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Full Length Research paper

Microbiological and physicochemical characterization of the natural fermented camel meat sausage

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In this study, fermentation of camel meat was followed by microbiological and physicochemical analyses. The sausages were characterized by the presence of lactic acid bacteria that resulted in a product with a final pH of about 5.06. No *Listeria monocytogenes*, *Salmonella* spp. and sulfite reducing clostridia were ever isolated from the raw materials or the fermented sausages during the maturation, underlining the safety of this product. The final water activity of the product was 0.91. Identification showed that the majority of lactobacilli isolated from MRS agar strains were assigned to *Lactobacillus plantarum*.

Key words: Biopreservation, spices, fermented sausages, physicochemical profile.

INTRODUCTION

The bacteria which play a significant role and commonly found in fermented sausages are lactic acid bacteria (Coppola et al., 1998). These microorganisms are used as starter cultures, promoting meat fermentation (Papamanoli et al., 2003). Lactic acid bacteria improve safety and stability of the product, enhance colour stability, prevent rancidity and release various aromatic substances (Coppola et al., 1998; Hammes et al., 1995; Nychas and Arkoudelos, 1990; Papamanoli et al., 2003). *Lactobacilli* are the predominant lactic acid bacteria and among them the most frequently isolated strains are *Lactobacillus curvatus*, *Lactobacillus sakei*, and *Lactobacillus plantarum* (Hammes, 1990; Schillinger and Liicke, 1987).

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. The strains selected as starter or protective cultures must have the most important technological properties and/or bacteriocin production capabilities (Hammes, 1990).

The aim of this study was to investigate the evolution of the different categories of the microorganisms in naturally fermented camel meat sausage, and physicochemical changes which occurred during fermentation. This investigation also seek to identify and characterize the desirable groups (lactic acid bacteria) in order to select the most suitable strains, according to their technological properties and antimicrobial activity against food borne pathogens, as starter or protective cultures.

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MATERIALS AND METHODS

Preparation of sausages

Sausages were manufactured, according to the standard practice applied without commercial starter cultures. Camel meat was cleaned of visible fat, the batches contained 90% lean meat and 10% fat (Soyer et al., 2005). Other ingredients were added as follows: 1.6% NaCl, 1.2% garlic, 0.5% glucose, 0.6% black pepper, 0.01% cardamom 0.01% *Laurus nobilis* and 0.01% mace of *Myristica fragrans*, 0.04% $NaNO_3$ and 0.01% $NaNO_2$. Meat and frozen adipose tissue were minced in a meat cut-ter (Tecmap, Barcelona, Spain), with an adjustable plate set at a hole of diameter of 12 mm, and then mixed with other ingredients in a mixer machine (model 35P, Tecnotrip S.A., Terrassa, Spain).

The mixture of product was stuffed into collagen casings (4 cm diameter; Colex 32 mm, Fibra S.A., Girona, Spain). Sausages were vacuum packaged in polyamidepolyethylene bags (Sacoliva, Castellar del Vallès, Spain). Sausages were hung in a climate chamber MLR.350 H (Sanyo Electric Company, OraGun, Japan) at 13°C and with a relative humidity of 90% for 7 days and reduced to 80% at 18°C till the end of the ripening process (28 days). Meats, spices

and the fermented sausages at 0, 3, 5, 7, 14 and 28 days were analyzed. Three samples were collected and used for the analyses.

Microbial analysis

After aseptically removing the casing, approximately 25 g of sausage were 10-fold diluted in 225 ml buffered peptone water (AES Laboratories, Combourg, France) and homogenized in a Masticator (model 400, Cooke Laboratories, Alexandria, VA, USA) for 1 min. Serial decimal dilutions were made and the following analyses were carried out on duplicate agar plates: (a) Total viable count on Plate Count Agar (PCA, Oxoid) (aerobic incubation at 30°C for 42 h); (b) *Enterobacteriaceae* by pour plating in violet red bile glucose agar (Merck, Darmstadt, Germany) with a double layer at 30°C for 24 h; (c) fecal *Enterococci* on Kanamycin Aesculin agar (Oxoid) incubated at 42°C for 24 h; (d) *Micrococcaceae* on Mannitol Salt Agar (Oxoid) incubated at 30°C for 48 h; (e) yeasts and moulds on Malt Extract Agar (Oxoid) supplemented with tetracycline (1 mg/ml, Sigma, Milan, Italy) incubated at 25°C for 48 – 72 h; (f) aerobic spore formers on Plate Count Agar (Oxoid) after pasteurization at 80°C for 10 min, incubated at 30°C for 48 h; (g) sulphite reducing *clostridia* on SPS Agar (Oxoid) incubated anaerobically at 37°C for 72 h; (h) for detection of *Salmonella*, pre-enrichment was done by suspending 25 g of sample in 225 ml buffered peptone water (Merck) followed by incubation at 37°C for 16 – 20 h and selective enrichment was done by transferring 0.1 ml of pre-enrichment culture in 10 ml Rappaport-Vassiliadis broth (RVS broth, Merck) followed by incubation at 42°C for 24 h and after incubation samples were streaked on modified BPLS agar, (Merck) (37°C for 24 h) and XLD agar (Merck) (37°C for 48 h); (i) for *Listeria* detection, enrichment was done by suspending 25 g of sample in 225 ml Fraser broth (Merck) followed by incubation at 30°C for 24 h, then, 0.1 ml of the culture enrichment were streaked on PALCAM agar (Merck) and incubated at 30°C for 48 h; (j) lactic acid bacteria (LAB) were enumerated by pour plating in Man, Rogosa and Sharpe (MRS) agar (Difco Laboratories, Detroit, MI, USA) at 30°C for 72 h in anaerobiosis (Oxoid jars with Anaero-Gen; Oxoid, Basingstoke, Hampshire, England). After counting, means and standard deviations were calculated.

Physicochemical analysis

The determination of moisture, ssh, proteins and fat was performed according to AOAC (2002). Water activity (Aw) was measured by Acqua Lab CX-2 (Decagon Devices, Inc. Pullman, Washington, USA), pH measurements were done by using a pH-electrode (Ingold, MGDX K57, Urdorf, Switzerland) connected to a pH-meter (Knick, type 763, Berlin, Germany).

Biogenic amines (tyramine, histamine, putrescine, cadaverine, phenylethylamine, tryptamine, agmatine, spermidine and spermine) were extracted with 0.6 N perchloric acid from sausages without casings during ripening, raw meat and spices. Thereafter, they were determined by ion-pair reverse-phase column high performance liquid chromatography with post-column derivatization with orthophthalaldehyde according to the procedure described by Hernàndez-Jover et al. (1996). Due to the typical loss of water content during the manufacturing process, the results of nitrogenous fractions and biogenic amine contents of samples, except for raw materials, were referred to dry matter (dm).

Isolation and characterization of lactic acid bacteria

Ten grams of camel sausage after 28 days of ripening were homogenized in 90 ml of 1/4 strength Ringer's solution (Lab M, Manchester, UK) for 2 min in a Stomacher 400 Lab Blender (Seward Medical, London, UK). Serial decimal dilutions in 1/4 strength Ringer's solution were prepared. *Lactobacillus* spp. were isolated in pour plates of MRS agar medium, incubated aerobically (30°C for 3 days). A total of 300 isolates were collected, randomly, from one representative high dilution MRS agar plate. The isolates were tested for cell morphology, gram reaction and catalase formation by dropping a 3% H_2O_2 solution directly onto each plate. All isolates were grown in MRS broth at 30°C for 24 h, purified by streaking on MRS agar and maintained in MRS broth plus 30% (w/v) glycerol (Merck) at - 20°C. The Gram-positive and catalase-negative strains were subjected to the following physiological and biochemical tests: gas (CO_2) formation from glucose, arginine hydrolysis, growth in 8% and 10% NaCl, growth at 4, 10, 15, 37 and 45°C, dextran production and fermentation of the following sugars: maltose, mannitol, melibiose, lactose, L-arabinose, sucrose, raffinose, cellobiose, trehalose, ribose, sorbitol, D-xylose and Galactose according to Sharpe (1979) using the miniplate method described by Jayne-Williams (1976). Sugar fermentation pattern was also determined using API 50 CHL (BioMerieux, Marcy l'Etoile, France) and the identification was performed by the computer program APILAB Plus. The 150 strains were selected after their grouping based on the results from physiological and biochemical tests and sugar fermentation. Growth at different temperatures was carried out in MRS broth after incubation for 72 h at 15, 37 and 45°C, and 7 – 10 days at 4 and 10°C. Gas formation from glucose was determined in modified MRS broth containing inverted tubes, with diammonium citrate replaced by ammonium sulphate (Serva) and incubation for 5 days at 30°C (Schillinger and Liicke, 1987). Hydrolysis of arginine was tested in modified MRS broth, without meat extract but with 0.3% (w/v) arginine (Serva, Heidelberg, Germany), diammonium citrate replaced by sodium citrate (Mallinckrodt, New York, USA) and low concentration of glucose (0.05% w/v) (Riedel de Haen, Seelze, Germany) (Hitchener, Egan, and Rogers, 1982; Schillinger and Liicke, 1987). Ammonia was detected after 72 h of incubation at 30°C, using Nessler's reagent (Merck). Growth in the presence of 8% and 10% (w/v) NaCl (Merck) was observed in MRS broth after incubation for 5 days at 30°C. Dextran production from sucrose was determined on modified MRS agar, in which glucose was replaced by 5% (w/v) sucrose (Serva), after 5 days of incubation at 30°C (Hitchener et al., 1982).

Statistical analysis

All experiments were replicated in four times and tests were triplicate. The results represent the mean ± standards deviations. Means were compared by Student's t-test. Differences were considered statistically significant when $P < 0.05$. Statistical analysis was conducted with SPSS 10.0 (SPSS, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Microbiological analysis of the raw materials and camel fermented sausage

The results of the microbiological analysis of the raw materials were in Table 1. Bacterial counts were relatively low, indicating a good hygienic quality of meat raw materials and spices. The results of the microbiological analysis of the naturally fermented sausage (Table 2) showed that the lactic acid bacteria constituted the major microflora of the sausages, since the cell numbers of the total viable count and MRS count were similar after the 3rd day of the fermentation. The population of lactic acid bacteria increased by day 5 – 7 and stayed constant during ripen-

Table 1. Results of the microbiological analysis carried out on raw materials used for the production of the natural fermented sausages studied.

Microbiological analysis	Meat		Spices	
	Mean	SD	Mean	SD
Total aerobic count	4.51	0.01	3.32	0.26
Micrococcaceae	2.25	0.25	np	
Lactic acid bacteria	4.23	0.32	np	
Yeasts	2.48	0. 57	< 1.00	na
Moulds	<2.00	na	< 1.00	na
Fecal enterococci	3.02	0.02	np	
Total enterobacteria	<1.00		<1.00	na
Aerobic spore formers	np		<1.00	
Sulfite reducing clostridia	Absent		np	
Listeria monocytogenes	Absent		Absent	
Salmonella spp.	Absent		Absent	

Values are expressed in log10 colony forming unit (cfu)/g. SD, standard deviation; n.a, not applicable; n.p, not performed aAbsent in 25 g of product

Table 2. Microbial growth (log cfu g^{-1} ± SD) of the fermented sausages.

Microbiological analysis	0		3		5		7		14		28	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total aerobic count	5.22	0.01	6.52	0.05	7.55	0.01	7.95	0.33	7.62	0.27	7.54	0.18
Micrococcaceae	2.32	0.18	3.21	0.05	4.11	0.22	5.06	0.36	4.26	na	4.12	na
Lactic acid bacteria	4.35	0.12	6.44	0.33	7.58	0.12	8.23	0.12	8.36	0.22	8.24	0.36
Yeasts	3.22	0.38	2.56	0.62	2.23	0.12	2.01	0.28	1.82	0.02	<1.00	n.a
Moulds	<2.00	n.a	<1.00	n.a	<1.00	n.a	<1.00	n.a	<1.00	n.a	<1.00	n.a
Fecal enterococci	3.89	0.39	2.77	0.40	1.65	0.06	1.12	0.64	<1.00	n.a	<1.00	n.a
Total enterobacteria	< 1.00	0.01	1.92	n.a	<1.00		<1.00		<1.00		<1.00	
Sulfite reducing clostridia	Absent		Absent		Absent		Absent		Absent		Absent	
Listeria monocytogenes	Absent		Absent		Absent		Absent		Absent		Absent	
Salmonella spp.	Absent		Absent		Absent		Absent		Absent		Absent	

Each number is the mean of three sausage samples. SD, standard deviation; n.a, not applicable

ripening. Because of the good adaptation of lactic acid bacteria to the meat environment and their faster growth rates which were displayed during fermentation and ripening of sausages, they became the dominant microflora (Drosinos et al., 2005). The Enterobacteria population was lower than 1 $\log_{10}$ cfu/g after stuffing. And they were eliminated at 5 days of ripening regardless of their initial population. This elimination was also due to subsequent action of spicing, spices such as *Elettaria cardamomum*, *L. nobilis* and *M. fragrans* (Chung et al., 2006; Dadalioglu and Evrendilek, 2004). Fecal enterococci were eliminated progressively with reached a value of < 1.00 $\log_{10}$ cfu/g. This observation confirms the strong competitive effect of lactic acid bacteria on the rest of the endogenous flora as is observed in other fermentations (Spyropoulou et al., 2001). Aerobic spore formers were eliminated after 3rd days of ripening. Yeasts and moulds their numbers decreased during fermentation. This observation reflects their poor competitiveness in the presence of actively growing aciduric bacteria. According to Samelis and Metaxopoulos (1998), yeasts appeared to be the main causative agent of spoilage. The disappearance of spoilage organisms is favoured by the low a_w values and predominating LAB in fermentation processes that exerts an antagonistic action on contaminating flora (Drosinos et al., 2005; Soyer et al., 2005). Micrococcaceae showed a load of 2.32 $\log_{10}$ cfu/g that increased during ripening. The average number at the end fermentation followed was about 4.12 $\log_{10}$ cfu/g, this reflecting their poor competitiveness in the presence of actively growing aciduric bacteria (Palumbo and Smith, 1977). Finally, sulphite reducing clostridia, *Salmonella*, and *Listeria* spp. were not detected after the stuffing and during the whole process of fermentation and ripening of sausages. The results obtained here are in agreement with previous studies (Kalalou et al., 2004).

Table 3. Mean values (% ± SD) of physicochemical parameters of the fermented sausages.

Physicochemical analysis	Control		Final product	
	Mean	SD	Mean	SD
Physical analysis				
pH	5.80	0.12	5.06	0.05
aw	0.96	0.02	0.91	0.24
Chemical analysis				
Moisture	76.05	0.22	66.54	0.18
Fat	10.11	0.69	20.70	0.18
Protein	21.51	0.05	19.13	0.27
Ash	1.10	0.14	5.04	0.63

Table 4. Mean (± SD) values of biogenic amine contents of mixture spices and raw meat (mg/kg fresh matter) and of sausage (mg/kg dm).

Biogenic amine	Spices (mg/kg fresh matter)		Raw meat (mg/kg fresh matter)		Sausage (mg/kg dm)	
	Mean	SD	Mean	SD	Mean	SD
Tyramine	18.44	0.12	< 0.3	n.a	16.06	0.44
Phenylethylamine	3.2	0.15	nd		nd	
Putrescine	11.23	0.04	nd		9.32	0.23
Cadaverine	2.31	0.02	nd		23.42	0.05
Agmatine	10.42	0.28	nd		nd	
Spermidine	27.52	0.01	2.72	0.26	10.27	0.09
Spermine	18.55	0.14	22.75	0.04	52.05	0.13

nd., not detected; n.a, not applicable.

Physicochemical analysis

The results of the physicochemical analysis of the fermented sausages at the end of the ripening time are reported in Table 3. The values of pH were about 5.06 in the final product. typical of low acidity sausages, and this was the result of the classical trend of microbial growth in the fermented sausages, where LAB are increasing in numbers at the very beginning of the fermentations, producing acids and a decrease in the pH, followed in the phases of maturation by the activity of *micrococci* that are able to neutralize the acids produced (Comi et al., 2005). The a_w showed a constant decrease during the maturation reaching values of 0.92, with moisture of 66.54%. The final value of the protein content was around 20%, while the final fat content was 20.70%. This increased was due to the effect of dehydratation (Comi et al., 2005). The ash content reached values of 5.04% at the end of the maturation. A much higher ash content, compared to raw meat was possibly resulted from salt and others additives added (Visessanguan et al., 2005).

Contents of biogenic amines of raw materials are shown in Table 4. In meat, the only amines present in significant amounts were the physiological polyamines spermidine and spermine, which conforms with the high hygienic quality of meat used for sausage elaboration, as do the microbial counts. Other biogenic amines were found in the spices, considerable levels of tyramine and lower levels of phenylethylamine were detected, this result was according with those found by Latorre-Moratalla et al (2007). However, the final quantitative contribution of these spices to the total biogenic amine pool in the stuff-fed sausage was insignificant. On the other hand, the aerobic counts in spices was 3.32 log (CFU/g), the occurrence of biogenic amines (especially aromatic amines and cadaverine) in spices may be indicative of contamination with amino acid decarboxylase positive microorganisms. In this sense, spices might have been vehicles of potentially aminogenic microorganisms to meat or eventually amino acid decarboxylase enzymes, which might have contributed to biogenic amine accumulation during the subsequent fermentation and ripening process (Latorre-Moratalla et al., 2007). During sausage manufacture, contents of physiological polyamines did not show significant changes ($p > 0.05$). No influence of starter inoculation product (Table 4). These data are in agreement with the hypothesis that spermidine and spermine in meat products are of endogenous origin, not being formed by microbial activity (Latorre-Moratalla et al., 2007). By contrast, the main biogenic amines associated with bacterial activity in fermented meat products (tyramine, putrescine and cadaverine) were influenced by starter culture in a

different manner depending on the amine (Table 4). Cadaverine found in sausages, which is usually associated with lysine-decarboxylase activity of undesirable Gram-negative bacteria (Miguélez-Arrizado and Vidal-Carou, 2006). Tyramine production was associated by the lysine- and ornithine-decarboxylase activity of contaminant bacteria (Latorre-Moratalla et al., 2007). Tyramine and cadaverine have been reported as major biogenic amines in other fermented products (Bover-Cid et al., 2000b, 2001b). In the present study, histamine was not found in sausage, the same phenomenon has been observed with other fermented sausages (Hernàndez-Jover et al., 1997a; Bover-Cid et al., 2000b). The fact that bacteria with the capacity to decarboxylate the histidine are uncommon in meat (Paulsen and Bauer, 1997) and the good hygienic quality of raw meat could explain the absence of this biogenic amine in these products. The aromatic mono amine phenylethylamine either does not appear; the amounts of this biogenic amine found in other fermented sausages were also very low, and rarely higher than 50 mg/kg (Paulsen and Bauer, 1997). The levels of tyramine and putrescine were lower; this result may be explained by the pH value (5.06) of the final product. So, this pH was lower and can prevent the development of decarboxylase positive bacteria, which were present in the raw material (Gonzàlez-Fernàndez et al., 2003). In this sense, Eitenmiller et al. (1978) stated that most of the wild bacteria in fermented natural sausage have a high capacity to decarboxylate tyrosine. Regarding the effect of sugar on biogenic amine contents in fermented sausages, Bower-Cid et al. (2001b) found contents of tyramine and cadaverine significantly higher in sausages without sugar in their formulation. Nevertheless, the sugar concentrations recommended to reduce biogenic amine production were slightly lower than the amounts used in this work. Latorre-Moratalla et al. (2007) reported that a mild pH values and higher free amino acid contents in sausages during fermentation favour biogenic amine production by micro-organisms. Biogenic amines production in this study were lower than previous work (Bover-Cid et al., 2001b) and agree with results obtained by Latorre-Moratalla et al. (2007); the aminogenesis in spontaneously fermented sausage was much more important, even when the hygienic quality of raw materials was optimal in both cases. In this cited work, the temperature of fermentation was considerably higher (17°C) than in the present study (13°C), and this may suggest that, besides the hygiene of raw materials and formulation, temperature might be a technologically important parameter to control the aminogenic activity of spontaneous fermenting microorganisms.

Identification of the isolated lactic acid bacteria

A total of 300 strains were identified. The 251 strains having thick, short, straight or curved rod or coccoid-rod shaped cells, indicated in Table 5 as rods, were regarded as belonging to the genus *Lactobacillus*. Based on the taxonomic criteria of Axelsson (1998), the strains, according to the results of gas and ammonia production, were characterized as: (i) facultatively heterofermentative lactobacilli (*Lactobacillus plantarum*, *L. delbrueïckii*, *L. pentosus*, *L. curvatus*, *L. sakei*, *L. paracasei* subsp. *paracasei*, *L. rhamnosus*), 222 strains; (ii) obligately heterofermentative arginine-positive lactobacilli (*L. brevis*), 2 strains; (iii) obligately heterofermentative arginine-negative cocci or coccoidrods (*Leuconostoc* species), 1 strains; and (iii) homofermentative arginine-positive cocci (*Lactococcus lactis* subsp. *Lactis*), 26 strains.

As indicated in Table 3, *lactobacilli* were by far the most prevalent microorganisms isolated from MRS agar plates (89.24% of the total isolates). Others were *Leuconostocs* (0.4%) and *Lactococcus lactis* subsp. *lactis* (10.35%). A notable observation was the low presence of *L. curvatus*, *L. delbrueïckii, L. sakei, L. rhamnosus, L. pentosus*, *L. paracasei* subsp. *paracasei* and *L. brevis* (5.57, 4.78, 2.39, 2.39, 1.99, 1.19 and 0.79%, respectively) and the high presence of *L. plantarum* (70.11%) and *Lc. lactis* subsp. *lactis* (10.35%). *L. plantarum*, commonly used as starter cultures (Montel, 1996) was isolated from the fermented meat products. *L. lactis* subsp. *lactis* has already been isolated from fermented products by Rodriguez et al. (1995). This result is different from those of other researchers (Papamanoli et al., 2003; Samelis et al., 1994; Samelis et al., 1998). According to these findings the dominant species found in Greek dry fermented sausages were *L. curvatus*/*L. Sakei* and only a few isolates were assigned to *L. plantarum*. Our results agree with those obtained by Drosinos et al, (2005) for traditional Greek dry salami sausages.

Phenotypic characterization of the isolated lactic acid bacteria

The phenotypic characteristics of the isolates are presented in Tables 5 and 6. The most frequently isolated lactic acid bacteria from dry sausages processed with different technologies are *L. sakei*, *L. curvatus* and *L. plantarum* (Hammes, 1990). The majority of the above microorganisms were capable of growing at a temperature of 4°C except *L. delbrueïckii* but not at 45°C, whereas all strains were able to grow at temperatures of 10, 15 and 37°C except *L. delbrueïckii* which grown well only at 37°C.

Also, *L. rhamnosus* strains grew in 10% NaCl, but the growth of *L. plantarum* in 10% NaCl was variable. Only one strains of *L. sakei* was able to grow in 10% NaCl. *L. sakei* strains were less salt-tolerant than *L. rhamnosus* and *L. plantarum* Drosinos et al. (2005). The majority of *L. sakei*, *L. pentosus* strains could deaminate arginine, but no *L. curvatus* possessed this capacity, in accordance with the typical characteristic of the species Drosinos et al. (2005). Several *L. plantarum* strains were capable of growing at 45°C and could hydrolyze arginine. Other authors (Papamanoli et al., 2003; Samelis et al., 1994) have reported the isolation of *L. plantarum* strains

Table 5. Physiological and biochemical tests used for the identification of lactic acid bacteria isolated from naturally fermented sausages.

Identification	No. of isolates	Cell morphology	CO_2 from glucose	NH_3 from arginine	Growth at (°C)					Growth in NaCl (%)		Dextran production
					4	10	15	37	45	8	10	
L. Plantarum	176 (70.11)	R	0	80 (45.45)	152 (86.36)	155 (87.5)	155 (87.5)	176 (100)	22 (12.5)	71 (40.34)	63 (35.79)	170 (96.59)
L. delbrueïckii	12 (4.78)	R	0	6 (50)	0	0	2 (16.66)	12 (100)	8 (66.66)	9 (75)	2 (16.66)	0
L. sakei	6 (2.39)	R	0	6 (100)	2 (33.33)	6 (100)	6 (100)	6 (100)	0	5 (83.33)	1 (16.16)	0
L. pentosus	5 (1.99)	R	0	5 (100)	4 (80)	5 (100)	5 (100)	5 (100)	4 (80)	4 (80)	3 (75)	1 (20)
L. curvatus	14 (5.57)	R	0	2 (14.28)	10 (71.42)	14 (100)	14 (100)	14 (100)	7 (50)	7 (50)	2 (14.28)	0
L. rhamnosus	6 (2.39)	R	0	2 (33.33)	6 (100)	6 (100)	6 (100)	6 (100)	3 (50)	6 (100)	6 (100)	0
Lb. paracasei subsp. *paracasei*	3 (1.19)	R	0	0	3 (100)	3 (100)	3 (100)	3 (100)	1 (33.33)	3 (100)	0	0
L. brevis	2 (0.79)	R	2 (100)	2(100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0	0
Ln. mesenteroides subsp. *mesenteroides*	1 (0.39)	C/CB	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	0	1 (100)
Lc. lactis subsp. *lactis*	26 (10.35)	C	0	21 (80.76)	21 (80.76)	21 (80.76)	21 (80.76)	21 (80.76)	0	10 (38.46)	0	0

The numbers indicate the positive strains in each character and in the brackets is the percentage. R, rods; C/CB, cocci/coccobacilli. L., Lactobacillus; Ln., Leuconostoc; Lc., Lacstococcus.

Table 6. Sugar fermentation pattern of the isolated lactic acid bacteria.

Species	Acid production from												
	D-maltose	D-mannitol	D-melibiose	D-lactose	L-Arabinose	D-saccharose	D-raffinose	D-cellobiose	D-trehalose	D-ribose	D-sorbitol	D-Xylose	D-Galactose
L.Plantarum	176 (100)	170 (96.59)	176 (100)	154 (87.5)	112 (63.63)	176 (100)	87 (49.43)	176 (100)	176 (100)	176 (100)	154 (87.5)	4 (2.27)	87 (49.43)
L.delbrueïckii	12 (100)	0	0	12 (100)	0	12 (100)	0	2 (16.66)	16 (100)	4 (33.33)	0	0	4 (33.33)
L. sakei	2 (33.33)	0	6 (100)	1 (16.66)	0	6 (100)	0	6 (100)	6 (100)	6 (100)	0	6 (100)	6 (100)
L. pentosus	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	3 (60)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)
L. curvatus	14 (100)	1 (7.14)	1 (7.14)	14 (100)	0	12 (85.71)	1 (7.14)	12 (85.71)	13 (92.95)	14 (100)	0	0	1 (7.14)
L . rhamnosus	6 (100)	6 (100)	4 (66.66)	6 (100)	1 (16..66)	6 (100)	1 (16..66)	6 (100)	6 (100)	6 (100)	6 (100)	0	6 (100)
Lb. paracasei subsp. *paracasei*	3 (100)	3 (100)	0	3 (100)	0	3 (100)	0	2 (66.66)	3 (100)	3 (100)	3 (100)	0	3 (100)
L. brevis	2 (100)	0	2 (100)	2 (100)	2 (100)	2 (100)	0	2 (100)	2 (100)	1 (50)	0	2 (100)	2 (100)
Ln. mesenteroides subsp. *mesenteroides*	1 (100)	0	1 (100)	1 (100)	1 (100)	1(100)	1 (100)	0	1 (100)	1(100)	0	1 (100)	1 (100)
Lc. lactis subsp. lactis	26 (100)	21 (80.76)	20 (76.92)	26 (100)	14 (53.84)	26 (100)	1 (3.84)	26 (100)	26 (100)	26 (100)	0	12 (46.15)	26 (100)

The numbers indicate the positive strains in each character and in the brackets is the percentage.

capable of growing at that temperature, and also isolation of strains capable of hydrolysing arginine (Coppola et al., 1998). Such differences in several physiological and biochemical properties, like growth at 45°C and different salt concentrations might be strain dependant (Sanchez et al., 2000). Finally, *L. sakei* or *L. curvatus* strains did not produce dextran from sucrose and only 6 out of 176 *L. plantarum* strains did so. The isolates identified as *L. curvatus* and *L. sakei* did not ferment xylose, mannitol, sorbitol and raffinose. Moreover, the former species was melibiose negative and maltose-positive, whereas the latter species showed the opposite characteristics. These were in accordance with those of other workers who classified such isolates as *L. curvatus* and *L. sakei* (Samelis et al., 1994; Schillinger and Liicke, 1987). *L. curvatus* strains were saccharose- and trehalose-positive, arabinose-negative and a high percentage (47.6%) fermented cellobiose. Drosinos et al. (2005) also found isolates of *L. curvatus* to be saccharose-positive and a high percenttage of the strains were trehalose and cellobiose-positive. *L. curvatus* strains, which are saccharose-positive and trehalose-variable, have often been isolated from meats (Korkeäla and Mäkelä, 1989). The *L. sakei* strains did not ferment maltose and lactose, a tendency very common among *L. sakei* strains of meat origin (Samelis et al., 1994; Schillinger and Liicke, 1987). Also, the strains were trehalose and cellobiose-positive and arabinose-negative. Similar results were found by Drosinos et al. (2005) during the characterization of the microflora of naturally fermented sausages. *L. plantarum* strains were able to ferment all the common carbohydrates. Species of *L. paracasei* subsp. *paracasei*, *L. rhamnosus*, *L. brevis*, *L. mesenteroides* subsp. *mesenteroides*, *L. delbrueïckii* and *Lc. lactis* subsp. *lactis* were also detected. These species have been, occasionally, isolated from meat (Noonpakdee et al., 2003; Rodriguez et al., 1995; Samelis et al., 1994).

Conclusion

It is very important to note that sugar fermentation profiles obtained by API 50 CHL, apart from excellent to good or acceptable identification (identification of one strain with ID > 85 – 95%), gave several doubtful and low discrimination profiles (identification of two or more strains with ID < 80%). Therefore, a clear species or subspecies assignment was not possible and the identification of the doubtful and low discrimination profiles was performed on the basis of other tests, such as cell morphology, growth at various temperatures, etc. Identification of lactic acid bacteria with large numbers of tests is tedious, time consuming and the results obtained, mostly by media with colorimetric changes, the evaluation of which is always subjective, are often difficult to interpret. Furthermore, determination of carbohydrate fermentations is not very convenient and may be misleading (Shaw and Harding, 1984). It is unsatisfactory to consider only carbohydrate fermentation patterns because variable fermentations often occur (Champomier et al., 1987). Andrigetto et al. (1998) found that some strains were wrongly assigned to species or subspecies, on the basis of sugar fermentation profiles obtained by API 50 CHL, which were totally different from those, obtained with other genotypic methods.

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