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Short communication

Amino acid-decarboxylase activity of bacteria isolated from fermented pork sausages

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Abstract

The occurrence of amino acid-decarboxylase activity in 92 strains of lactic acid bacteria, coagulase-negative staphylococci, and Enterobacteriaceae isolated from Spanish fermented pork sausages was investigated. The presence of biogenic amines in a decarboxylase synthetic broth was determined by ion-pair high performance liquid chromatography with *o*-phtalaldehyde post-column derivatization. Among the 66 lactic acid bacteria strains tested, 21 lactobacilli (in particular, *Lactobacillus curvatus*) and all 16 enterococci were amine producers. Tyramine was the main amine produced by these bacteria, although they also produced phenylethylamine, tryptamine, and/or the diamines putrescine and cadaverine. None of the lactic acid bacteria produced histamine. Coagulase-negative staphylococci were found to be negative amine-producers. Aromatic monoamines, apart from histamine, were not formed by Enterobacteriaceae. This family was responsible for cadaverine and putrescine production. The results obtained for biogenic amine production by bacteria in a synthetic medium suggest that amino acid-decarboxylase activity is strain dependent rather than being related to specific species.

Keywords: Amino acid-decarboxylase; Biogenic amines; Fermented sausages; Lactic acid bacteria; Enterobacteria

1. Introduction

The amount and type of biogenic amines (BA) formed depends on the nature of food and particularly on the kind of microorganisms present. Enterobacteriaceae and certain lactic acid bacteria (LAB) are particularly active in the production of BA (Beutling, 1996; Halász et al., 1994). These amine-producing microorganisms either may form part of the food associated population or may be introduced by contamination before, during or after processing of the food product. Therefore, microorganisms naturally present in raw materials, introduced throughout the processing or added as starter culture can critically influence BA production during the manufac-

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ture of fermented products (Maijala and Eerola, 1993; Bover-Cid et al., 1998, 2000).

During meat fermentation, microbial growth, acidification and proteolysis provide favourable conditions for BA production. The species of lactobacilli most commonly found in meat and meat products are *Lactobacillus sakei* and *L. curvatus*, which together with *L. bavaricus* and *L. plantarum* constitute the main microbial flora isolated from Spanish fermented sausages (Hugas et al., 1993). Other bacteria that can be found in relatively high numbers include enterococci (*E. faecalis* and *E. faecium*), which also contribute to the fermentation process. However, the presence of enterococci might also reflect a given level of contamination or a poor curing process (Holley et al., 1988). Salt-tolerant, nitrate-reducing coagulase-negative staphylococci are also detected in relatively high numbers in ripened meat products. *Staphylococcus xylosus* is the main species found in Spanish fermented sausages (Silla-Santos, 1998), although *S. carnosus* can also be used as a starter culture (Bover-Cid et al., 1999; Masson et al., 1996).

The aim of this study was to examine the occurrence of amino acid-decarboxylase activity of several strains of LAB, coagulase-negative staphylococci, and Enterobacteriaceae isolated from Spanish fermented pork sausages.

2. Material and methods

2.1. Bacterial strains and growth conditions

The microorganisms used in this study were obtained from the culture collections of the Meat Technology Centre (CTC-IRTA, Girona, Spain) and the Department of Nutrition and Food Science (University of Barcelona, Spain). A total of 92 bacterial strains including 66 LAB (50 lactobacilli and 16 enterococci), 10 coagulase-negative staphylococci, and 16 strain of Enterobacteriaceae isolated from different Spanish fermented sausages were tested.

LAB and enterococci were grown in MRS broth (Merck, Darmstadt, Germany) while staphylococci and enterobacteria were grown in Standard Nutritive broth (Merck). All bacteria were incubated at 30°C aerobically.

2.2. Determination of biogenic amine-forming capacity

Amino acid-decarboxylase activity of bacteria was assessed by the presence of BA in a decarboxylase broth described in a previous work (Bover-Cid and Holzapfel, 1999). The medium contained the precursor amino acids (0.5% tyrosine di-sodium salt and 0.25% L-histidine monohydrochoride, L-ornithine monohydrochoride, L-lysine monohydrochoride, L-phenylalanine, and L-tryptophan), pyridoxal-5-phosphate as a codecarboxylase factor, growing factors and buffer compounds. The pH of the medium was adjusted to 5.2 before autoclaving. The precursor amino acids were purchased from Sigma (St. Louis, MO, USA) while the other chemicals were from Merck.

Biogenic amines, tyramine (TY), histamine (HI), tryptamine (TRP), phenylethylamine (PHE), putrescine (PU), and cadaverine (CA) were determined by ion-pair high-performance liquid chromatography and post-column derivatization with *ortho*-phtaladehyde according to Hernández-Jover et al. (1996).

3. Results and discussion

Among the 66 LAB strains tested, 21 lactobacilli and all 16 enterococci strains were TY producers (Table 1). *L. plantarum* strains did not produce any BA while only a few *L. sakei* strains did. In contrast, *L. curvatus* (11 of 12 strains) and *Enterococcus* spp. (all 16 strains) produced BA, primarily TY, but also PHE. Accumulation of TY in the fermenting broth varied from 10 to 3000 mg/l, though it was usually higher than 500 mg/l. The production of PHE was generally lower, although more than 2000 mg/l were produced by one strain of *L. curvatus*. Production of TRP was only found in two strains of *L. curvatus*. In all cases, PHE and TRP were produced in conjunction with large amounts of TY. Formation of the diamines PU and, to a lesser extent, CA was observed in some cases of lactobacilli together with TY, although in a few strains PU production was independent of that of TY. Diamines were not produced by any enterococci. In any case, LAB tested did not show HI formation (results not shown).

Our results differ from those of Silla-Santos (1998) in which none of the lactobacilli (also isolated from

Table 1
Biogenic amine production (mg/l broth) by lactic acid bacteria and Enterobacteriaceae isolated from fermented sausages

Species	Strains tested	TY[a]	PHE	TRP	PU	CA
Lactic acid bacteria						
E. faecalis	11	11[b] (419–3736)[c]	11 (80–585)	ND	ND	ND
E. faecium	5	5 (474–4334)	5 (40–432)	ND	ND	ND
L. bavaricus	4	2 (458–551)	ND	ND	ND	ND
L. brevis	3	1 (516)	ND	ND	1 (418)	1 (14)
L. curvatus	12	11 (9–2986)	8 (2–2061)	2 (8–1321)	3 (37–906)	2 (19–29)
L. paracasei	1	1 (497)	ND	ND	ND	ND
L. plantarum	7	ND[d]	ND	ND	ND	ND
L. sakei	13	4 (15–2121)	ND	ND	ND	ND
Lactobacillus sp.	10	2 (446–507)	ND	ND	ND	1 (19)
Enterobacteriaceae						
C. freundii	1	ND	ND	ND	1 (43)	1 (15)
Enterob. cloacae	2	ND	ND	ND	2 (473–568)	2 (599–755)
K. oxytoca	1	ND	ND	ND	ND	1 (683)
Proteus vulgaris	5	ND	ND	ND	ND	ND
S. liquefaciens	4	ND	ND	ND	4 (474–758)	4 (591–971)
S. marcescens	1	ND	ND	ND	1 (471)	1 (594)
Serratia sp.	2	ND	ND	ND	1 (471)	1 (590)
Enterobacteriaceae	1	ND	ND	ND	1 (476)	1 (599)

[a] Tyramine (TY), phenylethylamine (PHE), tryptamine (TRP), putrescine (PU), cadaverine (CA).
[b] Number of positive strains.
[c] Concentration range of biogenic amine produced.
[d] Not detected.

Spanish fermented meat products) were shown to be amine-producers. The reasons to explain the lack of concordance between authors could be, firstly, the fact that amine formation is associated to specific strains but not to a species. Furthermore, the determination of the amino acid-decarboxylase activity of LAB may result in numerous false negative responses due to the acid production by LAB that counteracts the pH shift and, thus, not showing the colour change associated with a positive reaction. This can be overcome using buffer and acid-neutralising compounds in the decarboxylase media (Bover-Cid and Holzapfel, 1999). Moreover, the presence of the codecarboxylase factor (pyridoxal-5-phosphate) and the factors enhancing LAB growth in the synthetic differential plate medium used here might have enhanced the biogenic amine production activity of the microorganisms tested (Gale, 1946; Recsei et al., 1985).

In the present work, no BA formation was found in the strains of coagulase-negative staphylococci (results not shown). Straub et al. (1995) reported a remarkable potential for BA production in *S. carnosus* strains, but not for *S. xylosus*. Masson et al. (1996) only detected a weak tyramine production capacity for several species of staphylococci (*S. carnosus*, *S. xylosus*, *S. warneri*, and *S. saprophyticus*) of fermented sausage origin.

Most of the Enterobacteriaceae yielded CA and/or PU (Table 1). Strains belonging to *Enterobacter cloacae*, *Klebsiella oxytoca* and *Serratia* spp. produced large amounts of both diamines, reaching levels up to 500 mg/l of broth. *Citrobacter freundii* was a weak amino acid-decarboxylase bacterium (< 50 mg/l of CA, PU, and HI), while *Proteus vulgaris* did not form BA here. Histamine production is usually related to Enterobacteriaceae species (Halász et al., 1994), but in our study HI was formed in much lower amounts (< 60 mg/l) than diamines by most of the enterobacteria. None of the Enterobacteriaceae strains exhibited amino acid-decarboxylase activity towards tyrosine, phenylalanine, or tryptophan (Table 1).

Great variations have been reported in the kind and quantity of BA produced between different strains of the same species. This can be partially

explained by the factors that influence BA production such as temperature, pH, water activity, the presence of fermentable carbohydrates and the redox potential (Beutling, 1996). Likewise, strains with proteolytic activity may be more likely to produce BA in food systems since they yield a higher amount of precursor amino acids that can be decarboxylated. In this sense, for example, Straub et al. (1994) found a *L. curvatus* strain able to form TY from di- and tripeptides with tyrosine in a non-terminal position.

LAB are mainly associated with TY formation (especially in the case of enterococci). However, LAB not only yield TY since some LAB strains can also produce other aromatic amines such as PHE and TRP, and to a lesser extent the diamines PU and CA. Usually, the non-starter LAB are frequently recognised as being responsible for amine production in fermentation processes (Joosten and Northolt, 1987; Maijala and Eerola, 1993). In contrast, the formation of aromatic monoamines, other than that of HI, rarely occurs amongst Enterobacteriaceae, which are generally linked to CA and PU formation in meat products. Therefore, high levels of these diamines would indicate an excessive activity of undesirable microorganisms in raw materials or during the manufacture of fermented meat products.

Among the LAB species found in fermented pork sausages, *L. curvatus* strains appear to be the main producers of TY and, to a lesser extent, of PU, PHE, TRP, and CA. In contrast, *L. plantarum* and especially *L. sakei* would be better starter cultures considering their technological properties and also their general behaviour as amine-negative strains. Nevertheless, our results for BA production by microorganisms in a synthetic medium suggest that the capacity to produce amines might be strain dependent rather than being related to particular species. Therefore, when selecting a strain as a starter culture for meat and other food fermentation, its potential to form BA should be assessed. A positive reaction could be applied as an exclusion criterion.

The activity of amino acid-decarboxylase enzymes in a synthetic broth depends not only on the availability of amino acids, but also on the phase of bacterial growth, and on the microbial strain (Straub et al., 1994; Bover-Cid and Holzapfel, 1999). The factors influencing the ability of microorganisms to form BA during the manufacture of a fermented product (such as pH, water activity, nutrients, other food components, and technological conditions) should be studied in order to select those conditions that favour a proper fermentation process while limiting BA accumulation.

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