Food
Processing & Technology

Miranda et al., J Food Process Technol 2014, 5:6
http://dx.doi.org/10.4172/2157-7110.1000338

Short Communication Open Access

Technological Characterization of Lactic Acid Bacteria Isolated from Beef Stored on Vacuum-Packaged and Advanced Vacuum Skin Packaged System

Miranda JM[1]*, Samuel A[1], Nebot CG[1], Cepeda A[1], Franco CM[1] and Calo-Mata MP[2]

[1]*Laboratorio de Higiene Inspección y Control de Alimentos (LHICA), Dpto de Química Analítica, Nutrición y Bromatología, Facultad de Veterinaria, Universidad de Santiago de Compostela, E-2 7002 Lugo, Spain*

[2]*College of Biotechnology, Dpto de Química Analítica, Nutrición y Bromatología, Universidad de Santiago de Compostela, E-I 5782 Santiago, Spain*

Abstract

A total of 91 Lactic Acid Bacteria (LAB) isolated from meat packaged using traditional vacuum packaging (42 strains) or an advanced vacuum skin packaging system (49 strains) were characterised in terms of gas production, proteolytic and lipolytic activity, production of hydrogen peroxide, histamine and bacteriocin-like substances, and haemolytic activity. Thermal resistance of all isolated bacteria was also analysed. No differences were found for most parameters; with the exception of a higher production of bacteriocin-like substances in LAB isolated from meat packaged using advanced vacuum skin packaging than those obtained for isolated from meat packaged using traditional vacuum packaging. LAB isolated from advanced vacuum skin packaged meat had higher thermal resistance than LAB isolated from meat packaged with the traditional vacuum method, both at 55ºC (14.09 min vs. 11.17 min) and at 60ºC (6.87 min vs. 4.64 min). These data could contribute to an explanation for the longer shelf life of meat packed using advanced vacuum skin packaging compared to meat packed with traditional vacuum packaging.

Keywords: Packaging; Meat; Meat products; Spoilage organisms; Thermal processing; Lactic acid bacteria

Introduction

Fresh meat is one of the most perishable foods commercially available. Many factors influencing meat shelf life can promote spoilage, bacterial growth and oxidative processes during storage. These in turn provoke deterioration in the flavour, texture and colour of meat [1]. Maintenance of consumer-desired meat appearance is therefore of extreme importance in the retail sector [2], and is impacted by many factors, including temperature, oxygen exposure, light, microorganisms and enzymatic activity [3]. As previously illustrated, the impact of these factors on the stability of ground meat at point of sale is also significantly affected by package type and environment [4]. The meat industry is consequently required to utilise processing methods that provide a long shelf life for meats, as well as protective packaging methods [5,6].

Protective packaging films such as Traditional Vacuum Packaging (TVP), combined with refrigerated storage, have long been thought to help extend the shelf life of fresh meat by reducing microbial growth and oxidative rancidity [4]. Vacuum packaging can prevent the growth of food-borne pathogens and spoilage bacteria commonly present in meat, because the low oxygen supply restricts the growth of typical spoilage bacteria such as *Pseudomonas* [6]. Consequently, psychotropic and Microaerophilic Lactic Acid Bacteria (LAB) comprise the majority of the microbial population in vacuum-packaged meat [7]. LAB can reduce the presence of pathogenic bacteria by establishing a competitive environment that inhibits their growth. In some cases however, LAB have been reported to be the major spoilage bacteria in meat products after extended periods of storage [8]. Consequently, the shelf life of vacuum-packaged meat is generally limited by the number and composition of the predominating LAB flora.

Some meat industries have more recently employed a new packaging technique termed the Advanced Vacuum Skin Packaging (AVSP) system to help improve the shelf life of meat. The AVSP system involves instantaneous heating of an upper film at a high temperature immediately before its application to the meat surface. High temperature inactivate part of the bacteria present on the surface of the meat, and has been described as a feasible control measure to extend shelf life [9], while the close contact of the film with the meat surface prevents the formation of wrinkles and air pockets [10].

Previous studies have demonstrated that meat packaged using the AVSP system and subsequently refrigerated exhibits a slower microbial growth rate and a longer shelf life compared to meat packaged using the TVP system [4]. It was noted, however, that choice of packaging system has no specific effect on the composition of microbiota found in the meat [5]. Consequently, the main purpose of the present work was to characterise LAB isolated from TVP- and AVSP-packaged beef stored at 4°C in terms of antimicrobial activity, safety aspects and biotechnological potential. To the best of our knowledge, no data regarding technological characterisation of the LAB species found in meat packaged using the AVSP system compared to the TVP system are currently available.

Materials and Methods

Origin and isolation of LAB strains

To eliminate differences in LAB contamination of meat caused by external factors, all meat samples were obtained from animals slaughtered, processed, and packaged in the same slaughterhouse and quartering room by the same factory personnel (Cocarga, Pontevedra, Spain). A total of 50 beef samples (200 g each) were collected. Twenty-five samples each were packaged using the TVP system on a Vac-210 packing machine (Guasch, Barcelona, Spain), and the AVSP system on

***Corresponding author:** Jose Manuel Miranda Lopez, Laboratorio de Higiene Inspección y Control de Alimentos (LHICA), Dpto de Química Analítica, Nutrición y Bromatología, Facultad de Veterinaria, Universidad de Santiago de Compostela, E-2 7002 Lugo, Spain, Tel: +34982285900 Ext. 22407; Fax +34982254592; E-mail: josemanuel.miranda@usc.es

Received April 15, 2014; **Accepted** June 13, 2014; **Published** June 27, 2014

Citation: Miranda JM, Samuel A, Nebot CG, Cepeda A, Franco CM, et al. (2014) Technological Characterization of Lactic Acid Bacteria Isolated from Beef Stored on Vacuum-Packaged and Advanced Vacuum Skin Packaged System. J Food Process Technol 5: 338. doi:10.4172/2157-7110.1000338

Microorganism	Spanish reference	International reference
Brochothrix thermosphacta	CECT 847 T	ATCC 11509
Enterococcus faecalis	CECT 407	ATCC 23655
Escherichia coli	CECT 4076	CCUG 20570
Lactobacillus 30A	ND	ATCC 33222
Lactococcus lactis	CECT 539	ATCC 11454
Listeria monocytogenes	CECT 4031	NCTC 11994
Leuconostoc mesenteroides	CECT 4703	NCFB 963
Pseudomonas putida	CECT 385	ATCC 12842
Pseudomonas fluorescens	CECT 844	ATCC 13430
Salmonella enteritidis	CECT 4396	ATCC 29629
Staphylococcus aureus	CECT 4521	ATCC 35845
Yersinia enterocolitica	CECT 4315	ATCC 9610

Table 1: Reference strains employed for testing bacteriocin production in LAB isolated from meat packaged using TVP or AVSP systems (ATCC: American Type Culture Collection, USA; CECT: Spanish Type Culture Collection (Valencia, Spain); CCUG: Culture Collection University of Göteborg; NCBF: National Collection of Food Bacteria (UK); NCTC: UK National Collection of Type Cultures. ND: Not determined).

a Multivac R570 CD packing machine (Wolfertschwenden, Germany). After packaging, meat samples were stored at refrigeration temperatures (4°C) for a period of 10 days to allow the proliferation of specific LAB selected by each packaging method. LAB isolation was performed by aseptically extracting 25 g from each sample and diluting with an appropriate volume (1:9 w/v) of water containing 0.1% peptone (Merck, Darmstadt, Germany) and homogenising for 1 min in a masticator (AES, Combourg, France). After homogenisation, 1×10^{-3}-1×10^{-7} dilutions of homogenates were surface-plated on de Man, Rogosa and Sharpe (MRS) agar plates (Difco, Detroit, MI, USA). Plates were then incubated at 30°C for 72 h under micro-aerobic conditions generated using the AnaeroGen C system (Oxoid, Backingstoke, UK). After the incubation period, two presumptive LAB colonies of typically small, white and round appearance from each sample were randomly selected. The selected isolates were purified by three alternate subcultures on MRS broth (Oxoid) and in MRS agar (Difco). Afterwards, LAB species were identified using a PCR-based method as previously described [5]. After identification, all strains were maintained as frozen stocks in a mixture of MRS broth and 20% glycerol at -80°C.

Technological characterization of LAB

All isolates identified as LAB were technologically characterized in terms of production of gas from glucose fermentation, proteolytic and lipolytic activity, production of hydrogen peroxide and antimicrobial-like substances and tolerance to heat treatments. All analyses were performed in triplicate for each strain.

Gas production was analyzed by glucose fermentation test. This involved use of a medium containing glucose and NaCl at the concentrations usually found in dry fermented meat products (2%) with the addition of 1% tryptone, 0.4% yeast extract, 0.8% meat extract, 0.5% sodium acetate, and 0.2% triammonium citrate. Gas was collected in Durham tubes after 72 h of incubation at 30°C, and strains were considered as gas-producers if gas was detected. *Escherichia coli* (ATCC 25922) were used as a positive control.

Proteolytic activity of isolated strains was tested by analyzing the hydrolysis of milk casein on Trypticase Soy Agar (TSA; Panreac, Barcelona, Spain) supplemented with 1.5% skim milk. Plates were inoculated with the corresponding culture, and incubated in micro-aerobic conditions for 72 h at 37°C. Colonies surrounded by clear zones in the otherwise turbid culture medium were considered positive. *E. coli* (CECT 4076) was used as a positive control.

Lipolytic activity of isolated strains was determined on tributyrin agar plates (Merck) prepared as described by the manufacturer. Plates were inoculated with the corresponding culture and incubated in micro-aerobic conditions for 72 h at 37°C. Colonies surrounded by clear zones in otherwise turbid culture medium were considered positive. *Staphylococcus aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used as positive and negative controls respectively.

Production of hydrogen peroxide from each strain was determined by plating bacterial cells on MRS agar (Merck) containing 10 mL/L 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS; 50 mM, Sigma, St Louis, MO, USA) and 3 mL/L horseradish peroxidase (1.0 mg/mL; Sigma). The horseradish peroxidase was added after the medium temperature decreased to 50°C, as previously described [11]. The plates were inoculated with the corresponding culture, and after micro-aerobic incubation at 37°C for 72 h, plates were exposed for 3-4 h at room temperature before checking the results. The isolates showing crimson halos were considered hydrogen peroxide-positive.

The production of antimicrobial-like substances was tested by inoculating 20 mL of MRS broth with an overnight culture of each isolate (1% v/v) and incubating micro-aerobically at 30°C for 24 h. The cultures obtained were then centrifuged at 6500 *g* for 10 min to remove bacterial cells and the supernatant was subsequently sterilized by filtration through 0.22 µm filters (Millipore, Bedford, MA, USA). Anti-microbial activities of extracts were tested against the strains obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain) or from the American Type Culture Collection (ATCC) listed in table 1.

A disc diffusion method assay based on that described by Campos et al. [12] was used for the detection of antimicrobial activity. Twenty µL of cell-free culture extract were deposited on a non-impregnated 6 mm sterile disc (Oxoid), previously placed on Mueller-Hinton agar plates inoculated with 2 mL of a Gram-positive indicator strain, (approximately 1.5×10^{6} CFU/mL). Plates were left for 2-4 h at room temperature to allow diffusion before discs were placed. Plates were then incubated at 37°C for 24 h. The presence of a clear zone of inhibition around the disc was recorded as antimicrobial activity. Production of nisin by *Lactococcus lactis* (ATCC 11454) was used as a positive control for bacteriocin production.

Thermal tolerance is described by the thermal death time, or D value, which is the time in minutes required to achieve a 1-log reduction in bacterial counts. D values (min) D_{55} and D_{60} were calculated for each strain from the negative inverse slope of the straight portion of survival curves. These were obtained from a plot of the log colony count number of survivors versus their corresponding heating times [13]. For this purpose, pure LAB strains were grown at 30°C for 48 h on MRS agar plates. Two colonies were picked, placed in 0.5 mL Mueller-Hinton broth (Oxoid) and grown at 37°C with continual shaking to achieve a final concentration of approximately 1×10^{8} CFU/mL. Viable counts were determined prior to heating, by plating 0.1 mL serial 10-fold dilutions on MRS agar plates and incubating at 37°C for 48-72 h. Afterwards, a 0.1 mL aliquot of each serial 10-fold culture dilution was aseptically placed into sterile capillary tubes manually sealed with a propane torch. Capillary ends were sterile-sealed and subjected to rapid cooling in an ice bath (1-2°C), followed by incubation in a water bath (Memmert, Schwabach, Germany) at 55°C and 60°C for 1, 3, 5, 10 and 15 min. The temperature was monitored by a high-precision thermometer inserted into a sealed sterile Pasteur pipette containing 1 mL sterile water. Numbers of bacteria surviving in each capillary were estimated by culturing the contents on MRS agar plates at 37°C for 48-72 h. The killing effect was quantified by CFU and converted to log10

	TVP	AVSP	Total
Technological characteristics			
Glucose fermentation	2/42 (4.8)	2/49 (4.1)	4/91 (4.4)
Proteolytic activity	25/42 (59.5)	30/49 (61.2)	55/91 (60.4)
Lipolytic activity	3/42 (7.1)	7/49 (14.3)	10/91 (11)
Production of antimicrobial-like substances	0/42 (0)[a]	4/49 (8.2)[b]	4/91 (4.4)
H_2O_2 production	0/42 (0)	0/49 (0)	0/91 (0)
Safety aspects			
β-Haemolysin	4/42 (9.5)	5/49 (10.2)	9/91 (9.9)
Histamine production	2/42 (4.8)	2/49 (4.1)	4/91 (4.4)
Presence of HDC gene	0/42 (0)	0/49 (0)	0/91 (0)

Table 2: Technological characteristics and safety aspects of LAB isolated from meat packaged using TVP or AVSP systems (Results as expressed as: number of positive/number of strains (%). TVP: traditional packaging system. AVSP: advanced packaging system. [a,b]Values in the same row with different letters are significantly different)

	Number of strains				
Indicator strains	**Control***	**1**	**2**	**3**	**4**
Brochothrix thermosphacta (ATCC 11509)	11.0 ± 0.2	9.5 ± 0.1	NA	NA	NA
Enterococcus faecalis (ATCC 23655)	NA	NA	8.0 ± 0.2	NA	NA
Escherichia coli (CCUG 20570)	NA	NA	NA	NA	NA
Lactobacillus 30A (ATCC 33222)	NA	NA	NA	NA	NA
Listeria monocytogenes (NCTC 11994)	9.5 ± 0.1	12.0 ± 0.2	9.4 ± 0.5	11.0 ± 0.1	11.0 ± 0.1
Leuconostoc mesenteroides (NCFB 963)	NA	8.0 ± 0.1	NA	NA	NA
Pseudomonas putida (ATCC 12842)	NA	NA	NA	NA	NA
Pseudomonas fluoroscens (ATCC 13430)	NA	NA	NA	NA	NA
Salmonella enteritidis (ATCC 29629)	NA	NA	NA	NA	NA
Staphylococcus aureus (ATCC 35845)	10.0 ± 0.2	9.5 ± 0.5	9.0 ± 0.5	8.5 ± 0.5	8.5 ± 0.5
Yersinia enterocolitica (ATCC 9610)	NA	NA	NA	NA	NA

Table 3: Antimicrobial activity of extracellular extracts obtained from strains isolated from AVSP showing bacteriocin activity (*The nisin-producing strain *Lactococcus lactis* (ATCC 11454) was included as a positive control. Results are expressed as diameters of the inhibition zone and standard deviations in millimetres. AVSP: advanced packaging system; NA: No activity; ATCC: American Type *Culture Collection*, USA; CCUG: Culture Collection University of Göteborg; NCBF: National Collection of Food Bacteria (UK); NCTC: UK National Collection of Type Cultures)

to calculate reduction in the number of viable organisms after exposure to heat.

Safety investigation

The safety of isolated LAB was characterized in terms of haemolityc activity (production of haemolysin), histidine decarboxylase activity (production of histamine), and presence of the histidine descarboxylase (HDC) gene. All trials were done in triplicate for each strain.

Production of haemolysin was determined by plating actively-growing cells of LAB strains onto Columbia agar (BioMerieux, Marcy l'Etoile, France) supplemented with 5% (v/v) sheep blood. Plates were incubated aerobically at 37°C for 24–48 h. The haemolytic reaction was recorded by the presence of a clear zone of hydrolysis around the colonies (β-haemolysis), a partial hydrolysis and greening zone (α-haemolysis) or no reaction.

To monitor the production of histamine by histidine decarboxylase activity, overnight cultures of all bacterial strains were grown on histidine agar medium [14]. Color change from yellow to purple indicated the presence of histidine decarboxylase. *Lactobacillus* 30A (ATCC 33222) was used as a positive control.

Detection of the HDC gene was performed using DNA extracted from overnight MRS cultures and 1.5 mL of each revitalised culture, pelleted by centrifugation at 7 500 rpm for 10 min. Each pellet was resuspended in 180 µL of lysis buffer, [(20 mg/mL lysozyme in double-distilled H_2O), 20 mM Tris-HCl, 2 mM EDTA and 1.2% Triton X-100 (Sigma)]. After incubation at 37°C for 30 min, 25 µL of proteinase K (10 mg/mL, Sigma) was added, followed by incubation at 70°C for 30 min. Bacterial DNA was then purified from each extract using a DNA easy tissue minikit (QIAGEN, Valencia, CA, USA). Concentration of purified DNA was determined by measuring fluorescence after mixing with Hoechst-33258 (Sigma) on an LS-50B fluorometer (Perkin Elmer, Wellesley, MA, USA). PCR was performed using similar conditions to those previously described [15]. Amplification assays were carried out on a MyCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) using the following cycling parameters: 10 min for enzyme activation at 95°C followed by 30 cycles of 30 s at 95°C, 30 s at 52°C, and 2 min at 72°C; and a final extension step of 10 min at 72°C [16]. PCR products were analysed on 2.5% horizontal agarose (Pronadisa, Madrid, Spain) gels. *Lactobacillus 30A* (ATCC 33222) was used as a positive control.

Statistical analyses

The X^2 test was used to compare the technological and safety characteristics, and the D_{55} and D_{60} of LAB isolated from meat samples packaged using the TVP and AVSP systems. The differences were considered significant when the *p*-value was lower than 0.05. All statistical analyses were performed using Statgraphics version 5.0.1 software for windows (SAS Institute, North Carolina, USA).

Results

From a total of 100 potential LAB samples isolated from beef packaged using TVP or AVSP, 91 were positively identified as LAB by PCR. These results demonstrate that in global terms, the different characteristics of the packaging methods tested do not provide different LAB distribution profiles in samples isolated from beef packaged using either system. *Enterococcus* was the dominant genus of bacteria found in both types of packaged meat with a total of 49 isolates (53.9% of total),

followed by *Carnobacterium* (18 isolates, 19.8% of total), *Lactobacillus* (15 isolates, 16.4% of total) and *Leuconostoc* (9 isolates, 9.9% of total).

Most of the technological or safety properties investigated were not significant different between LAB isolated from TVP or AVSP systems (Table 2). Only four strains (4.4% of total) produced gas after 72 h of incubation at 30°C, whereas 55 of the strains investigated (60.4% of total) were able to hydrolyse casein, and therefore had proteolytic activity. On the other hand, lipolytic activity was only found in 10 LAB strains (11% of total). The production of antimicrobial-like substances was the only parameter which differed significantly between LAB isolated from TVP and those from AVSP (p=0.04). Bacteriocinogenic activity was only detected in four strains isolated from AVSP samples, which exhibited antagonistic effects on different specific pathogens and food spoilage (Table 3). All four strains produced antimicrobial-like substances active against *L. monocytogenes* (ATCC 11994) and *S. aureus* (ATCC 35845), whereas only one strain each was found to be active against *B. thermosphacta* (ATCC 11509), *E. faecalis* (ATCC 23655) and *Lc. mesenteroides* (NCFB 963), respectively.

On the other hand, none of the isolates were found to produce H_2O_2. With respect to haemolytic activity, β-haemolysis was detected in only nine isolates (9.9%), whereas α- or γ-haemolysis was not detected in any isolates. Additionally, the ability to form histamine from histidine was detected in four LAB strains (4.4%). However, these results were not confirmed by PCR detection of the HDC gene (Table 2).

With respect to heat resistance, significant reductions in viable bacterial counts when thermally stressed at 55°C and 60°C were observed for all bacteria tested. Table 4 lists the D values of all the species with a regression analysis (0:92<r ≤ 0:99), except in four strains with a regression analysis (0:86<r ≤ 0:89) at D_{60}. In general, it was found that strains isolated from the AVSP system were significantly more heat resistant at both temperatures than those strains isolated from the TVP system (p<0.05).

Discussion

Previous works reported that beef packaged under AVSP system showed different physic-chemical properties than those beef package under TVP system. Thus, it was reported than beef packaged under AVSP shows lower pH than those isolated from TVP, mainly after 26 days of packaging [4]. This fact could be related by lower LAB grown on beef packaged under AVSP system, and their consequent lower lactic acid production. Other work [10] revealed that beef packaged under AVSP system showed higher levels of lightness and redness than samples processed with TVP, as well as a better maintenance of general appearance. These results represent an important advantage of the AVSP system relative to TVP [10].

Most of the strains studied in this work did not produce gas from glucose. These results are in agreement with previous studies reporting that some strains of LAB are able to utilise ribose and gluconic acid as substrates for growth, and may produce organic acids and CO_2 [17].

Packaging		D values (min)		
system	Temp (°C)	Range	Mean ± SD	No. of strains
TVP	55	1.54–21.32	11.17 ± 3.34[a]	42
AVSP	55	1.56–40.55	14.09 ± 5.94[b]	49
TVP	60	0–13.41	4.64 ± 3.12[c]	42
AVSP	60	0.51–13.72	6.87 ± 2.61[d]	49

Table 4: Heat resistance values (D values) at 55ºC and 60ºC obtained for LAB isolated from meat packaged using the TVP or AVSP system ([a–d]Values in the same column with different letters are significantly different).

Gas production by LAB in meat has been described as an undesirable characteristic [18]. Consequently, the low number of gas-producing LAB in meat packaged using TVP or AVSP is advantageous.

Proteolytic and lipolytic activities, as well as antimicrobial activity and decarboxylation of amino acids, are among the most important and most studied technological and safety properties of LAB in food [19,20]. Most LAB isolates displayed proteolytic activity, although no significant differences were observed between TVP- and ASVP-isolated strains. The presence of proteolytic bacteria in packaged meat could affect the firmness of the meat. It is well known that meat aging is characterized by a significant softening of the meat, mainly arising from the effect of proteases on the myofibrilar protein fraction [10]. Previous works revealed that beef packaged under AVSP system showed higher firmness than those counterparts packaged under TVP, probably due to the fact that protease enzymes involved in tenderness have an optimum pH range for activity close to neutrality [4,10]. Thus, taking into account that in the present work it was found similar proteolytic activity in bacteria isolated from beef packaged under both systems, it seems suggest that differences in firmness reported by previous works are mainly causes by endogenous enzymes and not by proteolytic bacteria.

Although the presence of proteolytic bacteria in packaged meat could affect the firmness of the meat, proteolytic activity by meat microbiota may also be beneficial. Degradation of protein produces peptides and amino acids that may contribute to the development of flavour [21]. Our results are in agreement with previous studies which found that different species of LAB from different food origins have proteolytic activity [19,21]. In contrast, other studies have found that LAB isolated from meat products is only weakly proteolytic [18,20].

With respect to lipolytic activity, it was observed that most strains were not able to hydrolyse tributyrin, with only 10 strains showing a low lipolytic activity against tributyrin. These results are in accordance with others studies in meat products that also reported a weak capacity of LAB for lipolytic activity [18,19].

The potential of LAB to produce antimicrobial compounds has attracted much attention; as such compounds can be used to prevent food spoilage and to inhibit the growth of food pathogens [22]. The primary antimicrobial effect exerted by LAB is the production of lactic acid and the reduction of pH [23]. However, besides organic acids, LAB also produces a number of other inhibitory compounds such as bacteriocins. In this study, the presence of LAB that can produce antimicrobial-like substances was higher in meats packaged under the AVSP system than those packaged under the TVP system. Four strains, all isolated from meat packaged under AVSP system, displayed strong antimicrobial activity against pathogenic and spoilage bacteria (Table 3) with clearing zones of 8.0–19.5 mm. This increased number of bacteriocinogenic LAB in AVSP-packaged meat could explain its longer shelf life compared to TVP-packaged meat [10]. Enterococcal bacteriocins have been reported to be strong inhibitors of food-borne pathogens such as *L. monocytogenes*, *C. tyrobutyricum*, *C. perfringens* and *S. aureus* [23,24]. In this study, the antimicrobial substances produced by LAB isolates were active against Gram-positive food-borne pathogens such as *L. monocytogenes* and *S. aureus* in all cases. Use of these strains as starter or protective cultures, as well as a bio-preservative could be useful [22,25]. In contrast, activity against *B. thermosphacta*, *E. faecalis* or *Lc. mesenteroides* was only found in some isolates. Isolates were therefore unable to inhibit growth of the Gram-negative bacteria used in this study. This property is unusual and has only been reported in a few LAB-produced bacteriocins [24,26]. It has

previously been theorized that bacteriocins from LAB are low efficient against Gram-negative bacteria because the outer membrane blocks the bacteriocin target [27].

Absence of haemolytic activity is a key selection criterion for bacteria as starter strains for food use [28]. Although most LAB strains isolated from meat do not show haemolytic activity, some species of *Enterococcus* have been more frequently found to be haemolytic [29]. Consequently, *Enterococcus* must be checked carefully for the presence of haemolysins. Although 53.9% of strains isolated in this study belong to *Enterococcus* genus, fewer than 10% of isolates displayed β-haemolysis, a property that has not been frequently reported for LAB.

With respect to histamine production, none of the LAB isolated displayed histidine decarboxylase that was confirmed by the PCR method (presence of the HDC gene), although four strains were able to decarboxylate histidine in histidine agar media. Previous studies have also revealed low histamine production in LAB species such as *C. divergens* [30], and Pircher et al. [31] found that only 4.8% of *E. faecium* isolates from meat were capable of hydrolysing histidine.

Because of the association of LAB with spoilage in packaged meat and thermally-processed food [32], it is important to test these bacteria for thermal resistance. D values are extensively used to characterize the thermal resistance of common microorganisms in food [33,34]. Previously, it was reported that the D values for typical LAB at 60°C are in the range of 0.25-0.66 min [35]. Examples of D values at 60°C for organisms known to be heat resistant are 18.3-39.7 min for *E. faecium* in meat [36] and 2.53 min for *L. monocytogenes* in broth [37]. In contrast, no survivor cells were detected for *C. viridans* (ATCC BAA 336) when treated for 1 min, and no reduction in viable cell number was found for *E. faecalis* ATCC 7080 [37]. In the present work, we found that average D values at 55°C ranged from 11.17 min for LAB isolates from TVP samples to 14.09 min for LAB isolates from AVSP samples. Similarly, average D values at 60°C varied from 4.64 min for LAB isolates from TVP samples to 6.87 min for LAB isolates from AVSP samples. This could be explained by the fact that the AVSP system warms up the surface of the meat [10], possibly causing the death of heat-sensitive bacteria. It is generally assumed that the temperature of microorganisms influences their thermo-tolerance, as bacteria grown at higher temperatures show greater resistance to heat [38]. Moreover, it has been demonstrated that environmental conditions during bacterial growth are responsible for qualitative and quantitative changes in their membrane fatty acid profile. At higher temperatures, the saturated fatty acid content of the membrane increases and unsaturated fatty acid levels decrease [39]. These changes in membrane lipid composition mainly affect the fluidity of the cell membrane, with an increase in membrane fluidity corresponding to a decrease in thermal resistance [40]. The higher thermal resistance of LAB isol

ated from meats packaged using AVSP compared to TVP could also contribute to AVSP meat safety, as the surviving LAB produces organics acids that decrease meat pH and in some cases, produces bacteriocin-like antimicrobial substances.

Conclusions

This study has compared the technological characteristics of LAB isolated from two types of packaging systems (AVSP and TVP). Previous studies have revealed longer shelf life of meat packaged under the AVSP system compared to the TVP system but did not provide an explanation for this difference. Our results lead us to conclude that the LAB isolated from both packaging systems are not significantly different in their technological characteristics. The notable exception to this is the higher production of bacteriocin-like substances in LAB isolated from AVSP-packaged meat. On the other hand, we showed that LAB isolated from meat packaged using AVSP was more heat resistant than LAB isolated from TVP-packaged meat. Overall, these facts could explain the longer shelf life of meat packaged using the AVSP system compared to meat packaged with the TVP system.

References

1. Del Nobile MA, Conte A, Incoronato AL, Sevi OPA, Marino R (2009) New strategies for reducing the pork back-fat content in typical Italian salami. Meat Sci 81: 263-269.
2. Martinez B, Miranda JM, Vázquez BI, Fente CA, Franco CM, et al. (2012) Development of a hamburger patty with healthier lipid formulation and study of its nutritional, sensory, and stability properties. Food Bioprocess Tech 5: 200-208.
3. Hoyle PAR, Brashears MM, Woerner WD, Martin JN, Thompson LD, et al. (2012) Spoilage characteristics of traditionally packaged ground beef with added lactic acid bacteria displayed at abusive temperatures. J Anim Sci 90: 642-648.
4. Vázquez IB, Carreira L, Franco CM, Fente C, Cepeda A, et al. (2004) Shelf life extension of beef retail cuts subjected to an advanced vacuum skin packaging system. Eur Food Res Technol 218: 118-122.
5. Samuel A, Miranda JM, Guarddon M, Nebot CG, Calo-Mata MP, et al. (2011) Molecular characterization of lactic acid bacteria isolated from beef and stored using vacuum packaging and advanced vacuum skin packaging systems. CyTA-J Food 9: 335-341.
6. Cayré ME, Vignolo G, Garro O (2003) Modeling lactic acid bacteria growth in vacuum-packaged cooked meat emulsions stored at three temperatures. Food Microbiol 20: 561-566.
7. Samelis J (2006) Managing microbial spoilage in the meat industry. In food spoilage organisms ed. Blackburn, CDW. Woodhead Publishing Limited. Cambridge, UK.
8. Nattress FM, Jeremiah LE (2000) Bacterial mediated off-flavours in retail-ready beef after storage in controlled atmospheres. Food Res Int 33: 743-748.
9. Christiansen P, Nielsen EW, Vogensen FK, Brogren CIH, Ardö Y (2006) Heat resistance of *Lactobacillus paracasei* isolated from semi-hard cheese made of pasteurised milk. Int Dairy J 16: 1196-1204.
10. Barros VJ, Carreira L, Franco C, Vázquez BI, Fente C, et al. (2003) Microbiological and physiological properties of fresh retail cuts of beef packaged under an advanced vacuum skin systems and stored at 4ºC. J Food Prot 66: 2085-2092.
11. Marshall VM (1979) A note on screening hydrogen peroxide-producing lactic acid bacteria using a non-toxic chromogen. J Appl Bacteriol 47: 327-328.
12. Campos CA, Rodríguez O, Calo-Mata P, Prado M, Barros VJ (2006) Preliminary characterization of bacteriocins from *Lactococcus lactis*, *Enterococcus faecium* and *Enterococcus mundtii* strains isolated from turbot (*Psetta maxima*). Food Res Int 39: 356-364.
13. Hersom AC, Hulland ED (1980) Principles of thermal processing. In Canned foods. Thermal processing and microbiology. Churchill Livingstone, New York, USA.
14. Bover CS, Holzapfel WH (1999) Improved screening procedure for biogenic amine production by lactic acid bacteria. Int J Food Microbiol 53: 33-41.
15. Le JC, Lonvaud FA, Ten BB, Holstra H, van der Vossen JMBM (1995) Development of a detection system for histidine decarboxylating lactic acid bacteria based on DNA probes, PCR and activity test. J Applied Bacteriol 78: 316-326.
16. Marcobal A, de las Rivas B, Moreno-Arribas MV, Muñoz R (2005) Multiplex-PCR method for the simultaneous detection of histamine, tyramine and putrescine producing lactic acid bacteria in foods. J Food Prot 68: 874-878.
17. Leisner JJ, Laursen BG, Prévost H, Drider D, Dalgaard P (2007) *Carnobacterium*: positive and negative effects in the environment and in foods. FEMS Microbiol Rev 31: 592-613.
18. Benito MJ, Martín A, Aranda E, Pérez-Nevado F, Ruiz-Moyano S, et al. (2007)

Characterization and selection of autochthonous lactic acid bacteria isolated from traditional iberian dry-fermented Salchichón and Chorizo sausages. J Food Sci 72: 193-201.

19. Papamanoli E, Tzanetakis N, Litopoulou TE, Kotzekidou P (2003) Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. Meat Sci 65: 859-867.
20. Drosinos EH, Paramithiotis S, Kolovos G, Tsikouras I, Metaxopoulos I (2007) Phenotypic and technological diversity of lactic acid bacteria and staphylococci isolated from traditionally fermented sausages in Southern Greece. Food Microbiol 24: 260-270.
21. Bonomo MG, Ricciardi A, Zotta T, Parente E, Salzano G (2008) Molecular and technological characterization of lactic acid bacteria from traditional fermented sausages of Basilicata region (Southern Italy). Meat Sci 80: 1238-1248.
22. Foulquié MMR, Sarantinopoulos P, Tsakalidou E, De Vuyst L (2006) The role and application of enterococci in food and health. Int J Food Microbiol 106: 1-24.
23. Aymerich T, Holo H, Havarstein LS, Hugas M, Garriga M, et al. (1996) Biochemical and genetic characterization of enterocin A from *Enterococcus faecium*, a new antilisterial bacteriocin in the pediocin family of bacteriocins. Appl Environ Microbiol 62: 1676-1682.
24. Mareková M, Lauková A, Skaugen M, Nes I (2007) Isolation and characterization of a new bacteriocin, termed enterocin M, produced by environmental isolate *Enterococcus faecium* AL41. J Ind Microbiol Biot 34: 533-537.
25. Franz CMAP, Stiles ME, Schleifer KH, Holzapfel WH (2003) Enterococci in foods-a conundrum for food safety. Int J Food Microbiol 88: 105-122.
26. Caridi A (2002) Selection of *Escherichia coli*-inhibiting strains of *Lactobacillus paracasei* subsp. *Paracasei*. J Ind Microbiol Biot 29: 303-308.
27. Stevens KA, Sheldon BW, Klapes NA, Klaenhammer TR (1991) Nisin treatment for inactivation of *Salmonella* species and other Gram-negative bacteria. Appl Environ Microbiol 57: 3613-3615.
28. Giraffa G (1995) Enterococcal bacteriocins: their potential as anti-*Listeria* factors in dairy technology. Food Microbiol 12: 291-299.
29. Fifadara N, Radu S, Hassan Z, Beuchat LR, Rusul G (2003) Hemolytic and nonhemolytic vancomycin-resistant *Enterococcus faecalis* isolated from beef imported to Malaysia. J Food Prot 66: 1845-1850.
30. Masson F, Talon R, Montel RC (1999) Histamine and tyramine production by bacteria from meat products. Int J Food Microbiol 32: 199-207.
31. Pircher A, Bauer F, Paulsen P (2007) Formation of cadaverine, histamine, putrescine and tyramine by bacteria isolated from meat, fermented sausages and cheeses. Eur Food Res Technol 226: 225-231.
32. Aymerich T, Martín B, Garriga M, Hugas M (2003) Microbial quality and direct PCR identification of lactic acid bacteria and nonpathogenic staphylococci from artisanal low acid sausages. Appl Environ Microbiol 69: 4583-4594.
33. Martínez S, López M, Bernardo A (2003) Thermal inactivation of *Enterococcus faecium*: effect of growth temperature and physiological state of microbial cells. Lett Appl Microbiol 37: 475-481.
34. Reveron IM, Barreiro JA, Sandova AJ (2005) Thermal death characteristics of *Lactobacillus paracasei* and *Aspergillus niger* in Pilsen beer. J Food Eng 66: 239-243.
35. Franz CMAP, von Holy A (1996) Thermotolerance of meat spoilage lactic acid bacteria and their inactivation in vacuum-packaged vienna sausages. Int J Food Microbiol 29: 59-73.
36. Houben JH (2003) The potential of vancomycin-resistant enterococci to persist in fermented and pasteurised meat products. Int J Food Microbiol 88: 11-18.
37. Peirson MD, Guan TY, Holley RA (2003) Aerococci and carnobacteria cause discoloration in cooked cured bologna. Food Microbiol 20: 149-158.
38. Bussarin K, Sudip KR (2010) Induction of heat tolerance in autochthonous and allochthonous thermotolerant probiotics for application to white shrimp feed. Aquaculture 306: 302-309.
39. Nagamachi E, Shibuya S, Hirai Y, Matsushita O, Tomochika K, et al. (1991) Adaptational changes of fatty acid composition and the physical state of membrane lipids following the change of growth temperature in *Yersinia enterocolitica*. Microbiol Immunol 35: 1085-1093.
40. Logue CM, Sheridan JJ, McDowell DA, Blair IS, Hegarty T (2000) The effect of temperature and selective agents on the growth of *Yersinia enterocolitica* serotype O:3 in pure culture. J Appl Microbiol 88: 1001-1008.

Citation: Miranda JM, Samuel A, Nebot CG, Cepeda A, Franco CM, et al. (2014) Technological Characterization of Lactic Acid Bacteria Isolated from Beef Stored on Vacuum-Packaged and Advanced Vacuum Skin Packaged System. J Food Process Technol 5: 338. doi:10.4172/2157-7110.1000338