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Distribution of Aminogenic Activity among Potential Autochthonous Starter Cultures for Dry Fermented Sausages

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ABSTRACT

Any bacterial strain to be used as starter culture should have suitable characteristics, including a lack of amino acid decarboxylase activity. In this study, the decarboxylase activity of 76 bacterial strains, including lactic acid bacteria and gram-positive, catalase-positive cocci, was investigated. These strains were previously isolated from European traditional fermented sausages to develop autochthonous starter cultures. Of all the strains tested, 48% of the lactic acid bacteria strains and 13% of gram-positive, catalase-positive cocci decarboxylated one or more amino acids. Aminogenic potential was strain dependent, although some species had a higher proportion of aminogenic strains than did others. Thus, all *Lactobacillus curvatus* strains and 70% of *Lactobacillus brevis* strains had the capacity to produce tyramine and β-phenylethylamine. Some strains also produced other aromatic amines, such as tryptamine and the diamines putrescine and cadaverine. All the enterococcal strains tested were decarboxylase positive, producing high amounts of tyramine and considerable amounts of β-phenylethylamine. None of the staphylococcal strains had tyrosine-decarboxylase activity, but some produced other amines. From the aminogenic point of view, *Lactobacillus plantarum, Lactobacillus sakei,* and *Staphylococcus xylosus* strains would be the most suitable for use as autochthonous starter cultures for traditional fermented sausages.

Fermentation of traditional meat products usually relies on indigenous microflora and reflects the diversity of formulation and the manufacturing practices *(39)*. Lactic acid bacteria (LAB) and gram-positive, catalase-positive cocci (GCC^{+}) are the two bacterial groups that are used most often as fermentative microbiota in traditional sausages. LAB are usually the main fermenters (10^7 to 10^9 CFU/g) and are responsible for the typical acidification, with the consequent inhibition of spoilage and pathogenic bacteria *(2, 39)*. The species most commonly identified in these fermented meat products are *Lactobacillus sakei, Lactobacillus curvatus,* and *Lactobacillus plantarum (4, 32, 34)*. Enterococci, mainly *Enterococcus faecium,* also may constitute a large part of the microbiota of traditional fermented sausages, with levels close to 10^6 CFU/g *(2, 29, 39),* because these meat products have a relatively high pH and provide ideal conditions for survival and growth of these organisms *(18)*.

GCC^{+} are the second major bacterial group (10^6 to 10^8 CFU/g) in these sausages and contribute mainly to the color and development of flavor. *Staphylococcus xylosus, Staphylococcus saprophyticus,* and *Staphylococcus equorum* are the most common GCC^{+} species identified *(2, 36, 39)*. In some traditional fermented sausages, GCC^{+} levels, especially those of staphylococci, can be similar to or even greater than those of LAB. This feature differentiates these sausages from industrial products and may account for their appreciated sensory qualities *(2)*. However, indigenous microbiota and traditional manufacturing techniques do not always ensure acceptable hygienic quality of fermented sausages.

Biogenic amines are formed by the decarboxylation of their precursor amino acids by certain bacteria, including enterobacteria and enterococci but also lactobacilli and GCC^{+} *(38, 43)*. Large amounts of biogenic amines can accumulate in traditional fermented sausages *(20)*. The occurrence of large amounts of these substances is of concern in terms of the hygienic quality and safety of these products *(16, 38, 43)*. Therefore, control measures to minimize biogenic amine production are needed. Selected starter cultures have been used in experimental (pilot plant) and industrial production with variable success.

Knowledge of the indigenous microbiota usually present in traditional fermented sausages is essential for

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TABLE 1. *Occurrence of amino acid decarboxylase–positive strains among lactic acid bacteria and coagulase-negative staphylococci tested*

Species	No. of strains positive	No. of strains tested
Lactobacillus brevis	7	10
L. curvatus	4	4
L. fermentum	0	1
L. plantarum	0	3
L. sakei	0	15
Leuconostoc carnosum	2	2
L. mesenteroides	1	2
Weissella cibaria	0	1
Enterococcus faecium	7	7
E. hirae	1	1
Staphylococcus carnosus	1	1
S. epidermidis	1	2
S. equorum	0	4
S. haemolyticus	0	1
S. pasteuri	1	1
S. saprophyticus	0	2
S. simulans	0	1
S. succinus	0	1
S. xylosus	0	15
S. warneri	1	2

improving the hygienic quality and safety of these products. Specific strains isolated from the traditional products and adapted to the ecology of traditional fermentation (i.e., low temperatures) could be used as autochthonous starter cultures, thereby maintaining the typical sensory qualities of these sausages *(4, 40, 44)*. To reduce biogenic amine accumulation, the autochthonous starter culture must not be able to produce biogenic amines.

The main objective of the European project Tradisausage *(42)* was to improve the quality and safety of European traditional fermented sausages. In the frame of this project, the present study was conducted (i) to determine the amino acid decarboxylase activity of several strains of the dominant fermentative bacteria (LAB and GCC^+) isolated from traditional dry fermented sausages and (ii) to identify the best candidates for possible further use as autochthonous starter cultures to minimize the risk of biogenic amine accumulation in this type of food product.

MATERIALS AND METHODS

Bacterial strains. Decarboxylase activity was assessed for 76 strains of LAB (including lactobacilli, enterococci, *Leuconostoc,* and *Weissella*) and staphylococci, all isolated from several types of traditional fermented sausages. Table 1 summarizes the number of strains of each species studied. The strains examined were provided by the partners involved in the Tradisausage project (France, Spain, Portugal, Italy, Greece, and Slovakia) *(42),* who isolated and identified these strains by molecular methods *(2, 3, 14, 33, 45)*.

Determination of biogenic amine–forming capacity. To promote enzyme induction before the decarboxylase test *(5),* strains were subcultured four times at 30°C for 24 h in de Man Rogosa Sharpe broth (Oxoid, Cambridge, England) for LAB and in tryptic soy broth (Oxoid) for staphylococci. Both media contained 0.1% concentrations of the corresponding amino acid precursor (all from Merck, Darmstadt, Germany): L-tyrosine free base, L-histidine monochlorohydrate, L-ornithine monochlorohydrate, L-tryptophan, L-lysine monochlorohydrate, and L-phenylalanine. Broth cultures of all bacterial strains were then placed in a decarboxylase medium containing the precursor amino acids (0.5%), pyridoxal-5′-phosphate (Merck), and growing factors as previously described by Bover-Cid and Holzapfel *(5)* and incubated aerobically at 30°C for 4 days. The type and amount of biogenic amines produced were determined by high-performance liquid chromatography with postcolumn derivatization with *ortho*-phtaldialdehyde and fluorimetric detection following the procedure described by Hernández-Jover et al. *(17)*.

RESULTS AND DISCUSSION

Table 1 shows the amino acid decarboxylase–positive strains for all the species tested. Of the LAB strains, 48% produced one or more biogenic amines (11 *Lactobacillus,* 8 *Enterococcus,* and 3 *Leuconostoc* strains). Among lactobacilli, 100% of the *L. curvatus* strains and 70% of the *L. brevis* strains were biogenic amine producers. In contrast, none of the *L. sakei, L. fermentum,* or *L. plantarum* strains had amino acid decarboxylase activity. All *Enterococcus* strains (seven *E. faecium* and one *E. hirae*) were amino acid decarboxylase positive, as were three of the *Leuconostoc* strains tested (two *L. carnosum* and one *L. mesenteroides*). Only 13% of the *Staphylococcus* strains tested were amino acid decarboxylase positive.

The amino acid decarboxylase activities of LAB isolated from traditional fermented sausages are consistent with the results reported for other LAB isolated from various types of sausages *(3, 6, 12, 25, 26, 35, 37)*. Phenotypically, *L. brevis* and *L. curvatus* strains are usually associated with tyramine production in fermented meat products and in some cases with production of phenylethylamine, tryptamine, putrescine, and cadaverine *(3, 5)*. In contrast, *L. plantarum* and *L. sakei* strains are more frequently reported as nonaminogenic *(3, 6)*. Genes coding for tyrosine decarboxylase (*tdc* genes) have been identified in several strains of *L. brevis* (GenBank accession no. EF371897.1, EF371896.1, and AF446085.5) and *L. curvatus* (EF371895.1, AJ871286.1, AF354231.1, and AB086652.1). The partial sequence of *tdc* genes also has been described for an *L. plantarum* strain (EF178285.1). To our knowledge, the presence of *tdc* genes has not been described to date in any *L. sakei* strain. However, in *L. sakei* strain 23K, molecular techniques have confirmed that the absence of the *tdc* gene in its genome *(8)*.

Some studies have confirmed the ability of some *Leuconostoc* strains to form biogenic amines *(9, 15, 27),* while other *Leuconostoc* strains did not *(3, 5)*. In contrast, enterococci are extensively reported to have aminogenic potential, mainly as tyramine and phenylethylamine producers *(6, 25, 38)*. The *tdc* gene has been described in several strains of *Enterococcus faecalis* (AF371893, AE016830, and AF354231) *(10), E. hirae* (AY303667) *(11),* and *E. faecium* (EF371894 and AJ83966) *(21)*. In contrast to the tyrosine specificity of *L. brevis* decarboxylase *(28),* enterococci are nonselective for tyrosine and can

TABLE 2. *Quantification of biogenic amine production by decarboxylase-positive lactic acid bacteria and coagulase-negative staphylococci*

			Amine production (mg/liter)[a]				
Genus	Species	Strain	TY	PHE	TRP	PU	CA
Lactobacillus	*brevis*	LQC 0524	169.47	11.28			
		LQC 0528	148.74	6.84			
		LQC 0531	138.51	6.22			
		LQC 0537	142.62	8.84			
		LQC 0581	168.36	10.68			
		LQC 0588	148.35	6.46			
		LQC 0591	158.07	10.51			
	curvatus	IS02/F25	106.07	38.1			
		IS02/F26	76.55	15.06			
		P05/4	2,198.8	154.11		1,616.34	20.17
		P05/119	2,561.7	175.51		1,673.6	20.79
Leuconostoc	*carnosum*	S02/2M/1B	2,137.04	470.1			
		S02/F12	2,086.48	498.55			
	mesenteroides	LQC 0538	161.8	8.9			
Enterococcus	*faecium*	S02F11	2,867.4	535.5	8.5		
		S02/211	1,466.81	720.39	12.11		
		S02/223	1,006.47	555.14	11.87		
		S04 1M/2	2,429.68	440.67	8.83		
		S03 M1/2	2,133.22	674.13	13.02		
		S03F11	1,865.25	505.22	9.84		
		S01M122	2,227	578.26	9.37		
	hirae	IS02/Z30	159.8	79.6			
Staphylococcus	*carnosus*	P06/8		161.1	20.2		
	epidermidis	IS02/Z16				8.8	
	pasteuri	IS02/M5				227.3	8.1
	warneri	CTC6010				427.5	137.5

[a] Biogenic amines produced by each strain were analyzed in duplicate, and the relative standard deviation was always below 5%.

decarboxylate phenylalanine *(21)*. This finding is in agreement with the high frequency of simultaneous production of tyramine and phenylethylamine by enterococcal strains.

Staphylococcus species usually are described as weak or negative for decarboxylase activity *(6, 25, 36)*. Martín et al. *(23)* found this activity in only 35 of 240 strains, including strains of *S. xylosus, S. warneri, S. epidermidis,* and *S. carnosus*. Martuscelli et al. *(24)* reported that 50% of the *S. xylosus* strains tested were only weak producers of biogenic amines. However, some researchers have described staphylococci as having a remarkable potential to form biogenic amines *(26, 35, 37)*. The genetic potential for the tyrosine decarboxylase enzyme has been partially sequenced in an *S. epidermidis* strain (EF371899) and *S. xylosus (41)*.

In addition to determining whether various bacteria produce biogenic amines, the level of such production is also of interest. Table 2 shows the quantitative results for biogenic amine accumulation in the fermenting broth by the amine-positive strains. All LAB strains formed tyramine and β-phenylethylamine; the strongest tyrosine decarboxylase species were *E. faecium, L. carnosum,* and two strains of *L. curvatus,* all of which produced levels higher than 2,000 mg/liter in most cases. All of these strains also showed the capacity to produce moderate amounts of β-phenylethylamine (up to 1,000 mg/liter). In contrast, all strains of *L. brevis* and some of *L. curvatus* produced at least 10-fold lower amounts of tyramine and β-phenylethylamine. Decarboxylase-positive species of staphylococci did not produce tyramine. Depending on the species, these strains produced β-phenylethylamine, tryptamine, putrescine, and cadaverine. Usually the production of β-phenylethylamine and tryptamine is associated with high occurrence of tyramine *(36)*, but for *S. carnosus* the production of these amines was not related to that of tyramine. Although there was not a general trend, other authors also found this particular profile of amines produced by *S. carnosus (1, 12)*. *E. faecium* strains also produced low amounts of tryptamine, but this finding is consistent with the presence of tryptamine in fermented sausages when there are high amounts of tyramine. Putrescine and cadaverine production was less extensive; only two strains of *L. curvatus* and one of *Staphylococcus pasteuri* and *S. warneri* produced these diamines, especially putrescine (Table 2). In the present study, none of the species tested produced histamine. Histidine decarboxylase activity seems to be limited to some specific strains of contaminant species *(22, 30, 41)*. The results of the present work agree with other published data on decarboxylase activity of *Lactobacillus (3, 6, 7, 12, 31), Leuconostoc (31), Enterococcus (6, 13, 21),* and *Staphylococcus (23, 25)* strains found in fermented sausages.

On the basis of these results regarding biogenic amine production, enterococci and some strains of *Lactobacillus* usually found in dry fermented sausages (e.g., *L. curvatus*) would not be suitable candidates for starter cultures for traditional fermented sausages. In contrast, *L. sakei* and *L. plantarum* strains (among the LAB) and *S. xylosus* and *S. equorum* (among the GCC^{+}) would be the most appropriate candidates to be used as autochthonous starters. However, to maintain the sensory properties of traditional sausages, the use of more complex mixed starter cultures than those used in industrial procedures would be desirable. For this purpose, the contribution of other weak amine-producing bacteria, such as *L. brevis* or some strains of staphylococci, could be considered. *L. curvatus* also could be used, but the heterogeneous distribution of aminogenic potential among strains of this species confirms that amino acid decarboxylase activity is a strain-dependent property. Thus, the amino acid decarboxylase activity of any strain intended to be used as a starter culture must be tested case by case. The behavior of the selected strain(s) also must be assessed in the real product under the actual processing conditions. This was the aim of further studies carried out within the frame of the European Tradisausage project *(19, 40, 42)*.

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