (inoculated with bacteria extracted from cheese samples), inoculated these in 10 mL BHI, and then incubated the inoculated BHI at 30 °C for 12 h. Aliquots from these latter cultures were was used to inoculate 1 L BHI. These cultures were incubated for 8 h at 30 °C (OD 0.8—early stationary phase), the cells were harvested, and the membrane lipids were extracted from the cells as described below.

Extraction of total lipids

From each culture, cells pelleted by centrifugation (4 °C, 5877 *g*) were washed twice in phosphate buffer (pH 7.0). Total lipids (TL) were extracted with chloroform/methanol (2/1, v/v), and the extract was washed with 0.2 volumes of water (Folch et al. 1957). After phase equilibration, the lower chloroform layer (TL) was dried under nitrogen.

Methanolysis of lipids and gas chromatography

Each culture TL sample was converted to FA methyl esters (FAME) as previously described (Mastronicolis et al. 2006). FAME were separated using a Shimadzu gas chromatograph equipped with a split/splitless injector, flame ionization detector, capillary column DB-5 ms (Phenyl Arylene polymer virtually equivalent to 5 %-phenylmethylpolysiloxane; 30 m × 0.251 mm, film thickness of 0.25; Cat No. 1225532, Serial No. US3270652 H; J&W Scientific/Agilent Technologies Inc., Palo Alto, CA). Carrier gas (He) flow was 44 ml/min. The injector and detector were maintained at 250 and 280 °C, respectively. The oven temperature was ramped from 90 to 170 °C (70 °C min^{-1}) and from 170 to 210 °C (5 °C/min). The initial and final holding times were 1 and 2 min, respectively. Two standard mixtures were used: bacterial FAME (CP Matreya, Inc., State College, PA) and Marine oil FAME (Restek Corp., Middelburg, the Netherlands).

Identification and quantification of membrane phospholipids by thin-layer chromatography and phosphorus analysis

Thin-layer chromatography (TLC) analysis of phosphorus content was performed as previously described (Long and Staples 1961; Mastronicolis et al. 2010). Chloroform/methanol/acetic acid/water (50/25/6/2, v/v/v/v) was used for one-dimensional TLC [silica gel G60 aluminum chromatoplate (Merck Cat. No. 1.05554; Merck & Co., Kenilworth, NJ); thickness 0.25 mm; 10 × 9 × 10 cm]. Spots were visualized using iodine, ninhydrin, and phosphomolybdenum blue reagents (Dittmer and Lester 1964). The standards were purchased from Sigma Chemical Co. (St. Louis, MO)

Statistical analysis

All values were reported as the means of two experiments performed at different times, with two replicates at each time. The data were analyzed using analysis of variance. The *t* test for unpaired observations was tested at a confidence level of 95 %. Statistical analysis was carried out using STATISTICA 7 statistical software (Statsoft Inc., Tulsa, OK).

Results

Enumeration of *L. monocytogenes* in cheese samples.

The results on the enumeration of *L. monocytogenes* in cheeses made from milk inoculated at 10^5 CFU ml^{-1} are shown in Fig. 1.

In the first day after cheese-making, the counts in cheese samples prepared from milk inoculated with non-adapted cells (pCO) or acid-adapted cells (pLA) showed an increase of 2.38 and 2.30 $\log_{10}$ CFU g^{-1} respectively, compared to the unprocessed milk. A different pattern, a decrease of 1.79 $\log_{10}$ CFU g^{-1} was observed for cheese samples prepared from milk inoculated with benzalkonium chloride-adapted cells (pBC). During storage, *L. monocytogenes* counts were maintained at a stable level at the values reached on the first day.

Changes in *L. monocytogenes* membrane TL content

Bacterial cells were scraped from selected petri dishes [inoculated with bacteria extracted from cheese samples at 24 h (CO1, BC1, LA1), at 30 days (CO2, BC2, LA2), and at 60 days (CO3, BC3, LA3) after the curds had been drained] and cultured as described in the Culture preparation for analysis of membrane lipids section. Each culture was grown to the early stationary phase at 30 °C and their TL extracted. The ratio of TL to cell mass (wet cell weight, mg g^{-1}) and the lipid phosphorus content of each TL sample, expressed as a percentage, are shown in

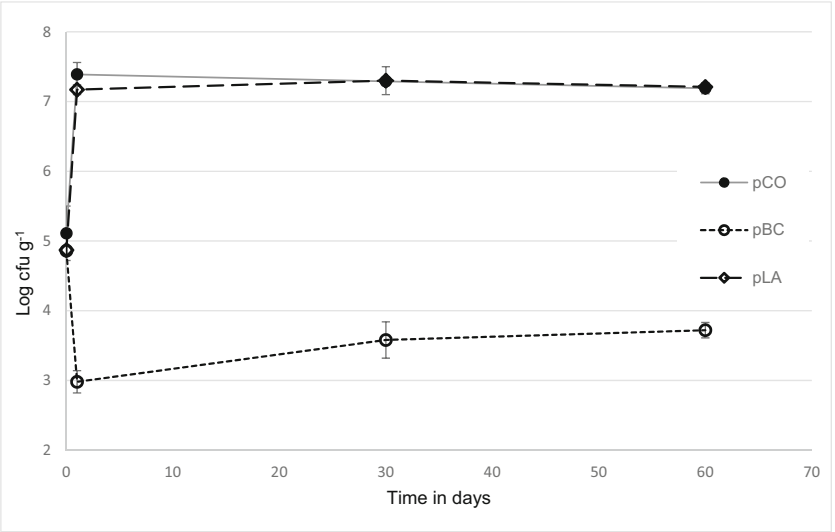

Fig. 1 *Listeria monocytogenes* counts in feta-type cheese samples during manufacture and storage at 5 °C. Cheese samples were prepared from milk inoculated with *L. monocytogenes* cells previously grown in brain-heart infusion broth (BHI) at 30 °C under optimal conditions (*pCO*, *filled circles*), at pH 5.5 due to addition of lactic acid, (*pLA*, *open diamonds*), or in presence of benzalkonium chloride 2.5 mg L^{-1} (*pBC*, *open circles*). For details on the culture conditions see Culture preparation for inoculation of milk section

Table 1. The TLC analysis of the TL content of each culture revealed patterns similar to those observed in our previous studies (Mastronicolis et al. 2008, 2010).

The phosphorus content of the lipids in cultures CO1, CO2, and CO3 was respectively 37.7, 29.2, and 23.4 % lower than that of the cells of the pre-inoculated culture pCO (all differences significant at $P<0.05$). In terms of phosphorus content, cultures CO1 and CO2 differed by 13.5 % (higher in CO1; $P<0.05$). For the culture BC1, the phosphorus content of the lipids in culture BC1 was 38.4 % lower than that of culture pBC ($P<0.05$). The difference in phosphorus content between cultures BC1 and BC2 was 12.6 % (higher in BC2; $P<0.05$), and between cultures BC2 and BC3, 34.9 % (lower in BC3; $P<0.05$). The percentage of phosphorus content was 54.8 % lower in culture BC3 than in culture pBC. The phosphorus content of the lipids in culture LA1 increased by 87.1 % compared with culture pLA, with an 8.6 % difference in values between cultures LA1 and LA2 (lower in LA2; $P<0.05$) and a 34.9 % difference between cultures LA2 and LA3 (higher in LA3; $P<0.05$).

Table 1 Ratio of total lipid content to wet cell mass and percentage phosphorus content of each *Listeria monocytogenes* sample of cells grown prior to inoculation either under optimal culture conditions, in the presence of benzalkonium chloride, or at pH 5.5, and from cells derived from bacteria extracted from matured cheese and grown under optimal conditions (series CO, BC and LA)

Culture of *Listeria monocytogenes*[a, b]	Ratio of TL to wet cell mass (mg g^{-1})	Phosphorus content of TL (%)
pCO	3.31 ± 0.28	3.19 ± 0.29
CO1	5.07 ± 0.11	1.99 ± 0.32
CO2	4.84 ± 0.01	2.25 ± 0.50
CO3	3.51 ± 0.28	2.44 ± 0.34
pBC	2.70 ± 0.13	2.81 ± 0.10
BC1	5.63 ± 0.87	1.73 ± 0.48
BC2	3.62 ± 0.48	1.95 ± 0.35
BC3	3.01 ± 0.47	1.27 ± 0.25
pLA	4.39 ± 0.65	1.14 ± 0.07
LA1	4.73 ± 0.02	2.13 ± 0.32
LA2	2.85 ± 0.12	1.95 ± 0.30
LA3	2.46 ± 0.32	2.58 ± 0.54

Values are presented as the mean ± standard deviation (SD) of two experiments performed on each TL/cell culture ($n=4$–6)

TL, Total lipids

[a] pCO, pLA, pBC, Cells grown under optimal conditions [brain-heart infusion broth (BHI) at 30 °C], at pH 5.5 (BHI, 30 °C), and in presence of benzalkonium chloride ((BHI, 30 °C), respectively

[b] The extraction was performed at 24 h (CO1, BC1, LA1), 30 days (CO2, BC2, LA2), and 60 days (CO3, BC3, LA3) after cheese production

Changes in FA profile of *L. monocytogenes*

Cells scrapped from selected petri dishes (inoculated with bacteria extracted from cheese samples) and subcultured in BHI under optimal conditions generally showed an FA profile which differed from that of the corresponding pre-inoculated cells (Fig. 2a–c). The BCFA content did not differ among cultures CO1, BC1, and LA1 (derived from cells extracted from cheese samples 24 h after cheese manufacture) (Fig. 3). In contrast, the unsaturated FA content did differ, with values of 15.29, 7.62 and 4.15 % for CO1, BC1, and LA1 cultures, respectively (Fig. 33a–c).The higher content of unsaturated straight chain FA (unSCFA) in the CO sample relative to the PCO sample was due to a remarkable 3.5-fold increase in C14:1 content in the CO sample (Fig. 2a). The cells of the CO2, BC2, and LA2 samples (derived from cells extracted from cheese samples after 30 days of storage) showed a similar FA profile, with SCFA content amounting to 22.26, 24.18, and 21.40 % respectively (Fig. 3a–c) and unSCFA content in the range of 5.45, 3.74, and 4.28 %, respectively. The cells of

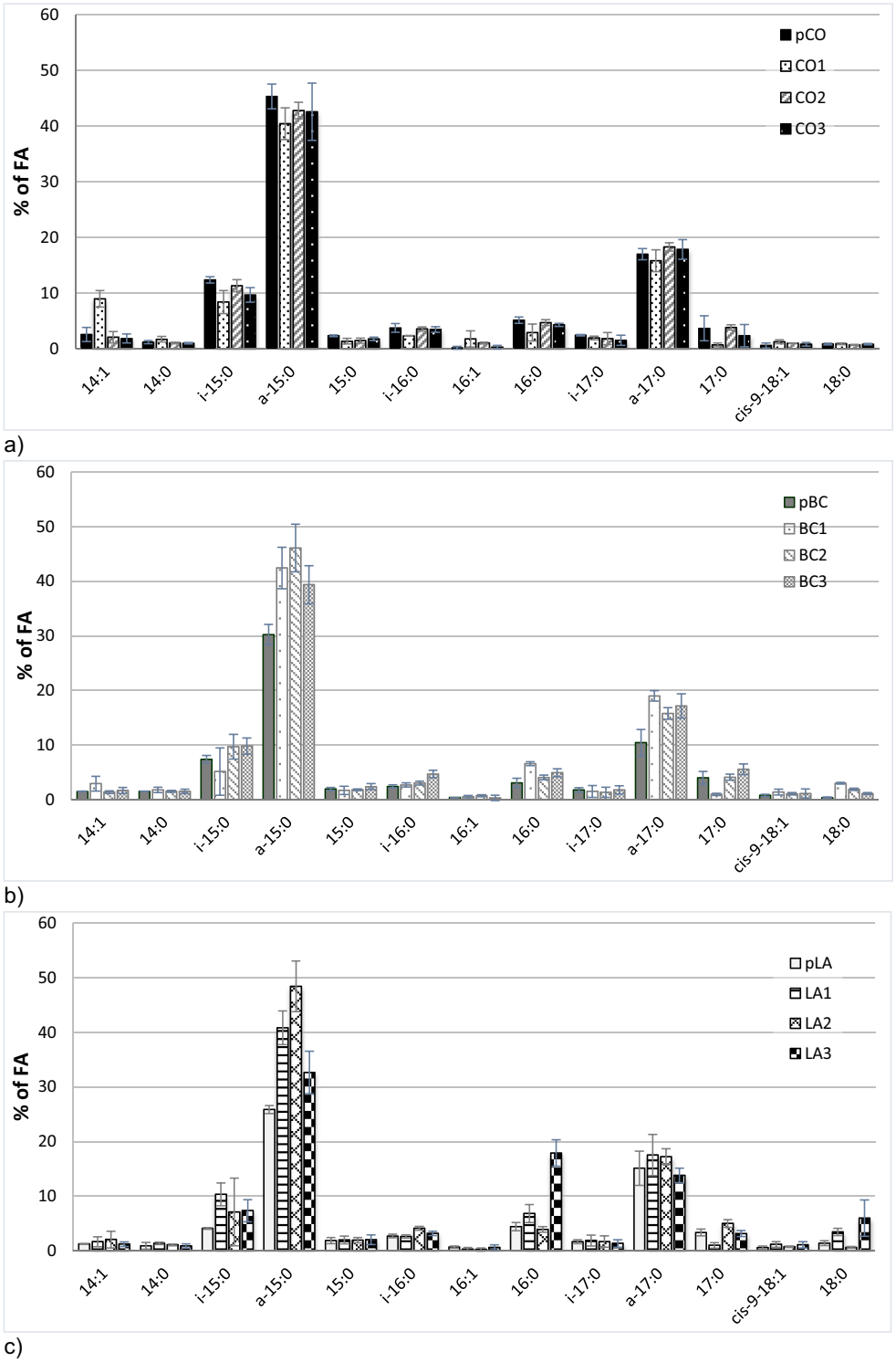

Fig. 2 Mean concentration [± standard deviation (*whiskers*)] of fatty acids (*x-axis*) in *L. monocytogenes* membrane lipids in cells grown before inoculation under optimal conditions (*pCO*), in the presence of benzalkonium chloride (*pBC*), or at pH 5.5 (*pLA*) and after being extracted from cells derived from bacteria extracted from cheese samples (series CO, BC, and LA; see footnote Table 1). The extraction was performed at 24 h (*CO1*, *BC1*, *LA1*), at 30 days (*CO2*, *BC2*, *LA2*), and at 60 days (*CO3*, *BC3*, *LA3*) after cheese production

cultures CO3, BC3, and LA3 (derived from cells extracted from cheese samples after 60 days of storage) showed a different trend in terms of changes in the FA profile. There were no statistically significant (95 % confidence level) changes in the FA profile of cultures CO3 and BC3, but cells of the LA3 culture showed an increased SCFA content, namely, 41.63 % of total FA (Fig. 3a–c). This increase is due to an increased saturated fatty acid (sSCFA) content, especially C16:0 which presented a content of 17.90 ± 2.43 % (Fig. 2c).

While for series CO and LA there were no changes in the ratio of BCFA C15:0 to C17:0, there was a decrease in this ratio in the BC series, from 3.15 in pBC cells to 2.32 in BC1 cells ($P < 0.05$), with a subsequent erratic trend of in this ratio over time (Fig. 4b). With respect to the ratio of iso to anteiso

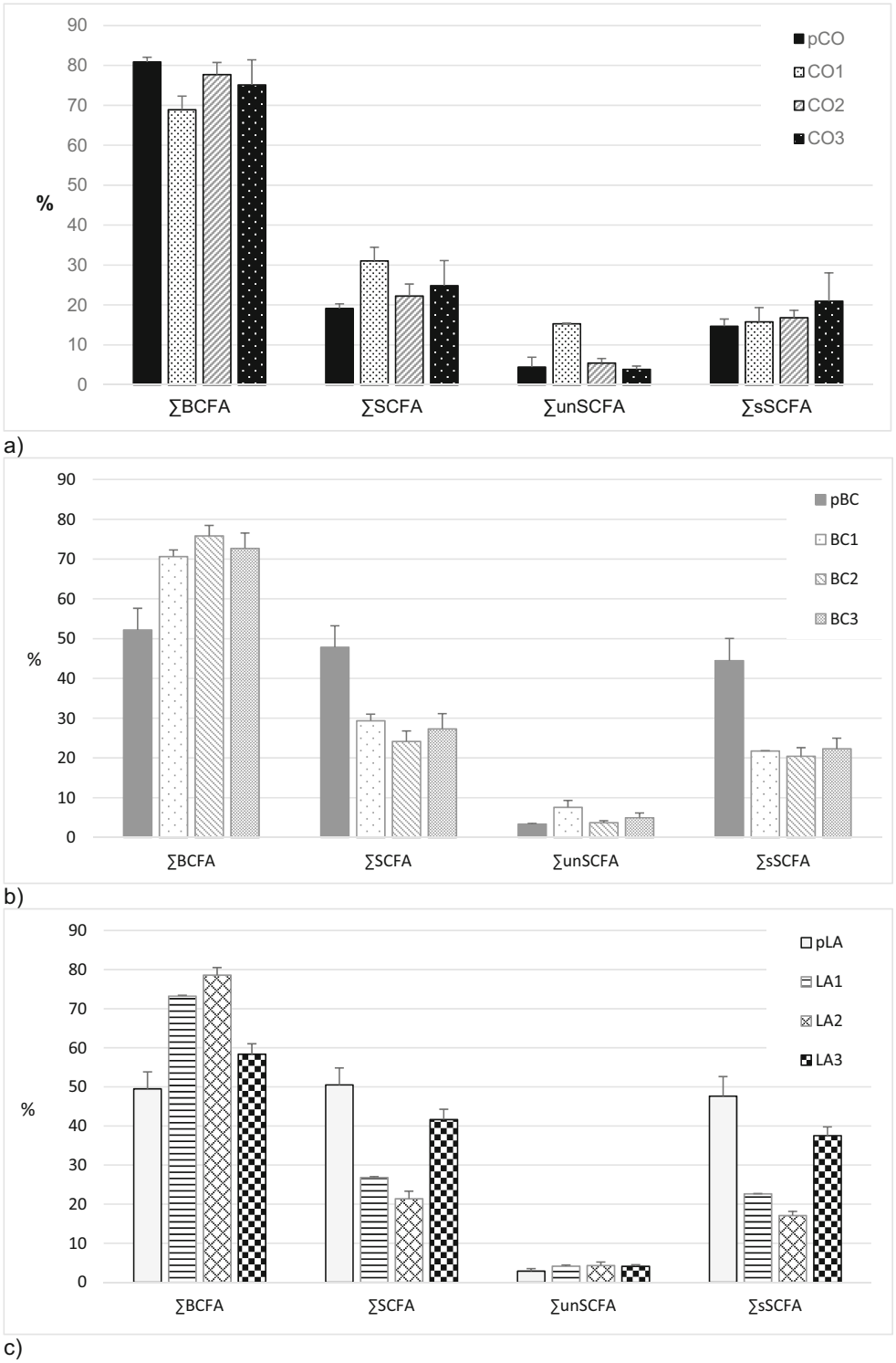

Fig. 3 Mean concentration [and standard deviation (*whiskers*)] of fatty acid classes [branched chain fatty acids (*BCFA*), straight chain fatty acids (*SCFA*), saturated straight chain fatty acids (*sSCFA*), and unsaturated straight chain fatty acids (*unSCFA*)] in *L. monocytogenes* membrane lipids in cells grown before inoculation under optimal conditions (*pCO*), in the presence of benzalkonium chloride (*pBC*), or at pH 5.5 (*pLA*) and in cells from series CO, BC and LA (derived from cells extracted from cheese samples). The extraction was performed at 24 h (*CO1*, *BC1*, *LA1*), at 30 days (*CO2*, *BC2*, *LA2*) and at 60 days (*CO3*, *BC3*, *LA3*) after cheese production (see Fig. 2 caption for more details)

FA, the major change seems to have happened with first 24 h, with a decrease in the ratio from 0.28 to 0.15 at the 24-h time-point ($P < 0.05$); thereafter, there was a trend towards increasing ratio values over time. At the last time-point of the analyses, which corresponded to the end of the storage period, the ratio equaled that of pre-inoculated cells. The ratio of sSCFA to unSCFA (∑sSCFA/∑unSCFA) for the sample series CO decreased over the first 24 h and thereafter showed a trend towards increasing, with the CO3 reaching a value higher than that that of pCO cells (Fig. 4a). The other two series of samples showed lower values relative to their respective pre-inoculated cells.

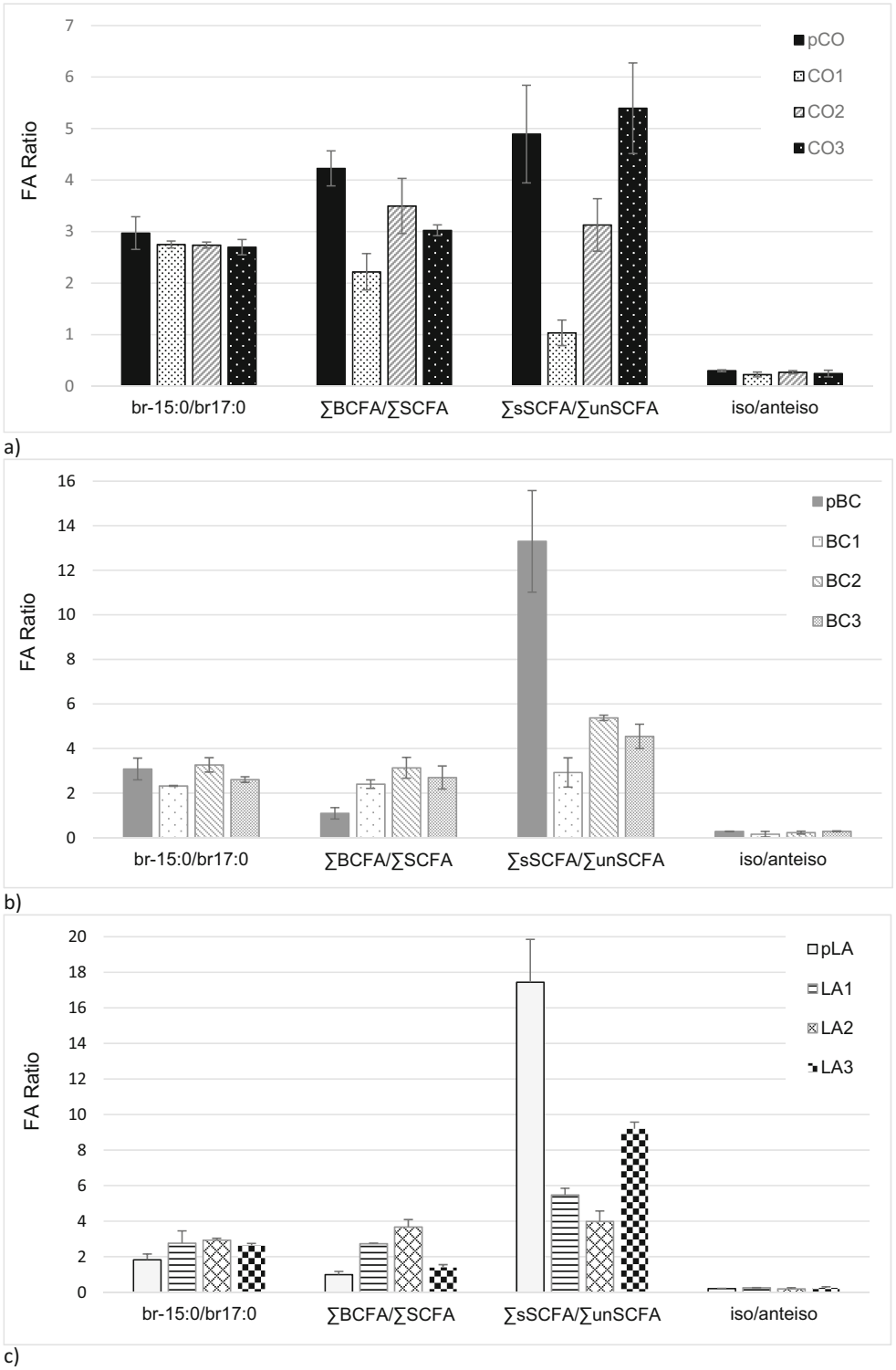

Fig. 4 Ratios of branched (*br*) chain fatty acids C15:0 to C17:0, (*br-C15:0/br-C17:0*), of total BCFA to total SCFA (∑*BCFA/*∑*SCFA*), of total unSCFA to total sSCFA (∑*unSCFA/*∑*sSCFA*) and of iso to anteiso BCFA (*iso/anteiso*) for cells before inoculation and for cultures derived from bacteria extracted from cheese samples at 24 h (*CO1*, *BC1*, *LA1*), at 30 days (*CO2*, *BC2*, *LA2*), and at 60 days (*CO3*, *BC3*, *LA3*) after cheese production

Discussion

Listeria monocytogenes behaves differently in different types of cheese depending on the different physico-chemical conditions during the manufacturing, ripening, and storage of the cheese [e.g., starter culture, acidity, temperature and duration of ripening and storage, moisture (a_w) and salt]. Papageorgiou and Marth (1989) reported that *L. monocytogenes* added to pasteurized milk was able to grow and increase by an average of 1.5 $\log_{10}$ CFU g^{-1} during the first 2 days of feta ripening at 22 °C. These authors confirmed that depending on strain, the pathogen decreased by only 1.28 to 3.07 $\log_{10}$ CFU g^{-1} during

the 90 days of storage at 4 °C, when the pH was as low as 4.30. These results led Papageorgiou and Marth (1989) to suggest that such growth is dependent on conditions at the early stage of cheese-making, when pH is >5.0. In contrast, Morgan et al. (2001) link the higher values to the physical entrapment of *L. monocytogenes* during the draining of the curd. The results of our study show that *L. monocytogenes* in the milk used for cheese-making may survive during the manufacturing and storage of the processed cheese, thereby supporting the conclusions of Papageorgiou and Marth (1989). However, in contrast to Papageorgiou and Marth (1989), who concluded that differences in strains have no impact on the behavior of the pathogen, we demonstrated a clear effect of inoculum history. We emphasize here that these results were achieved in the absence of starter culture and that the pH of the cheese did not fall to <5.5, which suggests that the decrease in *L. monocytogenes* counts observed in other studies could be due to the combined inhibitory effect of low pH and the activity of lactic starters (Genigeorgis et al. 1991).

We analyzed our results in a comparative manner, and the experimental conditions remained the same for all samples from the moment of extraction from cheese.

The results of our analyses of phosphorus in the lipids of the pre-inoculated cultures pCo, pBC, and pLA are consistent with those reported in earlier studies by our research group (Mastronicolis et al. 2010; Bisbiroulas et al. 2011). They demonstrate a clear change in percentage content of lipid phosphorus in cultures of cells derived from those extracted from cheese samples (series CO, LA, and BC) compared to pre-inoculated cultures. Changes in the phosphorus content of lipids suggests changes in the phospholipid composition of TL and may be due to changes in TL (changes in NL) or polar lipids (changes in glycolipids and aminolipids) (Mastronicolis et al. 2006, 2010). Specifically, the observed reductions in lipid phosphorus content of series CO and BC cells combined with the increase in the TL to wet cell ratio indicate a reduction in phospholipid content and a possible increase in NL. For the LA series, derived from cells extracted from cheese samples inoculated with acid-adapted cells, the increase in phosphorus content indicates an increased phospholipid content with or without a concomitant decrease in NL. Since changes in the lipid headgroup region of lipid bilayers could lead to modifications in the structure of transmembrane alpha-helices of proteins and, consequently, to changes in the packing of these helices, it is important to maintain the presence of charged lipids in the bilayer (Lopez et al. 2006). It has long been known that bacterial cells which have experienced different environmental histories may respond differently to current conditions. It may be that some of these history-dependent behavioral differences are physically necessary consequences of the prior history, leading some to argue that other, insignificant behavioral differences may be controllable—and therefore selectable—and even fitness-enhancing manifestations of memory (Wolf et al. 2008). Moderation of the phospholipid content may be part of a resistance mechanism, but several resistance mechanisms may contribute to a resistance phenotype, and levels of resistance vary according to the type of mechanisms present (Ming and Daeschel 1995; Verheul et al. 1997; Vadyvaloo et al. 2002).

It has been suggested that in *L. monocytogenes*, anteiso C15:0 coupled with unusually low amounts of sSCFA plays a critical role in providing an appropriate degree of membrane fluidity for growth at low temperatures (Annous et al. 1997). In our study, the cells (series CO) derived from *L. monocytogenes* cells which prior to inoculation were grown under optimal conditions (30 °C, 1 L BHI, pH 7) utilized the increase in SCFA that was synchronized with decreasing amounts of BCFA without altering the ratios of both BCFA C15:0 to BCFA C17:0 and iso to anteiso FA. These changes were reflected in the modifications of the BCFA to saturated (s)SCFA ratio (Fig. 4), and the cells maintained these changes during the whole experimetal period. The same was true for series LA. The difference between series CO and LA lies in the fact that while series CO showed an alteration in unsaturated FA content over time, series LA showed a change in the content of sSCFA (Fig. 2). We focused on the differences in the lipid composition and the FA profile among the series and over time and found that the patterns were not similar. For example, the pattern for sample CO1 was different from that for sample BC1; similarly, the pattern for sample LA1 differed from that of LA3. It is tempting to say that the patterns determined in the FA profiles of analyzed cultures are a memorized reflection of the changes that *L. monocytogenes* developed during the manufacturing and storage of feta cheese. The incorporation of higher SCFA proportions was observed in previous studies on *L. monocytogenes* and other bacteria under different stress conditions (Ming and Daeschel 1995; van Schaik et al. 1999; Chihib et al. 2005; Lopez et al. 2006; Giotis et al. 2007; Mastronicolis et al. 2010; Bisbiroulas et al. 2011).

We also followed cultures derived from cells that were initially cultured in the presence of benzalkonium chloride, an active antimicrobial agent present in many hand-sanitizers. The balance between fluidity and solid phase membrane is achieved not only by changes in SCFA (both unsaturated and saturated), but also by changes in the ratios of BCFAC15:0 to BCFA C17:0 ($P<0.05$), and iso to anteiso FA ($P<0.05$). The decrease in the BCFA C15:0 to BCFA C17:0 ratio is presented as the primary response of the FA adaptation in *L. monocytogenes* to low temperature (Mastronicolis et al. 1998; Chihib et al. 2003). This different way of "memorized" modifications observed in the FA profile of cells that were initially cultured in the presence of benzalkonium chloride (pre-inoculation) can also be linked with the different surviving trends shown by these cells

compared to those of the other two series. Whenever the state of a biological system is not determined solely by present conditions but also depends on its past history, we can say that the system has memory. Many different mechanisms can be used to maintain a memory of the past history of a bacterial cell. Memory is persistent, although not permanent (Casadesús and D'Ari 2002). This memory effect triggered by environmental conditions is probably an unexplored adaptation mechanism of bacterial pathogens (Grunert et al. 2014).

Overall, under the conditions of this study, the bacterium seemed to adopt different strategies to preserve the optimal fluidity and the functions of the membrane depending on the history of the inoculated cells and time of storage. The FA profile of *L. monocytogenes*' membrane lipids is dominated by the uneven-numbered branched fatty acids C15:0 and C17:0. Consequently, it is important for the bacterium to change the ratio of these FA in order to preserve the optimal fluidity and the functions of the membrane, as these changes will enable it to survive under harsh conditions and to overcome the hurdles posed by the manufacturing process and ripening of dairy products.

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Compliance with ethical standards The manuscript does not contain clinical studies or patient data.

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

References

Annous BA, Becker LA, Bayles DO, Labeda DP, Wilkinson BJ (1997) Critical role of anteiso-C15:0 fatty acid in the growth of *Listeria monocytogenes* at low temperatures. Appl Environ Microbiol 63: 3887–3894

Beales N (2004) Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. Compr Rev Food Sci Food Saf 3:1–20. doi:10.1111/j.1541-4337.2004.tb00057.x

Bisbiroulas P, Psylou M, Iliopoulou I, Diakogiannis I, Berberi A, Mastronicolis SK (2011) Adaptational changes in cellular phospholipids and fatty acid composition of the food pathogen *Listeria monocytogenes* as a stress response to disinfectant sanitizer benzalkonium chloride. Lett Appl Microbiol 52:275–280. doi:10.1111/j.1472-765X.2010.02995.x

Camilli A, Goldfine H, Portnoy DA (1991) Listeria monocytogenes mutants lacking phosphatidyl inositol specific phospholipase C are avirulent. J Exp Med 173:751–754. doi:10.1084/jem.173.3.751

Carpentier B, Chassaing D (2004) Interactions in biofilms between *Listeria monocytogenes* and resident microorganisms from food industry premises. Int J Food Microbiol 97:111–122

Casadesús J, D'Ari R (2002) Memory in bacteria and phage. BioEssays 24:512–518

Chihib NE, Ribeiro da Silva M, Delattre G, Laroche M, Federighi M (2003) Different cellular fatty acid pattern behaviours of two strains of *Listeria monocytogenes* scott A and CNL 895807 under different temperature and salinity conditions. FEMS Microbiol Lett 218:155–160

Chihib NE, Tierny Y, Mary P, Hornez JP (2005) Adaptational changes in cellular fatty acid branching and unsaturation of *Aeromonas* species as a response to growth temperature and salinity. Int J Food Microbiol 102:113–119

Diakogiannis I, Berberi A, Siapi E, Arkoudi-Vafea A, Giannopoulou L, Mastronicolis SK (2013) Growth and membrane fluidity of foodborne pathogen *Listeria monocytogenes* in the presence of weak acid preservatives and hydrochloric acid. Front Microbiol 4:152. doi:10.3389/fmicb.2013.00152

Dittmer JC, Lester RL (1964) A simple specific spray for the detection of phospholipids on thin-layer chromatography. J Lipid Res 5:126–127

Folch J, Lees M, Stanley-Sloane GH (1957) A simple method for the isolation and purification of total-lipids from animal tissues. J Biol Chem 226:497–509

Gandhi M, Chikindas ML (2007) *Listeria*: a foodborne pathogen that knows how to survive. Int J Food Microbiol 113:1–15

Genigeorgis C, Carniciu M, Dufulescu D, Farver T (1991) Growth and survival of *Listeria monocytogenes* in market cheeses stored at 4 °C to 30 °C. J Food Prot 54:662–668

Giotis ES, McDowell DA, Blair IS, Wilkinson BI (2007) Role of branched chain fatty acid in pH stress tolerance in *Listeria monocytogenes*. Appl Environ Microbiol 73:997–1001

Grunert T, Monahan A, Lassnig C, Vogl C, Müller M et al (2014) Deciphering host genotype-specific impacts on the metabolic fingerprint of listeria monocytogenes by FTIR spectroscopy. PLoS ONE 9(12):e115959. doi:10.1371/journal.pone.0115959

Kousta M, Mataragas M, Skandamis P, Drosinos EH (2010) Prevalence and sources of cheese contamination with pathogens at farm and processing levels. Food Control 21:805–815

Kozak J, Balmer T, Byrne R, Fisher K (1996) Prevalence of *Listeria monocytogenes* in foods: incidence in dairy products. Food Control 7:215–221

Lambert G, Kussell E (2014) Memory and fitness optimization of bacteria under fluctuating environments. PLoS Genet 10(9):e1004556. doi:10.1371/journal.pgen.1004556

Long C, Staples DA (1961) Chromatographic separation of brain lipids. Cerebroside and sulphatide. Biochem J 78:179–185

Lopes JA (1986) Evaluation of dairy and food plant sanitizers against *Salmonella typhimurium* and *Listeria monocytogenes*. J Dairy Sci 69:2791–2796

Lopez CS, Alice AF, Heras H, Rivas EA, Sanchez-Rivas C (2006) Role of anionic phospholipids in the adaptation of *Bacillus subtilis* to high salinity. Microbiology 152:605–616

Lunden J, Autio T, Markkula A, Hellstrom S, Korkeala H (2003) Adaptive and cross-adaptive responses of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants. Int J Food Microbiol 82:265–272

Mastronicolis S, German JB, Smith GM (1996a) Diversity of the polar lipids of the food-borne pathogen *Listeria monocytogenes*. Lipids 31:635–640

Mastronicolis SK, German JB, Smith GM (1996b) Isolation and fatty acid analysis of neutral and polar lipids of the food bacterium *Listeria monocytogenes*. Food Chem 57:451–456

Mastronicolis SK, German JB, Megoulas N, Petrou E, Foka P, Smith GM (1998) Influence of cold shock on the fattyacid composition of different lipid classes of the foodborne pathogen *Listeria monocytogenes*. Food Microbiol 15:299–306

Mastronicolis SK, Boura A, Karaliota A, Magiatis P, Arvanitis N, Litos C, Tsakirakis A, Paraskevas P, Moustaka H, Heropoulos G (2006) Effect of cold temperature on the composition of different lipid classes of the foodborne pathogen *Listeria monocytogenes*: focus

on neutral lipids. Food Microbiol 23:184–194. doi:10.1016/j.fm.2005.03.001

Mastronicolis SK, Arvanitis N, Karaliota A, Magiatis P, Heropoulos G, Litos C, Moustaka H, Tsakirakis A, Paramera E, Papastavrou P (2008) Coordinated regulation of cold-induced changes in fatty acids with cardiolipin and phosphatidylglycerol composition among phospholipid species for the food pathogen Listeria monocytogenes. Appl Environ Microbiol 74:4543–4549

Mastronicolis SK, Berberi A, Diakogiannis I, Petrova E, Kiaki I, Baltzi T, Xenikakis P (2010) Alteration of the phospho- or neutral lipid content and fatty acid composition in *Listeria monocytogenes* due to acid adaptation mechanisms for hydrochloric, acetic and lactic acids at pH 5.5 or benzoic acid at neutral pH. Antonie Van Leeuwenhoek 98:307–316. doi:10.1007/s10482-010-9439-z

Ming X, Daeschel MA (1995) Correlation of cellular phospholipid content with nisin resistance of *Listeria monocytogenes* Scott A. J Food Prot 58:416–420

Morgan F, Bonnin V, Mallereau M-P, Perrin G (2001) Survival of *Listeria monocytogenes* during manufacture, ripening and storage of soft lactic cheese made from raw goat milk. Int J Food Microbiol 64: 217–221

Ottaviani F, Ottaviani M, Agosti M (1997) Differential agar medium for *Listeria monocytogenes*. Quimper Froid Symposium Proceedings p 6. ADRIA Quimper France. 16–18 June 1997

Papageorgiou D, Marth E (1989) Fate of *Listeria monocytogenes* during the manufacture, ripening and storage of Feta cheese. J Food Prot 52:82–87

Papageorgiou DK, Bori M, Mantis A (1996) Growth of *Listeria monocytogenes* in whey cheeses: Myzithra, Anthotyros and Manouri during its sotrage at 5, 12 and 22 °C. J Food Prot 59: 1193–1199

Papageorgiou DK, Bori M, Mantis A (1997) Survival of *Listeria monocytogenes* in frozen ewe's milk and feta cheese curd. J Food Prot 60:1041–1045

Pereira da Silva E, Pereira De Martinis EC (2013) Current knowledge and perspectives on biofilm formation: the case of *Listeria monocytogenes*. Appl Microbiol Biotechnol 97:957–968

Portnoy DA, Jacks PS, Hinrichs DJ (1988) Role of hemolysin for the intrtacellular growth of Listeria monocytogenes. J Exp Med 167: 1459–1471

Tasara T, Stephan R (2006) Cold stress tolerance of *Listeria monocytogenes*: a review of molecular adaptive mechanisms and food safety implications. J Food Prot 69:1473–1484

Theodoridis A, Papageorgiou D, Abrahim A, Karaioannoglou P (2006) Fate of *Listeria monocytogenes* during the manufacture and storage of Chevre Metsovo and Pichtogalo chanion cheeses. Ital J Food Sci 18:51–61

Vadyvaloo V, Hastings JW, van der Merwe MJ, Rautenbach M (2002) Membranes of class IIa bacteriocin-resistant *Listeria monocytogenes* cells contain increased levels of desaturated and short-acyl-chain phosphatidylglycerols. Appl Environ Microbiol 68:5223–5230

van Schaik W, Gahan CG, Hill C (1999) Acid-adapted *Listeria monocytogenes* displays enhanced tolerance against the lantibiotics nisin and lacticin 3147. J Food Prot 62:536–539

Verheul A, Russell NJ, Van't Hof R, Rombouts FM, Abee T (1997) Modifications of membrane phospholipid composition in nisin-resistant *Listeria monocytogenes* scott A. Appl Environ Microbiol 63:3451–3457

Wolf DM, Fontaine-Bodin L, Bischofs I, Price G, Keasling J, Arkin AP (2008) Memory in microbes: quantifying history-dependent behavior in a bacterium. PLoS ONE 3(2):e1700. doi:10.1371/journal.pone.0001700