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International Journal of Food Microbiology 53 (1999) 33–41

International Journal of Food Microbiology

www.elsevier.nl/locate/ijfoodmicro

Improved screening procedure for biogenic amine production by lactic acid bacteria

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Received 20 May 1999; accepted 7 September 1999

Abstract

An improved screening plate method for the detection of amino acid decarboxylase-positive microorganisms (especially lactic acid bacteria) was developed. The suitability and detection level of the designed medium were quantitatively evaluated by confirmation of amine-forming capacity using an HPLC procedure. The potential to produce the biogenic amines (BA) tyramine, histamine, putrescine, and cadaverine, was investigated in a wide number of lactic acid bacteria (LAB) of different origin, including starter cultures, protective cultures, type strains and strains isolated from different food products. Also, several strains of Enterobacteriaceae were examined. Modifications to previously described methods included lowering glucose and sodium chloride concentrations, and increasing the buffer effect with calcium carbonate and potassium phosphate. In addition, pyridoxal-5-phosphate was included as a codecarboxylase factor for its enhancing effect on the amino acid decarboxylase activity. The screening plate method showed a good correlation with the chemical analysis and due to its simplicity it is presented as a suitable and sensitive method to investigate the capacity of biogenic amine production by LAB. Tyramine was the main amine formed by the LAB strains investigated. Enterococci, carnobacteria and some strains of lactobacilli, particularly of *Lb. curvatus*, *Lb. brevis* and *Lb. buchneri*, were the most intensive tyramine formers. Several strains of lactobacilli, *Leuconostoc* spp., *Weissella* spp. and pediococci did not show any potential to produce amines. Enterobacteriaceae were associated with cadaverine and putrescine formation. No significant histamine production could be detected for any of the strains tested.

Keywords: Decarboxylase activity; Biogenic amines (BA); Lactic acid bacteria (LAB); Enterobacteria

1. Introduction

Biogenic amines (BA) are organic basic compounds which occur in different kinds of food, such as fishery products, cheese, wine, beer, dry sausages and other fermented foods (ten Brink et al., 1990; Halász et al., 1994).

Several toxicological problems resulting from the ingestion of food containing relatively high levels of BA have been reviewed (i.e., ten Brink et al., 1990;

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0168-1605/99/$ – see front matter
PII: S0168-1605(99)00152-X

Mariné-Font et al., 1995). Histamine and tyramine have been the most studied BA due to the toxicological effects derived from their vasoactive and psychoactive properties. Histamine has been recognised as the causative agent of *scombroid poisoning* (histaminic intoxication), whereas tyramine has been related to food-induced migraines and hypertensive crisis in patients under antidepressive treatment with mono-amine oxidase inhibitor (MAOI) drugs. Alcohol and other BA, such as the diamines, putrescine and cadaverine, may boost the toxicity of the above amines. In addition, diamines are known to be potential precursors of carcinogenic nitrosamines, especially when nitrosable agents are present in food. Apart from these toxicological aspects, BA are of concern in relation to food hygiene. The occurrence of relatively high levels of certain BA has been reported as indicators of a deterioration process and/or defective elaboration (Karmas, 1981; Vidal-Carou et al., 1990).

In foods, BA are mainly generated by decarboxylation of the corresponding amino acids through substrate-specific enzymes of the microorganisms present in the food (ten Brink et al., 1990). The capability of BA formation has been described for several groups of microorganisms, mainly Enterobacteriaceae, *Pseudomonas* spp., enterococci and some other LAB (Halász et al., 1994). The occurrence and distribution of amino acid decarboxylase activity within different genera and species of the Enterobacteriaceae, mainly isolated from fish, has been extensively reported. By contrast, studies on the BA formation by LAB are still relatively scarce.

Several qualitative and quantitative methods to determine BA production by microorganisms have been described. Most of the screening procedures generally involve the use of a differential medium containing a pH indicator, such as bromocresol purple (Moeller, 1954; Niven et al., 1981; Choudhury et al., 1990). Usually, the media consist of a basal composition (peptone, yeast or meat extract, salt and/or glucose) to which the precursor amino acids are added (Table 1). A positive result is indicated by a change of the medium colour to

Table 1
Composition (%) of decarboxylase media according to the literature and of the improved medium

Component	Moeller (1954)	Niven et al. (1981)	Choudhury et al. (1990)	Joosten and Northolt (1989)	Maijala (1993)	Improved medium
Tryptone	0.5	0.5	0.5	0.5	0.5	0.5
Yeast extract	0.5	0.5	0.5	0.5	0.4	0.5
Meat extract	–	–	–	–	0.8	0.5
Sodium chloride	0.5	0.5	0.5	0.5	–	0.25
Glucose	–	–	0.05	0.1	–	0.05
Tween 80[a]	–	–	–	0.05	0.05	0.1
$MgSO_4$	–	–	–	0.02	0.02	0.02
$MnSO_4$	–	–	–	0.005	0.005	0.005
$FeSO_4$	–	–	–	0.004	0.004	0.004
Ammonium citrate	–	–	–	–	–	0.2
Thiamine	–	–	–	–	–	0.001
K_2PO_4	–	–	–	–	–	0.2
$CaCO_3$	0.1	0.1	–	0.01	0.01	0.01
Pyridoxal-5-phosphate	0.005	–	–	–	–	0.005
Amino acid	0.5–1.0	2.7 (His)	0.5 (0.005 for Tyr)	2.0	2.0	1.0
Bromcresol purple	0.001	0.006	0.005	0.006	0.006	0.006
Cresol red	0.0005	–	–	–	–	–
Agar	–	2	2	2	2	2
pH	6.0	5.3	5.5	5.0	5.3	5.3
Application	Enterobacteria	Enterobacteria from fish	*Lb. buchneri* *O. oenii*	Lactobacilli from cheese	LAB from meat products	LAB and enterobacteria

purple in response of the indicator to a pH shift. The pH change is dependent of the production of the more alkaline BA from the amino acids initially included in the medium.

Such indicator media have been used in several studies for the detection of amine-producing enterobacteria in fish and fish products (Chen et al., 1989). Yet, some reports (Baranowski, 1985; Roig-Sagués et al., 1997) have described the occurrence of false-positive reactions, due to the formation of other alkaline compounds. False-negative responses as a result of the fermentative activity of some bacteria which produce acid along with BA, constitute another major problem when screening LAB. In addition, some microorganisms, such as fastidious LAB, are not able to grow in these relatively simple media.

Modifications to these media have been reported by Joosten and Northold (1989) and Maijala (1993) in order to adapt the method to different purposes (Table 1). Joosten and Northold (1989) added some metal sulphates (Mg, Mn, and Fe) and Tween 80 to the Niven's medium (Niven et al., 1981) to enhance the growth of lactobacilli isolated from cheese. According to these authors, the contrast of the colour surrounding the colonies was improved by lowering the initial pH of the medium to 5.0 and by adding glucose. Optimal glucose concentration was 0.1%, as higher concentrations led to excessive lactic acid production, thereby counteracting the purple coloration. Tyrosine has low solubility (0.45 g/l at 25°C) and at the 2% concentration used, plates were not translucent and the characteristic colour change could not be used to detect tyramine producers. However, tyrosine-decarboxylating bacteria were surrounded by a clear halo, resulting from the disappearance of tyrosine precipitate. In spite of these modifications, Maijala (1993) reported that some non-starter LAB showed only weak growth response and were unable to produce a positive reaction. In order to facilitate the growth of meat LAB, decarboxylase agar of Joosten and Northold was modified by adding Lab-Lemco powder (meat extract) and omitting NaCl. According to Maijala (1993), glucose should be omitted because it may inhibit the appearance of the positive colour by causing a pH decrease as it is fermented to lactic acid by LAB.

Although these authors reported the suitability of the media to determine BA production by LAB, only few positive results were detected from a wide number of LAB tested, either due to insufficient growth or no change of colour. Therefore, the objectives of this study was to develop an improved screening method for the detection of amino acid decarboxylase-positive microorganisms (especially LAB). In addition, the suitability of the designed medium was evaluated by confirmation of the quantitative amine-forming capacity using an HPLC assay. The potential to produce BA (tyramine, histamine, putrescine and cadaverine) was investigated in a wide number of LAB, including starter cultures, protective cultures, type strains and strains isolated from different food products.

2. Material and methods

2.1. Bacterial cultures

A total of 177 strains of LAB, including lactobacilli, lactococci, enterococci and carnobacteria were used in this study. The strains were from the ATCC (American Type Culture Collection), BAFF (Bundesandtalt für Fleischforschung, Kulmbach, Germany), BFE (Federal Research Center for Nutrition, Karlsruhe, Germany), CTC (Meat Technology Center-IRTA, Girona, Spain), DNB (Department of Nutrition and Food Science, University of Barcelona, Spain), DSM (German Collection of Microorganisms, Braunschweig, Germany), and NCFB (National Collection of Food Bacteria, Reading, UK) collections. Also, 17 enterobacteriaceae strains isolated from fermented sausages were tested. Summarising information on the number of strains of each species studied is given in Table 2.

2.2. Activation of microbial cultures

In order to promote the enzyme induction before the actual screening test, LAB strains were subcultured 5 to 10 times in MRS broth (110661 Merck, Darmstadt, Germany), while Enterobacteriaceae strains were pregrown in Nutrient Standard broth (107882 Merck, Darmstadt, Germany), both containing 0.1% of each precursor amino acid (all from Merck, Darmstadt, Germany), comprising tyrosine free base, histidine monohydrochloride, ornithine

Table 2
List of species assayed using the improved decarboxylase screening medium: the number of strains positive for at least one BA is indicated with respect to the total number tested

Species	Strains	Species	Strains
Lb. acidophilus	2/5		
Lb. alimentarius	0/1	*Carnobacterium divergens*	10/10
Lb. amylovorus	0/2	*Carnobacterium gallinarum*	1/1
Lb. animalis	0/1	*Carnobacterium mobile*	0/1
Lb. bavaricus	2/4	*Carnobacterium piscicola*	14/14
Lb. bifermentans	1/1		
Lb. brevis	3/4	*Enterococcus durans*	1/1
Lb. brevis/buchneri	0/1	*Enterococcus faecalis*	15/15
Lb. buchneri	1/1	*Enterococcus faecium*	10/10
Lb. bulgaricus	1/1		
Lb. casei	1/1	*Leuconostoc carnosum*	2/2
Lb. cellobiosus	0/1	*Leuc. mesenteroides* ssp. *mesenteroides*	1/1
Lb. curvatus	12/15	*Leuc. mesenteroides* ssp. *cremoris*	0/1
Lb. delbrueckii ssp. *bulgaricus*	2/2	*Leuc. mesenteroides* ssp. *dextranicus*	0/1
Lb. delbrueckii ssp. *lactis*	0/1	*Leuc. mesenteroides*	0/1
Lb. fermentum	0/1		
Lb. gasseri	0/3	*Oenococcus oenii*	0/1
Lb. helveticus	0/1		
Lb. hilgardii	0/1	*Weissella confusa*	0/1
Lb. intestinalis	0/1	*Weissella viridescens*	0/1
Lb. johnsoni	1/4		
Lb. paracasei ssp. *paracasei*	1/6	*Pediococcus acidilactici*	0/1
Lb. paracasei ssp. *tolerans*	0/1	*Pediococcus pentosaceus*	0/1
Lb. plantarum	1/16		
Lb. reuteri	0/1	*Citrobacter freundii*	1/1
Lb. rhamnosus	0/5	*Enterobacter cloacae*	2/2
Lb. sakei	2/17	*Klebsiella oxytoca*	1/1
Lb. salivarius ssp. *salicinus*	0/1	*Proteus vulgaris*	0/5
Lactobacillus sp.	4/12	*Serratia liquefaciens*	4/4
		Serratia marcescens	1/1
Lactococcus lactis ssp. *lactis*	1/1	*Serratia* sp.	2/2
		Enterobacteriaceae	1/1
Streptococcus thermophilus	1/2		

monohydrochloride and lysine monohydrochloride, in addition to supplementation with 0.005% of pyridoxal-5-phosphate.

2.3. Screening medium

The medium designed was based on the Joosten and Northold (1989) medium and also on the MRS agar described by De Man et al. (1960) for the cultivation of LAB. The composition of the medium is shown in Table 1, in comparison with other decarboxylation media suggested earlier. Modifications to the Joosten and Northold medium consisted in reducing glucose concentration to 0.05% in order to avoid excessive acid production that might counteract the pH increase by the BA formation. Also, salt concentration was slightly lower. Metal sulphates, such as Mg (0.02%), Mn (0.005%), and Fe (0.004%), Tween 80 (0.1%), and ammonium citrate (0.2%) were included to enhance the growth of LAB. Since some LAB strains (especially heterofermentative LAB) require thiamine to grow, 0.001% of this vitamin was added. In order to improve the buffer effect and to neutralise the acid produced, 0.2% of *di*-potassium phosphate and 0.01% of calcium carbonate were added. Also, pyridoxal-5-phosphate was included to the decarboxylase medium (at 0.005%) since its presence as a cofactor for the decarboxylation reaction has a strong enhancing effect on the amino acid decarboxylase activity

(Gale, 1946; Recsei et al., 1985). The concentration of each amino acid was 1%. Bromocresol purple was used as pH indicator at 0.006%. The pH was adjusted to 5.3 and the medium was autoclaved for 10 min at 121°C to avoid excessive hydrolysis of the agar at the low pH (Niven et al., 1981).

All strains were streaked in duplicate on the decarboxylase medium plates with and without amino acids (as control) and were incubated for 4 days at 37°C, under aerobic and anaerobic conditions (MK3 Anaerobic Work Station, dw Scientific, Meintrup-Labortechnic, Länden, Germany) in parallel.

2.4. Confirmation of biogenic amine formation

Confirmation of the amine-forming capacity of each microbial culture was performed through a qualitative and quantitative chemical analysis of the BA (tyramine, histamine, putrescine and cadaverine) potentially formed in the fermenting broth. The strains, previously cultured in the MRS or Nutrient Standard broth (with the precursor amino acids and pyridoxal-5-phosphate added), were inoculated at 0.1% into a decarboxylase broth, formulated as the screening medium but without agar and containing 0.5% of tyrosine, 0.25% of histidine, ornithine, and lysine. The solubility problem of tyrosine was solved using tyrosine *di*-sodium salt, with a solubility of 50 g/l, thus achieving a constant concentration of available tyrosine in the medium in all the tubes. After incubating at 37°C for 4 days under aerobic and anaerobic conditions, 2 ml of fermenting broth were centrifuged (12 000 rpm/5 min), after which 1 ml of supernatant was extracted with 1 ml of 0.1 N HCl. It was centrifuged again (12 000 rpm/5 min), filtered through 0.45 μm (Millipore Corp., Bedford, MA, USA) and stored at −20°C until BA analysis.

Samples were analysed for the presence of BA by high-performance liquid chromatography (HPLC system from Waters Chromatography, Milford, MA) according to the method described by Hernández-Jover et al. (1996). The method is an ion-pair chromatography procedure based on the formation of ion-pairs between BA and octanesulfonic acid present in the mobile phase. Their separation as neutral compounds is carried out through a reverse phase column (Nova Pack C18, Waters). When BA are already separated, a post-column derivatization with *o*-phthalaldehyde (OPA) in presence of 2-mercaptoethanol leads to a high recovery because of a short time between the formation of the unstable derivatives and their detection. Detection was by fluorimetry with the excitation wavelength at 340 nm and the emission wavelength at 445 nm. The identification of the BA in the samples is made by the retention times according to the standards. The quantification is carried out by an external standard procedure through the calculation of a calibration curve with standard solutions at different concentrations ranging from 0.5 to 10 ppm.

2.5. Chemicals

All microbiological media and chemicals were from Merck (Darmstadt, Germany) except for BA standards that were from Sigma (St. Louis, MO, USA).

3. Results and discussion

3.1. Screening of decarboxylase activity

Table 2 shows the number of positive strains from the total number of strains tested by the screening procedure. Positive reactions, both on decarboxylase plates or in broth, were recorded when a purple colour occurred or tyrosine precipitate disappeared around the colonies or in the decarboxylase broth, respectively. From all the strains assayed and confirmed by HPLC analysis, no false-positive reaction was observed. On the other hand, three tyramine-producing strains of lactobacilli (*Lb. curvatus* CTC435, *Lb. sakei* CTC430, and *Lb. curvatus* CTC371) gave a negative response with the screening procedure. However, these false negatives were shown to be weak amine formers, producing only 18, 62 and 302 mg/l of tyramine in the medium, respectively. These concentrations were too low to cause the pH shift and the concomitant colour change, especially when considering the acid production of these LAB strains. Neither were they able to decarboxylate tyrosine to an extent at which a clear halo in the medium could be observed.

Limited information on the requirements and factors affecting amino acid decarboxylase capacity of microorganisms has been published so far. It seems important that the optimum pH to screen for

BA production should be slightly acid (5.0–5.5) in order to enhance the synthesis and activity of amino acid decarboxylase enzymes. It also appears practicable to use a pH which mimics the original food system or the product from which the organism has been isolated. The low pH may inhibit bacterial growth to some extent, especially in the case of Enterobacteriaceae (Niven et al., 1981). However, LAB are generally acid tolerant, and should grow sufficiently at pH around 5 used in the screening procedure.

Utilisation of glucose by bacteria could play a role in lowering the medium pH to a value more favourable level for BA production. Furthermore, Gale (1946) pointed out that BA are generally produced only when fermentable carbohydrate is present in the growth medium. The presence of glucose as energy source allows the medium to support the growth and the metabolic activity of fastidious bacteria such as LAB.

As is shown in Table 1, the earliest known decarboxylase differential medium of Moeller (1954) contained pyridoxal as cofactor of amino acid decarboxylation. None of the later modifications was formulated to include this component. Although some authors supplemented the medium with pyridoxine (Frank et al., 1985), it seems that the only active form of this B vitamin as codecarboxylase factor is the *o*-phosphorylated pyridoxal (Gale, 1946). The presence of pyridoxal-5-phosphate has a strong enhancing effect on the decarboxylase activity expression (Gale, 1946; Reesei et al., 1985) and it, therefore, appears to be an important ingredient of the screening medium.

In addition, some authors (Gale, 1946; Baranowski, 1985) have reported the inducible characteristic of amino acid decarboxylase enzymes. It seems that there is an adaptive formation of these enzymes in response to the presence of the specific substrate as well as to specific conditions during growth (i.e., acid environment). Therefore, their induction may be promoted by precultivation of a strain in a medium containing the amino acids and the cofactor.

According to Moeller (1954), strains of Enterobacteriaceae, must be protected from air during incubation in order to avoid false alkalisation at the surface of the medium. In the present work, no appreciable differences in the reaction under aerobic or anaerobic conditions were found among the LAB assayed. Yet, for Enterobacteriaceae strains, aerobic conditions always seemed to yield false-positive reactions, even on plates without any amino acid. Baranowski (1985) pointed out the importance of streaking microbial cultures on plates formulated without amino acids parallel to the screening plates in order to detect potential false-positive responses.

Other pH indicators, such as bromocresol green, chlorophenol red and combinations of both, were also tested. These indicators show the colour change at lower pH than bromocresol purple, and thus might be useful to detect BA formation by the acid producing LAB. However, a high number of false positives was found (data not shown) when using the above indicators. Chen et al. (1989) also observed higher false positive results using chlorophenol red than bromocresol purple.

The improved decarboxylase medium sufficiently supported the growth of the strains investigated. The simplicity of the method, the easy recognition of positive reactions, and the good correlation with the chemical analysis, suggest the suitability of the present medium as a superior screening method to investigate the capacity of LAB to produce BA.

3.2. Biogenic amine production by bacteria

Biogenic amine production has been most extensively studied with respect to histamine and tyramine, probably the two most important BA of bacterial origin in food, due to their toxicological effects. In the present work, the diamines putrescine and cadaverine were also investigated since they may potentiate the toxicity of the above amines, and they even might serve as indicators of poor hygienic quality in some food substrates (Mariné-Font et al., 1995).

Table 3 shows the qualitative and quantitative production of BA by LAB strains. Forty percent of the LAB strains were found to be tyramine formers (100% of the carnobacteria and the *Enterococcus* strains studied). Accumulation of tyramine in the fermenting broth showed a wide variation, from 20 to 5000 mg/l, being higher than 400 mg/l in most of the cases. Levels of tyramine formed were relatively high compared to those previously reported. This may be due to the composition of decarboxylase medium, the incubation time, and to the particular activity of the strains assayed. As in other studies

Table 3
Quantified (mg/l broth) biogenic amine production by lactic acid bacteria strains: determination was made after cultivation at 37°C for 4 days in decarboxylation broth[a]

Species	Strains tested	TY[b]		HI		PU		CA	
Carnobacterium divergens	10	(10)[c]	104–4483		–		–		–
Carnobacterium gallinarum	1	(1)	1504		–		–		–
Carnobacterium piscicola	14	(14)	159–2923		–		–		–
Enterococcus durans	1	(1)	610		–		–		–
Enterococcus faecalis	15	(15)	601–4986		–		–		–
Enterococcus faecium	10	(10)	379–4339		–		–		–
Lactobacillus acidophilus	5	(1)	498	(1)	22		–		–
Lactobacillus bavaricus	4	(2)	458–551		–		–		–
Lactobacillus bifermentans	1	(1)	561		–		–		–
Lactobacillus brevis	4	(2)	516–3589		–	(1)	481	(1)	14
Lactobacillus buchneri	1	(1)	7641		–		–		–
Lactobacillus bulgaricus	1	(1)	5135		–		–		–
Lactobacillus casei	1	(1)	3137		–		–		–
Lactobacillus curvatus	15	(13)	302–3494		–	(3)	37–906	(2)	18–30
Lactobacillus delbrueckii ssp. *bulgaricus*	2	(1)	641	(1)	23		–		–
Lactobacillus johnsonii	4	(1)	413		–		–		–
Lactobacillus paracasei ssp. *paracasei*	6	(1)	497		–		–		–
Lactobacillus plantarum	16	(1)	3331		–		–		–
Lactobacillus sakei	17	(3)	62–2121		–		–		–
Lactococcus lactis ssp. *lactis*	1	(1)	1380		–		–		–
Leuc. mesenteroides ssp. *mesenteroides*	1	(1)	135		–		–		–
Leuconostoc carnosum	2	(2)	3191–4590		–		–		–
Streptococcus thermophilus	2	(1)	5304		–		–		–
Lactobacillus sp.	12	(3)	446–2943		–		–	(1)	19

[a] Decarboxylation broth contained 0.5% tyrosine and 0.2% of histidine, ornithine and lysine, respectively.
[b] TY, tyramine; HI, histamine; PU, putrescine; CA, cadaverine.
[c] Number of strains which showed biogenic amine formation.

(Joosten and Northold, 1989; Leisner et al., 1994; Giraffa et al., 1995; Straub et al., 1995) *Lb. brevis*, *Lb. buchneri*, *Lb. curvatus*, *Leuconostoc carnosum*, *Carnobacterium* spp. and *Enterococcus* spp. were found to be the most intensive tyrosine decarboxylase species. Still, strains from other species also showed amine production. Only a few strains of lactobacilli produced putrescine and/or a small amount of cadaverine along with tyramine. Although some LAB have been reported to show intensive histidine decarboxylase activity (Joosten and Northold, 1989; Straub et al., 1995), no significant histamine production was observed in the LAB tested. In addition, several strains of lactobacilli, *Leuconostoc* spp., *Weissella* spp., and pediococci showed no decarboxylase activity. The results of BA production in a synthetic medium suggest that the capability to produce amines might be strain dependent rather than being related to specific species. However, this property seems to be more common among strains of particular species, e.g., *Lb. curvatus* as compared to *Lb. sakei* (see Table 2).

Several BA-forming strains are of importance in food fermentation. Some of the positive strains might be used as starter or protective cultures without knowledge about their potential to form BA (for instance, BA-positive strains of *Streptococcus thermophilus* for yoghurt production, numerous positive *Lb. curvatus* strains associated with sausage fermentation, and strains of *Ent. faecium* and *Ent. faecalis* of pharmaceutical products). Therefore, the inability to form BA needs also to be confirmed for microorganisms usually considered safe.

Most of the Enterobacteriaceae strains assayed, produced high quantities of cadaverine and putrescine, whereas no strain was found to produce tyramine (Table 4). Production of high amounts of histamine by enterobacteria in fish has been extensively reported (Taylor et al., 1978; Chen et al., 1989; Beutling, 1996), especially as causative agent

Table 4
Quantified (mg/l broth) biogenic amine production by Enterobacteriaceae strains: determination was made after cultivation at 37°C for 4 days in decarboxylation broth[a]

Species	Strains tested	TY[b]	HI		PU		CA	
Citrobacter freundii	1	–	(1)[c]	41	(1)	43	(1)	15
Enterobacter cloacae	2	–	(2)	18–29	(2)	473–568	(2)	599–755
Klebsiella oxytoca	1	–	(1)	38		–	(1)	683
Serratia liquefaciens	4	–	(2)	16–45	(4)	474–758	(4)	591–971
Serratia marcescens	1	–	(1)	16	(1)	474	(1)	594
Serratia sp.	2	–	(1)	29	(1)	471	(1)	590
Enterobacteriaceae	1	–		–	(1)	476	(1)	599

[a] Decarboxylation broth contained 0.5% tyrosine and 0.2% of histidine, ornithine and lysine, respectively.
[b] See Table 3.
[c] Number of strains which showed biogenic amine formation.

of *scombroid poisoning*. Yet, only minor formation of histamine was observed in the present study for the enterobacteria, which were originally isolated from meat products.

These results are in agreement with the fact that fermenting microorganisms (LAB) are occasionally associated with tyramine formation, although they can also contribute to the accumulation of other BA such as putrescine. Since LAB may constitute part of the spoilage association, they may also be responsible for tyramine accumulation during food spoilage. On the other hand, Enterobacteriaceae would be associated with cadaverine, putrescine, and histamine formation in meat products, mainly when a deterioration process occurs in either raw materials or end-products.

The negative result for biogenic amine production in laboratory media does not imply similar behaviour in a food product. It should be considered that food products are complex systems with a wide number of factors influencing microbial growth and activity. Little is known about the effect of different factors (such as glucose and NaCl concentration, additives, temperature, etc.) on BA production. This feature seems to be of special interest for fermenting food bacteria involved in fermentation. Furthermore, the influence of the redox potential and the shift from aerobic to anaerobic conditions on BA production in packaged commodities requires more attention. Also, interactive effects between BA and food associated microorganisms may provide another rewarding field of study.

4. Conclusions

A decarboxylase screening medium for LAB and Enterobacteriaceae has been modified. It enables improved detection of BA positive strains. The plate procedure still presents some limitations in terms of sensitivity in detecting BA forming microorganisms. However, compared to chromatographic analysis, the decarboxylase medium allows a rapid preliminary selection of strains with low decarboxylase activity, with a detection limit estimated around 350 mg/l.

BA production by bacteria has thus far been most extensively studied with respect to tyramine and histamine, probably the two most important BA of bacterial origin in food. In association with fermented foods, some LAB such as the enterococci (generally), carnobacteria, and some strains, particularly of *Lb. curvatus*, *Lb. brevis* and *Lb. buchneri*, have importance as tyramine producers. As food contaminants, the Enterobacteriaceae probably rank first as decarboxylase active bacteria mainly producing cadaverine and putrescine.

Acknowledgements

The authors gratefully acknowledged the financial support received by the EU Commission (Cost-Action 917: Biologically active amines in food; and FAIR CT-97-3078) and the 'Comisión Interministerial de Ciencia y Tecnología' (ALI95-0890-C04-

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