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Strains of *Staphylococcus* and *Bacillus* isolated from traditional sausages as producers of biogenic amines

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Histidine, lysine, ornithine, and tyrosine decarboxylase activities were tested in 38 strains of *Staphylococcus* (15 of *S. equorum*, 11 of *S. epidermidis*, 7 of *S. saprophyticus*, and 5 of *S. pasteuri*) and 19 strains of *Bacillus* (13 of *B. subtilis* and 6 of *B. amyloliquefaciens*) isolated from two Spanish traditional sausage varieties. The four decarboxylase activities were present in most of the strains studied, but some variability was observed between strains within each microbial species. Accumulation of putrescine and cadaverine was assessed in the culture media of the strains that displayed ornithine and lysine decarboxylase activities. The aminogenic potential of the strains was low, with amounts accumulated lower than 25 mg/L for the putrescine and than 5 mg/L for the cadaverine, with the exception of a strain of *S. equorum* that produced 1415 mg/L of putrescine, and of a strain of *S. epidermidis* that accumulated 977 mg/L of putrescine and 36 mg/L of cadaverine.

Keywords: decarboxylase activities, biogenic amines, *Staphylococcus, Bacillus*, putrescine, cadaverine, traditional sausages

INTRODUCTION

Biogenic amines are basic nitrogen compounds usually formed by decarboxylation of precursor amino acids (Janz et al., 1983; Halász et al., 1994; Silla Santos, 1996).

Formation of biogenic amines in foods is important for health and also for unfavorable effects on flavor (Suzzi and Gardini, 2003). Biogenic amines affect blood pressure, and excessive quantities in food can trigger migraines, gastric and intestinal problems, and allergic responses in sensitive people (Smith, 1980; Taylor, 1985; Stratton et al., 1991). These substances are especially dangerous in people being treated with monoaminooxidase enzyme inhibitors (Stratton et al., 1991).

During ripening of meat products, the proteins undergo degradation processes; large peptides are first generated and then degraded into oligopeptides, and these are in turn degraded to free amino acids. The free amino acids are then catabolized, giving rise to different compounds such as ammonia, α-ketoacids, methylketones, and amines.

In meat products, formation of biogenic amines is largely associated with the activity of microorganisms present in meat (Ten Brink et al., 1990; Shalaby, 1996; Paulsen and Bauer, 1997). Ripening of sausages provides conditions that are very favorable for the production of biogenic amines, due to the active growth of microbial populations, acidification, and proteolysis.

Different measures have been taken with the aim of preventing or minimizing formation of biogenic amines during the manufacture of raw-cured sausages, such as improved hygiene in production plants, the use of starter cultures formed by lactic acid bacteria with acidifying capacity, and the use of certain preservatives (Buncic et al., 1993; Maijala et al., 1993; Bover-Cid et al., 2000a,b; Suzzi and Gardini, 2003; Komprda et al., 2004; Lu et al., 2010). Although such practices usually reduce the production of biogenic amines, they do not totally prevent the production, and moreover, the increased proteolysis that results from the use of starter cultures may actually increase the availability of amino acids precursors.

Complete inhibition of biogenic amine formation during production of sausages, without any adverse effects, is desirable. However, production of biogenic amines is an extremely complex phenomenon that depends on several variables such as the growth kinetics of the microorganisms and their proteolytic and decarboxylase activities. In order to design strategies for specific inhibition of the production of these compounds, it is essential to obtain information about the potential production of biogenic amines by the microorganisms present in fermented meat products.

Of all the microbial groups present during the fermentation/ripening of the raw-cured sausages, the Enterobacteriaceae and the lactic acid bacteria have been abundantly studied as producers of biogenic amines. Information in the literature about the ability of the *Staphylococcus* species to produce biogenic amines is more reduced (Masson et al., 1996; Silla Santos, 1998; Martín et al., 2006; Drosinos et al., 2007; Bonomo et al., 2009; Even et al., 2010), and studies on the production of biogenic amines by *Bacillus* species are practically inexistent.

The objective of this research was to investigate the decarboxylase activity and the ability to produce biogenic amines "*in vitro*" by the species of *Staphylococcus* and *Bacillus* isolated from two Spanish traditional sausage varieties, with the aim of to elucidate the role of the microorganisms belonging to these two genera in the production of biogenic amines during the manufacture of the fermented and ripened sausages.

MATERIALS AND METHODS

BACTERIAL STRAIN IDENTIFICATION AND MOLECULAR TYPING

In this study, 38 strains of *Staphylococcus* (15 of *S. equorum*, 11 of *S. epidermidis*, 7 of *S. saprophyticus*, and 5 of *S. pasteuri*) and

19 strains of *Bacillus* (13 of *B. subtilis* and 6 of *B. amyloliquefaciens*) were used. The strains were isolated from 20 units of Androlla sausage and from 15 units of Botillo sausage (two Spanish traditional sausage varieties) at the end of the manufacturing process. Manufacture process and features of these two sausages have been previously described (Lorenzo et al., 2000). The strains were initially identified by classical methods in previous researches (García Fontán et al., 2007a,b) and their identity was confirmed prior carrying out the present work by sequentiation of the 16S rRNA gene, comparing the obtained sequences with those available in the database GenBank (National Center for Biotechnology Information, Bethesda, MD, USA).

Strains were molecular typed by $(GTG)_5$-PCR fingerprinting techniques. Genomic DNA extracted from each strain was subjected to rep-PCR analysis using the single oligonucleotide primer $(GTG)_5$ (Iacumin et al., 2006). Reactions were carried out in a final volume of 25 μL containing 12.5 μL of 2× ReddyMix 1.5 mM $MgCl_2$ (ABgene, Epsom, UK), 2 μL of extracted DNA, and 1 μM of $(GTG)_5$ primer. Amplifications were performed in a MyCycler thermal cycler (Bio-Rad, Hercules, USA). Initial denaturation (95°C, 2 min) was followed by 31 cycles of denaturation at 94°C for 3 s, a step at 92°C for 30 s, primer annealing at 40°C for 1 min and extension at 65°C for 8 min. The last cycle was followed by the final single extension step (65°C, 8 min).

Amplicons were separated by electrophoresis in a 1.5% agarose gel in buffer TBE 1× at 75 V for 2 h. After the run, gels were stained with ethidium bromide 1 μg/mL (Sigma-Aldrich, St. Louis, USA) for 30 min. The resulting fingerprints were visualized under UV light and digitally captured using the imaging system Gel Doc XR+ (Bio-Rad, Hercules, USA) and analyzed with the Quantity One software (Bio-Rad, Hercules, USA).

Strains were stored at −80°C in BHI broth (Oxoid Ltd., Basingstoke, Hampshire, UK), with 20% glycerol as a cryoprotective agent. Before use, the strains were reactivated by incubation in BHI broth at 37°C.

PREPARATION OF INOCULA

In order to prepare the inocula used in the quantitative analysis, firstly a correlation between the log CFU/mL and the Optical Density (at 650 nm) of the cultures was established for each strain by determining throughout the growth the O.D. and the log CFU/mL by plate counting in BHI agar (OXOID).

Samples of BHI broth cultures were collected after 24 h of incubation, the O.D. was measured (in order to calculate the number of CFU/mL), the cultures were centrifuged at 12000 × *g* and the cells were washed by resuspension in a solution of 0.85% NaCl and centrifugation at 12000 × *g* (three times). Finally, the cells were suspended in the 0.85% NaCl solution to provide inocula containing 10^9 CFU/mL.

PRELIMINARY QUALITATIVE TESTS FOR BIOGENIC AMINE PRODUCTION

As a preliminary test of the capacity of the bacterial strains to produce biogenic amines, the method described by Joosten and Northolt (1987) was used. The culture medium used contained tryptone (0.5%), yeast extract (0.5%), NaCl (0.5%), glucose (0.1%), Tween 80 (0.05%), $MgSO_4$ $7H_2O$ (0.02%), $CaCO_3$ (0.01%), $MnSO_4$ $4H_2O$ (0.005%), $FeSO_4$ $7H_2O$ (0.004%), bacteriological agar (2%), and purple bromocresol (0.006%) as pH indicator. The precursor amino acids of each biogenic amine (histidine, lysine, ornithine, and tyrosine) were added individually to the culture medium to a final concentration of 2%. The final pH was adjusted to 5.5 ± 0.1, the medium was sterilized and distributed in Petri dishes. Plates of the culture medium containing each one of the precursor amino acids were streaked, in order to obtain individual colonies, with each bacterial strain. The plates were incubated at 37°C and examined after 12, 24, 48, 72, and 120 h of incubation; a positive result was manifested by the appearance of a purple halo around the colonies.

QUANTITATIVE ANALYSIS OF THE BIOGENIC AMINES PRODUCED BY THE BACTERIAL STRAINS

In a previous study (Lorenzo et al., 2008), the different biogenic amines were quantified in the sausage units from which the microbial strains tested in the present work were isolated. We observed that in these sausages the putrescine and cadaverine were by far the major biogenic amines. In order to quantify the production of each biogenic amine (putrescine and cadaverine) by the different bacterial strains, in each bacterial strain, and for each individual precursor amino acid (ornithine and lysine), 2 tubes (5 mL each) of the culture medium (Joosten and Northolt, 1987) containing 2% of the corresponding individual precursor amino acid were each inoculated with 0.1 mL of a solution (0.85 g NaCl/L), containing 10^8 CFU. The tubes, with a final concentration of 2×10^7 CFU/mL, were incubated at 37°C for 72 h (previously, quantification of the biogenic amines was performed along 96 h of growth, showing that for most strains maximum accumulation took place after 72 h of incubation). After incubation, the O.D. was measured in one tube, and the corresponding biogenic amine was determined in the other. Firstly, 1 mL of 2 N HCl was added to the tube in order to stop microbial growth and decarboxylation. The content of the tube was then placed in a 25 mL volumetric flask, 1 mL of 1,7-diaminoheptane (internal standard) was added, and the final volume was made up with a 0.6 N $HClO_4$ solution. An aliquot (0.5 mL) of the mixture was then immediately placed in a tube, and 100 μL of 2 N NaOH (to make the solution more alkaline), 150 μL of a saturated solution of $NaHCO_3$, and 1 mL of dansyl chloride, were added consecutively. The tube was shaken gently, and placed in a water bath at 40°C for 45 min. In order to remove residues of dansyl chloride, 50 μL of ammonia were then added and the mixture was left to stand for 30 min. Finally, the volume was made up to 2.5 mL with acetonitrile and the mixture was filtered (0.25 μm).

Separation, identification, and quantification of the biogenic amines were carried out by HPLC, following the procedure described by Eerola et al. (1993), using the equipment and chromatographic conditions reported by Lorenzo et al. (2010).

A standard solution containing appropriate amounts of agmatine, tryptamine, 2-phenylethylamine, putrescine, cadaverine, histamine, tyramine, spermidine, spermine, and 1,7-diaminoheptane (as internal standard) was used to quantify the biogenic amines present in the samples.

All the samples and standards were injected at least in duplicate in different days. Repeatability tests were performed by injecting

a standard and a sample consecutively six times in a day. Reproducibility tests were also carried out by injecting the standard and the sample twice a day for 3 days, under the same experimental conditions. There were no significant differences ($P < 0.05$) in the results obtained in these tests.

The quantity of each biogenic amine was expressed in milligram per liter.

RESULTS

MOLECULAR TYPING OF THE *STAPHYLOCOCCUS* AND *BACILLUS* STRAINS ISOLATED FROM TRADITIONAL SAUSAGES

The obtained resulting fingerprints of the strains of *Staphylococcus* and *Bacillus* used in the present study demonstrated that the strains belonging to the same species were different. This is important, because some strains belonging to the same species came from the same sausage unit and could be the same.

DECARBOXYLASE ACTIVITY OF THE *STAPHYLOCOCCUS* AND *BACILLUS* STRAINS ISOLATED FROM TRADITIONAL SAUSAGES

The decarboxylase activity of the *Staphylococcus* strains tested is shown in **Table 1**. In this table, results reported in the literature for the same species were summarized. The *Staphylococcus* strains displayed a great variability in the histidine decarboxylase activity, with 7 of the 15 strains of *S. equorum* (46.67%), 9 of the 11 strains of *S. epidermidis* (81.82%), 6 of the 7 strains of *S. saprophyticus* (85.71%), and 4 of the 5 strains of *S. pasteuri* (80%) displaying this activity. The tyrosine decarboxylase, ornithine decarboxylase, and lysine decarboxylase activities were less variable. All strains of *S. saprophyticus* displayed tyrosine, ornithine, and lysine decarboxylase activities, whereas the frequency of the presence of these activities was slightly lower in strains of *S. equorum*, *S. epidermidis*, and *S. pasteuri*. Lysine was the amino acid decarboxylated with a higher frequency; the 100% of the strains of *S. equorum* and *S. saprophyticus*, the 91% of the strains of *S. epidermidis* and the 80% of the strains of *S. pasteuri* were able to decarboxylate this amino acid.

The decarboxylase activity observed in the *Bacillus* strains tested is shown in **Table 2**. Of the 13 strains of *B. subtilis* studied, 10 displayed histidine decarboxylase activity (76.92%), 12 tyrosine decarboxylase activity (92.31%), 8 ornithine decarboxylase activity (61.54%), and 8 lysine decarboxylase activity (61.54%). The strains of *B. amyloliquefaciens* showed less variable behavior; of the six strains studied, four were able to decarboxylate histidine (66.72%), and six were able to decarboxylate tyrosine, ornithine, and lysine (100%).

BIOGENIC AMINE ACCUMULATION AFTER 72 h OF GROWTH

The values of putrescine and cadaverine accumulated after 72 h of growth by the strains of *Staphylococcus* and *Bacillus*, respectively are shown in **Tables 3** and **4**.

The quantities of putrescine produced by the *Staphylococcus* strains tested ranged from 1.46 to 1415.05 mg/L in *S. equorum* (although one strain accumulated 1415.05 mg/L, the rest accumulated less than 25 mg/L) and from 0.37 to 977.13 mg/L in *S. epidermidis* (although one strain accumulated 977.13 mg/L, the rest accumulated less than 15 mg/L). The strains of *S. pasteuri*

Table 1 | Decarboxylase activity of the *Staphylococcus* strains isolated from traditional sausages in this work and from different sausages and other sources in previous studies.

Species	No. of strains	No. of strains positive	%	Bonomo et al. (2009)	Drosinos et al. (2007)	Even et al. (2010)	Martín et al. (2006)	Masson et al. (1996)	Silla Santos (1998)
HISTIDINE DECARBOXYLASE ACTIVITY									
Staphylococcus equorum	15	7	47	0	0	0	NT	NT	NT
S. epidermidis	11	9	82	NT	NT	3	14	NT	NT
S. saprophyticus	7	6	86	0	7	0	NT	0	<70
S. pasteuri	5	4	80	0	NT	NT	NT	NT	NT
TYROSINE DECARBOXYLASE ACTIVITY									
S. equorum	15	14	93	0	0	nt	NT	NT	NT
S. epidermidis	11	8	73	NT	NT	nt	0	NT	NT
S. saprophyticus	7	7	100	0	50	nt	NT	100	100
S. pasteuri	5	5	100	0	NT	nt	NT	NT	NT
ORNITHINE DECARBOXYLASE ACTIVITY									
S. equorum	15	14	93	0	0	0	NT	NT	NT
S. epidermidis	11	10	91	NT	NT	3	21	NT	NT
S. saprophyticus	7	7	100	0	16	3	NT	NT	<70
S. pasteuri	5	4	80	0	NT	NT	NT	NT	NT
LYSINE DECARBOXYLASE ACTIVITY									
S. equorum	15	15	100	86	0	0	NT	NT	NT
S. epidermidis	11	10	91	NT	NT	3	21	NT	NT
S. saprophyticus	7	7	100	50	50	3	NT	NT	<70
S. pasteuri	5	4	80	100	NT	NT	NT	NT	NT

(NT) Species not tested in the corresponding study. (nt) Decarboxylase activity not tested in the corresponding study.

also produced variable quantities of putrescine, with concentrations ranging between 1.19 and 12.39 mg/L. The strains of *S. saprophyticus* generally produced less putrescine, at less variable concentrations ranging from 0.43 to 1.91 mg/L.

Regarding the production of cadaverine, the quantities accumulated were again very variable within the strains of *S. equorum* (from 0.25 to 5.31 mg/L) and *S. epidermidis* (from 0.46 and 36.52 mg/L). A considerable variability was also registered within the strains of *S. saprophyticus* (from 0.58 to 4.79 mg/L) and *S. pasteuri* (from 0.40 to 4.33 mg/L).

The species that accumulated the highest amounts of putrescine and cadaverine were *S. equorum* and *S. epidermidis*. One of the strains of *S. equorum* accumulated 1415.05 mg/L of putrescine and one of the strains of *S. epidermidis* displayed a high aminogenic ability, accumulating 977.13 mg/L of putrescine or 36.52 mg/L of cadaverine after 72 h of growth in the culture medium.

The *Bacillus* strains in the present study also displayed a high degree of variability in their ability to produce putrescine and cadaverine. In the strains of *B. subtilis*, the quantities accumulated ranged from 0.39 to 18.43 mg/L for putrescine and from 0.43 to 4.29 mg/L for cadaverine. In the strains of *B. amyloliquefaciens*, the quantities ranged from 0.76 to 3.27 mg/L for putrescine, and from 0.53 to 3.07 mg/L for cadaverine.

Table 2 | Decarboxylase activity of the *Bacillus* strains isolated from traditional sausages in this work.

Species	No. of strains	No. of strains positive	%
HISTIDINE DECARBOXYLASE ACTIVITY			
Bacillus subtilis	13	10	77
B. amyloliquefaciens	6	4	67
TYROSINE DECARBOXYLASE ACTIVITY			
B. subtilis	13	12	92
B. amyloliquefaciens	6	6	100
ORNITHINE DECARBOXYLASE ACTIVITY			
B. subtilis	13	8	62
B. amyloliquefaciens	6	6	100
LYSINE DECARBOXYLASE ACTIVITY			
B. subtilis	13	8	62
B. amyloliquefaciens	6	6	100

DISCUSSION

Information in the literature concerning the amino acid decarboxylase activity in strains of the genus *Staphylococcus* is scarce and shows in general that microorganisms belonging to this genus are not significant possessors of these activities. Nonetheless, Silla Santos (1998) reported a high frequency of histidine, tyrosine, ornithine, and lysine decarboxylase activity in strains of *S. xylosus* and *S. saprophyticus* isolated from Spanish fermented sausages, which is in agreement with our results. Furthermore, Martín et al. (2007) observed ornithine and lysine decarboxylase activities in 57% of the strains of *S. xylosus* isolated from Iberian dry-cured sausages.

However, Drosinos et al. (2007) analyzed 300 staphylococci strains isolated from traditional fermented Greek sausages and observed that only a low proportion of strains displayed amino acid decarboxylase activity. The species with the highest proportion of strains that displayed histidine, tyrosine, ornithine, or lysine decarboxylase activity were *S. saprophyticus*, *S. simulans*, and *S. xylosus*, but within each species the proportion of strains that were positive for a specific amino acid decarboxylase activity was never

Table 3 | Values of accumulation of putrescine (mg/L) and cadaverine (mg/L) in the culture medium after 72 h of growth of the *Staphylococcus* strains isolated from traditional sausages.

Species	Putrescine				Cadaverine			
	No. of strains	Range of values*	Average	SD	No. of strains	Range of values*	Average	SD
Staphylococcus equorum	14	1.46–1415.05	112.67	374.92	15	0.25–5.31	1.85	1.59
S. epidermidis	10	0.37–977.13	100.58	308.01	10	0.46–36.52	5.83	10.91
S. saprophyticus	7	0.43–1.91	1.13	0.49	7	0.58–4.79	2.11	1.88
S. pasteuri	4	1.19–12.39	4.47	5.29	4	0.40–4.33	2.35	1.76

**Range of values of the strains.*

Table 4 | Values of accumulation of putrescine (mg/L) and cadaverine (mg/L) in the culture medium after 72 h of growth of the *Bacillus* strains isolated from traditional sausages.

Species	Putrescine				Cadaverine			
	No. of strains	Range of values*	Average	SD	No. of strains	Range of values*	Average	SD
Bacillus subtilis	8	0.39–18.43	3.14	6.19	8	0.43–4.29	1.30	1.50
B. amyloliquefaciens	6	0.76–3.27	1.89	0.94	6	0.53–3.07	1.40	1.11

**Range of values of the strains.*

greater than 50%. Martín et al. (2006) studying 239 *Staphylococcus* strains isolated from fermented sausages reported that only the 14.6% (35 strains) were able to decarboxylate one or more amino acids. In this same way, Even et al. (2010) working with 129 strains of coagulase-negative staphylococci isolated from various environments including cheeses and fermented sausages, observed that only 5 strains (∼6%) were able to produce detectable amounts of biogenic amines.

Bonomo et al. (2009) did not find any tyrosine or ornithine decarboxylase activity in any of the 37 staphylococci strains tested, and only observed histidine decarboxylase activity in two strains of *S. warneri*. As observed in the present study, lysine was the amino acid most frequent decarboxylated, and 62% of strains, belonging mainly to the *S. equorum* and *S. xylosus* species, were able to decarboxylate this amino acid. In the latter study the highest proportion of lysine-decarboxylating strains were in the *S. pasteuri* and *S. succinus* species.

Masson et al. (1996) did not observe histidine decarboxylase activity in any of the tested strains of *S. carnosus*, *S. xylosus*, *S. warneri*, and *S. saprophyticus* isolated from sausages. They observed tyrosine decarboxylase activity in all these strains, but the amounts of tyramine produced never achieved 40 μg/mL. Bover-Cid et al. (2001) did not observe any decarboxylase activity in any of the staphylococci strains tested.

Information regarding the amino acid decarboxylase activity of *Bacillus* strains isolated from meat products is very scarce. Roig-Sagués et al. (1996) analyzed four strains of *Bacillus* spp. isolated from *salchichón* (a Spanish traditional sausage) and found that some of the strains displayed histidine decarboxylase activity and were able to produce histamine, although in very low quantities (about 0.5 μg/mL).

There is some information about *Bacillus* strains isolated from salted and ripened Spanish anchovies. Hernández-Herrero et al. (1999) reported that *B. pumilus* was able to produce histamine, but in low quantities (12–17 μg/mL) and at low environmental NaCl concentrations (0.5–3% NaCl); this ability disappeared at higher concentrations of NaCl (10 and 20%).

Rodríguez-Jerez et al. (1994) analyzed 16 strains of *Bacillus* spp. isolated from Spanish salted semi-preserved anchovies and observed that none of the strains displayed ornithine or lysine decarboxylase activity; the percentage of strains that displayed histidine decarboxylase activity ranged from 75 to 81.25% depending on the culture medium (Niven or modified Niven) used in the test. The quantities of histamine produced ranged from 0 to 10.54 μg/mL.

The aminogenic potential of the *Staphylococcus* and *Bacillus* strains analyzed in the present study was generally low (quantities produced lower than 25 mg/L for putrescine and lower than 5 mg/L for cadaverine), with the exception of one strain of *S. epidermidis* and one of *S. equorum*, which produced higher quantities. These results therefore confirm that the amino acid decarboxylase activities are not particularly high in species of the genera *Staphylococcus* and *Bacillus*, especially when compared with other microbial groups such as Enterobacteriaceae or lactic acid bacteria, present in the fermented meat products (Bover-Cid et al., 2001; Lorenzo et al., 2010).

In the literature, there is little available information on the production of putrescine or cadaverine by *Staphylococcus* species. Martín et al. (2006) observed the production of variable quantities of putrescine (from 25 to >1000 mg/L) and cadaverine (from 25 to 1000 mg/L), being the strains of *S. epidermidis* the main producers. Even et al. (2010) also reported the production of variable quantities of putrescine (from 7 to 1499 mg/L) and cadaverine (from 3 to 140 mg/L).

In the literature, there is no information about putrescine and cadaverine production by species of the genus *Bacillus*, either from meat products or other different origin.

The high variability in biogenic amine production within strains belonging to the same species confirms previous findings (Bover-Cid and Holzapfel, 1999; Martín et al., 2006; Even et al., 2010) and again shows that the amino acid decarboxylase activity is a strain-dependant property. Although the aminogenic capacity of these two bacterial genera is not usually very high, since they can reach high counts in the sausages it is important to reduce their counts by the implementation of rigorous hygienic measures, in order to reduce the risk of accumulation of biogenic amines in the final products.

CONCLUSION

(a) Histidine, lysine, ornithine, and tyrosine decarboxylase activities were present in most of the strains of *Staphylococcus* and *Bacillus* isolated from Spanish traditional sausages. However, some variability was observed between strains, even within the same species.

(b) The production of putrescine and cadaverine by the species of *Staphylococcus* and *Bacillus* isolated from Spanish traditional sausages was in general low, with amounts accumulated lower than 25 mg/L for putrescine and lower than 5 mg/L for cadaverine. Only a strain of *S. equorum* produced 1415 mg/L of putrescine and a strain of *S. epidermidis* accumulated 977 mg/L of putrescine and 36 mg/L of cadaverine.

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REFERENCES

Bonomo, M. G., Ricciardi, A., Zotta, T., Sico, M. A., and Salzano, G. (2009). Technological and safety characterization of coagulase-negative staphylococci from traditionally fermented sausages of Basilicata region (Southern Italy). *Meat Sci.* 83, 15–23.

Bover-Cid, S., and Holzapfel, W. H. (1999). Improved screening procedure for biogenic amine production by lactic acid bacteria. *Int. J. Food Microbiol.* 53, 33–41.

Bover-Cid, S., Hugas, M., Izquierdo-Pulido, M., and Vidal-Carou, M. C. (2000a). Reduction of biogenic amine formation using a negative amino acid-decarboxylase starter culture for fermentation of "fuet" sausages. *J. Food Prot.* 63, 237–243.

Bover-Cid, S., Izquierdo-Pulido, M., and Vidal-Carou, M. C. (2000b). Influence of hygienic quality of raw material on biogenic amine production during ripening and storage of dry fermented sausages. *J. Food Prot.* 63, 1544–1550.

Bover-Cid, S., Hugas, M., Izquierdo-Pulido, M., and Vidal-Carou, M. C. (2001). Amino acid-decarboxylase activity of bacteria isolated from fermented pork sausages. *Int. J. Food Microbiol.* 66, 185–189.

Buncic, S., Paunovic, L., Radisic, D., Vojinovic, G., Smiljanic, D., and Baltic, C. (1993). Effects of gluconodeltalactone and *Lactobacillus plantarum* on the production of histamine and tyramine in fermented sausages. *Int. J. Food Microbiol.* 17, 303–309.

Drosinos, E. H., Paramithiotis, S., Kolovos, G., Tsikouras, I., and Metaxopoulos, I. (2007). Phenotypic and technological diversity of lactic acid bacteria and staphylococci isolated from traditionally fermented sausages in Southern Greece. *Food Microbiol.* 24, 260–270.

Eerola, S., Hinkannen, R., Lindfors, E., and Hirvi, T. K. (1993). Liquid chromatographic determination of biogenic amines in dry sausages. *J. AOAC Int.* 76, 575–577.

Even, S., Leroy, S., Charlier, C., Ben Zakour, N., Chacornac, J.-P., Lebert, I., Jamet, E., Desmonts, M.-H., Coton, E., Pochet, S., Donnio, P.-Y., Gautier, M., Talon, R., and Le Loir, Y. (2010). Low occurrence of safety hazards in coagulase negative staphylococci isolated from fermented foodstuffs. *Int. J. Food Microbiol.* 139, 87–95.

García Fontán, M. C., Lorenzo, J. M., Martínez, S., Franco, I., and Carballo, J. (2007a). Microbiological characteristics of Botillo, a Spanish traditional pork sausage. *LWT Food Sci. Technol.* 40, 1610–1622.

García Fontán, M. C., Lorenzo, J. M., Parada, A., Franco, I., and Carballo, J. (2007b). Microbiological characteristics of "androlla," a Spanish traditional pork sausage. *Food Microbiol.* 24, 52–58.

Halász, A., Baráth, A., Simon-Sarkadi, L., and Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. *Trends Food Sci. Technol.* 5, 42–49.

Hernández-Herrero, M., Roig-Sagués, A. X., Rodríguez-Jerez, J. J., and Mora-Ventura, M. T. (1999). Halotolerant and halophilic histamine-forming bacteria isolated during the ripening of salted anchovies (*Engraulis encrasicolus*). *J. Food Prot.* 62, 509–514.

Iacumin, L., Comi, G., Cantoni, C., and Cocolin, L. (2006). Molecular and technological characterization of *S. xylosus* isolated from naturally fermented Italian sausages by RAPD, Rep-PCR and Sau-PCR analysis. *Meat Sci.* 74, 281–288.

Janz, V. I., Scheiber, G., and Beutling, D. (1983). Die lebensmittelhygienische Bedeutung von Histamin und Tyramin. *Monatsh Veterinarmed* 38, 701–709.

Joosten, H. M. L. J., and Northolt, M. D. (1987). Conditions allowing the formation of biogenic amines in cheese. 1. Decarboxylative properties of some non-starter bacteria. *Neth. Milk Dairy J.* 41, 259–280.

Komprda, T., Smela, D., Pechova, P., Kalhotka, L., Stend, J., and Kleidus, B. (2004). Effect of starter culture, spice mix and storage time and temperature on biogenic amine content of dry fermented sausages. *Meat Sci.* 67, 607–616.

Lorenzo, J. M., Cachaldora, A., Fonseca, S., Gómez, M., Franco, I., and Carballo, J. (2010). Production of biogenic amines "in vitro" in relation to the growth phase by Enterobacteriaceae species isolated from traditional sausages. *Meat Sci.* 86, 684–691.

Lorenzo, J. M., Martínez, S., Franco, I., and Carballo, J. (2008). Biogenic amine content in relation to physicochemical parameters and microbial counts in two kinds of Spanish traditional sausages. *Arch. Lebensmittelhyg.* 59, 70–75.

Lorenzo, J. M., Michinel, M., López, M., and Carballo, J. (2000). Biochemical characteristics of two traditional Spanish dry-cured sausage varieties: Androlla and Botillo. *J. Food Compos. Anal.* 13, 809–817.

Lu, S., Xu, X., Zhou, G., Zhu, Z., Meng, Y., and Sun, Y. (2010). Effect of starter cultures on microbial ecosystem and biogenic amines in fermented sausage. *Food Control* 21, 444–449.

Maijala, R., Eerola, S., Aho, M. A., and Hirn, J. A. (1993). The effect of GDL-induced pH decrease on the formation of biogenic amines in meat. *J. Food Prot.* 56, 125–129.

Martín, A., Colín, B., Aranda, E., Benito, M. J., and Córdoba, M. G. (2007). Characterization of Micrococcaceae from Iberian dry-cured sausages. *Meat Sci.* 75, 696–708.

Martín, B., Garriga, M., Hugas, M., Bover-Cid, S., Veciana-Nogués, M. T., and Aymerich, T. (2006). Molecular, technological and safety characterization of Gram-positive catalase-positive cocci from sligtly fermented sausages. *Int. J. Food Microbiol.* 107, 148–158.

Masson, F., Talon, R., and Montel, M. C. (1996). Histamine and tyramine production by bacteria from meat products. *Int. J. Food Microbiol.* 32, 199–207.

Paulsen, P., and Bauer, F. (1997). Biogenic amines in fermented sausages: 2. Factors influencing the formation of biogenic amines in fermented sausages. *Fleischwirtschaft* 77, 32–34.

Rodríguez-Jerez, J. J., Mora-Ventura, M. T., López-Sabater, E. I., and Hernández-Herrero, M. (1994). Histidine, lysine and ornithine decarboxylase bacteria in Spanish salted semi-preserved anchovies. *J. Food Prot.* 57, 784–787, 791.

Roig-Sagués, A. X., Hernández-Herrero, M., López-Sabater, E. I., Rodríguez-Jerez, J. J., and Mora-Ventura, M. T. (1996). Histidine decarboxylase activity of bacteria isolated from raw and ripened Salchichón, a Spanish cured sausage. *J. Food Prot.* 59, 516–520.

Shalaby, A. R. (1996). Significance of biogenic amines to food safety and human health. *Food Res. Int.* 29, 675–690.

Silla Santos, M. H. (1996). Biogenic amines: their importance in foods. *Int. J. Food Microbiol.* 29, 213–231.

Silla Santos, M. H. (1998). Amino acid decarboxylase capability of microorganisms isolated in Spanish fermented meat products. *Int. J. Food Microbiol.* 39, 227–230.

Smith, T. A. (1980). Amines in food. *Food Chem.* 6, 169–200.

Stratton, J. E., Hutkins, R. W., and Taylor, S. L. (1991). Biogenic amines in cheese and other fermented foods: a review. *J. Food Prot.* 54, 460–470.

Suzzi, G., and Gardini, F. (2003). Biogenic amines in dry fermented sausages: a review. *Int. J. Food Microbiol.* 88, 41–54.

Taylor, S. L. (1985). *Histamine Poisoning Associated with Fish, Cheese, and Other Foods.* Geneva: World Health Organization, WPH/FOS 85, 1–47.

Ten Brink, B., Damink, C., Joosten, H. M. L. J., and Huis in't Veld, J. H. J. (1990). Occurrence and formation of biologically active amines in foods. *Int. J. Food Microbiol.* 11, 73–84.

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