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PAPER

Inhibition of *Listeria monocytogenes* by a formulation of selected dairy starter cultures and probiotics in an *in vitro* model

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ABSTRACT

Three strains of lactic acid bacteria (LAB) and a commercial probiotic were selected to evaluate their *in vitro* activity towards *Listeria monocytogenes*. The strains *Lactococcus lactis* ssp. *lactis*, strain 340, *L. lactis* ssp. *lactis*, strain 16; *Lactobacillus casei* ssp. *casei*, strain 208 and *Enterococcus faecium* UBEF-41 were inoculated into skim milk and brain heart infusion broth (BHI) to get an initial *Lactococcus: Lactobacillus: E. faecium* UBEF-41 ratio of 2:1:1 and a concentration of approximately 7 log cfu mL^{-1} until challenge vs. pathogen. *L. monocytogenes* ATCC 7644 was also inoculated in same media to get approximately 4 log cfu mL^{-1}. Growth curves in skim milk and BHI at 4, 10 and 30 °C, respectively were studied for: (i) LAB formulation; (ii) *L. monocytogenes* and (iii) LAB vs. *L. monocytogenes*. When challenged with LAB, at 30 °C in milk, *L. monocytogenes* was not detectable after day-3 and in BHI it decreased below log cfu mL^{-1} after day-5. At 10 and 4 °C, in both media, *L. monocytogenes* counts were always significantly lower ($p < .001$) than the counts of *L. monocytogenes* alone from day-2 for milk at 4 °C and BHI at 10 °C and from day-7 for BHI at 4 °C and milk at 10 °C. In conclusion, the proposed formulation was able to limit *L. monocytogenes in vitro* growth, even at refrigeration temperature.

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Introduction

The Centre for Disease Control and Prevention has been updating the list of multistate, foodborne outbreak investigations involving Listeria since 2006. Listeriosis, caused by *Listeria monocytogenes*, is a foodborne infection of public health concern due to its clinical severity and high fatality (Budiati et al. 2013).

In the US in 2016, multistate outbreaks of listeriosis were linked to frozen vegetables, raw milk and packaged salads (https://www.cdc.gov/listeria/outbreaks/). In Europe, in 2016, the Rapid Alert System for Food and Feed (RASFF) portal published 85 reports on the detection of *L. monocytogenes* in several products (meat and meat products, poultry meat, fish and fish products, milk and milk products, prepared dishes and snacks, herbs and spices), most of which were classified as 'serious risk' for consumers (https://webgate.ec.europa.eu/rasff-window/portal/?event=SearchByKeyword&StartRow=1).

In Italy in 2016, an outbreak of invasive listeriosis was associated with the consumption of head cheese (Marini et al. 2016). In the United Arab Emirates, no cases of listeriosis were reported in 2002 (Berger 2017). Therefore, while in the United Arab Emirates listeriosis have been rarely documented, the food imports trade from around the world is growing and it makes global the risks of contamination (Gohil et al. 1995, 1996).

Media coverages for recent warning, i.e. ice-cream contaminated with *L. monocytogenes* was recalled in the US and in other countries, including Qatar or collagen protein bar and Bite products recalled from 42 countries. The concerns regarding listeriosis are not only related to the high frequency of detection of *L. monocytogenes* due to the bacterial ecology, but also to the category of contaminated products (usually 'ready to eat products'), which are consumed without other previous treatment (Ajayeoba et al. 2016; Shimojima et al. 2016).

Listeria monocytogenes is a Gram-positive, rod-shaped, facultative anaerobic bacterium, ubiquitous and could be linked to several product categories as the alerts highlight (Lomonaco et al. 2015; Al-Nabulsi

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et al. 2016): effective strategies are essential not only to ensure food safety, but also to satisfy the health conscious consumers requirements for natural foods without chemical preservatives (Cenci-Goga et al. 2015).

The 'hurdle technology' has been applied to food preservation, as a gentle, but effective solution to preserve food quality, rheological properties and palatability of the product itself, ensuring a simultaneous control over the microbiota, which may allow the growth of microorganisms responsible for spoilage or poisoning (Pundhir and Murtaza 2015). The hurdle technology has been developed to decrease the use of preservatives, by a combination of factors ('hurdles'), in order to prevent the growth of undesired microbiota or to maintain it below acceptable levels during product shelf life (Leistner 2000).

The most important hurdles in food preservation are temperature, pH, water activity, redox potential, preservatives and competitive microbiota (e.g. lactic acid bacteria Leistner 2000). Food preservation implies that microorganisms are in a hostile environment for their survival.

Recent approaches are directed towards biocontrol using protective microflora, usually LAB, to inhibit the growth of pathogens and undesired microorganisms (Castellano et al. 2004). In fact, biopreservatives represent a hurdle, which consists of competitive microbiota, which are not only able to grow in the product without interfering with its characteristics, but at the same time, are also able to compete with pathogens, such as *L. monocytogenes* (Cizeikiene et al. 2013). For successful biopreservation, a LAB culture must survive during storage at refrigeration temperatures, compete with the relatively high, indigenous, microbial loads of raw meat, actively inhibit pathogenic and spoilage bacteria and should not alter the sensory properties of the product, except under temperature abuse conditions. The application of a LAB formulation with antimicrobial activity in food products might guarantee safety and quality without altering the characteristics of the product. To define the LAB formulation, it is important to consider the LAB and food characteristics, the technologies applied to it and the bacteria, against which LAB are expected to be active.

LAB play a key role in this respect (Cenci-Goga et al. 2008, 2012) and the properties of several strains have been studied (Djenane et al. 2005).

In an attempt to study the activity of the LAB formulation already used for salami production (Cenci-Goga et al. 2012, 2016), this research describes the effect of the LAB formulation on the fate of *L. monocytogenes* in an *in vitro* environment under different storage conditions.

Materials and methods

LAB formulation

The formulation of LAB used in the experiment consisted of three LAB, previously isolated from local, traditional cheeses made with raw ewes milk, manufactured in small scale dairy plants in the centre of Italy, together with a commercial probiotic. Bacterial strains were from the collection of the laboratory, Laboratorio di Ispezione degli Alimenti di Origine Animale and were classified as: *Lactococcus lactis* ssp. *lactis*, strain 340; *L. lactis* ssp. *lactis*, strain 16 and *Lactobacillus casei* ssp. *casei*, strain 208. Furthermore, *Enterococcus faecium UBEF-41* was isolated from the dietary supplement 'Enterelle' supplied by Bromatech Srl (Bromatech Srl, Natural micro food supplements, Milano, Italy). The strain *E. faecium* UBEF-41 has been further studied before inclusion in the starter formulation by sequencing and testing for antibiotic susceptibility by authors in previous studies (Cenci-Goga et al. 2016).

The characterisation and the acidifying activity of the formulation has been previously described by the authors (Cenci Goga et al. 1995; Clementi et al. 1998; Cenci-Goga et al. 2015).

Before the challenge test, freeze dried strains of *L. lactis* ssp. *lactis*, strain 340; *L. lactis* ssp. *lactis*, strain 16; *L. casei* ssp. *casei*, strain 208 were grown in de Man Rogosa Sharpe broth (MRS, OXOID, CM0359, Basingstoke, UK) at 30 °C for 48h and then spread on de Man Rogosa Sharpe agar (MRS agar, OXOID, CM1153) at 30 °C for 48h in microaerophilic conditions to check for purity. *E. faecium* UBEF-41 was grown in tryptic soy broth (TSB, BD Difco, Franklin Lakes NJ) at 37 °C for 48h on air and then spread on mEnterococcus agar (mENT, BD Difco) at 37 °C at 48h to check for purity. After incubation, the concentration for all the strains was approximately 9 log cfu mL^{-1}.

Challenge test and microbiological analysis

Before the challenge test, freeze dried *L. monocytogenes* ATCC 7644 was grown in brain heart infusion broth (BHI, BD Difco) and was incubated at 30 °C for 48h. *L. monocytogenes* need to be grown in a rich culture medium and BHI is the most commonly used non-selective media for cultivation of *Listeria* species (Jones and D'Orazio 2005). Later, *L. monocytogenes* was spread on Oxford agar (Listeria selective agar,

Table 1. Microbiological counts (log cfu mL^{-1}) at 4 °C for LAB, *E. faecium* UBEF-41 and *L. monocytogenes* in BHI and milk spiked with LAB only (LAB), *L. monocytogenes* only (Listeria) and with LAB + *L. monocytogenes* (Challenge).

		BHI						Milk					
		LAB		Listeria		Challenge		LAB		Listeria		Challenge	
	Day	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LAB	0	7.07	0.03	–	–	7.37	0.09	7.16	0.11	–	–	7.21	0.06
	2	7.17	0.10	–	–	7.19	0.07	7.28	0.11	–	–	7.26	0.09
	7	7.38	0.06	–	–	7.17	0.23	7.20	0.14	–	–	7.27	0.04
	14	7.29	0.11	–	–	7.20	0.10	7.37	0.06	–	–	7.24	0.08
	21	7.61^{a}	0.06	–	–	7.45^{b}	0.20	8.24^{a}	0.12	–	–	7.15^{b}	0.15
	28	7.06	0.14	–	–	7.06	0.22	7.69	0.20	–	–	7.36	0.10
Enterococcus faecium	0	7.10	0.10	–	–	7.09	0.03	7.18	0.10	–	–	6.93	0.06
	2	6.82	0.17	–	–	6.84	0.10	6.97	0.09	–	–	6.87	0.05
	7	6.79	0.18	–	–	6.95	0.13	7.19	0.01	–	–	7.12	0.07
	14	7.20	0.04	–	–	7.09	0.25	7.35	0.13	–	–	7.25	0.07
	21	7.17	0.18	–	–	7.33	0.02	8.17^{a}	0.07	–	–	6.04^{b}	0.01
	28	7.17	0.18	–	–	6.94	0.09	7.04	0.07	–	–	7.06	0.03
Listeria monocytogenes	0	–	–	4.47	0.19	4.02	0.34	–	–	4.66	0.01	4.64	0.12
	2	–	–	4.60	0.20	4.21	0.27	–	–	4.65^{a}	0.17	3.73^{b}	0.04
	7	–	–	5.96^{a}	0.07	3.67^{b}	0.06	–	–	5.33	0.07	5.03	0.05
	14	–	–	7.31^{a}	0.05	3.35^{b}	0.05	–	–	6.24	0.11	5.79	0.11
	21	–	–	8.98^{a}	0.08	3.34^{b}	0.03	–	–	7.09^{a}	0.09	5.90^{b}	0.11
	28	–	–	8.84^{a}	0.29	3.42^{b}	0.28	–	–	7.63^{a}	0.17	6.15^{b}	0.13

^{a,b}Different superscripts in the same row and within the same group (BHI or Milk) indicate significant different means ($p < .001$).
LAB: lactic acid bacteria.

Oxoid, with Listeria selective supplement) at 37 °C for 48h to check for purity. The concentration after incubation was approximately 10^{8} cfu mL^{-1}.

For the trial in skim milk, the LAB formulation was inoculated as pure cultures into 200 mL of skim milk (BD Difco) to get an initial *Lactococcus: Lactobacillus: E. faecium* UBEF-41 ratio of 2:1:1 and a concentration of approximately 10^{7} cfu mL^{-1} until challenge vs. pathogen. *L. monocytogenes*. ATCC 7644 was then inoculated into 200 mL of skim milk (BD Difco, 232100) to get an initial concentration of approximately 10^{4} cfu mL^{-1}.

For the trial in BHI, the LAB formulation was inoculated as pure cultures into 200 mL of BHI broth (BD Difco) with the same ratio *Lactococcus: Lactobacillus: E. faecium* UBEF-41 applied to skim milk and an initial concentration of approximately 10^{7} cfu mL^{-1} until challenge vs. pathogen. *L. monocytogenes*. ATCC was then inoculated into BHI broth (BD Difco) to get an initial concentration of approximately 10^{4} cfu mL^{-1}.

Growth curves for the following challenges were studied: (i) LAB formulation, (ii) LAB formulation vs. *L. monocytogenes* and (iii) *L. monocytogenes* alone. The challenges were carried out in skim milk and in BHI broth (BD Difco) at 30, 10 and 4 °C, respectively. The incubator (Sanyo MIR-153) maintained storage temperatures and the selected temperatures were monitored with a data logger.

Bacterial counts were recorded on day-0, 1, 2, 3, 4, 5 at 30 °C and on day-0, 2, 7, 9, 14, 18, 21 and 28 at 10 °C and 4 °C for LAB and *L. monocytogenes*.

Microbiological analyses were conducted with serial, decimal dilutions in triplicate, 0.1 and 0.01 mL samples of appropriate dilutions, which were poured or spread on selective agar plates. *Lactobacillus* spp. and *Lactococcus* spp. on MRS Agar were incubated at 30 °C for 48 h under microaerophilic conditions. Enterococci on mENT agar, were incubated at 37 °C for 48 h and *L. monocytogenes* were incubated on Oxford agar for 24 h at 30 °C. Sterility control was spread on Plate count agar (Oxoid) and were incubated for 48 h at 30 °C.

Sensitivity for the spread plate and for the pour plate was 10^{2} cfu g^{-1} and 10 cfu g^{-1}, respectively. The 95% confidence limit, as given by the classic formula $2s = 2\sqrt{x}$ (Adams and Moss 2000), ranged between ±37 and ±12% (i.e. plates with a number of colony forming unit (cfu) ranging from 30 to 300; Cenci-Goga et al. 2015).

Analysis of results

The arithmetic means of the triplicates was calculated for each sampling and then all the values (converted to log for microbiological analyses) were elaborated using GraphPad InStat, version 3.0b (La Jolla, CA) and graphs were obtained with GraphPad Prism version 6.0d (La Jolla, CA) for Mac OS X, respectively. The challenge was repeated for three times.

The $\log_{10}$ of the arithmetic means for all microbiological analyses was calculated for each of the three repetitions, following which all $\log_{10}$ data were analysed for variance with GraphPad InStat, version 3.0b,

Table 2. Microbiological counts (log cfu mL^{-1}) at 10 °C for LAB, *E. faecium* UBEF-41 and *L. monocytogenes* in BHI and milk spiked with LAB only (LAB), *L. monocytogenes* only (Listeria) and with LAB + *L. monocytogenes* (Challenge).

		BHI						Milk					
		LAB		Listeria		Challenge		LAB		Listeria		Challenge	
	Day	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LAB	0	7.07	0.03	–	–	7.37	0.09	7.16	0.11	–	–	7.21	0.06
	2	6.85	0.43	–	–	7.48	0.09	7.55	0.07	–	–	7.34	0.02
	7	8.39	0.01	–	–	8.41	0.09	8.64	0.09	–	–	8.56	0.31
	9	8.24	0.20	–	–	8.15	0.16	9.27	0.07	–	–	9.11	0.20
	14	8.42	0.10	–	–	8.29	0.08	9.01	0.14	–	–	8.92	0.28
	21	8.24	0.12	–	–	8.22	0.02	8.93	0.27	–	–	8.79	0.04
	28	8.82	0.13	–	–	8.49	0.44	9.05	0.17	–	–	9.06	0.11
Enterococcus faecium	0	7.10	0.10	–	–	7.09	0.03	7.18	0.10	–	–	6.93	0.06
	2	7.14	0.25	–	–	7.21	0.13	7.24	0.08	–	–	7.29	0.07
	7	7.66	0.04	–	–	7.94	0.29	8.63	0.10	–	–	8.59	0.13
	9	8.01	0.20	–	–	7.96	0.08	8.90	0.05	–	–	8.79	0.33
	14	8.21	0.13	–	–	8.11	0.20	8.59	0.02	–	–	8.51	0.21
	21	8.17	0.07	–	–	8.06	0.13	8.48	0.13	–	–	8.48	0.13
	28	8.76^{a}	0.13	–	–	8.04^{b}	0.01	8.36	0.10	–	–	8.27	0.03
Listeria monocytogenes	0	–	–	4.47	0.19	4.02	0.34	–	–	4.66	0.01	4.64	0.12
	2	–	–	5.81^{a}	0.16	3.73^{b}	0.04	–	–	5.68	0.08	5.33	0.16
	7	–	–	8.64^{a}	0.04	3.45^{b}	0.08	–	–	7.43^{a}	0.13	5.31^{b}	0.05
	9	–	–	8.94^{a}	0.14	3.54^{b}	0.20	–	–	7.75^{a}	0.35	5.38^{b}	0.04
	14	–	–	8.90^{a}	0.09	3.50^{b}	0.20	–	–	7.97^{a}	0.05	4.74^{b}	0.04
	21	–	–	8.63^{a}	0.20	3.51^{b}	0.23	–	–	8.06^{a}	0.08	3.03^{b}	0.05
	28	–	–	8.90^{a}	0.11	3.25^{b}	0.20	–	–	8.09^{a}	0.22	1.72^{b}	0.24

^{a,b}Different superscripts in the same row and within the same group (BHI or Milk) indicate significant different means ($p < .001$).
LAB: lactic acid bacteria.

for Mac OS X, followed by the Tukey Kramer multiple comparisons test.

Results and discussion

Tables 1, 2 and 3 show the microbiological counts at 4, 10 and 30 °C, respectively for LAB, *E. faecium* UBEF-41 and *L. monocytogenes* in BHI and milk spiked with LAB only (LAB), *L. monocytogenes* only (Listeria) and with LAB + *L. monocytogenes* (Challenge).

At 4 °C, when *L. monocytogenes* was spiked alone it reached values of 5.96 on day-7 and 8.98 log cfu mL^{-1} on day-21 in BHI and 5.33 on day-7 and 7.63 log cfu mL^{-1} on day-28 in milk, respectively. For the challenge at 4 °C, log reduction for *L. monocytogenes* at day-7 and day-28 in BHI was 2.29 and 5.42 log cfu mL^{-1}, respectively. The skim milk log reduction for *L. monocytogenes* at day-21 was found to be 1.68 log cfu mL^{-1}.

Refrigeration temperature is used to store several ready-to-eat products and at this temperature *L. monocytogenes* revealed its psychrotrophic character.

The comparison between growth in skim milk and in broth shows than at 4 °C LAB and *E. faecium* alone are not so influenced by the presence of *L. monocytogenes* while *L. monocytogenes* maintain higher value in skim milk instead of BHI during the challenge; this is possibly connected to the evidence that at 4 °C the LAB formulation maintain the same concentration throughout the 28 experimental days while *L. monocytogenes* find favourable conditions to grow.

When the temperature was at 4 °C the metabolism was seen to be slower and the LAB concentration was also seen to be less effective in inhibiting that at higher temperatures. At 10 °C, *L. monocytogenes*, when spiked alone, reached values of 8.64 on day-7 and 8.94 log cfu mL^{-1}on day-9 in BHI and 7.43 on day-7 and 8.09 log cfu mL^{-1} on day-28 in milk, respectively. For the challenge at 10 °C, all counts for *L. monocytogenes* from day-2 in BHI and from day-7 in milk were significantly lower. For the challenge at 10 °C, log reduction for *L. monocytogenes* at day-2 and day-28 in BHI was of 2.08 and of 5.65 log cfu mL^{-1}, respectively and at day-28 in skim milk the log reduction for *L. monocytogenes* was found to be 6.37 log cfu mL^{-1}.

At 10 °C in skim milk *L. monocytogenes* is more effectively inhibited considering that for the challenge at day-28 LAB reach 9.06 and in BHI 8.49 log cfu mL^{-1}, respectively.

At 30 °C, for the LAB vs. *L. monocytogenes* challenge in day-5, in BHI broth *L. monocytogenes* was 1.30 log cfu mL^{-1}, whereas in milk it was no longer detectable at day-3.

At 30 °C the LAB growth is faster and the high concentration level guarantees effective *L. monocytogenes* inhibition.

The differences between the results obtained between skim milk and BHI are mostly related to the

Table 3. Microbiological counts (log cfu mL^{-1}) at 30 °C for LAB, *E. faecium* UBEF-41 and *L. monocytogenes* in BHI and milk spiked with LAB only (LAB), *L. monocytogenes* only (Listeria) and with LAB + *L. monocytogenes* (Challenge).

		BHI						Milk					
		LAB		Listeria		Challenge		LAB		Listeria		Challenge	
	Day	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LAB	0	7.09	0.03	–	–	7.09	0.07	7.12	0.03	–	–	7.19	0.05
	1	8.25	0.08	–	–	8.03	0.08	8.25	0.04	–	–	8.29	0.04
	2	8.16	0.06	–	–	8.22	0.02	8.92	0.03	–	–	9.12	0.13
	3	7.74	0.18	–	–	7.99	0.08	9.16	0.05	–	–	8.90	0.13
	4	8.25^{a}	0.08	–	–	7.46^{b}	0.08	9.19^{a}	0.13	–	–	8.40^{b}	0.09
	5	8.04	0.04	–	–	7.97	0.04	9.30	0.07	–	–	9.14	0.18
Enterococcus faecium	0	6.90	0.01	–	–	6.90	0.11	6.91	0.07	–	–	6.91	0.07
	1	7.78	0.07	–	–	7.80	0.12	8.04	0.02	–	–	8.00	0.06
	2	7.77	0.11	–	–	7.64	0.07	7.91	0.06	–	–	7.91	0.04
	3	7.70	0.06	–	–	7.93	0.09	8.82	0.12	–	–	8.82	0.10
	4	7.55	0.33	–	–	7.93	0.09	8.10^{a}	0.08	–	–	8.83^{b}	0.10
	5	6.71^{a}	0.31	–	–	7.59^{b}	0.02	7.04^{a}	0.06	–	–	7.96^{b}	0.16
Listeria monocytogenes	0	–	–	4.32	0.02	4.10	0.03	–	–	4.30	0.07	4.32	0.16
	1	–	–	4.32	0.02	4.10	0.03	–	–	4.30	0.07	4.32	0.16
	2	–	–	7.18^{a}	0.04	3.46^{b}	0.11	–	–	7.31^{a}	0.03	3.60^{b}	0.12
	3	–	–	8.10^{a}	0.06	3.35^{b}	0.08	–	–	8.20^{a}	0.07	0.00^{b}	0.00
	4	–	–	8.01^{a}	0.08	2.88^{b}	0.03	–	–	8.05^{a}	0.12	0.00^{b}	0.00
	5	–	–	8.76^{a}	0.13	1.30^{b}	0.00	–	–	8.20^{a}	0.11	0.00^{b}	0.00

^{a,b}Different superscripts in the same row and within the same group (BHI or Milk) indicate significant different means ($p < .001$).
LAB: lactic acid bacteria.

higher ability for LAB to grow on milk (lower final pH, no buffering capacity and higher sugar content) with a more effective competitive growth compared to the growth on BHI.

The microbiological counts (Tables 1–3) for LAB and *E. faecium* showed no significant differences between the samples spiked with and without *L. monocytogenes,* except for incubation at 4 °C on day-21 (both BHI and milk), at 10 °C on day-28 (only in BHI and only for *E. faecium*), at 30 °C on day-4 for LAB (both in BHI and milk) and on day-4 and day-5 for *E. faecium* (both in BHI and milk). These results prove that the formulation used in this work was not affected by the presence of *L. monocytogenes* and that the two groups of LAB (lactobacilli and lactococci vs. enterococci) did not compete. The plateau was reached between day-2 and day-7 for LAB and for *E. faecium* and was maintained throughout the experiment at 4, 10 and 30 °C, respectively: this proves that the proposed formulation is balanced and consistent over time. *Listeria monocytogenes* is widely distributed in the environment and is frequently found in food. The incidence of listeriosis has been rising since the year 2000 in Europe (Marini et al. 2016). Due to the clinical severity of the illness, listeriosis is a major public health concern. Several psychrotrophic bacteria are able to grow at refrigeration temperatures if they are stored for a sufficient amount of time and they can be responsible for meat spoilage (Iulietto et al. 2015). Some strains of LAB are antagonistic against many microorganisms and might be useful as biopreservatives to increase meat shelf life and safety, by inhibiting spoilage and pathogenic bacteria. In particular, one approach to control foodborne listeriosis is to apply biopreservatives as competitors of the pathogen. To be effective, biopreservatives are required to grow under the same conditions as the target pathogen (Schillinger et al. 1991). The effect of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* used for yogurt made of camel milk was described towards *Escherichia coli* and *L. monocytogenes* at 4 and 10 °C, respectively (Al-Nabulsi et al. 2016). The counts of *L. monocytogenes* in camel milk were not different in the presence or absence of LAB until the day-14 when *L. monocytogenes* had a significant reduction in the presence of LAB at 4 and 10 °C, which might be attributed to the antimicrobial effects of LAB against *L. monocytogenes* (Cleveland et al. 2001).

Conclusions

The LAB formulation proposed in this study consists of psychrotrophic microorganisms, capable of developing under refrigerated conditions without modifying the organoleptic characteristics or spoil food products, as concluded in previous studies (Cenci-Goga et al. 2016).

In the proposed *in vitro model,* the ability of the LAB formulation to grow at 4, 10 and 30 °C both in milk and in BHI broth, was confirmed and maintained a concentration of almost always above 7 log cfu mL^{-1} throughout the entire experiment.

The application of the formulation of LAB and probiotics described in this study may provide an additional tool for preventing the growth and survival of potentially pathogenic psychrotrophic *Listeria* spp. strains.

The present formulation proved it was possible to achieve an enhanced inhibition of *L. monocytogenes*.

This study expand previous researches conducted on the effectiveness of the tested LAB formulation with particular emphasis on those products that can be contaminated by *L. monocytogenes* during food processes. We suggest to consider this LAB formulation as biopreservative to be added to for risk management of *L. monocytogenes* in dairy, meat and ready-to-eat products of plant origin.

Disclosure statement

No potential conflict of interest was reported by the authors.

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