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Short communication

Amino acid decarboxylase capability of microorganisms isolated in Spanish fermented meat products

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Abstract

Enterobacteria, lactic acid bacteria (LAB) and Gram-positive cocci were isolated from Spanish meat products. The most frequent species in the meat products studied were identified as *Lactobacillus sake*, *Lactobacillus plantarum* and *Lactobacillus curvatus* from De Man–Rogosa–Sharpe agar; *Staphylococcus xylosus*, *Staphylococcus saprophyticus* and *Micrococcus varians* from mannitol salt phenol-red agar; and *Hafnia alvei*, *Escherichia coli*, *Pseudomonas fluorescens*, *Enterobacter amnigenes* and *Enterobacter aerogenes* from violet red bile dextrose agar. The amino acid decarboxylase activity of the microorganisms isolated was assayed. Enterobacteria had higher amino acid decarboxylase activity than the other groups. LAB did not show any significant amino acid decarboxylase capability in this study. © 1998 Elsevier Science B.V.

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1. Introduction

In naturally fermented sausages the microflora varies, and so the biogenic amine content is variable. In fermented meat products, tyramine and histamine levels increase during the fermentation process (Maijala and Eerola, 1993). Lactic acid bacteria (LAB) are the fermenting microorganisms most frequently used in the meat industry. Some of these LAB have been identified as amino acid decarboxylase-positive and so capable of producing biogenic amines, e.g., *Lactobacillus buchneri*, *Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus hilgardii*, *Carnobacterium piscicola*, *Carnobacterium divergens* (Edwards et al., 1987; Tschabrun et al., 1990; Maijala and Eerola, 1993; Maijala et al., 1993).

Some spoiling microorganisms which grow on meat products, such as *Pseudomonas*, staphylococci, micrococci and enterococci, are histidine decarboxylase-positive, and possibly can form biogenic amines in meat products (Tiecco et al., 1986). Butturini et al. (1995) have also detected biogenic amines in salami produced by enterobacteria, especially *Escherichia coli*, *Enterobacter agglomerans*, *Citrobacter freundii*, *Proteus vulgaris*, *Klebsiella*

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ozaenae; and, less often, LAB, *Leuconostoc lactis*, *Lactobacillus plantarum*, *L. brevis*, *Lactobacillus fermentum*, *Lactococcus lactis*.

As certain biogenic amines have a negative effect on health and, on the other hand, the level of biogenic amines can, in some cases, be used as an indicator of quality in meat products (Halász et al., 1994), research should be undertaken in order to determine the relationship between microbial characteristics and the capability of these microorganisms to produce biogenic amines in meat products.

2. Material and methods

2.1. Samples

Spanish fermented meat sausages were purchased in the market. The types of sausages were salchichón, fuet and chorizo. Twelve samples of each type were analyzed. Each analysis was performed three times.

2.2. Microbiological analysis

Ten grams of sample were aseptically taken from the centre of the sausage, placed in a sterile stomacher bag and homogenized for 2 min in tryptone water (Merck, Darmstadt, Germany), using a Stomacher Lab-Blender 400 (Seward, London, UK). A series of decimal dilutions was prepared and inoculated in duplicate on De Man–Rogosa–Sharpe (MRS) agar (Merck), mannitol salt phenol-red (MSPR) agar (Merck) and violet red bile dextrose (VRBD) agar (Merck) incubated at 30°C for 48–72 h.

2.3. Identification of bacterial isolates

Between five to ten representative colonies of each pure culture of LAB isolated on MRS agar were examined for Gram reaction, morphology and catalase production. Biochemical tests for carbohydrate fermentation and esculin hydrolysis were performed with the API 50 CHL system (Bio Mèrieux, Marcy l'Etoile, France) at 30°C for 48 h. MRS broth, with inverted vials and citrate omitted, was used at 30°C for 5 days for CO_2 production from glucose. Growth at 45°C and 15°C was studied for 5 days in MRS broth. Arginine hydrolysis was detected by Nessler's reagent after incubation for 5 days at 30°C in modified MRS broth (Döring et al., 1988).

Five to ten representative colonies isolated from VRBD agar and MSPR agar were identified for each sample. The oxydase test and the API 20E system (Bio Mèrieux) were used for enterobacteria identification. Colonies grown on MSPR were transferred to agar medium P [tryptone 10 g/l (Difco, Detroit, MI, USA); yeast extract 5 g/l (Difco); NaCl 5 g/l (Panreac, Motplet and Esteban, Barcelona, Spain); glucose 1 g/l (Panreac); agar 15 g/l (Pronadisa, Hispanlab, Madrid, Spain)] and identified using the API STAPH system (Bio Mèrieux) for micrococci and staphylococci.

2.4. Amino acid decarboxylase activity

Media containing 40 g/l of plate count agar and 0.8 g of the corresponding amino acid were used. Preparation: 0.1 g yeast extract (Difco); 0.2 g tryptone (Difco); 0.4 g glucose (Panreac); 20 ml water; 0.8 g of the corresponding amino acid (L-lysine, Sigma, Sigma–Aldrich, Madrid, Spain; L-tyrosine, Sigma; L-histidine, Sigma; L-ornithine, Sigma); 0.8 ml bromocresol purple (Panreac) or bromothymol blue were added. The pH level was adjusted to 5.5 and afterwards the agar (0.6 g) was added to the medium. The same kind of medium but without amino acids was used in the control by the most probable number counts MPN series with the same number of tubes as with amino acid. The change from a yellow to a purple colour in the cultures was considered as a positive result.

3. Results and discussion

Table 1 shows the bacterial isolates from the dry sausages studied. As seen highest counts were observed for LAB and the lowest for enterobacteria.

In the studies performed to detect the amino acid decarboxylase capability of the bacteria isolated, 36 isolates of each microorganism identified were included (Table 2). *Enterobacter aerogenes* was histidine decarboxylase-positive in all the 36 assays performed; *E. coli* in 88% of the cases; *Staphylococcus xylosus* in 76%; *L. curvatus* and *Hafnia alvei* decarboxylated histidine only in 62% of the cases.

Table 1
Bacterial isolates from chorizo, fuet and salchichón samples and species identified

Meat	MSPR[a] agar		MRS[b] agar		VRBD[c] agar	
product	Species identified	C.f.u./g	Species identified	C.f.u./g	Species identified	C.f.u./g
Chorizo	(8)[d] *S. xylosus*	3.1×10^{3}–1.6×10^{5}	(8) *L. sake*	6.3×10^{3}–6.1×10^{8}	(3) *E. coli*	10
	(4) *S. xylosus*/*M. varians*	2.9×10^{4}–2.4×10^{5}	(3) *L. sake*/*L. curvatus*	7.0×10^{7}–6.6×10^{8}	(1) *H. alvei*/*E. coli*	1.4×10^{4}
			(1) *L. curvatus*	4.4×10^{8}		
Fuet	(12) *S. xylosus*	1.6×10^{4}–2.6×10^{6}	(8) *L. plantarum*	1.8×10^{5}–3.6×10^{8}	(5) *P. fluoresscens*	7.7×10^{2}–3.5×10^{3}
			(2) *L. plantarum*/*L. sake*	1.6×10^{8}–6.0×10^{8}	(3) *S. liquefaciens*	1.6×10^{3}–4.0×10^{3}
			(2) *L. sake*	4.5×10^{7}–8.6×10^{7}	(2) *H. alvei*	1.9×10^{3}–2.0×10^{3}
Salchichón	(9) *S. xylosus*	9.0×10^{2}–1.6×10^{5}	(8) *L. plantarum*	1.5×10^{4}–1.3×10^{8}	(2) *E. aerogenes*	10–35
	(3) *S. xylosus*/*saprophyticus*	3.4×10^{3}–1.3×10^{5}	(4) *L. plantarum*/*L. sake*	2.1×10^{6}–2.3×10^{7}	(2) *Enterobacter amnigenes*	10
					(1) *E amnigenes*/*H. alvei*	1.8×10^{2}

[a] Mannitol salt phenol-red; [b] De Man–Rogosa–Sharpe; [c] violet red bile dextrose; [d] numbers between parenthesis are the samples with positive identification of the species mentioned.

The remaining microorganisms showed hardly any histidine decarboxylase activity. Tyrosine was decarboxylated in 100% of the assays by *Pseudomonas fluorescens*, *Serratia liquefaciens* and *Staphylococcus saprophyticus*. Ornithine was decarboxylated in 100% of the assays by *E. aerogenes*, *E. coli*, *P. fluorescens*, *S. liquefaciens* and *Micrococcus varians*. Lysine was decarboxylated by *E. aerogenes*, *P. fluorescens*, *E. coli* and *S. liquefaciens* in 100% of the assays.

These results do not agree with those obtained by von Beutling (1993), who detected that *E. coli* and *Serratia* spp. did not decarboxylate tyrosine, whereas *Lactococcus* did. In studies on fermented meat products, Maijala et al. (1993) identified *H. alvei* and *S. liquefaciens* with histidine decarboxylase activity. These strains are well known as traditional amine producers in fish and dairy products (Maijala et al., 1993). Moreover, histidine decarboxylase activity has been detected in *P. fluorescens* by Tiecco et al. (1986). Normally, the enterobacteria have been linked with the formation of biogenic amines in fish (Taylor et al., 1989) and in meat in conjunction with *Pseudomonas* spp. and *Carnobacteria* spp. (Slemr, 1981; Edwards et al., 1987). In the present work LAB did not decarboxylate amino acids except for *L. curvatus*, which showed a slight histidine decarboxylase activity (results not shown). This can be seen as positive from the viewpoint of their use as starters. However, Butturini et al. (1995) indicate that cadaverine and putrescine are produced in fermented sausage without detectable presence of enterobac-

Table 2
Amino acid decarboxylase capability of microorganisms isolated in meat products

	Amino acids			
	Histidine	Tyrosine	Ornithine	Lysine
L. sake	−	−	−	−
L. plantarum	−	−	−	−
L. curvatus	−	−	−	−
E. aerogenes	+	−	+	+
Enterobacter amnigenes	ND	ND	ND	ND
E. coli	+ / −	−	+	+
H. alvei	−	−	−	−
P. fluorescens	−	+	+	+
S. liquefaciens	−	+	+	+
M. varians	−	−	+	−
S. xylosus	+ / −	−	−	−
S. saprophyticus	−	+	−	−

Data are the average of 36 assays; ND, not determined; +, 100% positive; + / −, 70% positive; −, less than 70% positive.

teria, but with a flora totally composed of LAB. Moreover, *L. curvatus* has been referred to as an amine producing bacteria in meat products (Edwards et al., 1987; Tschabrun et al., 1990). *L. plantarum* has been identified as a strain capable of producing histamine in Swiss cheese (Edwards and Sandine, 1981; Stratton et al., 1991). The production of biogenic amines in vitro has been demonstrated with *L. curvatus*, *Staphylococcus carnosus* and *M. varians*, but not with *Lactobacillus sake* (Chander et al., 1989; Straub et al., 1995), all of which are typical of meat products. *Micrococcus* spp. and *Staphylococcus* spp. have been identified as histidine decarboxylase-positive in fermented sausage by Tiecco et al. (1986), who established a close relationship between bacterial and amine content because both parameters undergo a parallel evolution throughout the sausage ripening process.

The great variety of microbial flora counts in the meat products studied, together with the differences obtained in the results of the tests of their amino acid decarboxylase capability, makes it hard to establish a close relationship between microbiological quality and the possible presence of biogenic amines.

However, enterobacteria seem to be stronger producers of biogenic amines than LAB, micrococci and staphylococci according to the present results.

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