Brazilian Journal of Microbiology (2007) 38:547-552
ISSN 1517-8382

PHENOTYPIC CHARACTERIZATION AND SPECIES-SPECIFIC PCR OF PROMISING STARTER CULTURE STRAINS OF *LACTOBACILLUS PLANTARUM* ISOLATED FROM NATURALLY FERMENTED SAUSAGES

Maristela Cortez Sawitzki[1,4]; Ângela Maria Fiorentini[1,2]; Fábio Cristiano Angonesi Brod[1,2]; Caroline Tagliari[1]; Teresinha Marisa Bertol[3]; Ana Carolina Maisonnave Arisi[1,*]; Ernani Sebastião Sant'Anna[1]

[1]Departamento de Ciência e Tecnologia de Alimentos, Centro de Ciências Agrárias, Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil; [2]Universidade Regional do Noroeste do Estado do Rio Grande do Sul, Departamento de Biologia e Química, Santa Rosa, RS, Brasil; [3]EMBRAPA SUÍNOS e AVES, Concórdia, SC, Brasil; [4]Universidade Estadual do Rio Grande do Sul, Ibirubá, RS, Brasil

Submitted: October 04, 2006; Returned to authors for corrections: March 05, 2007; Approved: July 18, 2007.

ABSTRACT

The purpose of the present work was to characterize promising starter culture strains of *Lactobacillus plantarum* isolated from naturally fermented artisanal sausage manufactured in the northwestern region of Rio Grande do Sul state, Brazil. From 127 isolates of homofermentative, Gram-positive and catalase-negative lactic acid bacteria, ten isolates were randomly selected and the phenotypic characterization and species-specific PCR were performed. Genomic DNA from each isolated strain and from the reference strains *L. plantarum* ATCC 8014 and *L. pentosus* ATCC 8041 were amplified using two pairs of *L. plantarum* species-specific primers (16/Lpl and LbP11/LbP12). The results of the phenotypic characterization and species-specific PCR indicated that five out of ten isolates were *Lactobacillus plantarum.*

Key-words: fermented sausage; *L. plantarum*; phenotypic characterization; PCR

INTRODUCTION

Modern food biotechnology has moved a long way since ancient time of empirical food fermentations. The addition of desirable microorganisms (starter culture) to meat products attend different purposes such as: to improve safety (inactivation of pathogens), to improve stability (extension of shelf life by inhibiting undesirable changes brought about by spoilage microorganisms or abiotic reactions), to provide diversity (modification of the raw material to obtain new sensory properties) and to provide health benefits through positive effects on the intestinal microbiota (14). A starter culture can be defined as a microbial preparation containing a large numbers of cells of at least one type of microorganism to be added to a raw material to produce a fermented product by accelerating and steering its fermentation process (13).

According to Drosinos *et al.* (9), generally, starter cultures consist of lactic acid bacteria, Gram-positive catalase-positive cocci, yeasts and moulds, depending on the sausage type. Lactic acid bacteria possess the main role in this microbial consortium; they affect both the technological properties and the microbial stability of the final product through the production of lactic and acetic acids and the consequent pH decrease.

A further requirement of a starter or protective culture is that it must be able to contribute to the desirable changes in the meat products. These desirable changes should be brought about better by the starter culture than by the biota present on the meat. The reduction of the pH by the lactic acid secreted by lactic acid bacteria is one example of these desirable changes (11). The abilities of the lactic acid bacteria, in particular lactobacilli, to reduce the pH and to produce bacteriocins prevent the growth of pathogenic and spoilage microorganisms,

*Corresponding Author. UFSC - CCA - Dep. de Ciência e Tecnologia de Alimentos - Rod. Ademar Gonzaga, 1346 cep 88034-001 Florianópolis, SC - Brasil. Tel.: (48) 3331-5382. E-mail: arisi@cca.ufsc.br

improving the safety and conservation of meat products (2,6,8,10,15,16,22).

Among lactobacilli, *Lactobacillus curvatus, L. sakei* and *L. plantarum* are the species most widely isolated from naturally fermented meat products (1-3,9,17,19,25). However, meat fermentation by the natural lactic acid microbiota can sometimes fail, resulting in products of poor quality; for this reason, the addition of starter cultures has been recommended and has become common in the manufacture of several types of fermented sausages. In the production of traditional fermented sausages, it is important to use starter cultures consisting of lactobacilli isolated from local products which are well adapted to the particular product and to the specific production technology and which contribute to the generation of the traditional flavor of the product (4).

The aim of the present work was to characterize *Lactobacillus plantarum* isolated from naturally fermented artisanal sausage produced in the northwestern region of the Rio Grande do Sul state, Brazil, as a first stage in order to investigate the technological properties (as starter culture) of lactic acid bacteria isolated from local products.

MATERIAL AND METHODS

Bacterial strains and growth conditions

Aiming to select few promising starter cultures strains, only ten strains (codified: AJ2, AL2, R2, AF5, AD3, AN3, AM2, C5, AP3 and AB4) were randomly selected from 127 strains of lactic acid bacteria homofermentative, Gram-positive and catalase-negative. These strains were obtained of a total of 168 strains of lactic acid bacteria isolated from 42 samples of artisanal sausage, from the first 7 days of the fermentation, manufactured without the addition of starter culture, from 21 different origins (household scale), in the northwestern region of the Rio Grande do Sul state.

Reference strains of the closely related species *Lactobacillus plantarum* and *Lactobacillus pentosus* were acquired from the Collection André Tosello Foundation: *L. plantarum* ATCC 8014 and *L. pentosus* ATCC 8041. All strains were grown on MRS agar (Merck) plates incubated anaerobically at 37ºC for 48 h. They were sub-cultured twice (1% inoculum, 24 h, 37ºC) in 10 mL MRS broth and kept frozen at -50ºC in the presence of 20% glycerol.

Phenotypic characterization

The ten selected strains and the reference strains were tested for gas production from glucose (tested in MRS broth at 37ºC for 48 h) and catalase activity. Gram-staining and cell morphology was observed using an optical microscope (23).

Growth at different temperatures was observed on MRS agar plates after 3 days of incubation at 8ºC and 45ºC. Growth at different pH values was observed after 3 days of incubation at 37ºC on MRS agar plates adjusted with HCl (1M) to pH 3.9 and 9.6. Growth at different salt concentrations was observed after 3 days of incubation at 37ºC on MRS agar plates added with 7% and 10% NaCl.

For the fermentation test, swab of each culture of *Lactobacillus* isolated and each type strain grown on MRS agar plates (incubated anaerobically at 37ºC for 48 h) was suspended in API 50 CHL medium (API systems, BioMérieux). Using sterile pipette, homogenized suspension of the cells in the medium was distributed into each of the 50 wells on the 50 CH strips. All wells were overlaid with sterile paraffin oil (Merck) to affect anaerobiosis. Strips were moistened and covered as recommended by the manufacturer and incubated at 37ºC. Changes in color from violet were monitored daily for 5 days. The strips were read after the incubation time and each test was interpreted: positive (+) samples were denoted by a change in color to yellow; no change in color indicated negative (-) samples; and the samples that changed to another color were doubtful (?). The first strip was used as a control well. An esculin hydrolysis (revealed by a change to darker color or black) was represented by a positive sign while a negative sign represented no change.

DNA isolation

Reference strains (*L. plantarum* ATCC 8014 and *L. pentosus* ATCC 8041) and ten selected strains were submitted to PCR analysis. A 1.0 mL aliquot of each overnight cultures (grown in MRS broth at 37ºC) was centrifuged at 13,000x*g* for 2 min at room temperature to pellet cells. Bacterial DNA was isolated by Wizard® Genomic DNA Purification Kit (Promega) using mutanolysin (18) according to the 'Isolation of Genomic DNA from Gram Positive and Gram Negative Bacteria Protocol' provided by the manufacturer.

Primers and PCR conditions

Genomic DNA of each strain isolates and of each reference strains *L. plantarum* ATCC 8014 and *L. pentosus* ATCC 8041 were submitted to PCR using *L. plantarum* and *L. pentosus* species-specific primers.

Two primer pairs (Invitrogen) were used to identify *L. plantarum*. The PCR specific identification of *L. plantarum* was performed using the primers LbP11 (5'AATTGAGGCA GCTGGCCA3') and LbP12 (5'GATTACGGGAGTCCAAGC3') according to Quere *et al.* (18) or the primers 16 (5'GCTGGATC ACCTCCTTTC3') and Lpl (5'ATGAGGTATTCAACTTATG3') according to Berthier and Ehrlich (5). The PCR specific identification of *L. pentosus* was performed using the primer pair 16 (5'GCTGGATCACCTCCTTTC3') and Lpe (5'GTATTC AACTTATTAGAACG3') according to Berthier and Ehrlich (5).

Twenty-five µL of reaction mixture containing 80 ng of bacterial DNA, 0.5 µM of each primer and 10 µL of the Eppendorf Master Mix (2.5x) that corresponds to final concentration in the PCR reaction of 1.25 U Taq DNA

Polimerase, 50 mM KCl, 30 mM Tris-HCl, 1.5 mM Mg^{2+}, 0.1% Igepal-CA630 and 200 µM of each dNTP.

Amplifications were carried out in a Minicycler™ (MJ Research, Inc. Watertown, MA) with the following programs: for primers 16/Lpl and 16/Lpe, denaturation at 94ºC for 5 min followed by 30 cycles of 94ºC for 1 min, 53ºC for 45 s, 72ºC for 1 min; final extension at 72ºC for 5 min. For primers LbP11/LbP12, denaturation at 95ºC for 5 min followed by 45 cycles of 94ºC for 30 s, 54ºC for 1 min, and 72ºC for 1 min; final extension at 72ºC for 10 min.

Gel electrophoresis was carried out by applying 15 µL of PCR products to 2.5% agarose gel. Gels were run for 50 min at 80 V in TEB (tris-borate-EDTA) electrophoresis buffer. A DNA molecular weight marker 50 bp DNA Ladder (Promega) was used as standard. Gels were visualized by UV illumination after ethidium bromide staining.

RESULTS AND DISCUSSION

The identification of the *Lactobacillus* species that dominate the microbiota of fermented sausages is an important step in the development of new starter cultures for meat fermentation (4). In the present study among the 168 strains of lactic acid bacteria isolated, 127 strains (75.6%) were characterized as lactic acid bacteria homofermentative, Gram-positive and catalase-negative. Among the 127 strains, ten isolates were randomly selected and the phenotypic characterization and species-specific PCR were performed.

The homofermentative lactobacilli are suitable as starter cultures for sausage because they produce only lactic acid from the sugars available, while the heterofermentative lactobacilli produce acetic acid, ethanol and carbon dioxide in addition to lactic acid (11). The higher concentrations of acetic acid result in a pungent off-flavor and the formation of higher amounts of carbon dioxide leads to the development of cavities of different sizes (7). All ten random selected isolates were able to grow at pH 3.9 and 9.6 at 37ºC on MRS agar. Four isolated strains (AL2, AP3, AD3 and AM2) were able to grow on MRS agar, supplemented with 7% NaCl and one isolate (C5) was able to grow on MRS agar supplemented with 10% NaCl. All isolates were able to grow on MRS agar at 8ºC, but only three isolates (C5, AN3 and AM2) were able to grow at 45ºC (Table 1).

In meat fermentation the physiological activity of microorganisms brings about desirable changes which decisively determine the character of a product. The ability of strains to grow at pH 3.9 is a significant factor because the most important change brought by lactic acid bacteria in a ripened meat product is the lowering of the pH by the secretion of lactic acid (11). The decrease of pH in sausage causes various effects and interactions. The most important effects are coagulation of the meat proteins; all reactions necessary for color formation and improvement of the stability (7). Due to decreased water-binding capacity of the meat proteins, the acidification accelerates drying out, and thus shortens the processing time (12).

NaCl tolerance is another significant factor for choosing a strain as starter culture in dried fermented products (21). A strain able to grow in 6.5% NaCl might result from a selection of strain resistant to the high salt concentration during sausage processing. NaCl-sensitive strains, present at the beginning of the process, would stop growing when NaCl concentration becomes too high (1).

The interpretation of the fermentation profiles (Table 2) was facilitated by the use of the data base "API-WEB" (BioMérieux) in which the identification of an organism is accompanied by the following information: the percentage of identification (% id), that is, an estimate of how closely the profile corresponds to the stated taxon relative to all the other taxa in the data base.

The results of the phenotypic characterization suggests the *L. plantarum* reference strain and the strains AJ2, AD3, AN3 and AM2 as *L. plantarum* with % id ≥ 96.7; the strain AL2 as *L. plantarum* with 72% and as *L. pentosus* with 27.3%; the strains AF5, C5 and AP3 as *Pediococcus pentosaceus* with % id ≥ 96.5. For the R2 strains, a clear species or subspecies assignment was not possible because of the doubtful profile of sugar fermentation, evidenced by the % id for *L. plantarum* and *L. pentosus*. The strain AB4 presented doubtful phenotypic characterization as *L. pentosus* and *Lactococcus lactis* ssp *lactis.*

PCR analysis using species-specific primers for *L. plantarum* were carried out for the ten isolated strains. Using 16/Lpl primers, the *L. plantarum* type strain and seven isolated strains (AJ2, AL2, R2, AF5, AD3, AN3 and AM2) gave a PCR product of approximately 220 bp (Fig. 1), as reported by Berthier & Ehrlich (5). Using LbP11/LbP12 primers, the *L. plantarum* type strain and the same seven isolated strains gave a PCR product of 250 bp (Fig. 2), as reported by Quere *et al*. (18). Using species-specific primers for *L. pentosus* 16/Lpe, amplification was observed only for *L. pentosus* type strain, isolated strains did not give any amplification (data not shown). According to Berthier and Ehrlich (5), the 16/Lpl and 16/Lpe primers allow distinguishing the closely related species *L. plantarum* and *L. pentosus*. These primers are complementary to variable sequences in the 16S/23S DNA spacer regions of *L. plantarum* and *L. pentosus*.

A clear identification of species, especially within the genus *Lactobacillus*, may sometimes be difficult using phenotypic methods such as sugar fermentation patterns due to an increasing number of lactic acid bacteria species which vary on a small number of biochemical characteristics (18). Reenen and Dicks (20) conclude that similar reactions of fermentation of sugars are not enough for phylogenetic classification of *L. plantarum* and *L. pentosus*, because significant similarity exists between the two species in these reactions. Although phenotypic techniques such as the API 50CHL system are still being taken as powerful tools capable of discriminating among the species of *Lactobacillus*, the use of genetic methods for

Table 1. Biochemical and physiological characteristics of lactic acid bacteria isolates and reference strains.

Isolates	*L.plantarum* ATCC 8014	AJ2	AL2	AB4	R2	AP3	C5	AF5	AD3	AN3	AM2	*L.pentosus* ATCC 8041
CO_2 from glicose	-	-	-	-	-	-	-	-	-	-	-	-
Production H_2S	-	-	-	-	-	-	-	-	-	-	-	-
Grow at 7% NaCl	-	-	+	-	-	+	-	-	+	-	+	-
Grow at 10% NaCl	-	-	-	-	-	-	+	-	-	-	-	-
Grow at pH 3.9	+	+	+	+	+	+	+	+	+	+	+	+
Grow at pH 9.6	+	+	+	+	+	+	+	+	+	+	+	+
Grow at 8ºC	+	+	+	+	+	+	+	+	+	+	+	+
Grow at 45ºC	-	-	-	-	-	-	+	-	-	+	+	-
Sugar fermentation:												
Glycerol	-	-	+	?	+	?	?	-	-	?	-	-
Erythritol	-	-	-	-	-	-	-	-	-	-	-	-
D-Arabinose	-	-	-	-	-	-	-	?	-	-	-	-
L-Arabinose	?	+	+	+	+	+	+	+	-	+	+	+
Ribose	+	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	-	?	-	-	-	-	-	+	-	-	?	+
L-Xilose	-	-	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	?	-	-	-	-
β-Methyl-xylose	-	-	-	-	-	-	-	-	-	-	-	-
Galactose	+	+	+	+	+	+	+	+	+	+	+	+
D- Glucose	+	+	+	+	+	+	+	+	+	+	+	+
D-Frutose	+	+	+	+	+	+	+	+	+	+	+	+
D-Manose	+	+	+	-	+	+	+	+	+	+	+	+
L-Sorbose	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	?	?	?	-	?	-	-	?	?	-	?	-
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	-	+	?	?	-	+	-	+	+
Sorbitol	+	+	+	-	+	-	-	-	-	-	+	+
β-Methyl-D-mannoside	?	-	-	-	-	-	-	-	-	-	-	-
β-Methyl-D-glucoside	+	?	-	-	-	-	-	?	-	-	?	+
N Acetyl glucosamine	+	+	+	+	+	+	+	+	+	+	+	+
Amygdaline	+	+	+	?	+	+	+	+	+	+	+	+
Arbutine	+	+	+	+	+	+	+	+	+	+	+	+
Esculine	-	-	-	+	+	+	+	+	+	+	-	+
Salicine	+	+	+	+	+	+	+	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	?	+	?	?	?	+	+	+	+
Melibiose	+	+	+	+	+	+	+	+	+	+	+	+
Saccharose	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	?	+	-	+	+	+	+	+	+
Insuline	-	-	-	-	-	-	-	-	-	-	-	-
Melizitose	+	+	+	-	-	-	-	-	+	-	+	?
D - Raffinose	+	+	+	?	+	?	?	-	+	?	+	-
Amidon	-	-	-	?	-	-	-	-	?	-	-	-
Glycogene	-	-	-	-	-	-	?	-	?	-	-	-
Xylitol	-	-	-	-	-	-	-	-	-	-	-	-
β-Gentibiose	+	+	+	-	+	+	+	+	+	+	+	-

D - Turanose	-	+	+	-	+	-	-	-	+	-	+	-
D - Lyxose	-	-	-	-	-	-	-	-	-	-	-	-
D - Tagalose	-	+	-	?	+	+	?	+	+	?	+	+
D -Fucose	-	-	-	-	-	-	-	-	-	-	-	-
L - Fucose	-	?	-	-	-	-	-	?	-	-	?	-
D -Arabitol	-	?	?	-	-	-	-	-	-	-	?	-
L - Arabitol	-	-	-	-	-	-	-	-	-	-	-	-
Gluconate	-	-	+	-	-	-	-	-	?	-	-	?
2 ceto -gluconate	-	-	-	-	-	-	-	-	-	-	-	-
5 ceto - gluconate	-	-	-	-	-	-	-	-	-	-	-	-

- means negative result, + means positive result and ? means doubtful result as described in material and methods.

Table 2. Phenotypic and molecular characterization of lactic acid bacteria isolated from naturally fermented sausages and reference strains ATCC.

Strains	API 50 CHL		
	Percentage of identification	Phenotypic characterization	Molecular characterization
L. plantarum ATCC 8014	99.5	*L. plantarum*	*L. plantarum*
AJ2	96.7	*L. plantarum*	*L. plantarum*
AL2	72.027.3	*L. plantarum* *L. pentosus*	*L. plantarum*
AB4		*P. pentosaceus* *L. lactis ssp lactis*	Not characterized
R2	89.410.4	*L. pentosus* *L.plantarum*	*L. plantarum*
AP3	97.7	*P. pentosaceus*	Not characterized
C5	98.7	*P. pentosaceus*	Not characterized
AF5	96.5	*P. pentosaceus*	*L. plantarum*
AD3	98.7	*L. plantarum*	*L. plantarum*
AN3	98.7	*L. plantarum*	*L. plantarum*
AM2	96.7	*L. plantarum*	*L. plantarum*
L. pentosus ATCC 8041	90.2	*L. pentosus*	*L. pentosus*

Lactobacillus taxonomy has become the backbone for a reliable identification (24).

The phenotypic characterization corroborates with species-specific PCR for the *L. pentosus* ATCC 8041 and the *L. plantarum* ATCC 8014 reference strains and for the isolated strains AJ2, AL2, AD3, AN3 and AM2, but not for the strains AF5 and R2 (Table 2). Further analysis is necessary to identify AF5 and R2 strains. Thus, the five strains AJ2, AL2, AD3, AN3 and AM2 isolated from naturally fermented artisanal sausage and identified as *L. plantarum* could be used as starter cultures, AL2, AD3 and AM2 are the most promising ones because they are salt tolerant. 16S rDNA sequencing will be applied to confirm the identification of AJ2, AL2, AD3, AN3 and AM2 strains.

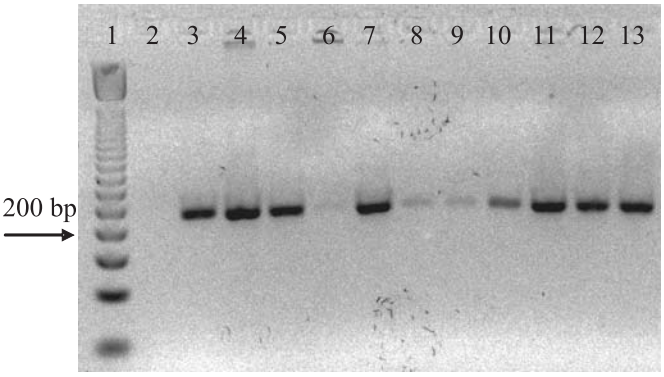

Figure 1. PCR products obtained from lactic acid bacteria isolates using 16/Lpl primers (2.5% agarose gel). Lane 1: 50 bp ladder (Promega); lane 2: negative control (water); lane 3: *L. plantarum* ATCC 8014; lane 4: AJ2; lane 5: AL2; lane 6: AB4; lane 7: R2; lane 8: AP3; lane 9: C5; lane 10: AF5; lane 11: AD3; lane 12: AN3 and 13: AM2.

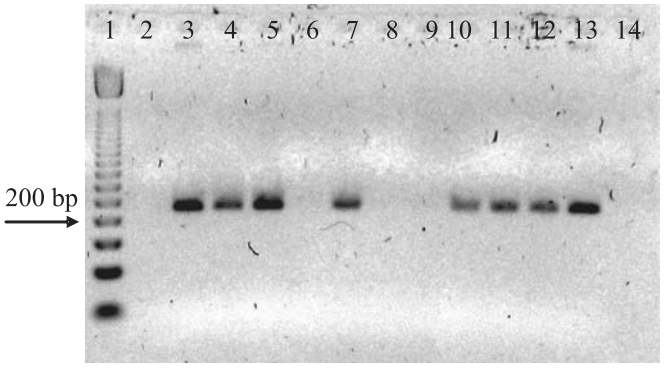

Figure 2. PCR products obtained from lactic acid bacteria isolates using Lbp11/Lbp12 primers (2.5% agarose gel). Lane 1: 50 bp ladder (Promega); lane 2: negative control (water); lane 3: *L. plantarum* ATCC 8014; lane 4: AJ2; lane 5: AL2; lane 6: AB4; lane 7: R2; lane 8: AP3; lane 9: C5; lane 10: AF5; lane 11: AD3; lane 12: AN3; lane 13: AM2; lane 14: *L. pentosus* ATCC 8041.

ACKNOWLEDGEMENTS

This work was supported by EMBRAPA, UNIJUÍ and UFSC, Brazil. CT was supported by IC fellowship from CNPq, Brazil.

RESUMO

Caracterização fenotípica e por PCR espécie-específica de cepas promissoras como cultivos iniciadores de *Lactobacillus plantarum* isolados de embutidos cárneos fermentados naturalmente

O objetivo do presente trabalho foi caracterizar cepas promissoras como cultivos iniciadores de *Lactobacillus plantarum* isoladas de embutidos cárneos fermentados naturalmente produzidos na região noroeste do Rio Grande do Sul, Brasil. Das 127 bactérias ácido láctica homofermentativas, Gram-positivo e catalase-negativo isoladas, dez foram aleatoriamente selecionadas e a caracterização fenotípica e a PCR espécie-específica foram realizadas. DNA genômico das cepas isoladas e das cepas de referência *L. plantarum* ATCC 8014 e *L. pentosus* ATCC 8041 foram amplificadas utilizando-se dois pares de iniciadores espécie-específicos para *L. plantarum* (16/Lpl e LbP11/LbP12). Os resultados da caracterização fenotípica e da PCR espécie-específica permitiram a identificação como *Lactobacillus plantarum* de cinco cepas das dez selecionadas.

Palavras-chave: embutido fermentado, *L. plantarum*, caracterização fenotípica, PCR

REFERENCES

1. Ammor, S.; Rachman, C.; Chaillou, S.; Prévost, H.; Dousset, X.; Zagorec, M.; Dufour, E.; Chevallier, I. (2005a). Phenotypic and genotypic identification of lactic acid bacteria isolated from a small-scale facility producing traditional dry sausages. *Food Microbiol.*, 22: 373-382.
2. Ammor, S.; Dufour, E.; Zagorec, M.; Chaillou, S.; Chevallier, I. (2005b). Characterization and selection of *Lactobacillus sakei* strains isolated from traditional dry sausages for their potential use as starter culture. *Food Microbiol.*, 22: 529-538.
3. 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.
4. Andriguetto, C.; Zampese, L.; Lombardi, A. (2001). RAPD-PCR characterization of lactobacilli isolated from artisanal meats plants and traditional fermented sausages of Veneto region (Italy). *Lett. Appl. Microbiol.*, 33: 26-30.
5. Berthier, F.; Ehrlich, S.D. (1998). Rapid species identification within two groups of closely related lactobacilli using PCR primers that target the 16S/23S rRNA spacer region. *FEMS Microbiol. Lett.*, 161: 97-106.
6. Bromberg, R.; Moreno, I.; Zaganini, C.L.; Delboni, R.R.; Oliveira, J. (2004). Isolation of bacteriocin-producing lactic acid bacteria from meat and meat products and its spectrum of inhibitory activity. *Brazil. J. Microbiol.*, 35: 137-144.
7. Buckenhüskes, H.J. (1993). Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. *FEMS Microbiol. Rev.*, 12: 253-272.
8. Dejenane, D.; Martínez, L.; Blanco, D.; Yangüela, J.; Beltrán, J.A.; Roncalés, P. Effect of lactic acid bacteria on extension of shelf life and growth of *Listeria monocytogenes* in beef steaks stored in CO_2-rich atmosphere. *Braz. J. Microbiol.*, 36: 405-412, 2005.
9. Drosinos, E.H.; 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.
10. Fiorentini, A.M.; Sant'Anna, E.S.; Porto, A.C.S.; Mazo, J.Z.; Franco, B.D.G.M. (2001). Influence of bacteriocins produced by *Lactobacillus plantarum* BN in the shelf-life of refrigerated bovine meat. *Braz. J. Microbiol.*, 32: 42-46.
11. Geisen, R.; Lücke, F.K.; Kröckel, L. (1992). Starter and protective cultures for meat and meat products. *Fleischwirtsch*, 72: 894-898.
12. Jessen, B. (1995). Starter cultures for meat fermentation. In: Campbell – Platt; Cook, P.E. *Fermented meats*. London: Blackie Academe Professional, 130-159.
13. Leroy, F.; Vuyst, L.D. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci. Technol.*, 15: 67-78.
14. Lücke, F.-K. (2000). Utilization of microbes to process and preserve meat. *Meat Sci.*, 56: 105-115.
15. Lücke, F.-K. (1985). Mikrobiologische Vorgänge bei der Herstellung von Rohwurst und Rohschinken. *Band 5 der Kulmbacher Reihe*, S, 85-102.
16. Maciel, J.F.; Teixeira, M.A.; Moraes, C.A.; Gomide, L.A.M. (2003). Antibacterial activity of lactic cultures isolated of Italian salami. *Braz. J. Microbiol.*, 34 (suppl.1): 121-122.
17. Parente, E.; Griego, S.; Crudele, M.A. (2001). Phenotypic diversity of lactic acid bacteria isolated from fermented sausages produced in Basilicata (Southern Italy). *J. Appl. Microbiol.*, 90: 943-952.
18. Quere, F.; Deschamps, A.; Urdaci, M.C. (1997). DNA probe and PCR –specific reaction for *Lactobacillus plantarum*. *J. Appl. Microbiol.*, 82: 783-790.
19. Rantsiou, K.; Drosinos, E.H.; Gialitaki, M.; Urso, R.; Krommer, J.; Gasparik-Reichardt, J.; Toth, S.; Metaxopoulos, I.; Comi, G.; Cocolin, L. (2005). Molecular characterization of *Lactobacillus* species isolated from naturally fermented sausages produced in Greece, Hungary and Italy. *Food Microbiol.*, 22: 19-28.
20. Reenen, C.A.V.; Dicks, L.M.T. (1996). Evaluation of numerical analysis of Random Amplified Polymorphic DNA (RAPD)-PCR as a method to differentiate *Lactobacillus plantarum* and *Lactobacillus pentosus*. *Curr. Microbiol.*, 32: 183-187.
21. Rovira, A.; Nieto, J.C.; Rodriguez, A.; Reguera, J.I.; Gonzáles, Z. (1997). Characterization and selection of lactobacilli isolated from Spanish fermented sausages. *Microbiol.*, 13: 201-208.
22. Samelis, J.; Stavropoulos, S.; Kakouri, A.; Metaxopoulos, J. (1994). Quantification and characterization of microbial populations associated with naturally fermented Greek dry salami. *Food Microbiol.*, 11: 447-460.
23. Schillinger, U.; Lücke, F.K. (1987). Identification of lactobacilli from meat products. *Food Mcrobiol.*, 4: 199-208.
24. Vandamme, P.; Pot, B.; Gillis, M.; DeVos, P.; Kersters, K.; Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol. Rev.*, 60: 407-438.
25. Vogel, R.F.; Lohmann, M.; Nguyen, M.; Weller, A.N.; Hammes, W.P. (1993). Molecular characterization of *Lactobacillus curvatus* and *Lactobacillus sake* isolated from sauerkraut and their application in sausage fermentations. *J. Appl. Bacteriol.*, 74: 295-300.