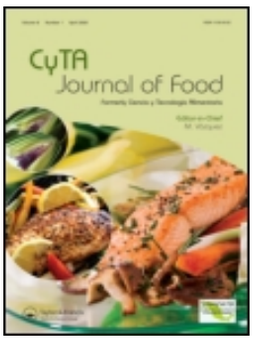

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Molecular characterization of lactic acid bacteria isolated from beef and stored using vacuum-packaging and advanced vacuum skin packaging systems

Caracterización molecular de bacterias ácido-lácticas aisladas a partir de carne de ternera envasada al vacío de modo tradicional y mediante un sistema avanzado

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A total of 91 lactic acid bacteria (LAB) were isolated from 50 beef samples, 25 packaged by traditional vacuum packaging and 25 packaged using advanced vacuum skin packaging. The isolated LAB were identified using 16S rRNA sequencing, whereas randomly amplified polymorphic DNA (RAPD-PCR) and cluster analysis were used for typing the LABs. Ten different species of LAB were identified and assigned to the following species: *Enterococcus gilvus* (22 isolates), *Enterococcus faecium* (9 isolates), *Enterococcus casseliflavus* (8 isolates), *Enterococcus faecalis* (4 isolates), *Enterococcus malodoratus* (3 isolates), *Enterococcus devriessei* (3 isolates), *Lactobacillus sakei* (15 isolates), *Carnobacterium divergens* (12 isolates), *Carnobacterium maltaromaticum* (5 isolates), and *Leuconostoc mesenteroides* (8 isolates). The RAPD profile bands observed were not significantly discriminatory, with exceptions of *E. casseliflavus, E. faecalis*, and *E. faecium*, which suggests that the type of packaging system used had no specific effect on the selection of most microbiota in the meat after packaging.

Keywords: RAPD; 16S rRNA; meat; beef; *E. gilvus*; lactic acid bacteria; vacuum-packaging

Un total de 91 bacterias ácido-lácticas (LAB) fueron aisladas a partir de 50 muestras de carne de ternera, de las cuales 25 fueron envasadas al vacío de modo tradicional y 25 fueron envasadas mediante un sistema de vacío avanzado. Los aislamientos fueron identificados a través de la secuenciación del ADN ribosomal 16S, mientras que la caracterización de las LAB se realizó mediante la amplificación al azar del ADN polimórfico (RAPD-PCR) y su posterior análisis cluster. Fueron identificadas cepas pertenecientes a diez especies bacterianas diferentes, en concreto: *Enterococcus gilvus* (22 aislamientos), *E. faecium* (9 aislamientos), *E. casseliflavus* (8 aislamientos), *E. faecalis* (4 aislamientos), *E. malodoratus* (3 aislamientos), *E. devriessei* (3 aislamientos), *Lactobacillus sakei* (15 aislamientos), *Carnobacterium divergens* (12 aislamientos), *C. maltaromaticum* (5 aislamientos) y *Leuconostoc mesenteroides* (8 aislamientos). Los perfiles de bandas de ADN obtenidas no revelaron diferencias significativas, con las excepciones de *E. casseliflavus, E. faecalis* y *E. faecium*, sugiriendo que el tipo de envasado no tiene un efecto específico en la selección de la mayor parte de la microbiota presente en la carne envasada.

Palabras clave: RAPD; 16S rRNA; carne; ternera; *E. gilvus*; bacterias ácido-lácticas; envasado al vacío

Introduction

The increased globalization of food markets often results in long distances between production and commercialization areas. This increased globalization, combined with consumer demand for high quality products, has required the meat industry to utilize processing methods that provide a long shelf life for meat and meat products (Barros-Velázquez et al., 2003). The usefulness of 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 (Davies, 1995; Vázquez et al., 2004). Under TVP, the oxygen supply is restricted, and the gas phase is determined by the rate of gas permeation through the film and the rate of oxygen consumption. Consequently, these changes have a selective effect on the microbial population (Cayré, Garro, & Vignolo, 2005) because the low oxygen supply restricts the growth of some typical spoilage bacteria, such as *Pseudomonas* (Cayré, Vignolo, & Garro, 2003). Consequently, lactic acid bacteria (LAB), which are of a psychotropic and microaerophilic nature become the major spoilage

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population in vacuum-packaged and, subsequently, refrigerated meats (Cayré et al., 2005, Samelis, 2006). As a consequence, the spoilage caused by LAB in vacuum-packaged meats is especially important after extended periods of storage (Nattress & Jeremiah, 2000; Yost & Nattress, 2002).

More recently, some meat industries have 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 the instantaneous heating of an upper film at a high temperature immediately before its descent over the meat surface. The tight disposition of the film over the meat surface prevents the formation of wrinkles and air pockets (Barros-Velázquez et al., 2003). Previous studies have demonstrated that beef packaged using the AVSP system and subsequently refrigerated exhibits a slower microbial growth rate and has a considerably longer shelf life compared to samples processed using a TVP system (Barros-Velázquez et al., 2003; Vázquez et al., 2004). Another important finding reported by these authors is that the heating of the upper film in the AVSP system does not completely eliminate initial LAB contamination from the surface of the meat. Consequently, LAB-induced spoilage also occurs in meat packaged using this system.

Taking into account that LAB can be present in meat packaged using both the TVP and AVSP systems, the longer shelf life of meat packaged using the AVSP system may be the result of a different LAB spoilage microbiota compared to that present in meat packaged using TVP. LAB present in vacuum-packaged meat usually belongs to the genera *Lactobacillus, Leuconostoc, Carnobacterium* or *Enterococcus* (Jones, 2004). These LAB do not all have the same spoilage activities. For this reason, it is important to know which LAB cause spoilage to meat packaged using the TVP and AVSP systems.

Because many LAB have similar nutritional and growth requirements, their identification using conventional phenotypic methods, such as sugar fermentation patterns, is difficult. These techniques yield ambiguous results, are time-consuming and unreliable, and may render misidentifications (Tamang et al., 2008). Consequently, a number of molecular methods have been used to identify LAB strains isolated from meat and to study their biological diversity (Audenaert et al., 2010; Nieminen et al., 2011). The most frequently used methods for this purpose are the following: hybridization or species-specific amplification, which use probes and primers targeted towards the 16S rRNA sequence (Albano et al., 2009); amplification and sequence analysis of the 16S rRNA and 16S/23S rRNA spacer region; and cluster analysis of electrophoretic fragments obtained by randomly amplified polymorphic DNA (RAPD-PCR) analysis (Albano et al., 2009; Corsetti, Settanni, Valmorri, Mastrangelo, & Suzzi, 2007; Tsai, Lai, Yu, & Tsen, 2010; Urso, Comi, & Cocolin, 2006).

The aim of this work was to determinate the LAB populations of beef packaged using the TVP or AVSP systems in order to investigate if the better shelf life and physicochemical properties exhibited by meats packaged using the AVSP (Barros-Velázquez et al., 2003; Vázquez et al., 2004) are related to the selection of different LAB populations. For this purpose, LAB isolated from meats packaged using TVP or AVSP were indentified by amplification of 16S rRNA using universal primers and by sequencing the PCR products (Balcázar et al., 2007; Catzeddu, Mura, Parente, Sanna, & Farris, 2006). RAPD-PCR and cluster analyses of the patterns obtained were performed to acquire more detailed information about the strains. To the best of our knowledge, this is the first study on the characterization and comparison of LABs isolated from beef packaged using the TVP and AVSP systems.

Materials and methods

Reference strains and culture conditions

The reference strains used in this study (Supplementary Table 1) were obtained from the Spanish Type Culture Collection (CECT) or the Agriculture Research Council-Research Centre for Forage and Dairy Productions (CRA-FLC, Lodi, Italy). All strains were grown in De Man, Rogosa and Sharpe (MRS) culture agar (Difco Laboratories, Detroit, MI, USA) at 30°C for 72 h under micro-aerobic conditions, which were generated using the AnaeroGen C system (Oxoid, Basingstoke, UK) in anaerobic jars.

Origin and isolation of the LAB strains

In order to eliminate differences in LAB meat contamination caused by external factors, all meat samples were obtained from animals slaughtered, beaked-up, and packaged in the same slaughterhouse and quartering room, and using the same factory personnel (Cocarga, Pontevedra, Spain). A total of 50 beef samples (200 g each) were employed. Twenty-five samples were packaged using either the AVSP system, which was performed on a Multivac R570 CD packing machine (Multivac, Wolfertschwenden, Germany) or the TVP system, on a Vac-210 packing machine (Guasch, Barcelona, Spain). After packaging, the samples were stored at refrigeration temperatures ($\leq$4°C) for a period of at least 10 days in order to allow for the proliferation of specific LABs selected by both packaging methods.

For LAB isolation, 25 g of each sample type was aseptically extracted and diluted with an appropriate volume (1/9 w/v) of 0.1% peptone water (Merck, Darmstadt, Germany) and homogenized for 1 min in a masticator (AES; Combourg, France). After homogenization, the samples were diluted in 0.1% peptone water, and 10^{-3} to 10^{-7} dilutions of homogenates were surface plated on MRS agar plates (Difco). These

plates were then incubated at 30°C for 72 h under micro-aerobic conditions generated using the Anaero-Gen C system (Oxoid). After the incubation period, two typical white, small and round presumptive LAB colonies from each sample were randomly selected ($n = 100$). The isolates were purified by three alternate subcultures on MRS broth (Oxoid) and in MRS agar (Difco). Afterwards, the isolates were primarily characterized as LAB because the strains were non-motile, Gram-positive, catalase-negative, and oxidase-negative. After primary characterizations, and for further identification, all strains were maintained as frozen stocks in a mixture of MRS broth and 20% glycerol at −80°C.

Preparation of the samples for PCR

LAB strains were incubated overnight at 30°C in 10 ml of MRS broth. Genomic DNA was extracted from 1.5 ml of each culture, which was pelleted by centrifugation at 7500 rpm for 10 min. Each pellet was re-suspended in 180 μl of lysis buffer. This lysis buffer was prepared in 10 ml aliquots and had a final concentration of 20 mg/ml of lysozyme (in double-distilled water), 20 mM Tris–HCl (pH 8.0), 2 mM EDTA and 1.2% Triton X-100. All the reagents were purchased from Sigma (St. Louis, MO, USA). After an incubation step at 37°C for 30 min, 25 μl of proteinase K (10 mg ml^{-1}) (Sigma) was added, which was followed by incubation at 70°C for 30 min. Bacterial DNA was purified from each extract using a DNeasy Tissue mini Kit (Qiagen Inc., Valencia, CA, USA). The concentration of the purified DNA extract was determined by measuring fluorescence, which resulted from mixing with Hoechst 33258 reagent (Sigma) using a LS 50B fluorimeter (Perkin Elmer, Wellesley, MA, USA).

Genotypic identification by 16S rRNA gene sequence analysis

Molecular characterization of the LAB was performed through the detection of 16S rRNA sequences using a set of universal primers, which consisted of P8FPL (5′-AGTTTGATCCTGGCTCAG-3′) and P806R (5′-GGACTACCAGGGTATCTAAT-3′) (McCabe, Zhang, Huang, Wagar, & McCabe, 1999), which generated a 834 bp fragment. All amplification assays were composed of 100 ng of template DNA, 25 μl of a master mix (BioMix, Bioline Ltd., London, UK) and 25 pmol of each oligonucleotide primer to achieve a final volume of 50 μl. The amplification conditions were as follows: an initial denaturing step of 5 min at 94°C followed by 30 cycles of denaturation at 94°C for 60 s, annealing at 55°C for 60 s, extension at 72°C for 60 s, and a final extension step at 72°C for 15 min. All PCR assays were performed on a MyCycler Thermal Cycler (BioRad Laboratories, Hercules, CA, USA). The PCR products were resolved by electrophoresis in horizontal 2.0% (w/v) agarose gels (MS-8, Pronadisa, Madrid, Spain) and visualized by ethidium bromide staining. Prior to sequencing, the PCR products were purified by means of an ExoSAP-IT kit (GE Healthcare, Amersham Biosciences, Uppsala, Sweden). Direct sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The same primers used for PCR were used for sequencing both strands of the PCR products. Sequencing reactions were analyzed in an automatic sequencing system (ABI 3730XL DNA Analyzer, Applied Biosystems).

Sequence and phylogenetic analyses

The DNA sequences were carefully reviewed using Chromas software (Griffith University, Queensland, Australia). Sequence identities were validated by alignments performed with the assistance of the CLUSTAL-X program (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997). Sequences were submitted to the National Center for Biotechnology Information (NCBI, Bethesda, USA) for similarity searches against GenBank. The BLAST 2 program (Altschul et al., 1997) was used to determine sequence similarities.

All strains that showed divergent RAPD-PCR profiles were subjected to phylogenetic analysis. Phylogenetic analyses were conducted using the sequences from the 834-bp DNA fragments, which were generated using the P8FPL and P806R primers. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1 (Kumar, Tamura, & Nei, 2004) using different models for distance calculations (Kimura, Tajima-Nei, Tamura, three parameters) and two possible tree reconstruction methods (neighbor-joining and minimum evolution).

Random amplified polymorphic DNA-PCR analysis

To evaluate the biological diversity at the intra-specie level of the strains, isolates obtained from each packaged system were submitted to RAPD-PCR with primer M13. RAPD-PCR was performed using 100 ng of template DNA and 25 μl of a master mix (BioMix). The M13 primer (5′-GAGGGTGGCGGTTCT-3′), as described by Andrighetto, Zampese, & Lombardi (2001), was added at 1 μmol/l for a final volume of 50 μl. Amplification reactions were performed using a thermal cycler from Applied Biosystems (GeneAmp-PCR System 2700). The reaction conditions that were used for the PCR with the M13 primer were as follows: initial denaturation at 94°C for 5 min, followed by 33 cycles of denaturation at 94°C for 60 s, annealing at 45°C for 1 min, extension at 72°C for 1 min 30 s, and a final extension at 72°C for 15 min. A volume of 10 μl of PCR products was separated and visualized using

horizontal 1.5% agarose gels (MS-8, Pronadisa, Madrid-Spain), in a solution of 1xTAE (Tris-acetate-EDTA) buffer and ethidium bromide (10 mg/ml) with electrophoresis at 80 V. The 123-bp DNA ladder (DNA Ladders D 5042, Sigma) was used as a size marker. To check for reproducibility, PCR assays were always performed in triplicate. Each reaction was also tested using negative controls.

Computer analysis of RAPD-PCR profiles

Stained gels were photographed with a DC290 Zoom digital camera (Kodak, Edinburgh, UK) under UV light (HEROLAB UVT–20 ME GMBH Laborgeräte, Germany). The molecular weight of each band from the gel image was measured with the help of FragSize software version 1.0.0.3 (Massachusetts Uwel University, USA). A range of molecular weights was assigned, and the presence or absence of each band in the corresponding range was recorded as one or zero, respectively.

Statistical analysis

The mean log colony forming units (CFU) LAB per gram values in meats packaged using the TVP and AVSP systems were compared using a Student's *t*-test. The X^2-test was used to compare the different proportions of LAB species in meats packaged using the TVP and AVSP systems. The differences were considered significant when the *P*-value was lower than 0.05. The unweighted pair group method with arithmetic averages (UPGMA) was used for elaboration of the dendrogram clusters. All statistical analyses were performed using Statgraphics version 5.0.1 software for windows (SAS Institute, North Carolina, USA).

Results

The average log CFU/g counts of LAB obtained from meat samples packaged using the TVP (7.4 ± 3.21) and AVSP systems (7.2 ± 2.08) indicated that the packaging system used did not lead to significant differences in the average LAB log CFU/g counts in meat samples.

Of the 100 LAB isolated from the meats, 9 could not be recuperated after the freezing step. Thus, only 91 LABs were identified using PCR methods, with 49 were isolated from meat packaged using the AVSP system and 42 isolated from meat packaged using the TVP system. After partial 16S rRNA sequencing and sequence similarity searches in the NCBI databases, the isolates were classified into 10 species (Supplementary Table 2). *Enterococcus* was the dominant genus in both types of packaged meat with a total of 49 isolates (53.9% of the total), followed by *Carnobacterium* (18 isolates, 19.8% of the total), *Lactobacillus* (15 isolates, 16.4% of the total) and *Leuconostoc* (9 isolates, 9.9% of the total).

The *Enterococcus gilvus* was the predominant species and comprised 24.2% of the isolates (22 out of 91), *Lactobacillus sakei* compromised 16.5% of the isolates (15 out of 91), and *Carnobacterium divergens* compromised 13.2% of the isolates (12 out of 91). The remaining strains were the following: *Enterococcus faecium* 9.9% (9 isolates), *Leuconostoc mesenteroides* 9.9% (9 isolates), *Enterococcus casseliflavus* 8.8% (7 isolates), *Carnobacterium maltaromaticum* 6.6% (6 isolates), *Enterococcus faecalis* 4.4% (4 isolates), and *Enterococcus devriessei* and *Enterococcus malodoratus* (3.3% each).

The results indicate that in global terms the special characteristics of the packaging methods tested do not provide different distribution profiles of LAB isolated from beef packaged using the AVSP or TVP system. In comparing specific species, only *L. mesenteroides* was more frequently isolated from meat packaged using the TVP system compared to the AVSP system ($P = 0.045$).

The observed phylogenetic relationships (Supplementary Figure 1) did not shown any evidence for higher similarities for strains isolated from meat packaged using the same packaging system. RAPD patterns with three to nine bands were obtained. Thus, strains isolated from meat packaged using TVP presented RAPD-PCR profiles closely related to strains isolated using AVSP, whereas some isolates belonging to the same species and isolated from the same packaging system (e.g. *E. casseliflavus* 4–6 and 4–7) showed RAPD-PCR profiles phylogenetically less related.

In global terms, it was found to be a good correlation between phylogenetic analysis (Supplementary Figure 1) and cluster analysis of RAPD-PCR patterns (Supplementary Figures 2 and 3), because most strains identified as the same specie by 16S rRNA partial sequencing were grouped in the same cluster by RAPD-PCR analysis. Discrepancies were obtained only in case of two strains (33-2 TVP and 30-4 AVSP, cluster IV) (Supplementary Figure 3), those were identified as *C. divergens* by 16S rRNA partial sequencing, and were grouped in the same cluster as *C. maltaromaticum* by RAPD-PCR analysis.

RAPD-PCR patterns with three to nine bands were obtained (Supplementary Figure 4). The reproducibility of the RAPD-PCR, which was verified in independent experiments, was obtained in four replicated patterns using a type strain (results not shown) and was found to be higher than 90%. The similarities of the relationships among the patterns are shown in the dendrogram in Supplementary Figure 2. The cluster analysis at the 50% distance level for defining species showed six main clusters for the *Enterococcus* group: cluster I comprised 11 different profiles belonging to *E. gilvus*, whereas clusters II, III, IV, V, and VI comprised all remaining *Enterococcus* strains and their type strains. For another LAB species, four clusters (labeled

I to IV) were detected. Cluster I comprised the *L. sakei* type strain and 15 isolates that formed 12 different profiles. Cluster II comprised the *L. mesenteroides* type strain and nine isolates which formed eight different profiles. Cluster III comprised the *C. divergens* type strain and 10 isolates that consisted of 8 different profiles. Finally, the remaining eight isolates formed eight different band profiles, which were grouped in cluster 4 with the *C. maltaromaticum* type strain. The data obtained by the RAPD fingerprinting technique did not demonstrate a selective effect for the packaging system used. However, it was possible to analyze the sub-clusters separately to observe that the majority were formed by isolates from the same packaging system.

Generally, microorganisms isolated from meats packaged using the same system were distributed all along the dendrogram. This distribution shows that the packaging system did not produce a specific selection of isolates. However, it was observed that the clusters designated III, V, and VI, which are related to the *E. devriesei, E. faecium*, and *E. casseliflavus* species, respectively, ended up grouping isolates from both the AVSP and TVP packages separately. These grouped isolates had 100% (1/1 from TVP and 2/2 from AVSP), 100% (6/6 from AVSP and, 3/3 from TVP) and 100% (3/3 from TVP and 5/5 from AVSP) accuracy, respectively. The remaining isolates from the AVSP and TVP did not form separate clusters.

Discussion

This study was primarily aimed at characterizing the LAB populations isolated from two types of beef samples, which were derived from either AVSP-packaged or TVP-packaged meat. Contrary to the results obtained in the present work, it has previously been reported that meats packaged using an AVSP system display lower LAB counts compared to meats packaged using a TVP system (Barros-Velázquez et al., 2003). However, it should be noted that in this cited work, significant differences were only found after 40 days of packaging.

Identifications using partial 16S rRNA sequencing with a universal primer did not allow us to distinguish between closely related species. This was due to the fact that some strains identified as *C. divergens* by partial 16S rRNA sequencing were grouped in the same cluster as *C. maltaromaticum* after RAPD-PCR and cluster analysis.

Based on the identification results, it was possible to show that according to the results previously reported by other authors (Chenoll, Macian, Elizquivel, & Aznar, 2006; Jones, 2004; Yost & Natress, 2002), populations from the genera *Enterococcus, Lactobacillus, Leuconostoc*, and *Carnobacterium* characterized the ecology of both types of packaged meats. In agreement with previous studies, *Enterococcus* was the dominant genus in meats packaged using either the TVP or AVSP systems. This finding emphasizes that the four genera isolated in this work are the most well-adapted bacteria to a vacuum-packaged environment (Björkroth, Minna, Peter, & Hannu, 2005; Pennacchia, Ercolini, & Villani, 2011).

E. gilvus was the most dominant species (22 isolates). To the best of our knowledge, this is the first study to report the presence of this species in beef. This species was first described after isolation from human clinical specimens (Tyrrell et al., 2002) and was recently isolated from cheese (Zago, Bonvini, Carminati, & Giraffa, 2009) and poultry meat (Koort, Coenye, Vandamme, Sukura, & Bjork-roth, 2004), as well as from the hands of workers and traditional Spanish fermented pork sausages (Martín, Corominas, Garriga, & Aymerich, 2009). However, our results are not in agreement with the majority of the scientific literature related to this topic, which indicate *E. faecalis* and *E. faecium* as the primary enterococcal species that are most frequently isolated from meats as well as other foods (Björkroth et al., 2005, Gomes et al., 2008). Taking into account that *E. gilvus* is typically isolated from humans (Tyrrell et al., 2002; Martín et al., 2009), and the fact that meat packaged using both TVP and AVSP were handled by the same factory personnel, it is reasonable to assume that the high presence of this enterococcal species was related to contamination during meat processing. After screening the phylogenetic tree, the farthest distance between the enterococcal species was found from *E. gilvus*, to *E. faecalis* or *E. faecium* groups. This fact completely excludes any potential misidentification of *E. gilvus* with respect to the *E. faecalis* or *E. faecium* species.

A number of molecular techniques can be used for DNA fingerprinting and the characterization of LAB. RAPD-PCR is the most widely used, especially due to the rapidity and reliability of this method (Ehrmann & Vogel, 2005; Riboldi, de Mattos, Guedes Frazzon, d'Azevedo, & Frazzon, 2008). In this study, the M13 primer was used to classify LAB, which is in agreement with results reported by other authors that have successfully used a single primer and RAPD analyses to classify and type LAB (Bonomo, Ricciardi, Zotta, Parente, & Salzano, 2008; Kostinek et al., 2005; Urso et al., 2006). LAB strains with identical RAPD-PCR profiles were found for *E. faecium*, *E. gilvus*, *C. divergens*, *L. mesenteroides*, and *L. sakei*.

The *E. gilvus* species had a very good adaptive profile for both of the employed packaging systems. This adaptivity is reflected by the fact that it has showed the highest genetic variability of any of the identified species and by the fact that isolates belonging to this species exhibited an identical RAPD-PCR profile in meats processed using the two packaging systems. This finding seems to suggest that *E. gilvus* was a frequent species among the LAB population of the meat before its packaging and that this species showed a remarkable ability to survive in the meat packaged using both methods during the refrigerated storage.

Previous works (Barros-Velazquez et al., 2003; Vázquez et al., 2004) have reported that beef packaged using the AVSP system has a longer shelf life compared to meat packaged using the TVP system. The LAB identification results from this study did not indicate a high discrepancy between the LAB populations selected by the different packaging methods. Only in the case of *L. mesenteroides* was there a higher proportion of LAB isolated from meat packaged using the TVP system. This finding may suggest that this species has an important role in meat spoilage with regard to this packaging system. It should be pointed out that Pennacchia et al. (2011) reported that among the LAB implicated in the spoilage of vacuum-packaged meats, *Leuconostoc* spp. have a special involvement.

With the exception of *E. gilvus*, *C. divergens* and *L. mesenteroides*, none of the other isolates that belong to the same species showed identical RAPD-PCR profiles for both packaging methods. However, identical profiles for the same packaging system on different samples were found for a few of *E. gilvus* strains. These results indicate that depending on the packaging method, the results do not clearly specify the selection for certain LAB strains. From our point of view, it is necessary to closely investigate the specific microbial characteristics of interest in food technology with regard to the LAB present in this class of products. This investigation would supply new information on the role of LAB selected by the AVSP packaging system in the preservation of meat.

Supplementary material

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