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Molecular and phenotypic characterization of *Lactobacillus curvatus* isolated from handmade Brazilian salami

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17 *Lactobacillus curvatus* and one *Lactobacillus brevis* isolate were obtained from salami. The isolates were previously selected according to the desirable characteristics regarding technological criteria. Identification was based primarily on the biochemical carbohydrate assimilation profile, and later by amplified 16S rDNA restriction analysis (ARDRA). Due to inconsistencies in the results found by the identification methods employed, the 16S rRNA gene was partially sequenced and the result confirmed identity of *L. curvatus*, as predicted by ARDRA, which shows that this molecular technique is efficient in identifying the species. All isolates of *L. curvatus* were characterized by the molecular technique of random amplified polymorphic DNA (RAPD). In order to analyze the relationships existing between the isolates, taxonomic dendrograms were obtained based on molecular profile, what was suitable for the typing. The molecular techniques are efficient in the identification of *Lactobacillus* sp. isolates, which can be complementary or even substitute biochemical techniques. Thus, these molecular methods offer a fast and economic way for the identification and characterization of lactic acid bacterial with a potential use as starter cultures in the elaboration of handmade fermented products.

Key words: 16S amplified ribosomal DNA restriction analysis (ARDRA), random amplified polymorphic DNA (RAPD), lactobacilli, microbial genetics.

INTRODUCTION

Fermented meat products are evaluable on sensory, chemical and microbiological properties. The primary function of lactic acid bacteria (LAB) in meat fermentation is the rapid production of lactic acid which leads to growth inhibition of pathogenic microorganisms, increasing shelf life and favoring the development of a unique flavor (Rantsiou et al., 2006; Samelis et al., 1994). These safety and quality parameters give additional advantages to the

use of LAB in food processing (Leroy et al., 2006; Harris et al., 1989).

Among the technological criteria considered as desirable characteristics for microorganisms used as culture starters, are the vigorous growth in concentrations of sodium chloride, growth at high temperatures, antimicrobial activity against pathogens, absence of pathogenic activity, and usually the microorganisms that meet these criteria are the LAB, specially the lactobacilli (Cocolin and Rantsiou, 2007; Leroy et al., 2006; Työppönen et al., 2003; Samelis et al., 1994).

Another critical step in obtaining a new starter culture is the isolation and identification of the microorganisms

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presents in spontaneous fermentation of fermented product (Rantsiou et al., 2006), which is important to identify the microorganisms as it is desired to track the presence of microorganisms during fermentation (Tsai et al., 2010).

The microorganism identification by biochemical, chemotaxonomic and other related methods is not sufficient for classification (Giraffa et al., 2008; Najjari et al., 2008), therefore, molecular techniques are utilized to assist in the identification of microorganisms (Soto et al., 2010).

The sequencing and comparison of sequences of the 16S rDNA gene have been used for species identification (Balcázar et al., 2007; Olsen and Woese, 1993). It is a very reliable method for sorting and identifying bacterial species, which, by means of alignments, is able to predict results with a high degree of precision and accuracy (Cilia et al., 1996), despite some disadvantages for other molecular methods and limitation (Mohania et al., 2008).

Another possible analysis for 16S rRNA genes is the amplified ribosomal DNA restriction analysis (ARDRA) (Giraffa et al., 1998). This highly discriminatory technique has been shown by several studies to be suitable for the discrimination of different genera of LAB such as *Enterococcus*, *Pediococcus*, *Lactococcus*, *Lactobacillus* and *Bifidobacterium* (Soto et al., 2010; Collado and Hernández, 2007) and of *Lactobacillus* species including *Lactobacillus plantarum*, *Lactobacillus sakei*, *Lactobacillus lactis*, *Lactobacillus curvatus*, *Lactobacillus salivarius*, *Lactobacillus delbrueckii* and others (Soto et al., 2010; Najjari et al., 2008; Miteva et al., 2001).

Moreover, some molecular techniques can be used for the characterization and differentiation of isolates of the same species of *Lactobacillus*. Among the most important molecular strategies available are PCR amplification with specific primers as denaturing gradient gel electrophoresis (DGGE), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and qRT-PCR (Mohania et al., 2008; Rodas et al., 2005). PCR-based methods, particularly the random amplified polymorphic DNA (RAPD), are suited for the low cost, rapid and efficient intraspecific analysis of *Lactobacillus* strains from foodstuffs (Berthier't and Ehrlich, 1999; Mahenthalingam et al., 2009). The aim of this study was to obtain new isolates of lactic acids bacteria from naturally fermented meats, selected for having potential use as starter culture, and to determine the taxonomic and molecular profile of *L. curvatus* using ARDRA and RAPD methods.

MATERIALS AND METHODS

Strains, culture conditions and phenotypic identification

The isolates of lactic acid bacteria selected for this study were collected from 20 small-scale facilities producing naturally fermented meat product (salami) without the addition of starter cultures, obtained according to Samelis et al. (1994). The

microorganisms were incubated at 30°C/48 h under microaerophilic conditions. Cells from colonies growing on MRS agar (Sigma-Aldrich) were Gram-stained and tested for catalase activity. Gram-positive and catalase-negative rod-shaped organisms were evaluated for their ability to inhibit one or more of the foodborne bacteria: *Listeria monocytogenes* Scott A, *Salmonella enterica* subsp. *enterica* serovar *enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus*, and two closely related LAB: *Lactobacillus bulgaricus* and *Leuconostoc mesenteroides*. Samples of *Lactobacillus* spp. used were: *Lactobacillus sakei* ATCC 15521; *Lactobacillus plantarum* ATCC 8014; *Lactobacillus casei* ATCC 393; *Lactobacillus acidophilus* ATCC 4356; *Lactobacillus delbrueckii* subsp. *lactis* ATCC 7830; *Lactobacillus brevis* ATCC 367; *Lactobacillus fermentum* ATCC 9338, *Lactobacillus lindneri* and *Lactobacillus curvatus* L442.

Technological characteristics

Selected isolates were evaluated for some desirable characteristics related to technological criteria: gas production from glucose, tolerance to temperature extremes (temperature abuse) and salt tolerance (Työppönen et al., 2003; Ying et al., 2010). After tests, the viability of each culture was assessed based on the resulting colonies obtained on MRS agar and quantified by the number of such colonies forming units (CFU.g⁻¹).

Antimicrobial activity and biochemical identification

After culture on MRS broth, each bacteria was removed by centrifugation for 10 min at 7000 g. The pH supernatant was adjusted to 6.0 and checked for antimicrobial activity by agar well diffusion assay (Harris et al., 1989). The wells punched on soft agar seeded with 1% of an overnight grown indicator strain were filled with 40 µl of culture supernatant. Positive control: 40 µl of nisaplin (Danisco, UK) was diluted in HCl 0.02 N for a final concentration of 100 IU (International Units). The plates were incubated overnight at optimal temperature for each indicator strain and after incubation, the diameters of the inhibitory zones were measured. The isolates of *Lactobacillus* spp. with technological characteristics desirable and with inhibitory activity to at least one of the indicator organisms (*L. monocytogenes*, *S. enteritidis*, *S. typhimurium* and *S. aureus*), 18 altogether, were identified based on carbohydrate fermentation patterns obtained with API 50 CH (bioMérieux, France). All strains were sub-cultured on MRS broth and kept frozen at -20°C in MRS broth supplemented with 10% glycerol until use.

Identification by amplified 16S rDNA restriction analysis (ARDRA)

The isolates selected with desirable performance were also identified by ARDRA technique. DNA extraction was performed using extraction kit Whatman FTA® Classic Card (USA) as previously described (Bextine et al., 2004). Amplification of the 16S rRNA gene (*rrs* gene) was performed using a 50 µl mix (10X PCR buffer with MgCl₂ 1.5 mM, 0.25 mM dNTP, 2.5 U of Taq polymerase (Invitrogen), 50 pmol of each primer and DNA template) and the universal primers: FD1 (5'-AGAGTTTGATCCTGGGTCAG-3') and RP2 (5'-GGCTACCTTGTACGACTT-3') (Weisburg et al., 1991). The samples were subjected to the following program: 35 cycles of 94°C (1 min), 50°C (1 min) and 72°C (1 min and 30 s) with PCR thermal cycler HBSP02110 (Thermo Electron Corp.).

14 µl of the amplification product of each *Lactobacillus* isolate and standard *Lactobacillus* species were digested with restriction endonucleases. 10 units of Alu I, Hha I and Hinf I restriction enzymes (Invitrogen) were added to each reaction. This mixture

was incubated at 37°C for 2 h. The restriction fragments were separated by electrophoresis on 3% agarose gel with 0.5 µg/ml of ethidium bromide in the electrophoresis tank, using 0.5 X TBE buffer at 2 Vcm⁻¹ for 4 h. A DNA molecular weight marker, 100-bp DNA ladder (Ludwig Biotec), was used as standard. The gel was observed under UV light and a digital image was captured (Photo Capt Software version 12.5 for Windows - Vilber Lourmat) for analysis.

Analysis by random amplified polymorphic DNA (RAPD)

The RAPD profiles of selected isolates of *L. curvatus* were determined. The PCR mixture contained 2 mM MgCl₂, 0.25 mM dNTP, 2.5 U of *Taq* polymerase (Invitrogen), PCR buffer (Invitrogen), 50 pmol of primer and DNA template. Five different primers were tested: P1254 (CCGCAGCCAA), OPB-17 (AGGGAACGAG), 23L (CCGAAGCTGC), OPA-4 (AATCGGGCTG) and OPB-15 (CCAGGGTGTT). Amplification was performed as previously described (Betancor et al., 2004) using the following program (with some modifications): 4 cycles of 94°C for 4 min, 37°C for 4 min and 72°C for 4 min; then 35 cycles of 94°C for 30 s, 37°C for 1 min, and 72°C for 2 min, followed by a final 10 min at 72°C. The PCR products (15 µl of each sample) were loaded on 1.5% agarose gel and electrophoresed for 2 h at 3 Vcm⁻¹. The digital image was captured as described previously. To confirm the reproducibility of the method, each strain was assayed three times.

Phylogenetic data analysis

The dendrograms were constructed using Jaccard's similarity coefficient from the similarity matrix based on the binary code of the molecular profile data by unweighted pair group method analysis (UPGMA) for bootstrapping. Each phenogram was reconstructed 2000 times by repeated sampling with replacement using computer program WinBoot (Yap and Nelson, 1996).

DNA sequencing

The amplification products of the 16S rRNA gene were purified with the PCR Clean-Up Gel Extraction® (Macherey-Nagel/MN) kit and the amplicon was partially sequenced by ACTGene Análises Moleculares LTDA (Ludwig Biotec/Brazil) with the Big Dye Terminator v3.1 Cycle sequencing kit (Applied Biosystems) on the ABI-PRISM 3100 Genetic Analyzer (Applied Biosystems). The sequences were compared with the sequences deposited in the GenBank database using the BLAST algorithm (Altschul et al., 1997). In this study, the sequences were deposited in the GenBank database using the BankIt (web-based data submission tool: <http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank>).

RESULTS

The microorganisms were selected according to technological criteria, with the lactobacilli isolation being emphasized. At the first screening, 120 lactic acid bacteria were obtained from naturally fermented salami. Aiming to work only with *Lactobacillus* species, 50 isolates that were presented as Gram positive, catalase negative bacilli, medium-sized to slightly elongated were selected. These were tested for their desirable technological characteristics. The heterofermentative character was exclusionary for this study, and only three

of the 50 isolates analyzed were found to be gas producers. Therefore, based on the products of glucose metabolism, 94% of the strains were found to be homofermentative.

Regarding tolerance to extreme temperature of the 50 isolates initially selected, 21 showed viability after growth test at 10, 20 and 45°C and great growth at extreme temperature (Table 1), showing desirable feature as starter cultures. Furthermore, for tolerance to salt, at the concentrations tested, only one isolate showed no growth at 4%, but especially for 8% salt concentration, 31 showed good growth (Table 1), and 15 isolate did not remain viable after the test.

The isolates selected were also characterized for antimicrobial activity, determining the potential production of substance like bacteriocin. By using well diffusion assay, we observed that 20 isolates presented inhibitory activity to at least one of the isolates selected in this study (Table 1).

Based on phenotypic characterization of the 50 LAB isolates initially tested, only 18 showed concomitant technological characteristics favorable for use in fermented products as starter culture, thus these 18 LAB isolates were biochemically and molecularly identified.

From the biochemical identification, according to the carbohydrate fermentation tests, interestingly, most LAB isolates were identified as *Lactobacillus curvatus* (85.71%, isolates L1, L3, L5, L7, L8, L9, L11, L14, L15, L17, L18, L21, L31, L32, L40 and L47), one was identified as *L. lindneri* (7.14%, isolate L2), and one was identified as *L. brevis* (7.14%, isolate L10).

To confirm the results, these isolates were also identified by the ARDRA method. The restriction profile of the amplified fragment of the 16S rRNA gene was obtained for the standard *Lactobacillus* species, using three enzymes recognizing 4 bp: Alu I, Hinf I and Hha I. The best results for species differentiation were obtained with Alu I restriction enzyme, which showed patterns more visible in agarose gel (Figure 1). A dendrogram was constructed by UPGMA (Figure 1) using a numerical analysis of the genotypic profiles.

The ARDRA technique of 18 isolates based on 16S rRNA gene patterns found with Alu I, Hinf I and Hha I, allowed us to identify the species of the lactobacilli. The results were similar to those achieved by biochemical methods, except those of the 2L strain, identified as *L. lindneri* by biochemical identification, and its ARDRA profile was similar to the other *L. curvatus* isolates (Figure 2) and standards of *L. curvatus*. To confirm the identification of the L2 strain and the other predicted *L. curvatus*- isolates L1, L3 and L5, the 16S rRNA genes was partly sequenced.

These sequences were aligned with those from GenBank using the BLAST algorithm and deposited in the GenBank database: 1L strain (accession number JN382073), L2 strain (accession number JN382074), L3 strain (accession number JN811554) and L5 strain

Table 1. Technological criteria for strains of lactobacilli isolated from fermented meat products.

Isol	Gas	Temp (10 to 45°C)	Salt (8%)	Inibit.	Isol	Gas	Temp (10 to 45°C)	Salt (8%)	Inibit
<u>L1</u>	-	+	+	+	L26	-	-	-	-
<u>L2</u>	-	+	+	+	L27	-	-	-	-
<u>L3</u>	-	+	+	+	L28	-	-	-	-
L4	-	-	+	+	L29	-	-	-	-
<u>L5</u>	-	+	+	+	L30	-	-	+	-
L6	-	-	-	-	<u>L31</u>	-	+	+	+
<u>L7</u>	-	+	+	+	<u>L32</u>	-	+	+	+
<u>L8</u>	-	+	+	+	L33	+	-	-	-
<u>L9</u>	-	+	+	+	L34	-	-	-	-
<u>L10</u>	-	+	+	+	L35	-	-	-	-
<u>L11</u>	-	+	+	+	L36	-	-	-	-
L12	-	-	+	+	L37	+	-	-	-
L13	-	+	-	-	L38	-	-	+	-
<u>L14</u>	-	+	+	+	L39	-	-	+	-
<u>L15</u>	-	+	+	+	<u>L40</u>	-	+	+	+
L16	-	-	-	-	L41	-	-	+	-
<u>L17</u>	-	+	+	+	L42	-	-	+	-
<u>L18</u>	-	+	+	+	L43	-	-	+	-
L19	-	+	-	-	L44	-	-	+	-
L20	-	+	-	-	L45	+	-	+	-
<u>L21</u>	-	+	+	+	L46	-	-	+	-
L22	-	-	-	-	<u>L47</u>	-	+	+	+
L23	-	-	-	-	L48	-	-	+	-
L24	-	-	-	-	L49	-	-	+	-
L25	-	-	-	-	L50	-	-	-	-

The isolates selected for presents desirable characteristics related to technological criteria are underlined. The signs (+) indicate the desired or undesirable (-) profile of isolates (*Isol*) for: gas production, tolerance to temperature extremes (Temp), tolerance to salt (Salt) and inhibitory activity of foodborne bacteria (Inibit).

(accession number JN811555). Partial sequencing of the 16S rRNA 5' gene of these isolates confirmed that they were *L. curvatus* as previously identified by restriction profile, which shows that the methodology employed is able to identify the corresponding species. Moreover, sequencing was able to differentiate *L. curvatus* even from the closely related *L. sakei* species.

Aiming to distinguish molecularly, each *L. curvatus* isolate and obtaining a typing on the basis of their DNA profiles, the RAPD method was used. The OPB 17 primer (Figure 3) in particular was more effective for the differentiation of 17 *L. curvatus* isolates from fermented meat products and generated DNA fragments ranging from approximately 2.0 to 0.1 kbp, providing the molecular profile from which a dendrogram was constructed by UPGMA with numerical analysis of the profiles to group the isolates by similarity. RAPD (with OPB 17 primer) provided evidence of a clear molecular diversity between the *L. curvatus* isolated in this study and presents an effective way to distinguish these

isolates or monitor the presence of each one when used as culture starters.

DISCUSSION

Lactic acid bacteria have a great potential for use as probiotic (Gusils et al., 1999) and food biopreservative (Carvalho et al., 2006). The use of these microorganisms as starter cultures in the elaboration of various fermented products would increase the safety of the consumption of these products with the ability to produce antagonistic substances for many pathogenic microorganisms (Kim et al., 2008; De Martinis et al., 2002).

In this study, 18 selected isolates of LAB (17 *L. curvatus* and 1 *L. brevis*) showed desirable technological characteristics. Among these characteristics for microorganisms used as culture starters are the vigorous growth in concentrations of sodium chloride (up to 6%), growth at temperatures of 20 to 43°C, antimicrobial

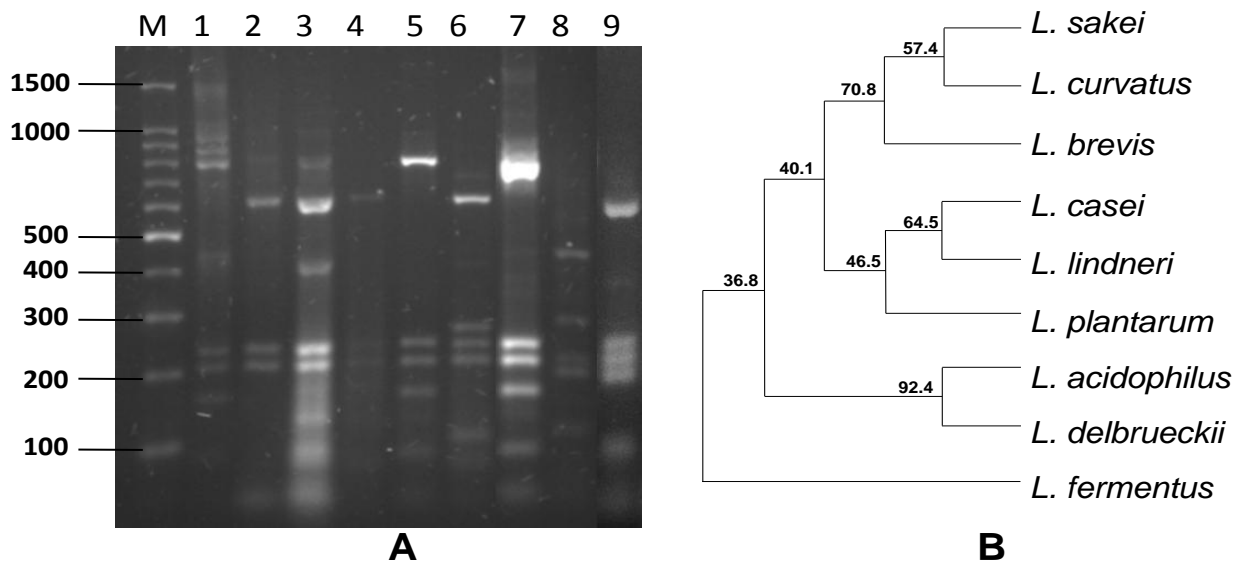


Figure 1. 16S-ARDRA molecular profile of *Lactobacillus* patterns. (A) Electrophoretic analysis of Alu I restriction profile of amplified fragment of 16S rRNA gene (ARDRA) of standard species, where: M is the marker of molecular weight 100 bp DNA ladder and lines 1 to 9, respectively, are *L. sakei*, *L. casei*, *L. acidophilus*, *L. delbrueckii*, *L. brevis*, *L. plantarum*, *L. curvatus*, *L. fermentus* and *L. lindneri* isolates. (B) Dendrogram constructed by UPGMA using Jaccard's coefficient based on ARDRA patterns of Panel A. The values in the groups indicate the percentage recorded for a particular branch based on 2,000 bootstrap replications using Winboot software.

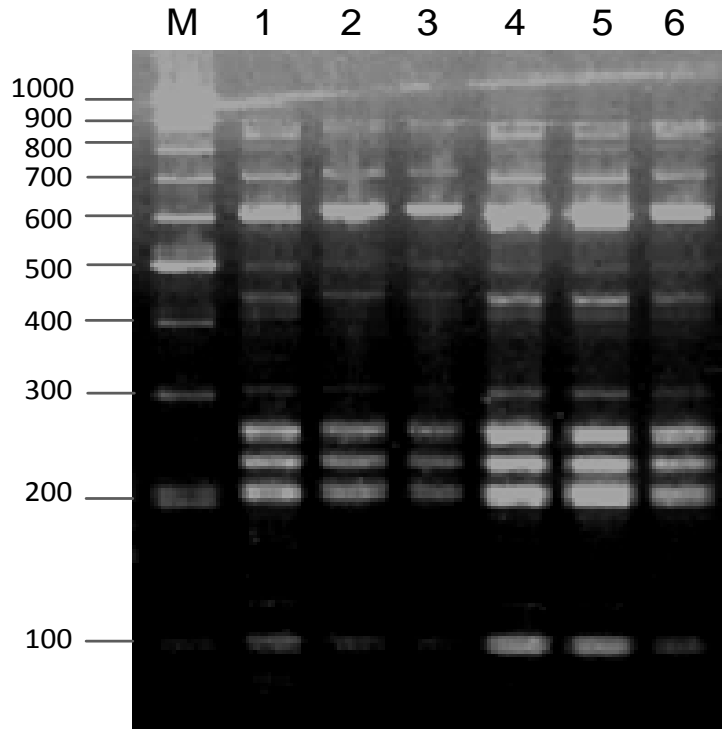


Figure 2. Identification by ARDRA profile of salami *Lactobacillus* spp. isolates. Electrophoresis in 3% agarose gel of amplified fragment of 16S rRNA gene digested with Alu I restriction enzymes, where: M is the marker of molecular weight 100 bp DNA ladder and the lines 1 to 6, respectively, are the restriction profile of *Lactobacillus* isolates 1L, 2L, 3L, 5L, 7L and 8L.

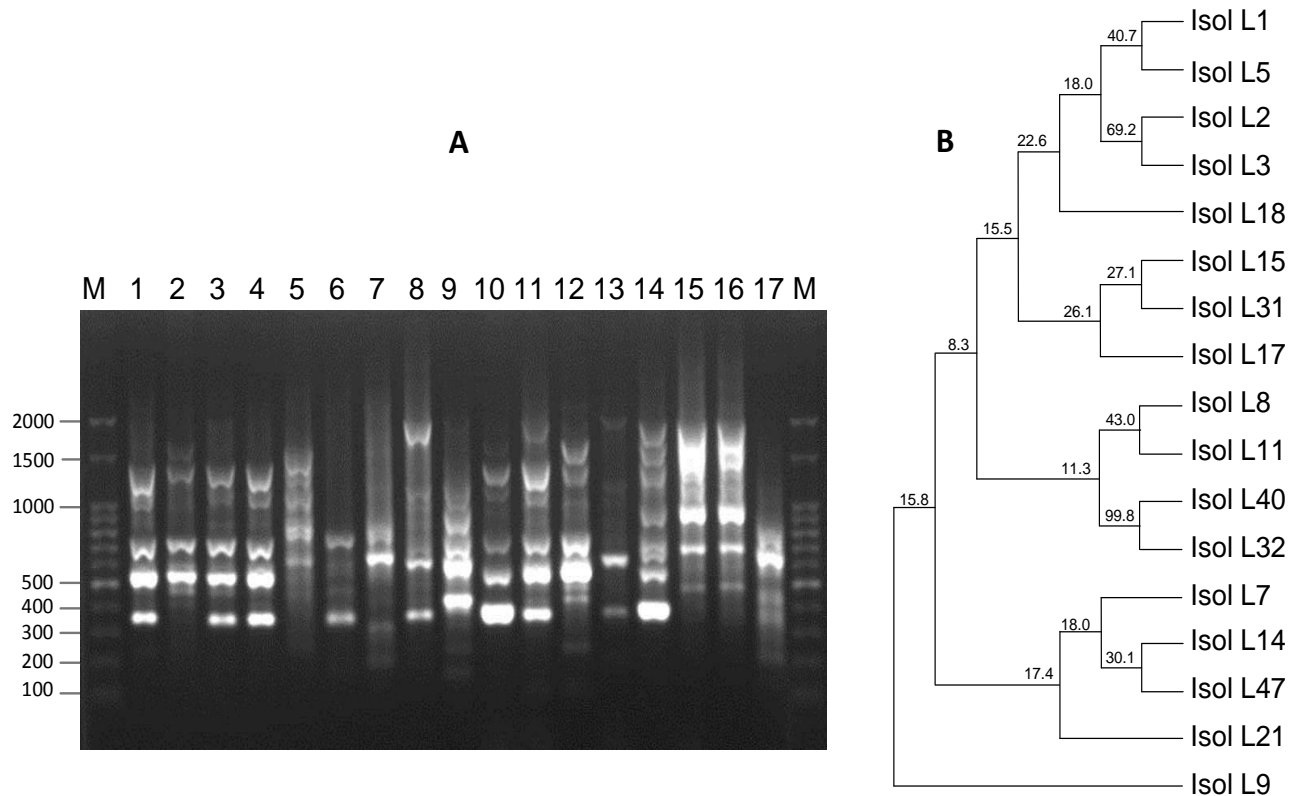


Figure 3. RAPD typing of *L. curvatus* isolates of salami. (A) Electrophoretic analysis of the amplification profile of the RAPD method using the OPB17 primer of seventeen *L. curvatus* isolates where: M is the marker of molecular weight 100 bp DNA ladder and the lines 1 to 17, respectively, are *L. curvatus* isolates 1L, 2L, 3L, 5L, 7L, 8L, 9L, 11L, 14L, 15L, 17L, 18L, 21L, 31L, 32L, 40L and 47L. (B) Dendrogram constructed by UPGMA using Jaccard's coefficient based on RAPD patterns of Panel A. The values found in the groups indicate the percentage recorded for a particular branch based on 2,000 bootstrap replications using Winboot software.

activity against pathogenic microorganisms and absence of pathogenic activity and others (Cocolin and Rantsiou, 2007; Samelis et al., 1994; Työppönen et al., 2003, Leroy et al., 2006). Specifically, the temperature is possibly the most important factor affecting the viability and development of microorganisms. Bacteria need to be sufficiently resistant to high temperatures since raw fermented sausages and salami usually undergo a smoking process (Moeller et al., 2010). The lag phase, growth rate, final number of cells, nutritional requirements, enzymes and chemical composition of cells are affected by temperature. Chilling (temperatures between 1 and 7°C) and freezing may have lethal effects, but these depend on the microorganism in question, the micro-environment and the storage conditions (Ying et al., 2010). Likewise, salt is one of the first obstacles to the growth of microorganisms in salami, acting also in the solubilization and diffusion of the myofibrillar proteins of meat by forming a gel between the meat particles and between the meat and fat (Työppönen et al., 2003).

The isolates also showed antimicrobial activity. According to Ogunbanwo et al. (2003), the inhibition

occurs after the stationary phase, due to the production of endogenous extracellular proteinases that can decrease the activity of bacteriocins. *L. curvatus* isolated from fermented sausages or salami showed inhibitory activity towards *L. monocytogenes* and were able to produce bacteriocins (Cocolin and Rantsiou, 2007).

Beyond the desirable technological characteristics of starter culture used as food biopreservative, it has easy and fast tools for identifying and typing microorganisms (Mohania et al., 2008; Tsai et al., 2010; Carvalho et al., 2006). Thus, the molecular method has demonstrated an important approach for *Lactobacillus* spp. and other LAB characterizations (Mohania et al., 2008).

Especially, ARDRA method is able to rapidly identify Gram positive bacteria and to identify and differentiate between various *Lactobacillus* species (Tsai et al., 2010; Rodas et al., 2005; Sánchez et al., 2005; Miteva et al., 2001). Collado and Hernández (2007) and Giraffa et al. (1998) also used the ARDRA method with API 50 CHL biochemical system to identify *Lactobacillus* and other LAB. The API system was not useful for the identification of the *Bifidobacterium* genus, *L. delbrueckii* and *L.*

acidophilus species because some strains showed variable use of carbohydrate fermentation patterns at different times (Collado and Hernández, 2007; Giraffa et al., 1998).

Though ARDRA method is highly discriminatory and is a reproducible tool for the identification of *Lactobacillus* species (Miteva et al., 2001; Najjari et al., 2008), in our results, no differences were found in the restriction profiles of *L. curvatus* and *L. sakei* 16S rRNA genes obtained with the three restriction endonucleases (Alu I, Hha I and Hinf I), likely due to their phylogenetic proximity (Berthier't and Ehrlich, 1999; Rodas et al., 2005). This indicates limitation of the technique for differentiation of these species.

Thereby, the region 5', specifically, of the 16S rRNA gene was sequenced because, as observed in *in silico* analysis, according to Soto et al. (2010), its earliest 450 nucleotides contained the sequences with the differences necessary to differentiate and identify LAB species (data not shown). At this sequence of the 16S rRNA gene are included the variable regions V1 and V2; these regions provide strong evidence for the identification of *Lactobacillus*, including *L. sakei* and *L. curvatus* among others (Rantsiou et al., 2006; Balcázar et al., 2007).

The overall data confirmed the need for combined phenotypic and molecular approaches for identifying natural isolates including the sequencing of genes that easily differentiate closely related species. In these cases, when the identification by phenotypic tests is often ambiguous or to distinguish *L. curvatus* and *L. sakei* species, a complementary identification by partial sequencing of the 16S rRNA gene is necessary (Rantsiou et al., 2006; Mohania et al., 2008).

However, for use as starter cultures besides the identification, species is necessary for differentiating closely related strains of LAB associated with food (Mahonia et al., 2008), so the determination of specific provenience and differentiation of the inoculant of natural strains is required (Rantsiou et al., 2006). The use of RAPD methods enables the discrimination of most genotypes on the basis of their DNA profiles (Berthier't and Ehrlich, 1999). As suggested, the RAPD-PCR proved to be a convenient method for fingerprinting and therefore a good tool for the typing of *Lactobacillus* spp. strains (Sánchez et al., 2005; Mahenthiralingam et al., 2009).

Our RAPD results using OPB17 primer and specific amplification conditions presented great potential for typing and reproducibility of *L. curvatus* isolates from handmade salami.

However, the use of appropriate methods and careful optimization of conditions determine the level of discrimination and its final usefulness (Markiewicz et al., 2010). In this case, the use of more than one complementary method is essential for achieving more reliable and accurate results both for species determination and for the characterization of isolates of the same species, combining phenotypic identification

techniques and molecular methods as needed.

Conclusion

In conclusion, the isolation of LAB present in spontaneous fermentation of fermented salami with desirable technological criteria is a viable alternative for obtaining new microorganisms with potential use as starter cultures to improve the quality of this food types. To identify these microorganisms, ARDRA of 16S rRNA gene gave results comparable to specific *Lactobacillus* species and therefore could be useful for rapid identification of isolates, and the use of partial sequencing of the 16S rRNA gene also appears to be a accessory tools as phylogenetic marker for the accurate discrimination and identification of *Lactobacillus* species. The molecular profiles obtained by RAPD methods might also be useful for intraspecies discrimination of *L. curvatus* isolates which monitor their presence in fermentation and enable the use of these microorganisms as starter culture in the production of salami, a Brazilian handmade fermented meat product, aggregating value and quality.

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