LWT - Food Science and Technology 163 (2022) 113579

Contents lists available at ScienceDirect

LWT

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

Selection and characterization of lactic acid bacteria with activity against *Listeria monocytogenes* from traditional RTE ripened foods

Irene Martín [a,*], Alicia Rodríguez [a], Alberto Alía [a], Remigio Martínez [b], Juan J. Córdoba [a]

[a] Higiene y Seguridad Alimentaria, Instituto Universitario de Investigación de Carne y Productos Cárnicos, Facultad de Veterinaria, Universidad de Extremadura, Avda. de Las Ciencias. S/n, 10003, Cáceres, Spain
[b] TECAL, Instituto Universitario de Investigación de Carne y Productos Cárnicos, Facultad de Veterinaria, Universidad de Extremadura, Avda. de Las Ciencias, S/n, 10003, Cáceres, Spain

ARTICLE INFO

Keywords:
Lactic-acid bacteria
Anti-*Listeria monocytogenes* activity
PFGE analysis

ABSTRACT

Lactic-acid bacteria isolated throughout the ripening process from traditional RTE soft cheeses and dry-cured fermented sausages were characterized and selected for their anti-*L. monocytogenes* activity. For this, the LAB isolates were first screened for their activity against this pathogen in agar spot assay and further evaluated in cheese and dry-cured fermented sausage models simulating conditions of ripening of these products. From a total of 371 LAB isolates, 84 showed anti-listerial activity in agar spot assay and only 10 of them were selected for reducing *L. monocytogenes* counts in food models. The selected LAB strains belonged to the species *Enterococcus faecium, Lacticaseibacillus casei, Lacticaseibacillus paracasei, Lactilactobacillus sakei* and *Lactococcus garvieae.* All of them have at least one gene encoding known bacteriocins. The most active strains, *Lc. casei* 116 and *Ll. sakei* 205, provoked reductions higher than 2 log cycles of *L. monocytogenes* levels in food models, respectively. The selected LAB strains may be utilized to control *L. monocytogenes* throughout the ripening process in ripened soft cheeses and dry-cured fermented sausages. The combination of 16S rRNA sequencing with PFGE analysis with restriction *Sgs*I and *Not*I enzymes could be useful to control the implantation of these selected strains throughout the ripening process.

1. Introduction

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive, facultatively anaerobic, rod-shaped intracellular bacterium (Filipello et al., 2017; Rodriguez et al., 2021). Due to its resistance to high salinity and low pH environments, ability to form biofilms, and growth at low temperatures, *L. monocytogenes* is found in raw, unpasteurized milk or cheeses, although in the last decade, other foods have also been involved in several outbreaks, including meatloaf, smoked fish, fermented raw sausages, or vegetables, especially in ready-to-eat (RTE) processed foods (Shamloo et al., 2019; Smith et al., 2018). The presence of *L. monocytogenes* in ripened products such as traditional RTE dry-cured fermented sausages and cheeses is of great public health concern because it causes listeriosis. The invasive form of this disease has a high mortality rate and even if patients survive, severe neurological sequelae may possess (García et al., 2020). Undoubtedly, the control of *L. monocytogenes* in traditional RTE ripened foods constitutes a challenge for fermented food-related industries.

An alternative to control this pathogenic bacterium in these RTE products is the use of selected lactic-acid bacteria (LAB) with anti-*L. monocytogenes* activity as a protective culture (Siedler et al., 2019; Singh, 2018). These antimicrobial properties of LAB are attributed to different modes of action including competition for nutrients and/or space and the production of one or more antimicrobial active metabolites such as organic acids (mainly lactic and acetic acids), hydrogen peroxide and also other compounds, such as bacteriocins (Vieco-Saiz et al., 2019; Yap et al., 2021). They have been widely used in traditional fermentation processes since they are Generally Recognized As Safe (GRAS) and have important properties, such as inhibition of the growth of *L. monocytogenes* (Reis et al., 2012). Before using LAB in traditional RTE ripened foods, it is necessary a selection of strains adapted to these products with activity against *L. monocytogenes*. LAB can be found naturally in different food products (Carr et al., 2002; Gajbhiye & Kapadnis, 2016) including dry-cured fermented sausages and ripened

* Corresponding author.
E-mail address: iremartint@unex.es (I. Martín).
URL: http://higiene.unex.es/ (I. Martín).

https://doi.org/10.1016/j.lwt.2022.113579
Received 8 February 2022; Received in revised form 4 May 2022; Accepted 16 May 2022
Available online 19 May 2022

cheeses (Perin et al., 2017). However, not all LAB strains present as natural contamination are capable of surviving in these traditional ripened foods have anti-*L. monocytogenes* activity. Thus, it is necessary to evolve an appropriate methodology for the selection and characterization of active LAB against *L. monocytogenes* adapted to traditional ripened products. In this work, the methodology used to isolate and characterize LAB with anti-*L. monocytogenes* activity in both traditional dry-cured fermented sausage and in traditional soft-ripened cheeses industries will be described.

The objective of this work was to isolate, select and characterize LAB from traditional RTE soft-ripened cheeses and dry-cured fermented sausages throughout their ripening process with anti-*L. monocytogenes* activity.

2. Materials and methods

2.1. Listeria monocytogenes culture and conditions

To evaluate the anti-*L. monocytogenes* activity from LAB isolates in agar spot assay and LAB strains in food models, the strain of *L. monocytogenes* S7-2 (serotype 4b) was used. This strain belongs to the National Institute of Agricultural and Food Research and Technology (INIA) collection (Madrid, Spain) and was isolated from ripened foods. To prepare the *L. monocytogenes* inoculum, 100 μL of a stock culture (stored in Brain Heart Infusion (BHI) broth (Pronadisa, Madrid, Spain) containing 20% (w/v) glycerol at −80 °C) was sub-cultured twice onto BHI broth at 37 °C for 24 h. To check the level of inoculation, serial dilutions were plated onto Chromagar™ Listeria agar plates (Scharlab, Madrid, Spain) and incubated at 37 °C for 48 h.

2.2. Obtention of LAB isolates in ripened cheeses and dry-cured fermented sausages industries

This study was carried out in 12 factories of traditionally Protected Designation of Origin (PDO) soft-bodied cheeses from "Torta del Casar" (A, B and C), "Queso de la Serena" (D, E, F and G) and "Queso Ibores" (H, I, J, K and L) and 3 dry-cured fermented meat sausages factories (M, N and O). In cheese industries, samples for LAB isolation were aseptically taken from milk, curd and half-ripened and ripened cheeses. In the dry-cured fermented sausages factories, samples for LAB isolates were aseptically taken from the meat mixture before stuffing and the product at the middle and end of ripening. Additionally, work and equipment surfaces were sampled with sterile swabs (Aes Chemunex, France).

All taken samples, including swabs and food products, were put into sterile stomacher bags under suitable conditions and transported under refrigerated conditions to the lab to be processed. Then, 10 g of products or swabs were mixed with 90 mL of 1% (w/v) peptone water (Conda, Spain) and homogenized in a Stomacher device (Seward, model 400 Circulator, UK) at 300 rpm for 2 min. Several decimal dilutions were prepared in 1% (w/v) sterile peptone water for LAB enumeration on Man Rogosa Sharpe agar (MRS; Conda) and incubated at 30 °C for 48 h, under microaerophilic conditions (ISO 15214:1998). The characteristic colonies in the MRS agar were picked from the agar plates, re-streaked to obtain pure culture and preliminarily characterized as LAB isolates according to the Gram stain reaction, morphology in MRS plates and microscopic observation. All LAB isolates were cultured in MRS broth (Conda) and stored at −80 °C in the same medium with 10% (v/v) glycerol (Fisher Scientific, USA).

2.3. Screening of anti-L. monocytogenes activity of LAB isolates

The anti-*L. monocytogenes* activity of all LAB isolates obtained was firstly tested by using the agar spot assay. For this, LAB isolates were grown under microaerophilic conditions in 10 mL of MRS broth and incubated for 48 h at 30 °C. After incubation, the cultures were centrifuged for 3–5 min at 10,000 rpm. Subsequently, the pellet obtained was resuspended in 200 μL MRS broth and 10 μL was spotted onto the surface of BHI agar (Conda). Then, these plates were overlaid with 10 mL of BHI agar containing 10^6 CFU/mL of *L. monocytogenes* S7-2. The anti-listerial activity was determined by observation of clearing zones of inhibition around LAB spot. Only LAB isolates showing zones greater than 2 mm of inhibition were selected.

2.4. Identification of selected LAB isolates by 16S rRNA sequencing

The selected LAB isolates were inoculated in a MRS broth medium and then incubated at 30 °C for 24 h. For each isolate studied, the DNA was extracted according to the instructions of the commercial DNA extraction MasterPure™ Complete DNA and RNA Purification Kit (Epicentre, Madison, WI, USA). The obtained DNA was eluted in 35 μL of TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA; Epicentre) and kept at −20 °C until required. The DNA concentration (ng/μL) and purity (A_{260}/A_{280} ratio) were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). Then, the identification of the LAB strains was performed by sequencing analysis of the 16S rRNA region according to the methodology proposed by Walter et al. (2000). Sequence analysis of PCR products was carried out by the Applied Bioscience Techniques Service of the University of Extremadura (STAB, Badajoz, Spain). The sequence results obtained were aligned with the nucleotide sequence database in GenBank using the BLAST tool from NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were analyzed and 97% similarity was used as the criterion for species identification.

2.5. Evaluation of anti-L. monocytogenes activity of selected LAB strains in food models

2.5.1. Preparation of food models and inocula of tested microorganisms

The LAB strains preliminarily selected by their anti-*L. monocytogenes* activity in agar spot assay were further evaluated for their efficiency to inhibit this pathogen in food models. For this, model systems relied on "Torta del Casar" soft cheese and dry-cured fermented sausage were used. The conditions used were those that these products usually have at the first stages of the ripening process when microorganisms could be inoculated into the products.

To prepare "Torta del Casar" cheese model system, ripened cheeses were firstly lyophilized in a freeze dryer (Labconco®, USA). Then, the "Torta del Casar" cheese-based agar was carried out by autoclaving 20 g of bacteriological agar (Pronadisa, Spain) in 500 mL of deionized water for 20 min at 121 °C (103 kPa) and mixed with 200 g of freeze-dried "Torta del Casar" cheese and 12.5 g of trisodium citrate dehydrate (Scharlab, Spain) heated in 400 mL of deionized water at 100 °C for 1 h (Gori et al., 2007; Martín et al., 2022). Finally, the pH was adjusted to 6.0 using lactic acid by using a pH meter (Crison, Spain) and the water activity (aw) value was checked to confirm a value of 0.975 by an aw meter (Novasina AG, Switzerland). This was done before cooling down the medium up to 45–50 °C in a water bath with shaken vigorously prior to pouring 15 mL into 25 cm^2 Petri plates.

The dry-cured fermented sausage model system was prepared with dry-cured fermented "salchichón", as the main constituent, lyophilized in a freeze dryer from Labconco®. Next, the dry-cured fermented "salchichón"-based agar was prepared by autoclaving 250 g/L of lyophilized dry-cured fermented sausage "salchichón" and 20 g/L of bacteriological agar. The pH was adjusted to 5.5 with lactic acid and the aw was found to be 0.98 by using a pH meter (Crison) and an aw meter (Novasina AG), respectively. After autoclaving for 20 min at 121 °C, the culture medium was cold down up to 45–50 °C and vigorously shaken prior to pouring into 25 cm^2 Petri plates.

For preparing the inoculum of *L. monocytogenes* S7-2, the working cultures of this microorganism were consecutively grown twice in BHI broth at 37 °C for 24 h each. The concentration of the fresh cultures was adjusted to reach a concentration of 4 log CFU/mL in the inoculum.

The inoculum of each one of the selected LAB strains was prepared after microaerophilic conditions growing twice in 10 mL of MRS broth at 30 °C each. After the incubation, the cultures were centrifuged for 3–5 min at 10,000 rpm. Subsequently, the pellet obtained was resuspended in 200 μL MRS broth and diluted with MRS to reach a concentration of 4 log CFU/mL to prepare the inoculum.

2.5.2. Experimental settings

L. monocytogenes S7-2 and the selected LAB strains were inoculated at the same time in "Torta del Casar" cheese-based agar or dry-cured fermented-based agar depending on the LAB industry (cheese or dry-cured fermented sausage) origin, to simulate conditions found by LAB strains in ripened foods when *L. monocytogenes* reaches these products by contamination. For this, the inoculum of *L. monocytogenes* 4b (50 μL) and LAB strains (in a total volume of 50 μL) were inoculated to obtain final concentrations of approximately 3 log CFU/cm^2 to reach level of *L. monocytogenes* slightly higher than those allowed in European Union (Regulation (EC) nº 1441/2007). Samples were incubated for 7 days at 7 °C. This temperature was used to simulate cooling temperature according to the maximum temperature allowed for the "Torta del Casar" cheese and dry-cured fermented sausages at the first stages of ripening (Regulation (EC) nº 853/2004). All assays were conducted in quintuplicate.

2.5.3. Enumeration of inoculated microorganisms

Levels of inoculated microorganisms (*L. monocytogenes* and LABs) were determined at days 0 and 7 of incubation time. For this, inoculated "Torta del Casar" cheese-based agar and dry-cured fermented sausage-based agar were mixed with 30 mL of 1% (w/v) peptone water and homogenized in a Stomacher device at 300 rpm for 1 min. Decimal serial dilutions of the homogenate were subsequently carried out in 1% (w/v) peptone water and then 100 μL of the cell suspensions were spread onto Chromagar™ *Listeria* agar plates to determine *L. monocytogenes* counts and on MRS for LAB counts. Plates of Chromagar™ *Listeria* agar were incubated at 37 °C for 24–48 h and characteristic *L. monocytogenes* colonies (green colonies with a surrounded opaque halo) were counted, and results expressed as log CFU/cm^2. MRS plates were incubated at 30 °C for 48 h, under microaerophilic conditions (ISO 15214:1998) and results were expressed as log CFU/cm^2. Those LAB strains showing the highest activity against *L. monocytogenes* were further selected for the next assays.

2.6. Detection of genes encoding known bacteriocins

The LAB strains showing the highest anti-*L.monocytogenes* activity in food model systems were tested by PCR based on genes related to bacteriocins production (nisin, lacticin 481, lactococcins A, B, G, Q and 972, brevicin, plantaracins A, E, F, S, NC8 and pediocin PA) production. For this, total genomic DNA from overnight cultures of these LAB strains was extracted according to the instructions of the MarterPure™ Complete DNA and RNA Purification kit (Epicentre). The DNA obtained was eluted in TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA; Epicentre) and kept at −20 °C until required. The DNA concentration (ng/μL) and purity (A_{260}/A_{280} ratio) were determined using a 1.5 μL aliquot on a NanoDrop spectrophotometer (Thermo Fisher Scientific).

All primers used in the PCR reactions (Table 1) were previously reported for bacteriocin gene detection in LAB (Alegría et al., 2010; Ho et al., 2018; Holo et al., 2001; Maldonado et al., 2004; Rodríguez et al., 2000; Stephens et al., 1998; Xie et al., 2011). They were purchased from Sigma. For amplification of DNA amplicons from 44 to 1110 bp (Table 1), 50 μL reaction mixtures containing 2.5 mM $MgCl_2$, 1 μL reaction buffer, 100 μM each deoxynucleoside triphosphate (dNTP), 100 pmol of each primer, 5 U of Taq DNA polymerase (Thermo Fisher Scientific), and 250 ng of genomic DNA of selected LAB strains as the template were used, with a thermal cycler MASTER CYCLER® (Eppendorf). Several protocols were optimized for each gene encoding known bacteriocins (Table 2). After amplification, PCR products were separated by electrophoresis on 1.5% (w/v) agarose gels using 1x Tris acetate EDTA (Scharlab S.L., Spain) at 90 V for 1 h. Gels were stained with 3 μL Red Safe Nucleic Acid Staining Solution (iNtRon Biotechnology, Korea). DNA bands were visualized with a UV transilluminator Gel Doc 2000 Image Documentation System (Bio-Rad Laboratories, USA) and then photographed by means of the camera GeneSnap and analyzed by the software equipment GeneTools (Syngene, UK). A DNA molecular size marker of 0.05–1.5 kbp (NZYTech Lda., Portugal) was used to determine the size of the PCR products.

Table 1
Primers used for PCR amplification of the bacteriocin-related genes.

Target gene	Primers' name	Primer sequence (5′-3′)	Size amplicon (bp)	References
Nisin	nisZ-prom-F	CTCGACGATACCATCACTCTTC	1010	Ho et al. (2018)
	nisP3	TCTTTCCCATTAACTTGTACTGTG		
Lacticin 481	lact-481-F	TCTGCACTCACTTCATTAGTTA	366	Rodríguez et al. (2000)
	lact481-R	AAGGTAATTACACCTCTTTTAT		
Lactococcin 972	lcn972-F	TTGTAGCTCCTGCAGAAGGAACATGG	312	Alegría et al. (2010)
	lcn972-R	GCCTTAGCTTTGAATTCTTACCAAAAG		
Lactococcin G and Q	lactGQ-F	GAAAGAATTATCAGAAAAAG	620	Alegría et al. (2010)
	lactGQ-R	CCACTTATCTTTATTTCCCTCT		
Lactococcin A and B	lcnAB-F	GAAGAGGCAATCAGTAGAG	771	Alegría et al. (2010)
	lcnA-R	GTGTTCTATTTATAGCTAATG		
	lcnB-R	CCAGGATTTTCTTTGATTTACTTC		
Brevicin	brevicin 174A-F	GTCTTAAATGCTAGGCTTGTCA	766	Noda et al. (2015)
	brevicin 174A-R	CTGGCAAGACAAACGGTTAG		
Plantaracin A	pnlA-F	TAGAAATAATTCCTCCGTACTTC	573	Xie et al. (2011)
	pnlA-R	ATTAGCGATGTAGTGTCATCCA		
Plantaracin EF	plnEF-F	TATGAATTGAAAGGGTCCGT	516	Xie et al. (2011)
	pnlEF-R	GTTCCAAATAACATCATACAAGG		
Pediocin PA	pediocin PA-1-F	AAAGATACTGCGTTGATAGG	1120	Xie et al. (2011)
	pediocin PA-1-R	GAGAAGCCATGCTGAAAG		
Plantaracin NC8	pnlNC8-F	GGTCTGCGTATAAGCATCGC	159	Maldonado et al. (2004)
	pnlNC8-R	AAATTGAACATATGGGTGCTTTAAATTCC		
Plantaracin S	pnlS-F	GCCTTACCAGCGTAATGCCC	320	Stephens et al. (1998)
	pnlS-R	CTGGTGATGCAATCGTTAGTTT		
Plantaricin W	pnlW-F	TCACACGAAATATTCCA	44	Holo et al. (2001)
	pnlW-R	GGCAAGCGTAAGAAATAAATGAG		

Table 2
Optimized conditions used for PCR amplification of the bacteriocin-related genes.

Target gene	Stages		
	Stage 1 (1 cycle)	Stage 2 (30 cycles)	
Nisin	95 °C, 6 min	95 °C, 30 s	50 °C, 30s
Lacticin 481			
Lactococcin 972			
Lactococcin G and Q			
Lactococcin A and B			
Brevicin		96 °C, 1 min	56 °C, 30 s
Plantaracin A		94 °C, 30s	55 °C, 1 min
Plantaracin EF			54 °C, 1 min
Pediocin PA			50 °C, 1 min
Plantaracin NC8		94 °C, 1 min	60 °C, 1 min
Plantaracin S			60 °C, 1 min
Plantaricin W			55.5 °C,1 min

All PCR reactions have a final extension step consisting in 72 °C for 5 min.

2.7. Final characterization of the selected LAB strains with anti-*L. monocytogenes* by Pulsed-field Gel Electrophoresis typing

The most active LAB strains against *L. monocytogenes* were further characterized by Pulsed-field Gel Electrophoresis (PFGE) digesting the DNA with the restriction *Sgs*I and *Not*I enzymes (Thermo Fisher Scientific) and the subsequent electrophoresis on the Chef Mapper® XA Pulsed Field Electrophoresis System (Bio-Rad Laboratories). The protocol described by PulseNet (Graves & Swaminathan, 2001) was used with some modifications. Bacterial cells embedded in agarose Seakem® Gold (Lonza, Switzerland) (plugs) with lysozyme (Sigma-Aldrich, USA) (20 mg/mL) were lyzed in lysis buffer (50 mM Tris:50 mM EDTA, pH 8.0, 1% sarcosine, 0.1 mg/mL proteinase K [Thermo Fisher Scientific]) by incubating for 2 h at 55 °C in a water bath with agitation. The plugs were then washed twice in sterile ultrapure water and 4 times in TE buffer. The digestion of each DNA sample in the plugs was carried out with the *Sgs*I (10 U/μL) and *Not*I (10 U/μL) enzymes for 3 h at 37 °C. The digestion of the control *Salmonella enterica* subsp. *enterica* serovar *Braenderup* (ATCC® BAA-664™) was carried out with the enzyme Xbal (10 U/μL). The PFGE was performed in 1% (w/v) agarose Seakem® Gold in TBE buffer (0.45 M TRIS, 0.45 M boric acid, 10 mM EDTA) in the Chef Mapper® XA Pulsed Field Electrophoresis System at 6 V/cm and 14 °C for 17 h with switch times of 1 s–15 s. Images were obtained with a Gel Doc 2000 Image Documentation System (Bio-Rad Laboratories), after staining with SYBR™ Gold Nucleic Acid Gel Stain (10.000X; Invitrogen, USA) for 15 min. The combined *Sgs*I/*Not*I PFGE pulsotypes were analyzed using the BioNumerics software (Applied Maths, Belgium).

2.8. Statistical analyses

The statistical treatment was carried out using the software IBM SPSS Statistic version 20 (IBM, USA). For the statistical analysis of data, LAB evaluated conditions were used as independent variables. The counts of *L. monocytogenes* (log CFU/cm^2) were analyzed as dependent variables. Once dependent and independent variables of the analysis were determined, a normal distribution of obtained data was studied by using the Shapiro Wilk test. Subsequently, analyses were conducted using the Mann–Whitney test (Mann & Whitney, 1947) and statistical significance was established at $p \leq 0.05$.

3. Results

3.1. Preliminary identification of LAB isolates and screening of anti-*L. monocytogenes* activity

A total of 371 isolates (189 obtained from cheese factories and 182 from dry-cured meat industries) were preliminarily considered as LAB. Most of these isolates were obtained from products at different stages of ripening (initial, half, and final maturation stages).

From these LAB isolates, 84 showed inhibition zones (from 2 to 12 mm) against *L. monocytogenes* S7-2 in the initial screening using the agar spot assay. Of these, 52 were derived from cheese factories and 32 were isolated in the dry-cured fermented sausages industries (Table 3). In all of the sampled industries, active isolates were obtained, except in factory E, where no LAB isolates with anti-*L. monocytogenes* activity were found.

3.2. Molecular identification of selected LAB isolates by 16S rRNA sequencing

The 84 selected LAB isolates with anti-*L. monocytogenes* activity were identified by 16S rRNA sequencing. All of them belonged to the species *Enterococcus durans, Enterococcus faecium, Enterococcus hirae, Leuconostoc pseudomesenteroides, Leuconostoc mesenteroides, Lacticaseibacillus casei, Lactiplantibacillus plantarum, Lacticaseibacillus paracasei, Lactilactobacillus sakei, Lactococcus garvieae* and *Lactococcus lactis* (Table 3). The most frequent species isolated in the 12 raw ewe's milk cheeses factories tested was *Le. mesenteroides* while *Ll. sakei* was the predominant species between the isolates of the 3 dry-cured fermented sausages industries. The A cheese factory and the M dry-cured fermented sausage industry were the industries where more diversity of active species was found (Table 3).

3.3. Selection of LAB strains by their anti-*L. monocytogenes* activity on food models

Initial levels of both inoculated microorganisms (*L. monocytogenes* and LAB) were found around 3 log CFU/cm^2 at day 0 of inoculation and all of the tested LAB strains reaching values of about 5 log CFU/cm^2 after incubation of the inoculated food models for 7 days at 7 °C (data not shown).

The growth/reduction of *L. monocytogenes* during the incubation time on the corresponding food model system depended on the inoculated LAB. Thus, it was observed that only 5 out of the 52 strains isolates from cheese industries had a significant ($p \leq 0.05$) reduction of *L. monocytogenes* compared to the control without LAB inoculation (Fig. 1). These strains were *Lc. casei* 31, *Lc. casei* 116, *Lco. garvieae* 151, *Lco. garvieae* 156 and *Lc. paracasei* 185, observing the highest inhibition of *L. monocytogenes* in "Torta del Casar" cheese-based agar when co-inoculated with *Lc. casei* 116 (approximately of 2.35 log CFU/cm^2). The same occurs with LAB strains from dry-cured fermented sausage industries since only 5 out of the 32 strains tested showed a significant reduction of *L. monocytogenes* counts compared to the control (Fig. 1). These strains were *Lc. paracasei* 13, *E. faecium* 188, *Ll. sakei* 197, *Ll. sakei* 204 and *Ll. sakei* 205. The highest inhibition of *L. monocytogenes* growth (2.5 log CFU/cm^2) was found with *Ll. sakei* 205. These 10 LAB strains were selected and further investigated for bacteriocin encoding gene detection.

3.4. Detection of bacteriocin structural genes

All the 10 selected LAB strains showed amplicons of the expected size for at least one of the genes encoding known bacteriocins (Table 4). Most of these strains amplified the gene pediocin PA. However, no PCR products were obtained with the specific primers based on lacticin 481, brevicin and plantaracin W (Table 4). *Lc. casei* 116 and *Lco. garvieae* 151, isolated in cheese industries, showed the highest number (5) of amplicons (Table 4). Thus, in *Lc. casei* 116 the genes encoding nisin, lactacins A, B, G and Q and pediocin PA were detected, while in *Lco. garvieae* amplicons of the appropriate sizes for lactacins 972, B, G and Q, plantaracin EF and pediocin PA were found. It is also remarkable that the strains *Ll. sakei* 31, from cheese origin, and *Lc. paracasei* 13, from fermented sausage origin, showed amplicons for 3 of the bacteriocin

Table 3
Distribution of the lactic-acid bacteria isolates from cheese and meat industries showing anti-*Listeria monocytogenes* activity in the agar plot assay.

Identification	Cheese												Dry-cured fermented sausages			Total
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
Enterococcus durans	1	2	–	–	–	–	–	–	–	–	–	–	–	3	–	6
Enterococcus faecium	1	1	–	–	–	–	–	–	–	–	–	–	2	3	2	9
Enterococcus hirae	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
Leuconostoc pseudomesenteroides	1	–	–	–	–	2	–	–	–	–	–	–	1	–	–	4
Leuconostoc mesenteroides	-	1	3	–	–	1	12	–	–	2	–	–	–	–	1	20
Lacticaseibacillus casei	6	–	1	–	–	–	–	–	1	–	–	–	–	1	–	9
Lactiplantibacillus plantarum	–	–	–	1	–	–	1	–	–	–	–	–	1	–	–	3
Lacticaseibacillus paracasei	1	1	5	–	–	–	–	–	–	–	1	–	1	–	–	9
Lactilactobacillus sakei	–	–	–	–	–	–	–	–	–	–	–	–	–	5	6	11
Lactococcus garvieae	1	1	–	–	–	–	–	–	–	–	–	–	2	3	–	7
Lactococcus lactis	–	–	–	–	–	–	–	3	–	1	–	1	–	–	–	5
Total isolates	11	6	9	1	0	3	13	3	1	3	1	1	8	15	9	84
	52												32			

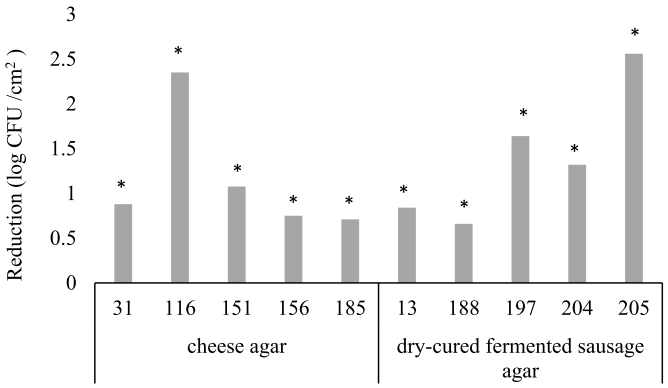

Fig. 1. Reduction of the levels of *L. monocytogenes* counts in food models (cheese-based agar and dry-cured fermented sausages-based agar) after incubation for 7 days at 7 °C. *Lacticaseibacillus casei* 31, *Lacticaseibacillus casei* 116, *Lactococus garvieae* 151, *Lactococus garvieae* 156 and *Lacticaseibacillus paracasei* 185, *Lacticaseibacillus paracasei* 13, *Enterococcus faecium* 188, *Lactilactobacillus sakei* 197, *Lactilactobacillus sakei* 204 and *Lactilactobacillus sakei* 205. Asterisks indicate significant differences ($p \leq 0.05$) with respect to the control.

encoding genes tested in this work (Table 4).

3.5. Final characterization by PFGE of the selected strains with anti-*L. monocytogenes* activity

The selected LAB strains showing the highest activity in food model systems were further characterized by the PFGE restriction analysis (Fig. 2). PFGE profiles with the restriction *Not*I and *Sgs*I enzymes of the most active LAB strains (*Ll. sakei* 31, *Lc. casei* 116 and *Lco. garvieae* 151 from cheese origin and *Ll. sakei* 197, 204 and 205 isolated from dry-cured fermented sausages industries), are shown in Fig. 2. The PFGE profiles obtained with *Not*I showed 8–16 restriction fragments (depending on the strains), between 33 and 668 Kpb, that allow a clear differentiation between all of the tested strains. The enzyme *Sgs*I generated PFGE profiles of restriction fragments that ranged from 10 to 16 depending on the strains (Fig. 2), which also allow the differentiation of the selected LAB strains.

4. Discussion

In this study, isolation, selection, and characterization of autochthonous LAB strains from traditional soft cheeses and dry-cured fermented sausages were carried out to be considered as bioprotective cultures for their ability to inhibit the *L. monocytogenes* growth. Traditional ripened foods constitute rich ecological niches for screening LAB with anti-*L. monocytogenes* activity (Cocolin et al., 2007; Xiraphi et al., 2008). A total of 371 isolates obtained from different steps of processing of these products were preliminarily characterized as LAB. Although a higher number of cheese factories than meat industries was tested, the number of LAB isolates obtained was very similar. These LAB isolates were firstly tested for the anti-*L. monocytogenes* activity using a screening method based on agar spot assay. This method has been reported as appropriate for the screening of LAB strains with antimicrobial activities (Albano et al., 2007; Balouiri et al., 2016; Campagnollo et al.,

Table 4
Bacteriocin encoding genes of the ten selected LAB with the highest anti-*L. monocytogenes* activity.

	LAB strains	Target gene												
		nisin	lact481	lcn972	lcnG and Q	lcnA	lcnB	brevi	plnA	plnEF	PedioPA	pnlNC8	pnlS	pnlW
Cheeses	*Ll. sakei* 31	–	–	–	–	–	–	–	–	X	X	–	X	–
	Lc. casei 116	X	–	–	X	X	X	–	–	–	X	–	–	–
	Lco. garvieae 151	–	–	X	X	–	X	–	–	X	X	–	–	–
	Lco. garvieae 156	–	–	–	–	–	–	–	–	–	–	X	X	–
	Lc. paracasei 185	–	–	–	–	–	–	–	–	–	–	–	X	–
Dry-cured fermented sausages	*Lc. paracasei* 13	–	–	–	–	–	–	–	X	–	X	X	–	–
	E. faecium 188	X	–	–	–	–	–	–	–	–	–	–	–	–
	Ll. sakei 197	–	–	–	–	–	–	–	–	–	–	–	X	–
	Ll. sakei 204	–	–	–	–	–	–	–	–	X	X	–	–	–
	Ll. sakei 205	–	–	–	–	–	–	–	–	–	X	–	–	–

Nisin (nisin), lact481 (lacticin 481), lcn972 (lactococcin 972) lcnG and Q (lactococcin G, Q), lcnA(lactococcin A), lcnB (lactococcins B), brevi (brevicin), plnA (plantaracin A), plnEF (plantaracin EF), pedioPA (pediocin PA), pnlNC8 (plantaracin NC8), pnlS (plantaracin S) and pnlW (plantaracin W).

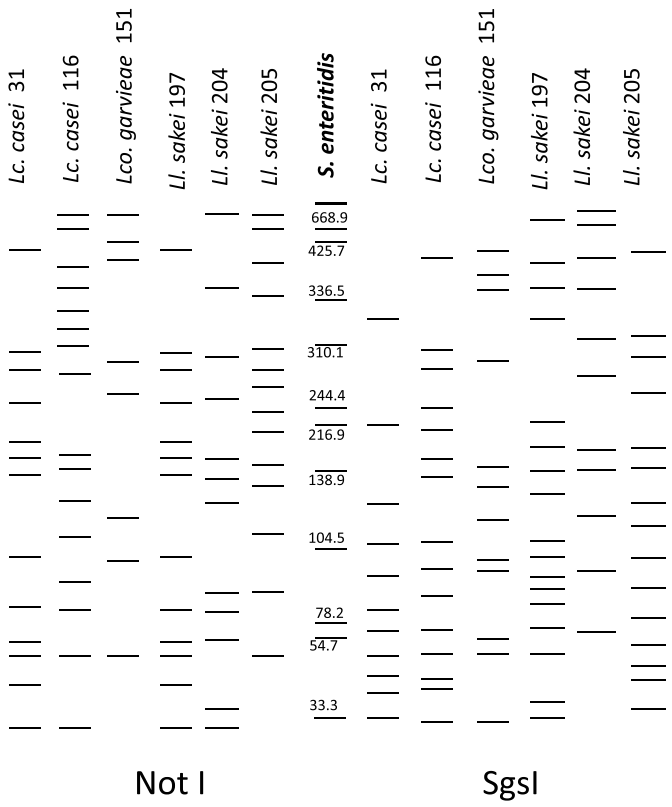

Fig. 2. Pulsed Field Gel Electrophoresis (PFGE) profiles of selected lactic-acid bacteria by using the restriction enzymes *Not*I and *Sgs*I. *Lacticaseibacillus casei* 31, *Lacticaseibacillus casei* 116, *Lactococus garvieae* 151, *Lactilactobacillus sakei* 197, *Lactilactobacillus sakei* 204 and *Lactilactobacillus sakei* 205. *Salmonella enterica* subsp. *enterica* serovar *Braenderup* was used as control.

2018). 22.64% of the LAB isolates tested showed inhibition zones of *L. monocytogenes* higher than 2 mm, which could be because of competition for nutrients and space and/or production of antimicrobial compounds such as lactic acid and other organic acids, ethanol, diacetyl, carbon dioxide, hydrogen peroxide and bacteriocins (Kasra-Kermanshahi & Mobarak-Qamsari, 2015). The percentage of LAB isolates with anti-listerial activity was in the range of active strains reported with the agar spot assay in different foods (Fontana et al., 2015; Macaluso et al., 2016). When the origin of active isolates was analyzed, a higher percentage was obtained in cheese industries (27.51%) than in dry-cured fermented sausages factories (17.58%), in spite of the number of LAB isolates tested from the two types of industries was similar (189 from cheese factories and 182 from meat industries). It is possible that the ecological niche of ripened cheeses made with raw milk, usually with relative high levels of microbial contamination, favors that native LAB strains develop antimicrobial activity to be competitive in this food environment (Arqués et al., 2014).

When the selected active LAB isolates were characterized by 16S rRNA sequencing a total of eleven different LAB species was found. This method has been reported for the appropriate characterization of LAB strains isolated from ripened foods (Domingos-Lopes et al., 2017). All the species found have been extensively reported with antimicrobial activity (Mohammed & Çon, 2021; Pellegrino et al., 2019; Rodríguez et al., 2000). Most of the eleven active species were found in both types of industries, except *Lc. lactis* and *E. hirae* which were only detected in cheese factories, while *Ll. sakei* was only found in dry-cured fermented sausages factories, probably because this species is more adapted to this specific ecological niche (Nomura et al., 2006; Zagorec & Champomier-Vergès, 2017).

All the 84 different LAB strains selected by their anti-listerial activity belonging to the described eleven species were further tested by anti-*L. monocytogenes* activity in food models. Only 10 of the 84 LAB strains tested showed activity in the assays of co-inoculation in the food models tested. In the cheese model the most active LAB strain was *Lc. casei* 116 showing a reduction of 2.35 log CFU/cm^2 of *L. monocytogenes* while in dry-cured fermented "salchichón" model *Ll. sakei* 205 achieved a reduction of 2.5 log CFU/cm^2. Reductions in counts of this pathogen of 2 log CFU/mL or cm^2 have also been reported with selected LAB strains in milk and cheese agar medium (García et al., 2020; Martín et al., 2022). Panebianco et al. (2021) reported reductions of *L. monocytogenes* levels from 0.5 to 1 log CFU/g both in vitro and in soft cheese by using selected *Ll. sakei* and *Lp. plantarum*. Although many LAB strains were selected for their ability to inhibit *L. monocytogenes*, in vitro assays, not all of them have been effective in real RTE ripened food systems (Martín et al., 2021; Panebianco et al., 2021). The food models used in the present work simulate conditions of temperature, water activity and pH conditions of cheese and dry-cured fermented sausages. This step in the selection of active LAB is of utmost importance to discard those strains lacking activity or with low effectiveness in the processing or storage conditions of ripened foods. After this evaluation 10 LAB strains able to reduce *L. monocytogenes* in conditions of ripening of cheeses and dry-cured fermented sausages, were obtained. These 10 LAB strains were investigated for genes encoding known bacteriocins. The results justify the reduction of *L. monocytogenes* counts in the food models since all these strains have at least one gene encoding known bacteriocins. Selected LAB strains with anti-microbial activity isolated from ripened products have been reported to produce different bacteriocins such as nisin, lactococcins and pediocins (Alegría et al., 2010; Salas et al., 2017; Xie et al., 2011). In the present work, *Lc. casei* 116 showing the highest activity in the cheese model amplified the genes encoding nisin, lactacins A, B, G and Q and pediocin PA. Regarding active LAB strains from dry-cured fermented sausages origin, the most active strain in the "salchichón" model was *Ll. sakei* 205 that only amplified the gene encoding Pediocin PA, probably because the activity of this strain is not only due to the production of bacteriocins, but also, to the production of organic acids, diacetyl, carbon dioxide, ethanol or hydrogen peroxide (Kasra--Kermanshahi & Mobarak-Qamsari, 2015). In addition, other active strains such as *Lc. paracasei* 13 showed amplicons for 3 of genes encoding bacteriocins tested in this work.

Considering the activity in the food models and the presence of genes encoding known bacteriocins, 6 LAB strains including *Ll. sakei* 31, *Lc. casei* 116 and *Lco. garvieae* 151 from cheese origin and *Ll. sakei* 197, 204 and 205 isolated from dry-cured fermented sausages industries, were selected as the most active strains against *L. monocytogenes*. Thus, the procedure followed in the present work including first screening in agar spot assay and further selection in food models allowed the selection of 6 LAB actives strains active against *L. monocytogenes*, all of them possessing genes encoding known bacteriocins. These strains could be proposed to be used in ripened cheeses or dry-cured fermented sausages to control *L. monocytogenes*. Food models allow a faster and easier selection of active LAB strains against *L. monocytogenes* in comparison with evaluation in real foods since it is not necessary to carried out ripening process under controlled conditions of the inoculated real foods. The LAB strains selected by their activity in foods models showed activity against *L. monocytogenes* in real foods. In fact, the most active strain *Ll. sakei* 205 produced a reduction higher than 2 log CFU/g of *L. monocytogenes* counts during ripening of dry-cured fermented "salchichón" elaborated according to the traditional processing (Martín et al., 2021) and the most active strain *Lc. casei* 116 has been reported to reduce 2 log CFU/g of *L. monocytogenes* levels during ripening "Torta del Casar" soft cheese following an industrial process (unpublished data). This reduction could be sufficient to guarantee the elimination of *L. monocytogenes* throughout the processing of RTE ripened foods at the level of hypothetic contamination (usually below 2 log CFU/g). This allows to minimize the risk of listeriosis and meet the microbiological criteria for this pathogen of ripened foods during their shelf-life as

established by The European Union (Regulation (EC) nº 1441/2007). In addition, the selected LAB strains in this work have belonged to species with the recognition of "Qualified Presumption of Safety" (QPS) from the European Food Safety Authority (EFSA).

To evaluate the implantation of the selected actives strains throughout the food ripening process it is necessary a characterization method that allows distinguishing to the strain level. In this work, further characterization of the 6 selected strains was carried out by PFGE analysis using the restriction *Sgs*I and *Not*I enzymes. With these two enzymes, clear differentiation of the 6 selected strains was obtained. This technique but using the restriction enzyme *Sfi*I has been reported to allow differentiation of some LAB species at strain level (Adesulu-Dahunsi et al., 2021). The combination of 16S rRNA sequencing with PFGE with restriction *Sgs*I and *Not*I enzymes allows successful intraspecific differentiation to be used to control the implantation of these selected strains throughout the ripening process.

5. Conclusion

The procedure followed including first screening in agar spot and further selection in food models allowed selection of 6 LAB active strains actives against *L. monocytogenes*, all of them with genes encoding known bacteriocins. The most active strains *Lc. casei* 116 and *Ll. sakei* 205 provoked reductions higher than 2 log cycles of *L. monocytogenes* in cheeses and dry-cured fermented sausages models, respectively. Considering the safety and antimicrobial potential of the selected LAB, they may be utilized to control *L. monocytogenes* throughout the ripening process in ripened soft cheese and dry-cured fermented sausages. The combination of 16S rRNA sequencing with PFGE analysis using restriction *Sgs*I and *Not*I enzymes could be useful to control the implantation of these selected strains throughout the ripening process.

CRediT authorship contribution statement

Irene Martín: Methodology, Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing. **Alicia Rodríguez:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration. **Alberto Alía:** Methodology, Writing – review & editing. **Remigio Martínez:** Methodology, Writing – review & editing, All authors have read and agreed to the published version of the manuscript. **Juan J. Córdoba:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work has been funded by the INIA throughout the projects RTA-2017-00027-C03-03 and the Junta de Extremadura and FEDER by IB16149. GR15108 and GR18056. I. Martín is supporting by a predoctoral fellowship from the Spanish Ministry of Education, Culture and Sports (FPU 16/05303).

References

Adesulu-Dahunsi, A. T., Sanni, A. I., & Jeyaram, K. (2021). Diversity and technological characterization of *Pediococcus pentosaceus* strains isolated from Nigerian traditional fermented foods. *Lebensmittel-Wissenschaft & Technologie, 140*, Article 110697. https://doi.org/10.1016/j.lwt.2020.110697

Albano, H., Oliveira, M., Aroso, R., Cubero, N., Hogg, T., & Teixeira, P. (2007). Antilisterial activity of lactic acid bacteria isolated from "Alheiras" (traditional Portuguese fermented sausages): In situ assays. *Meat Science, 76*(4), 796–800. https://doi.org/10.1016/j.meatsci.2007.01.019

Alegría, Á., Delgado, S., Roces, C., López, B., & Mayo, B. (2010). Bacteriocins produced by wild *Lactococcus lactis* strains isolated from traditional, starter-free cheeses made of raw milk. *International Journal of Food Microbiology, 143*(1–2), 61–66. https://doi.org/10.1016/j.ijfoodmicro.2010.07.029

Arqués, J. L., Rodríguez, E., Langa, S., Landete, J. M., & Medina, M. (2014). Antimicrobial activity of lactic acid bacteria in dairy products and gut: Effect on pathogens. *BioMed Research International*. https://doi.org/10.1155/2015/584183

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharmaceut. Anal., 6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Campagnollo, F., Margalho, L., Kamimura, B., Feliciano, M., Freire, L., Lopes, L., Alvarenga, V., Cadavez, V., Gonzales-Barron, U., Schaffner, D., & Sant'Ana, A. (2018). Selection of indigenous lactic acid bacteria presenting anti-listerial activity, and their role in reducing the maturation period and assuring the safety of traditional Brazilian cheeses. *Food Microbiology, 73*, 288–297. https://doi.org/10.1016/j.fm.2018.02.006

Carr, F. J., Chill, D., & Maida, N. (2002). The lactic acid bacteria: A literature survey. *Critical Reviews in Microbiology, 28*(4), 281–370. https://doi.org/10.1080/1040-840291046759

Cocolin, L., Foschino, R., Comi, G., & Grazia Fortina, M. (2007). Description of the bacteriocins produced by two strains of *Enterococcus faecium* isolated from Italian goat milk. *Food Microbiology, 24*(7–8), 752–758. https://doi.org/10.1016/j.fm.2007.03.001

Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuff. Official Journal of the European Communitites, 1441..

Domingos-Lopes, M. F. P., Stanton, C., Ross, P. R., Dapkevicius, M. L. E., & Silva, C. C. G. (2017). Genetic diversity, safety and technological characterization of lactic acid bacteria isolated from artisanal Pico cheese. *Food Microbiology, 63*, 178–190. https://doi.org/10.1016/j.fm.2016.11.014

Filipello, V., Gallina, S., Amato, E., Losio, M. N., Pontello, M., Decastelli, L., & Lomonaco, S. (2017). Diversity and persistence of *Listeria monocytogenes* within the Gorgonzola PDO production chain and comparison with clinical isolates from the same area. *International Journal of Food Microbiology, 245*, 73–78. https://doi.org/10.1016/j.ijfoodmicro.2017.01.012

Fontana, C., Cocconcelli, P. S., Vignolo, G., & Saavedra, L. (2015). Occurrence of antilisterial structural bacteriocins genes in meat borne lactic acid bacteria. *Food Control, 47*, 53–59. https://doi.org/10.1016/j.foodcont.2014.06.021

Gajbhiye, M. H., & Kapadnis, B. P. (2016). Antifungal-activity-producing lactic acid bacteria as biocontrol agents in plants. *Biocontrol Science and Technology, 26*(11), 1451–1470. https://doi.org/10.1080/09583157.2016.1213793

García, M. J., Ruíz, F., Asurmendi, P., Pascual, L., & Barberis, L. (2020). Searching potential candidates for development of protective cultures: Evaluation of two *Lactobacillus* strains to reduce *Listeria monocytogenes* in artificially contaminated milk. *Journal of Food Safety, 40*(1). https://doi.org/10.1111/jfs.12723

Gori, K., Mortensen, H. D., Arneborg, N., & Jespersen, L. (2007). Ammonia production and its possible role as a mediator of communication for *Debaryomyces hansenii* and other cheese-relevant yeast species. *Journal of Dairy Science, 90*(11), 5032–5041. https://doi.org/10.3168/jds.2006-750

Graves, L. M., & Swaminathan, B. (2001). PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. *International Journal of Food Microbiology, 65*(1–2), 55–62. https://doi.org/10.1016/S0168-1605(00)00501-8

Ho, V. T. T., Lo, R., Bansal, N., & Turner, M. S. (2018). Characterisation of *Lactococcus lactis* isolates from herbs, fruits and vegetables for use as biopreservatives against *Listeria monocytogenes* in cheese. *Food Control, 85*, 472–483. https://doi.org/10.1016/j.foodcont.2017.09.036

Holo, H., Jeknic, Z., Daeschel, M., Stevanovic, S., & Nes, I. F. (2001). Plantaricin W from *Lactobacillus plantarum* belongs to a new family of two-peptide lantibiotics. *Microbiology, 147*(3), 643–651. https://doi.org/10.1099/00221287-147-3-643

ISO 15214. (1998). *Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of mesophilic lactic acid bacteria-Colony-count technique at 30 degrees C*.

Kasra-Kermanshahi, R., & Mobarak-Qamsari, E. (2015). Inhibition effect of lactic acid bacteria against food born pathogen, *Listeria monocytogenes*. *Appl. Biotechnol. Food, 2*(4), 11–19. https://doi.org/10.22037/afb.v2i4.8894

Macaluso, G., Fiorenza, G., Gaglio, R., Mancuso, I., & Scatassa, M. L. (2016). In vitro evaluation of bacteriocin like inhibitory substances produced by lactic acid bacteria isolated during traditional Sicilian cheese making. *Ital. J. Food Saf., 5*(1), 20–22. https://doi.org/10.4081/ijfs.2016.5503

Maldonado, A., Jiménez-Díaz, R., & Ruiz-Barba, J. L. (2004). Induction of plantaricin production in *Lactobacillus plantarum* NC8 after coculture with specific Gram-positive bacteria is mediated by an autoinduction mechanism. *Journal of Bacteriology, 186*(5), 1556–1564. https://doi.org/10.1128/JB.186.5.1556-1564.2004

Mann, H. B., & Whitney, D. (1947). On a test of whether one of two random variables is stochastically larger than the other. *The Annals of Mathematical Statistics, 2*(5), 347–370. http://projecteuclid.org/euclid.aop/1176996548.

Martín, I., Rodríguez, A., Alía, A., Martínez-Blanco, M., Lozano-Ojalvo, D., & Córdoba, J. J. (2022). Control of *Listeria monocytogenes* growth and virulence in a traditional soft cheese model system based on lactic acid bacteria and a whey protein hydrolysate with antimicrobial activity. *International Journal of Food Microbiology, 361*, Article 109444. https://doi.org/10.1016/j.ijfoodmicro.2021.109444

Martín, I., Rodríguez, A., Sánchez-Montero, L., Padilla, P., & Córdoba, J. J. (2021). Effect of the dry-cured fermented sausage "salchichón" processing with a selected

Lactobacillus sakei in *Listeria monocytogenes* and microbial population. *Foods, 10*(4). https://doi.org/10.3390/foods10040856

Mohammed, S., & Çon, A. H. (2021). Isolation and characterization of potential probiotic lactic acid bacteria from traditional cheese. *Lebensmittel-Wissenschaft & Technologie, 152*(April), Article 112319. https://doi.org/10.1016/j.lwt.2021.112319

Noda, M., Miyauchi, R., Danshiitsoodol, N., Higashikawa, F., Kumagai, T., Matoba, Y., & Sugiyama, M. (2015). Characterization and mutational analysis of a two-polypeptide bacteriocin produced by citrus Iyo-derived *Lactobacillus brevis* 174A. *Biological and Pharmaceutical Bulletin, 38*(12), 1902–1909. https://doi.org/10.1248/bpb.b15-00505

Nomura, M., Kobayashi, M., Narita, T., Kimoto-Nira, H., & Okamoto, T. (2006). Phenotypic and molecular characterization of *Lactococcus lactis* from milk and plants. *Journal of Applied Microbiology, 101*(2), 396–405. https://doi.org/10.1111/j.1365-2672.2006.02949.x

Panebianco, F., Giarratana, F., Caridi, A., Sidari, R., De Bruno, A., & Giuffrida, A. (2021). Lactic acid bacteria isolated from traditional Italian dairy products: Activity against *Listeria monocytogenes* and modelling of microbial competition in soft cheese. *Lebensmittel-Wissenschaft & Technologie, 137*(July 2020), Article 110446. https://doi.org/10.1016/j.lwt.2020.110446

Pellegrino, M. S., Frola, I. D., Natanael, B., Gobelli, D., Nader-Macias, M. E. F., & Bogni, C. I. (2019). In vitro characterization of lactic acid bacteria isolated from bovine milk as potential probiotic strains to prevent bovine mastitis. *Probiotics. Antimicrob. Protein, 11*(1), 74–84. https://doi.org/10.1007/s12602-017-9383-6

Perin, L. M., Savo Sardaro, M. L., Nero, L. A., Neviani, E., & Gatti, M. (2017). Bacterial ecology of artisanal Minas cheeses assessed by culture-dependent and -independent methods. *Food Microbiology, 65*, 160–169. https://doi.org/10.1016/j.fm.2017.02.005

Regulation (EC) No 853/. (2004). *Of the European parliament and of the council*. Official Journal of the European Union. Issue L139).

Reis, J. A., Paula, A. T., Casarotti, S. N., & Penna, A. L. B. (2012). Lactic acid bacteria antimicrobial compounds: Characteristics and applications. *Food Engineering Reviews, 4*(2), 124–140. https://doi.org/10.1007/s12393-012-9051-2

Rodríguez, E., González, B., Gaya, P., Nuñez, M., & Medina, M. (2000). Diversity of bacteriocins produced by lactic acid bacteria isolated from raw milk. *International Dairy Journal, 10*(1–2), 7–15. https://doi.org/10.1016/S0958-6946(00)00017-0

Rodriguez, C., Taminiau, B., García-Fuentes, E., Daube, G., & Korsak, N. (2021). *Listeria monocytogenes* dissemination in farming and primary production: Sources, shedding and control measures. *Food Control, 120*, Article 107540. https://doi.org/10.1016/j.foodcont.2020.107540

Salas, L. M., Mounier, J., Valence, F., Coton, M., Thierry, A., & Coton, E. (2017). Antifungal microbial agents for food biopreservation—a review. *Microorganisms, 5*(3), 1–35. https://doi.org/10.3390/microorganisms5030037

Shamloo, E., Hosseini, H., Moghadam, A. Z., Larsen, H. M., Haslberger, A., & Alebouyeh, M. (2019). Importance of *Listeria monocytogenes* in food safety: A review of its prevalence, detection, and antibiotic resistance. *Iranian Journal of Veterinary Research, 20*(4), 241–254.

Siedler, S., Balti, R., & Neves, A. R. (2019). Bioprotective mechanisms of lactic acid bacteria against fungal spoilage of food. *Current Opinion in Biotechnology, 56*, 138–146. https://doi.org/10.1016/j.copbio.2018.11.015

Singh, P. (2018). Recent approaches in food bio-preservation-a review. *Open Veterinary Journal, 8*(1), 2226–4485. https://doi.org/10.4314/ovj.v8i1.16

Smith, A., Moorhouse, E., Monaghan, J., Taylor, C., & Singleton, I. (2018). Sources and survival of *Listeria monocytogenes* on fresh, leafy produce. *Journal of Applied Microbiology, 125*(4), 930–942. https://doi.org/10.1111/jam.14025

Stephens, S. K., Floriano, B., Cathcart, D. P., Bayley, S. A., Witt, V. F., Jiménez-Díaz, R., Warner, P. J., & Ruiz-Barba, J. L. (1998). Molecular analysis of the locus responsible for production of plantaricin S, a two-peptide bacteriocin produced by *Lactobacillus plantarum* LPCO10. *Applied and Environmental Microbiology, 64*(5), 1871–1877. https://doi.org/10.1128/aem.64.5.1871-1877.1998

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., & Drider, D. (2019). Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Frontiers in Microbiology, 10*(57). https://doi.org/10.3389/fmicb.2019.00057

Walter, J., Tannock, G. W., Tilsala-Timisjarvi, A., Rodtong, S., Loach, D. M., Munro, K., & Alatossava, T. (2000). Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. *Applied and Environmental Microbiology, 66*(1), 297–303. https://doi.org/10.1128/AEM.66.1.297-303.2000

Xie, Y., An, H., Hao, Y., Qin, Q., Huang, Y., Luo, Y., & Zhang, L. (2011). Characterization of an anti-*Listeria* bacteriocin produced by *Lactobacillus plantarum* LB-B1 isolated from koumiss, a traditionally fermented dairy product from China. *Food Control, 22*(7), 1027–1031. https://doi.org/10.1016/j.foodcont.2010.12.007

Xiraphi, N., Georgalaki, M., Rantsiou, K., Cocolin, L., Tsakalidou, E., & Drosinos, E. H. (2008). Purification and characterization of a bacteriocin produced by *Leuconostoc mesenteroides* E131. *Meat Science, 80*(2), 194–203. https://doi.org/10.1016/j.meatsci.2007.11.020

Yap, P.-C., MatRahim, N.-A., AbuBakar, S., & Lee, H. Y. (2021). Antilisterial potential of lactic acid bacteria in eliminating *Listeria monocytogenes* in host and ready-to-eat food application. *Microbiology Research, 12*(1), 234–257. https://doi.org/10.3390/microbiolres12010017

Zagorec, M., & Champomier-Vergès, M.-C. (2017). *Lactobacillus sakei:* A starter for sausage fermentation, a protective culture for meat products. *Microorganisms, 5*(3), 56. https://doi.org/10.3390/microorganisms5030056