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Tyramine degradation and tyramine/histamine production by lactic acid bacteria and *Kocuria* strains

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Abstract

Of 53 strains of lactic acid bacteria and *Kocuria*, screened for production or degradation of biogenic amines, 29 *Kocuria varians* and four strains of *Enterococcus faecalis* produced tyramine and, at lower concentrations, histamine. In contrast, *Lactobacillus* strains that did not possess amino acid decarboxylase activity degraded tyramine. The greatest tyramine oxidase activity was present in the strains *L. casei* CRL705 (98% degradation) and CRL678 (93%) as well as in *L. plantarum* CRL681 (69%) and CRL682 (60%).

Introduction

Biogenic amines, low molecular weight organic bases, can be formed and degraded as a result of microbial activity. They are usually produced by amino acid decarboxylases:

$$R - CHNH_2 - COOH \rightarrow R - CH_2 - NH_2 + CO_2.$$

Biogenic amines in foods are of concern in relation to both food spoilage and food safety. They are generated either as the result of endogenous amino acid decarboxylase activity in raw food materials or by the growth of decarboxylase-positive microorganisms under conditions favourable to enzyme activity (Silla Santos 1996, Halász *et al.* 1994). Biogenic amines are natural components of many fermented and non-fermented foods of animal and vegetable origin and may represent a food poisoning hazard, specially in conjunction with additional promoting factors such as monoamine oxidase inhibitors or alcohol (Silla Santos 1996). In addition, diamines are potential precursors of carcinogenic nitrosamines specially when nitrosable agents are present as in meat products (Scanlan 1983).

The active growth of several microbial populations, acidification and the proteolysis during dry sausage fermentation, makes this environment particularly favourable to biogenic amine formation (Bover-Cid *et al.* 1999). The main bacterial cultures for meat fermentation are lactic acid bacteria and the Gram positive, catalase positive cocci, including micrococci and staphylococci (Hugas & Monfort 1997). The production of biogenic amines in meat and meat products has often been related to lactic acid bacteria (Maijala & Eerola 1993, Paulsen & Bauer 1997). However, *Staphylococcus carnosus* also has a potential to form amines (Straub *et al.* 1995). Since physiological inactivation of biogenic amines can be achieved by microorganisms possessing amine oxidase activity, several studies have been carried out to evaluate this activity in different microbial species from fermented foods (Leuschner *et al*. 1998, Martuscelli *et al.* 2000). Mono- and di-amino oxidases are present in higher organisms and in bacteria (Yamashita *et al.* 1993). Amine oxidases catalyse the oxidative deamination of primary amines:

$$R - (CH_2) - NH_2 \rightarrow R - CHO + H_2O_2 + NH_3.$$

Among the microorganisms exhibiting this activity, strains of *Micrococcus varians*, reclassified as *Kocuria varians* by Stackebrandt *et al.* (1995), used for

sausage fermentation oxidized tyramine under aerobic conditions, whereas *S. carnosus* did not (Leuschner & Hammes 1998). Even though amine oxidase activity was found among some dairy and meat isolates (Voigt & Eitenmiller 1978, Leuschner *et al.* 1998) little is known about amine degradation by lactic acid bacteria.

The object of this study was to examine the potential of starter strains from artisanal fermented sausages to form or degrade biogenic amines and to select strains suitable for preventing an amine accumulation in the final product.

Materials and methods

Bactrial strains and culture conditions

Strains of *Lactobacillus plantarum* (11), *Lactobacillus casei* (8), *Pediococcus acidilactici* (1), *Enterococcus faecalis* (4) and *Kocuria varians* (29) used in this study were isolated from artisanal fermented sausages produced in Argentina (Vignolo *et al*. 1986) and belong to the collection of CERELA-CONICET. *Micrococcus varians* LTH 1540 was kindly supplied by Dr Hammes (Institute for General Food Technology and Food Microbiology, University of Hohenheim, Germany) and was used as an oxidase positive-control. Strains, stored at −70 °C, were activated, before use in Man–Rogosa–Sharpe broth for lactic acid bacteria and in a medium composed (w/v) of 0.3% meat extract and 0.5% peptone, pH 6.6, for *Kocuria* and *Enterococcus* and incubated 18 h at 30 °C. Cells were recovered by centrifugation, washed with 20 mM sodium phosphate buffer and this suspension was used for the inoculum.

Qualitative detection of biogenic amine producers

The ability to produce biogenic amines by decarboxylation of the corresponding amino acid used as a precursor (L-histidine and L-tyrosine) was tested according to the method of Joosten & Northolt (1989). The plates with the agar medium, supplemented with histidine or tyrosine at 20 mg l^{-1} were spotted with the active strain and incubated anaerobically at 30 °C for 2–5 days. Growth of decarboxylating strains was easily recognizable because of their purple halo in the yellow medium. To corroborate the production of biogenic amines, the test was carried out in a broth media, the supernatant was collected, deproteinized and analysed by HPLC.

Tyramine degradation

Resting cells (from overnight cultures), recovery by centrifugation, were washed with 20 mM sodium phosphate buffer (pH 7.3) and the pellet was resuspended in the same buffer supplemented with 2.5 mM of tyramine.HCl (ICN Biomedicals Inc., Ohio, USA). The cell suspensions (20 ml), adjusted to a turbidity of 6 at 560 nm (=0.06 g dry cell) were incubated in a 100 ml flask at 30 °C for 96 h with shaking at 200 r.p.m. Samples were taken (at 0 and 96 h) and added to an equal amount of 1 M HCl, centrifuged (10 000 × *g*) and the supernatant frozen until HPLC analysis.

Analytical methods

HPLC analyses were carried out with a C18 Novapack column, 60 Å, 4 μm (Phenomenex, Torrance, USA). Prior to analysis, samples were diluted with 0.4 M sodium borate (pH 10) at a 1:10 ratio and filtered through a 0.22 μm filter. Solvents used for the separation were A: Na_2HPO_4/NaH_2PO_4 (0.1 M, pH 7.3)/methanol/tetrahydrofuran (80:20:1, by vol) and B: methanol/Na_2HPO_4-NaH_2PO_4 (80:20, v/v.). Elution gradient was: 60% A:40% B, 8 min; 20% A:80% B, 18 min; 100% B, 10 min and 60% A:40% B, 12 min. The samples were derivatized prior to column injection by adding 50 μl reagent (200 mg *o*-phthaldialdehyde; 9 ml methanol; 1 ml 0.4 M sodium borate pH 10; and 160 μl 2-mercaptoethanol) to 50 μl diluted sample. The derivatization time was 1 min and 20 μl of the derivative solutions were immediately injected. The flow rate was 1 ml min^{-1} and the column effluent was monitored by UV absorption at 340 nm.

Results and discussion

Fifty-three strains isolated from artisanal fermented sausages were screened for the ability to produce biogenic amines (Table 1). The production of both amines, histamine and tyramine, was common among the *Kocuria* isolates (29/29). All the strains decarboxylated histamine and only four of them failed to decarboxylate tyrosine. Of the lactic acid bacteria, *Enterococcus* strains (4/4) formed tyramine but not histamine in contrast with *Lactobacillus* (19/19) and *Pediococcus* (1/1) strains that produced neither histamine nor tyrosine.

The amino acid decarboxylase-positive *Kocuria* strains can contribute to histamine and tyramine pro-

Table 1. Strains exhibiting the potential to form tyramine and histamine in the qualitative test after incubation of 5 days at 30 °C.

Strains	Tyramine	Histamine	Strains	Tyramine	Histamine
Kocuria varians			GV801	+++	+
GV311	+++	++	GV803	+++	+
GV312	−	+	GV804	+++	+
GV313	−	+	GV811	+++	+
GV314	−	+	GV812	+++	+
GV381	+++	+	GV821	+++	+
GV382	+++	+	GV822	+++	+
GV411	+++	+	GV823	+++	+
GV412	+++	+	GV824	+++	+
GV413	+++	+	GV831	+++	+
GV414	+++	+	GV832	+++	+
GV415	+++	+	*Micrococcus varians*		
GV515	+++	+	LTH1540	−	+
GV516	+++	+	*Enterococcus faecalis*		
GV610	+++	+	CRL1066	+++	−
GV620	+++	+	CRL1067	+++	−
GV630	+++	+	CRL1068	+++	−
GV711	+++	+	CRL1069	+++	−
GV712	−	+			

Biogenic amine production: (+++), > 10 mg l^{-1}; (++), 2–10 mg l^{-1}; (+), < 2 mg l^{-1} and (−), negative. *L. plantarum*, *L. casei* and *P. acidilactici* strains were all negative.

duction, in particular during the first stages of sausage fermentation when the conditions are optimal for *Staphylococcus*/*Koccuria* growth. The potential of the assayed *Kocuria* strains to form histamine and tyramine was in marked contrast to the findings of Martuscelli *et al.* (2000) who reported that only 50% of *Stahylococcus xylosus* isolates from Italian artisanal fermented sausages were positive for amine formation. On the other hand, amino acid decarboxylase activity among lactic acid bacteria from meat products was reported by Masson *et al.* (1996, 1999) and Straub *et al.* (1994). With the exception of *Enterococcus* strains, *Lactobacillus* and *Pediococcus* used in this study did not produce histamine and tyramine in the assayed conditions while *E. faecium* strains showed to have tyrosine decarboxylase activity forming tyramine. Eitenmiller *et al.* (1978) also attributed tyramine content in sausage to *E. faecalis*. These results have been corroborated by HPLC analysis (data not shown).

When the ability to catabolize tyramine in five *Lactobacillus* strains, one *Pediococcus acidilactici* and five *Kocuria varians* strains was investigated, different tyramine degradation levels were obtained (Table 2). A remarkably high potential to degrade tyramine was exhibited by *L. casei* CRL705 and CRL678

Table 2. Degradation of tyramine (expressed as percentage) by lactic acid bacteria and *Kocuria* strains after incubation of 96 h in 20 mM sodium phosphate buffer (pH 7.3) in the presence of 2.5 mM tyramine.

Strains	Degradation (%)
Lactobacillus casei	
CRL705	98
CRL678	93
Lactobacillus plantarum	
CRL681	69
CRL682	60
Kocuria varians	
GV313	40
GV314	39
GV712	43
GV803	45
Micrococcus varians	
LTH1540	43

L. casei CRL717, *P. acidilactici* CRL937, and *K. varians* GV312 did not degrade tyramine.

with 98% and 93% of tyramine breakdown, respectively. In a less extention, *L. plantarum* CRL681 and CRL682 showed tyramine oxidase activity lowering the initial amine concentration by 69% and 60%, respectively. When *Kocuria varians* strains were assayed, the potential for amine degradation was found to be lower when compared to *Lactobacillus* oxidase activity. *K. varians* GV313, GV314 and GV712 (decarboxylase-negative strains) showed 40%, 39% and 43% of tyramine degradation respectively, these results being similar to *M. varians* LTH 1540 (43%) used as oxidase-positive control. On the other hand, *K. varians* GV803 that catabolized 45% of tyramine present in the medium, was also capable to decarboxylate tyrosine, conversely strain GV312 which was not able to decarboxylate tyrosine did not degrade tyramine.

The potential for amine breakdown is a common characteristic amongst the starter strains tested in this work. These findings do not agree with Leuschner *et al.* (1998) who reported histamine and tyramine oxidase activities to be low or absent in lactic acid bacteria while *Micrococcus* spp. exhibited a high activity for amine catabolism with *M. varians* LTH1540 having the highest tyramine oxidase activity. Nevertheless, amine oxidase activities displayed by *L. casei* and *L. plantarum* have to be further tested in real systems. In fact, high tyramine oxidase activity in broth, may have a limited effect on tyramine breakdown during sausage fermentations. Leuschner & Hammes (1998) studying tyramine degradation by micrococci during ripening of fermented sausages reported only a slight decrease of final tyramine concentration when a combination of *L. curvatus* (tyramine decarboxylase positive) and *M. varians* (oxidase positive) was inoculated. Even when differential tyramine degradation rates were observed in the centre of the sausage and beneath the casing because oxygen diffusion, amine oxidase enzymes were still operating in the conditions characterizing the ripening of fermented sausages (Leuschner *et al.* 1998).

Conclusion

The use of highly competitive starter cultures able to degrade biogenic amine content (oxidase positive) and/or not to produce them (amino acid decarboxylase negative) in association with high quality raw materials and good manufacturing practices, constitutes the best way to obtain products with the typical sensorial properties but with reduced health risks. These biochemical activities, in particular oxidase activity, can be considered as a criteria for the selection of strains for sausage fermentation.

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