Characterization and selection of *Lactobacillus plantarum* species isolated from dry fermented sausage reformulated with camel meat and hump fat

Lobna Mejri1 · Mnasser Hassouna1

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**Abstract** A total of 29 strains of *Lactobacillus plantarum*, isolated from traditional dry fermented camel sausages and identified by biochemical and molecular methods, were characterized by their technological and safety properties including acidifying capacity, proteolytic activity, and antimicrobial and antibiotic resistance in order to select the most suitable for use as starter cultures in combination with coagulase-negative staphylococci to improve and standardize the safety and quality of sausages. All the *Lb. plantarum* were able to hydrolyze casein and some strains showed very weak lipolytic activity. Moreover, all isolates showed acidifying activity by reducing pH to less than 4.3 at 15 and 25 °C after 72 h. Related to safety aspects, the totality of *Lb. plantarum* was resistant to tetracycline, gentamicin, and kanamycin. A large majority of strains exhibited antimicrobial activities against *Listeria monocytogenes*, *Staphylococcus aureus*, and *Aeromonas hydrophila*.

**Keywords** Antibiotic resistance · Antimicrobial activity · Camel meat · Fermented sausage · *Lactobacillus plantarum* · Selection

**Introduction**

The camel is a good source of meat in areas where the climate adversely affects other animal’s production efficiency (Kadim et al. 2006). Camel meat is healthier as they produce carcasses with less fat as well as having less levels of cholesterol in fat than other meat animals (El-Faer et al. 1991; Dawood and Alkanhal 1995; Kadim et al. 2013). Camel meat is also used for remedial purposes for diseases such as hyperacidity, hypertension, pneumonia, and respiratory disease as well as an aphrodisiac (Kurtu 2004; Kadim et al. 2008). Reformulation of meat products based on processing strategies has drawn the attention of meat manufacturers seeking to develop innovative meat products that promote better consumer health (Jimenez-Colmenero et al. 2010). Moreover, a number of studies have examined possibilities for reformulation of fermented sausages using different meat types, such as goat and sheep (Stajic et al. 2013) and mutton (Zhao et al. 2011). The potential of camel meat has received increased attention, although more focus has been paid to nutritional value of camel meat than to its use in fermented sausages.

The fermentation of sausages involves the participation of mainly lactic acid bacteria (LAB), coagulase-negative staphylococci (CNS), and, less importantly, yeast and molds (Ruiz-Moyano et al. 2009). The most frequent LAB species present in fermented sausage processes are *Lb. sakei*, *Lb. curvatus*, and *Lb. plantarum*. However, in some instances, the contribution of Enterococci seems to be also relevant (Comi et al. 2005). LAB plays an important role in the formation of lactic acid by fermenting carbohydrates, and hence could contribute to the safety of the process. Moreover, the lactic acid production contributes to the formation of the texture and in the acid taste (Landeta et al. 2013). As a consequence of this process, the muscle protein coagulates, resulting in the slice ability, firmness, and cohesiveness found in the final product. Also LAB may inactivate pathogens through the production of antimicrobial compounds such as lactic acid, acetic acid, hydrogen peroxide, carbon dioxide, and bacteriocins (Aymerich et al. 2000).
LAB originating from fermented meat are particularly well adapted to the ecology of meat fermentation and thus should be considered for selection as starter cultures (Drosinos et al. 2005; Aro Aro et al. 2010; Zhao et al. 2011). The most promising bacteria for starter cultures are those which are isolated from the indigenous microflora of traditional products. These microorganisms are well adapted in the meat environment and are capable of dominating the microflora of products. *Lb. plantarum* is one of the most used species in addition to *Lb.* sakei, *Lb.* curvatus, *Lb.* pentosus, *Lb.* casei, *Pc.* pentosaceus, and *Pc.* acidilactici (Essid et al. 2009). Generally, LAB from fermented sausages have been traditionally identified based on simple physiological, biochemical, and chemotaxonomic methods. Although valuable from a practical point of view, results obtained by these methods are not always sufficient to characterize strains to species level, mainly within species from genera *Lactobacillus* (Ammor et al. 2005) and *Enterococcus* (Velasco et al. 2004). Despite that the main role of LAB in fermented meat products is related with lactic acid production, LAB possess additional relevant characteristics that need to be taken into account in order to select them as starter cultures. The main objective of this work was to characterize *Lb. plantarum* strains isolated from dry fermented camel sausages and the selection of the most suitable strains according to their technological characteristics and safety properties.

**Materials and methods**

**Bacterial strains and growth conditions**

The strains of *Lb. plantarum* used in this study were isolated from a traditional fermented sausage prepared with camel meat (75 %) and hump fat (25 %) without using microbial starter cultures. Four productions were prepared under the same conditions. Four samples were taken from various regions of the south of Tunisia (camel breeding). All the strains were isolated and purified on MRS agar (Man Rogosa and Sharpe) (Pronadisa, Sevilla, Spain) at 30 °C during 24 h under anaerobic conditions.

**DNA extraction**

DNA was extracted using NucleoSpin® Tissue kit (Macherey–Nagel, Düren, Germany) according to the manufacturer’s instructions. Lysis was achieved by incubation of the cultured cells in a proteinase K-740506 (Macherey–Nagel) at a final concentration of 30 mg/1.35 mL. Appropriate conditions for binding of DNA to the silica membrane in the NucleoSpin® Tissue Columns were created by the addition of chaotropic salts and ethanol to the lysate. The binding process was reversible and specific to nucleic acids. Contaminants were removed by subsequent washing with two different buffers. Pure genomic DNA was finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

**Taxonomical identification of *Lb. plantarum* strains**

The PCR-specific reaction of *Lb. plantarum* was performed using the primers M13 (5′GAGGGTGCGGCTCT 3′). PCR was performed in a final volume of 25 μL containing 5 μL of each primer (10 μM), 1 μL of deoxynucleoside triphosphate (dNTP) at a concentration of 10 mM, 2 μL of MgCl₂ (25 mM), 5 μL of 5X PCR buffer, 5 U of Taq polymerase (Promega, Madison, USA), and 10.75 μL of sterile ultrapure water. After the denaturing step for 5 min at 95 °C, 45 amplification cycles (1 min at 95 °C, 1 min at 36 °C, and 4 min at 72 °C) were performed. The size and the quality of DNA were checked by 1.2 % (w/v) agarose gel electrophoresis in TBE buffer (90 mM Tris–borate, 2 mM EDTA; pH 8.2) with a 1-kb DNA MW marker. The gel was stained with ethidium bromide and the bands were visualized under UV light. The DNA profiles were analyzed using the Bionumerics system (Bio-Numerics 2.50, UPGMA Pearson Correlation, Applied Maths, Sint-Martens-Latem, Belgium). The Sequencing of the 16S rRNA gene of the *Lb. plantarum* was carried out. The primers used for amplification of the 16S rRNA gene were fD1 (5′AGAGTTTGATCCTGGCTCAG 3′) and rD1 (5′TAA GAGGTTGATCCAGGCC 3′) used to obtain 1500 bp PCR amplicons (Weisburg et al. 1991; Winker and Woese 1991). The PCR was carried out as follows: one cycle of initial heating at 94 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 90 s, annealing at 62 °C for 90 s, and extension at 72 °C for 120 s. PCR products were separated by electrophoresis (1 h at 85 V) on 1 % (w/v) agarose gel electrophoresis and the DNA was visualized under UV light after staining with ethidium bromide (0.5 μg/mL). In certain instances where it was unable to discriminate among species, amplification and sequencing of the rpoA was carried out using two primers rpoA-21-F (5′ATGATYARTTTRATTGGAAAACC 3′) and rpoA-23-R (5′ ACHGTRTRATDCCDGCCG 3′) (Naser et al. 2005). PCR reactions were composed of 36.8 mL sterile ultrapure water, 5 mL of PCR buffer (with MgCl₂), 5 mL of dNTP (1.25 mmol L⁻¹), 0.5 mL (21 mmol L⁻¹) each of both forward primer and reverse primer, 0.2 mL AmpliTaq DNA polymerase (Promega), and 2 mL template DNA. The thermal program consisted of 5 min at 95 °C, 3 cycles of 1 min at 95 °C + 2 min 15 s at 46 °C + 1 min 15 s at 72 °C, 30 cycles of 35 s at 95 °C + 1 min 15 s at 46 °C + 1 min 15 s at 72 °C, and a final 7 min at 72 °C. Identification of isolates at the genus and species levels was
carried out by performing a search in GenBank database using the Basic Local Alignment Tool (BLAST) program (Altschul et al. 1997).

**Phenotypic characterization of *Lb. plantarum***

Cell morphology, Gram staining reaction, and catalase activity were examined. Gram-positive, catalase-negative isolates were checked for gas production from glucose in MRS broth (Pronadisa) containing Durham tubes (Greco et al. 2005). Sugar fermentation patterns of LAB isolates were determined using the API 50 CH system (BioMérieux, Craponne, France) and the identification of the LAB strains was performed using the computer program APIWEB database software (BioMérieux). Strains were stored at −20°C in MRS Broth containing 20% glycerol.

**Acidification activity**

The acidification activity was tested according to Ammor et al. (2005) on SB medium designed for simulating some conditions of sausage manufacture. The SB medium contained 10% meat extract (Bio-Rad, Marnes-la-Coquette, France), 2% glucose (Chemi-pharma, Bardo, Tunisia), 2.5% NaCl (Chemi-pharma), 1% Bacto-peptone (Pronadisa), pH 6.5. A defined volume of an overnight culture of each strain (MRS broth, 30°C, A600 = 0.5) was used to inoculate 75 mL of SB medium to obtain an A600 of 0.05. pH values were recorded after 4, 8, 12, 24, 48, 72, and 96 h of incubation at 15, 25, and 37°C using a pH meter (WTW portable pH meter pH 315i/SET. Wissenschaftlich).

**Catalase activity**

The catalase activity was determined according to a previous method of Landeta et al. (2013) and Essid et al. (2007). The strains were incubated in MRS broth for 24 h at 30°C, then 5 mL of culture (resting cells with an A600 = 1.0) was centrifuged, and the resulting pellet was mixed with 1.5 mL of 60 mM H2O2 in 20 mM phosphate buffer pH 7.0. Catalase activity was measured spectrophotometrically at 240 nm after 3 min of incubation at room temperature. Results were expressed in arbitrary units (µmol of degraded H2O2/min/mL of cells with A600 = 1.0).

**Proteolytic activity**

Proteolytic activity of LAB was carried out using two methods. The first method was performed on MRS medium supplemented with 4% gelatin and 10% skimmed milk. Production of gelatinase was manifested by the appearance of a zone of turbidity around the colonies after incubation for 16–18 h at 30°C (Cariolato et al. 2008), while the hydrolysis of caseins was determined by measurement of the area of the clear zone surrounding the inoculated spots after incubation for 72 h at 30°C. In the second method, the proteolytic activity was performed by the absorbance measured at 440 nm using azocasein and was expressed as ΔOD440 × h⁻¹ × mg⁻¹ dry weight (Boulares et al. 2012).

**Lipolytic activity**

Lipolytic activity was initially determined using a method previously described by Essid et al. (2007). An overnight culture of each strain (MRS broth, 24 h, 30°C) was centrifuged at 13,000 g and the pellet was resuspended in 20 mM phosphate buffer, pH 7.0. Each cell suspension (10 µL) was inoculated on a spot at the surface of the MRS plates supplemented with 1% of Tween 80, Tween 20, and tributyrin. After incubation at 30°C for 48 h, the lipolytic activity was determined by the appearance of precipitated zones surrounding the spots in which the diameter was measured in mm. Lipolytic activity was also performed by titration method as follows: 1 mL of an overnight culture of each strain was inoculated into 10 mL of broth containing 1% tryptone, 0.5% yeast extract, and 3% NaCl, pH 7.0, supplemented with 4% (w/v) of fat (Mauroilfo et al. 2004). After incubation at 30°C for 7 days, the lipid fractions were extracted by adding 10 mL of petroleum ether (Merck). The free fatty acids of the upper phase (lipid extract) were titrated with NaOH (0.1 N) using 1% phenolphthalein as an indicator. The lipolytic activity was determined by the following equation: (a × N × 28.2)/g, where a is the mL of NaOH used in the titration, N is the normality of NaOH, 28.2 is the percent equivalent weight of oleic acid, and g is the weight of fat in the sample.

**Antimicrobial activity**

The antimicrobial activity of each strain of *Lb. plantarum* was determined using the well diffusion method described by Papamaloni et al. (2003) and Anyogu et al. (2014), against six undesirable strains: *Salmonella arizonae* (ATCC 25922), *Aeromonas hydrophila* (ATCC 7966), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (DH5alpha, Institute Pasteur of Tunisia), *Listeria monocytogenes* (ATCC 070101121), and *Staphylococcus aureus* (ATCC 25923). Twenty milliliters of nutrient agar (Pronadisa) was inoculated with 100 µL of an overnight culture of the indicator organism and poured into a Petri dish. After solidification, wells were made and filled with 50 µL of supernatant from 24-h cultures of test organisms (LAB). Plates were held at 4°C for about 3–4 h to aid radial diffusion, before being incubated at 30°C for 24–48 h,
depending on indicator organism. The appearance of a clear zone surrounding the wells indicates antimicrobial activity.

**Antibiotic susceptibility**

Susceptibility to antibiotics was determined using a disk diffusion test as described by Landeta et al. (2013). The antibiotic disks were placed onto Mueller–Hinton agar (Pronadisa) plates and overlaid with 3 mL of MRS soft agar broth (0.7 % agar, p/v) inoculated with 150 μL of the cultures with a cell concentration corresponding to 0.5 McFarland turbidity standard. After incubation at 30 °C for 24 h, the diameter of inhibition halos around the colonies was measured. Sensitivity or resistance was determined according to the recommendation of the National Committee for Clinical Laboratory Standard (NCCLS 2002). The antibiotic disks used were ampicillin (10 μg), cefotaxime (30 μg), chloramphenicol (30 μg), gentamicin (30 μg), kanamycin (30 μg), piperacillin + tazobactam (110 μg), trimethoprim + sulfamethoxazole (25 μg), ticarcillin (75 μg), penicillin G (10 IU), tetracycline (30 μg), and nalidixic acid (30 μg).

**Statistical analysis**

Statistical analyses were performed using SPSS version 17 software (SPSS, Chicago, IL, USA). Statistical significance for differences was determined at 5 % probability level.

**Results**

**Identification of isolates**

One hundred thirteen Gram-positive and catalase-negative LAB strains were isolated from traditional dry fermented camel sausage. Rod-shaped cells were observed in 80 isolates (70.8 %) and classified as lactobacilli which 29 strains were identified as *Lb. plantarum*. These strains showed differences in fermentation abilities of some sugars by the API 50 CH system. No strain produces gas from glucose. To ensure definitive identification, these strains were subjected to species-specific PCR amplification; only one band at almost 1500 bp was observed for all strains (Fig. 1). On the basis of 16S rRNA gene sequences, all *Lb. plantarum* form the same branches (Fig. 2). Strains had at least 99 % 16S rRNA gene sequence similarity. Comparisons of rpoA and 16S rRNA indicated that the rpoA gene correlated well with the 16S rRNA gene sequences.

**Acidification activity**

At 15 and 25 °C (temperatures usually used for meat fermentation), the strains reduced the pH in SB broth to below 4.3 at 72 and 48 h, respectively. No significant difference (*p > 0.05*) was observed between strains for each temperature tested (data not shown).

**Catalase activity**

In this study, catalase activity was analyzed using a spectrophotometric assay measuring the μmol of degraded H₂O₂ (Table 1). All the analyzed strains showed weak catalase activity. No significant difference (*p > 0.05*) was observed among strains.

**Proteolytic activity**

In order to know the proteolytic activity of the *Lb. plantarum* strains from fermented sausages, two different assays were used: agar plate method and spectrophotometric method. The agar plate assay for the detection of proteolytic activity revealed that the majority of isolates were able to hydrolyze caseins. The strains P2Lbp12, P4Lbp25, and P4Lbp26 showed the highest proteolytic activity with the agar plate method (5.49 ± 0.88, 5.17 ± 0.76, and 5.20 ± 0.26 mm, respectively) (Table 1). On the other hand, none of the strains analyzed showed proteolytic activity using gelatine as a substrate. Moreover, the extracellular proteolytic activity using the spectrophotometric method on azocasein substrate was very poor for all strains of *Lb. plantarum* (Table 1).

**Lipolytic activity**

Lipolytic activity, by the agar method, showed that the majority of the strains could hydrolyze Tween 20; the strain P3Lbp18 had the largest halo surrounding the wells (6.07 ± 2.08 mm). Only 14 strains could hydrolyze Tributyrin, and the largest halo was given by the strain P2Lbp12 (7.66 ± 0.16 mm). Tween 80 was hydrolyzed by only 10 strains and the highest activity was observed for the strain P3Lbp18 (4.66 ± 0.38 mm) (Table 1). Lipolytic activity was also assessed by the titration method, and no significant differences for all strains (*p > 0.05*) were found between the two types of fat (Table 1). The majority of strains could hydrolyze camel and beef fat. P2Lbp13, P3Lbp21, and P4Lbp25 had higher lipolytic activities.

**Antibacterial activity**

The agar well diffusion method was used to assess the antimicrobial activity of *Lb. plantarum* isolated from...
fermented sausage. Their antimicrobial properties were tested against six major foodborne pathogenic bacteria. The ability of the strains to inhibit pathogenic indicator bacteria varied according to the indicator organism. Table 2 shows the results for the antimicrobial activity of the isolates in terms of the diameter of the zone of inhibition. All the strains showed strongest inhibition against L. monocytogenes, S. aureus, and A. hydrophila. The strain P2Lbp14 had the largest zone of inhibition against all the indicator pathogens tested; its activity was highest against L. monocytogenes (19 ± 1.3 mm) and least for Escherichia coli (10 ± 1.1 mm).

**Antibiotic resistance**

Antibiotic resistance of *Lb. plantarum* was determined using the disk diffusion method (Table 3). All isolates were resistant to gentamicin, kanamycin, and tetracycline. Only 10 strains (34 %) were resistant to ampicillin. The majority of *Lb. plantarum* isolates (89 %) were susceptible to trimethoprim + sulfamethoxazole and 7 % of isolates are susceptible to chloramphenicol.

**Discussion**

LAB originally isolated from traditional sausages are probably the best candidates for improving the microbiological safety of these foods, because they are well adapted to the conditions in sausages and should therefore be more competitive than LAB from other sources. Their identification based on biochemical tests has proven to be difficult and time-consuming. These phenotypic methods are limited in terms of both their discriminating ability and accuracy. Benito et al. (2008) reported that the LAB strains isolated from Iberian dry fermented sausages, biochemical identification by API 50 CHL showed different errors at genus and species levels. For this reason, molecular methods have been developed allowing the accurate identification of LAB from meat products (Landeta et al. 2013).
Despite that the main role of LAB in fermented meat products is related to lactic acid production, LAB possess additional relevant characteristics that need to be taken into account in order to select them as starter cultures.

The most important characteristic for potential starter strains is their ability to acidify their environment rapidly, as the acid production and the accompanying pH decrease give a specific aroma and extend the lag phase of sensitive organisms including foodborne pathogens (Kostinek et al. 2005). At 15 °C, the *Lb. plantarum* strains produced acid more rapidly than the *Lb. sakei* strains studied by Ammor et al. (2005). Klinberg et al. (2005) showed that *Lb. plantarum* and *Lb. pentosus* strains showed faster acidification in a meat model (24 °C) compared to *Lb. sakei*, *Lb. casei*, and *Pc. pentosaceus* which are usually used as meat starters. The temperature of incubation affects the rapidity of the acidification, which can affect the time of fermentation: the faster the pH decreases, the shorter the drying process (Arnau et al. 2007).

Most lactobacilli are able to form hydrogen peroxide by oxidizing lactate and thus increasing rancidity and the

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**Table 1** Technological properties of *lactobacillus plantarum* strains

| Strains | Catalase\(^a\) | Proteolytic activities\(^b\) | Lipolytic activities\(^c\) |
|---------|----------------|-----------------------------|---------------------------|
|         | M.A | G.A | Azocasein\(^d\) | T20 | T 80 | Tributyrin | Boeuf fat\(^e\) | Camel fat\(^f\) |
| P1 Lbp1 | 1.03 | ++ | – | 0.31 | ++ | – | ++ | 13.6 | 11.28 |
| P1 Lbp2 | 1.39 | ++ | – | 0.40 | – | – | – | 13.6 | 11.28 |
| P1 Lbp3 | 1.87 | ++ | – | 0.34 | ++ | – | ++ | 15.62 | 17.62 |
| P1 Lbp4 | 1.29 | – | – | 0.37 | ++ | – | ++ | 15.71 | 16.21 |
| P1 Lbp5 | 1.49 | ++ | – | 0.41 | – | – | – | 12.92 | 9.16 |
| P1 Lbp6 | 0.96 | ++ | – | 0.24 | ++ | – | ++ | 14.50 | 14.10 |
| P1 Lbp7 | 1.70 | ++ | – | 0.32 | ++ | – | – | 12.92 | 9.16 |
| P1 Lbp8 | 1.87 | – | – | 0.23 | ++ | – | +++ | 12.92 | 9.16 |
| P1 Lbp9 | 1.63 | – | – | 0.29 | ++ | – | – | 1.12 | 3.52 |
| P2 Lbp10 | 1.96 | – | – | 0.24 | ++ | ++ | – | 13.60 | 11.28 |
| P2 Lbp11 | 1.39 | ++ | – | 0.32 | ++ | – | – | 11.12 | 14.10 |
| P2 Lbp12 | 1.19 | ++ | – | 0.36 | ++ | ++ | +++ | 14.50 | 19.74 |
| P2 Lbp13 | 1.59 | – | – | 0.40 | – | – | – | 23.60 | 21.15 |
| P2 Lbp14 | 1.78 | ++ | – | 0.45 | ++ | ++ | – | 14.50 | 14.10 |
| P3 Lbp15 | 0.91 | ++ | – | 0.37 | ++ | ++ | – | 16.30 | 14.10 |
| P3 Lbp16 | 1.69 | – | – | 0.43 | ++ | – | – | 6.75 | 4.23 |
| P3 Lbp17 | 1.42 | – | – | 0.24 | ++ | ++ | ++ | 14.50 | 11.28 |
| P3 Lbp18 | 1.24 | ++ | – | – | +++ | ++ | – | 14.50 | 14.10 |
| P3 Lbp19 | 1.00 | – | – | 0.39 | ++ | ++ | – | 11.35 | 8.46 |
| P3 Lbp20 | 1.85 | ++ | – | – | – | – | – | 13.60 | 16.21 |
| P3 Lbp21 | 1.94 | ++ | – | 0.31 | – | – | ++ | 24.50 | 24.67 |
| P3 Lbp22 | 1.07 | ++ | – | 0.55 | ++ | ++ | – | 12.70 | 11.28 |
| P4 Lbp23 | 1.94 | ++ | – | 0.34 | ++ | ++ | +++ | 5.17 | 5.33 |
| P4 Lbp24 | 1.95 | ++ | – | 0.34 | ++ | – | – | 7.87 | 10.23 |
| P4 Lbp25 | 1.97 | ++ | – | 0.56 | ++ | ++ | ++ | 17.42 | 23.26 |
| P4 Lbp26 | 2.22 | ++ | – | 0.54 | – | – | – | 15.62 | 17.62 |
| P4 Lbp27 | 1.83 | ++ | – | 0.51 | + | ++ | +++ | 14.50 | 14.10 |
| P4 Lbp28 | 1.79 | – | – | 0.17 | ++ | ++ | ++ | 2.25 | 7.05 |
| P4 Lbp29 | 2.06 | ++ | – | 0.10 | ++ | – | ++ | 16.30 | 19.74 |

*Lbp* *Lactobacillus plantarum*, *P1* first production (samples were taken from Medenine, south of Tunisia); *P2* second production (samples were taken from Gabes, south of Tunisia); *P3* third production (samples were taken from Tataouine, south of Tunisia); and *P4* fourth production (samples were taken from Kebili, south of Tunisia)

\(^a\) Catalase activity

\(^b,c\) Proteolytic and lipolytic activity. MA (Milk Agar), GA (Gelatin Agar), T20 (Tween 20), T80 (Tween 80), Tributyrin: (−) negative; (+) 0.1–2 mm; (+++) 2–6 mm; (++++) 6–10 mm; (+++++) >10 mm

\(^d,e,f\) Values are means of the three replicates; standard deviations are in the range [±0.00 to ±2.50]

\(^e,f\) % of oleic acid
discoloration of the final meat product. In our research, all the analyzed *Lb. plantarum* strains showed weak catalase activity. Our result is in agreement with Ammor and Mayo (2007) which reported that some LAB involved in meat fermentation, such as *Lb. sakei*, *Lb. plantarum*, *Lb. pentosus*, and *Pa. acidilactici*, possess weak catalase activity as compared to the constitutive catalase of CNS which is active in meat products hydrolyzing the hydrogen peroxide produced.

Several aromatic substances and organic acids are released by protease and lipase activity from microorganisms. Proteolysis and lipolysis influence both texture and flavor development due to the formation of low-molecular weight compounds (free fatty acids, peptides, etc.). All the *Lb. plantarum* strains could hydrolyze casein and showed weak activity using azocasein. Similar results were reported by Papamaloni et al. (2003). On the other hand, Baruzzi et al. (2005) found that strains of *Lb. sakei*, *Lb. curvatus*, and *Lb. casei*, which can also be used as starters, exhibit weak proteolytic activity on lyophilized pork meat. Montel et al. (1998) reported that despite that *Lactobacillus* species are weakly proteolytic in general, some *Lb. casei*, *Lb. plantarum*, *Lb. curvatus*, and *Lb. sakei* strains actively contribute to the hydrolysis of the sarcoplasmic proteins, and several peptidase activities have been reported in *Lb. sakei*, *Lb. curvatus*, and *Lb. plantarum* isolated from sausages (Fadda et al. 1999).

Occasional descriptions of lipolytic activity from LAB strains have also been reported. For example, Silva Lopes

| Strains | Indicator strains |
|---------|-------------------|
|         | *Listeria Monocytogenes* | *Staphylococcus aureus* | *Escherichia coli* | *Salmonella arizonae* | *Aeromonas hydrophila* | *Pseudomonas aeruginosa* |
| P1 Lbp1 | –                  | +++               | –                | +++                 | +++             | –                     |
| P1 Lbp2 | –                  | +++               | ++               | +++                 | +++             | –                     |
| P1 Lbp3 | ++++              | +++               | +                | +++                 | +++             | ++                    |
| P1 Lbp4 | –                  | –                 | –                | +++                 | +++             | –                     |
| P1 Lbp5 | ++                | +++               | –                | +++                 | +++             | –                     |
| P1 Lbp6 | ++                | +++               | ++               | +++                 | +++             | ++                    |
| P1 Lbp7 | ++++              | +                 | ++               | +++                 | +++             | ++                    |
| P2 Lbp8 | +++               | +++               | +++              | +++                 | +++             | ++                    |
| P2 Lbp9 | ++++              | +++               | ++               | +++                 | +++             | +                     |
| P2 Lbp10 | +++             | +++               | –                | +++                 | +++             | +                     |
| P2 Lbp11 | +++              | +++               | ++               | +++                 | +++             | –                     |
| P2 Lbp12 | +++              | +++               | –                | +++                 | +++             | ++                    |
| P2 Lbp13 | +++              | +++               | ++               | +++                 | +++             | ++                    |
| P2 Lbp14 | +++              | +++               | +++              | +++                 | +++             | ++                    |
| P3 Lbp15 | ++              | +++               | –                | +                   | –               | –                     |
| P3 Lbp16 | +++              | +++               | +++              | +++                 | +++             | ++                    |
| P3 Lbp17 | +++              | +++               | +++              | +++                 | +++             | ++                    |
| P3 Lbp18 | +++              | +++               | +++              | +++                 | +++             | ++                    |
| P3 Lbp19 | –                | +++               | –                | +++                 | +++             | –                     |
| P3 Lbp20 | +++              | ++                | ++               | +++                 | +++             | +                     |
| P3 Lbp21 | +++              | +++               | –                | +++                 | +++             | +                     |
| P3 Lbp22 | +++              | +++               | +++              | +++                 | +++             | ++                    |
| P4 Lbp23 | –                | +++               | –                |+++                 |+++             |–                     |
| P4 Lbp24 | –                | +++               | ++               |+++                 |+++             |+                     |
| P4 Lbp25 | +++              | ++                | +                |+++                 |+++             |+                     |
| P4 Lbp26 | +++              | +                 | ++               |+++                 |+++             |+                     |
| P4 Lbp27 | +++              | +++               | +                |+++                 |+++             |+                     |
| P4 Lbp28 | +++              | +++               | ++               |+++                 |+++             |+                     |
| P4 Lbp29 | +++              | +++               | –                |+++                 |+++             |+                     |

Table 2: Antimicrobial activity of *Lb. plantarum* against pathogenic bacteria

Area of haloes surrounding the wells = \[ \sqrt{R^2 - r^2} \]; \( R \) radius of the halo; \( r \) radius of the colony

– Negative reaction; + area of inhibition zone <200 mm\(^2\); ++ area of inhibition zone between 200 and 400 mm\(^2\); +++ area of inhibition zone between 400 and 600 mm\(^2\); ++++ area of inhibition zone >600 mm\(^2\)
Table 3 Antibiogram determined by antibiotic disks of 29 selected isolates of Lactobacillus plantarum

| Strains        | Antibiotics |
|---------------|-------------|
|               | Am  | CTX | C  | CN  | K   | TNP | SXT | TC  | TE  | NA  |
| P1 Lbp1       | R   | I   | R   | R   | S   | S   | R   | R   |     |     |
| P1 Lbp2       | R   | I   | I   | R   | R   | S   | S   | R   |     |     |
| P1 Lbp3       | R   | I   | R   | R   | I   | S   | R   | S   |     |     |
| P1 Lbp4       | R   | I   | I   | R   | R   | S   | S   | R   | I   |     |
| P1 Lbp5       | R   | I   | R   | R   | S   | S   | R   | S   |     |     |
| P1 Lbp6       | R   | I   | R   | R   | S   | S   | R   | S   |     |     |
| P1 Lbp7       | R   | I   | R   | R   | I   | S   | S   | R   |     |     |
| P1 Lbp8       | S   | I   | R   | R   | S   | S   | I   | R   |     |     |
| P1 Lbp9       | R   | I   | R   | R   | R   | S   | S   | R   |     |     |
| P2 Lbp10      | R   | R   | R   | R   | S   | S   | R   | S   |     |     |
| P2 Lbp11      | S   | I   | R   | R   | S   | I   | S   | R   |     |     |
| P2 Lbp12      | R   | I   | R   | R   | S   | S   | I   | R   |     |     |
| P2 Lbp13      | S   | I   | I   | R   | R   | I   | S   | R   | I   |     |
| P2 Lbp14      | S   | S   | I   | R   | R   | S   | S   | R   |     |     |
| P2 Lbp15      | S   | S   | I   | R   | R   | S   | S   | S   |     |     |
| P3 Lbp16      | R   | R   | S   | R   | R   | S   | I   | R   |     |     |
| P3 Lbp17      | R   | S   | I   | R   | R   | S   | S   | R   | I   |     |
| P3 Lbp18      | S   | R   | I   | R   | R   | S   | S   | R   | I   |     |
| P3 Lbp19      | R   | S   | I   | R   | R   | I   | S   | S   | I   |     |
| P3 Lbp20      | S   | R   | S   | R   | R   | S   | S   | R   |     |     |
| P3 Lbp21      | R   | R   | I   | R   | R   | S   | R   | R   |     |     |
| P3 Lbp22      | S   | S   | I   | R   | R   | S   | S   | S   |     |     |
| P4 Lbp23      | S   | S   | R   | R   | R   | I   | S   | S   | R   |     |
| P4 Lbp24      | S   | S   | I   | R   | R   | S   | S   | S   | R   |     |
| P4 Lbp25      | S   | S   | I   | R   | R   | S   | S   | R   |     |     |
| P4 Lbp26      | S   | S   | I   | R   | R   | S   | S   | R   |     |     |
| P4 Lbp27      | S   | S   | R   | R   | R   | S   | S   | R   |     |     |
| P4 Lbp28      | S   | S   | R   | R   | R   | I   | S   | S   | S   |     |
| P4 Lbp29      | S   | S   | S   | R   | R   | I   | S   | S   | S   |     |

Am: ampicillin (10 µg), CTX: cefotaxime (30 µg), CN: chloramphenicol (30 µg), K: kanamycin (30 µg), TNP: piperacillin + tazobactam (110 µg), SXT: trimethoprim + sulfamethoxazole (25 µg), TC: ticarcillin (75 µg), TE: tetracycline (30 µg), NA: nalidixic acid (30 µg). 

et al. (1999) described that Lb. plantarum DSMZ 12028, isolated from fermented sausage, produces a lipase during the meat fermentation releasing free fatty acids.

LAB isolated from traditional sausages are probably the best candidates for improving the microbiological safety of these foods, because they are well adapted to the conditions in sausages and should therefore be more competitive than LAB from other sources. Therefore, 29 Lb. plantarum strains were screened for exhibition of antagonistic activities against indicator microorganisms. Our results showed that the majority of isolates exhibited antagonistic effects against Gram-positive bacteria. These results were in accordance with previous results of Nieto-Lozano et al. (2002) who found that strains of Lb. plantarum had inhibitory activity against S. aureus and not against P. aeruginosa, E. coli, and Salmonella spp. Moreover, many studies confirm that Lb. plantarum strains have anti-L. monocytogenes activity (Nieto-Lozano et al. 2002; Papamaloni et al. 2003; Klinberg et al. 2005). The strains of Lb. plantarum could inhibit Gram-positive bacteria (S. aureus, L. monocytogenes) better than Gram-negative bacteria (Salmonella arizonae, E. coli, P. aeruginosa) as found by Ammor et al. (2005) and Albano et al. (2007). In general, the outer membrane of Gram-negative bacteria may protect the cytoplasmic membrane from the action of antimicrobial compounds (Gao et al. 1999).

Clinical use of antibiotics has achieved a significant reduction in the morbidity and mortality associated with infectious diseases. The food chain is becoming a possible way of dissemination of antibiotic resistance among bacterial populations of animals and humans. Many species of lactobacilli become vectors of antibiotic resistance genes. In this study, the susceptibility of Lb. plantarum strains to different antibiotics was tested. Our results partially confirm those of Nguyen et al. (2007), who found that Lb. plantarum strains were resistant to tetracycline, gentamicin, and penicillin. Gevers et al. (2003) reported that, in general, LAB isolated from fermented dry sausages were resistant to tetracycline, gentamicin, and penicillin.

All strains of Lb. plantarum had good acidifying activity; however, they showed some differences in antimicrobial, proteolytic, and lipolytic activities. Strains that can be used as starters to produce fermented sausage are the strain P2Lbp14, which had the best antagonistic activity, and strain P3Lbp18 could be selected for its high lipolytic activity, and the strains P2Lbp12 and P4Lbp25 should be selected because of their good proteolytic activity.

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