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Biogenic Amine Formation in Fermented Beverages: Targets, Tools and Triumphs

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ABSTRACT

Biogenic amines (BA) are mainly produced in food and beverages such as sherry products, cheese, wine and fermented sausages or vegetables by bacteria metabolism via the activity of specific amino acid decarboxylases or as a spontaneous chemical reaction. The formation of biogenic amines in food and beverages depends on the quantities of free amino acids and the presence of microorganisms with decarboxylase activities. Due to their physiological activity, these molecules are of concern in relation to food safety and spoilage. Histamine and tyramine have been the most studied because of their implication in food poisoning. The improvement of molecular tools, usually based on PCR techniques, has allowed a fast and sensitive characterization of the majority of bacteria producing BA. For instance, the sequence of tyrosine, ornithine, lysine and histidine decarboxylase genes have been determined and primers for detection of tyramine- and histamine-producing bacteria in food have been developed. Furthermore, real time PCR approaches have been undertaken in order to identify viable but not cultivable (VNC) BA-producing bacteria. Moreover, the characterization of starter cultures possessing amine oxidase activity (AO) to control or reduce the accumulation of biogenic amines is a worthwhile goal in order to reduce BA production in fermented beverages.

Keywords: amine oxidase, *hdc*, *odc*, real time PCR

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INTRODUCTION

Biogenic amines (BA) are organic bases with aliphatic, aromatic or heterocyclic structures that can be found in several foods, in which they are mainly produced by microbial decarboxylation of amino acids, with the exception of physiological polyamines (Silla Santos 1996). BA are undesirable in all foods and beverages because they may induce headaches, respiratory distress, heart palpitation, hyper or hypotension, and several allergenic disorders (Silla Santos 1996). Phenylethylamine and tyramine, at concentrations of 3 and 25-40 mg l^{-1} respectively, are reported to cause migraines and hypertension (Soufleros *et al*. 1998). Histamine is the most toxic and its effect can be enhanced by other amines such as putrescine and agmatine by preventing its oxidation (Silla Santos 1996; Soufleros *et al*. 1998).

However, human sensitivity varies according to the individual detoxifying activities of some enzymes involved in biogenic amine metabolism, such as histamine methyltransferase or others, less specific, such as monoaminoxidase and diaminoxidases. These enzymes are inhibited by several types of drugs such as the neuromuscular blocking drugs d-tubocurarine, pancuronium and alcuronium and ethanol (Sattler *et al*. 1985). As a consequence of this synergistic action, the simultaneous consumption of fermented foods and beverages causes disorders, even if each separate product might not be considered as hazardous (Lanvoud-Funel 2001). BA accumulation in foods requires the availability of precursors (i.e. amino acids), the presence of microorganisms with amino acid decarboxylases, and favourable conditions for their growth and decarboxylating activity (ten Brink *et al*. 1990). In general, histamine (HIS), putrescine (PUT), cadaverine (CAD), tyramine (TYR), tryptamine (TRY), 2-phenylethylamine (PHE), spermine (SPM) and spermidine (SPD) are the most important BA in foods (**Table 1**). Histidine, tyrosine, tryptophane, lysine, phenylalanine and ornithine are precursors for the formation of HIS, TYR, TRY, CAD, PHE and PUT, respectively. Both SPE and SPD can be produced from arginine metabolism together with agmatine. BA can be found as a consequence

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Invited Mini-Review

Table 1 Precursors and enzymes involved in biogenic amines formation in food.

Biogenic amines	Precursors	Enzymes (Abbreviation)
Histamine	Histidine	Histidine decarboxylase (HDC)
Tyramine	Tyrosine	Tyrosine decarboxylase (TDC)
Putrescine	Ornithine	Ornithine decarboxylase (ODC)
Putrescine	Agmantine	Agmantine deiminase (AgD)
Agmantine	Arginine	Arginine decarboxylase (ADC)
Cadaverine	Lysine	Lysine decarboxylase (LDC)
Tryptamine	Tryptophane	Tryptophane decarboxylase (TDC)
2-phenylethylamine	Phenylalanine	Phenylalanine/tyrosine decarboxylase (PAL/TDC)

of amino acid decarboxylase metabolic microbial activity in foods such as wine, fermented meat and fish products, cheeses and fermented vegetables (Maijala *et al*. 1995; Silla Santos 1996; Moreno-Arribas *et al*. 2000; Suzzi and Gardini 2003; Landete *et al*. 2005a).

Food-fermenting lactic acid bacteria (LAB) are generally considered to be not toxinogenic or pathogenic. Some species of LAB, however, can produce BA. Many LAB belonging to the genera *Lactobacillus*, *Enterococcus*, *Carnobacterium*, *Pediococcus*, *Lactococcus* and *Leuconostoc* are able to decarboxylate amino acids (Bover-Cid and Holzapfel 1999; Lonvaud-Funel 2001). Strains of lactobacilli belonging to the species *L. buchneri*, *L. alimentarius*, *L. plantarum*, *L. curvatus*, *L. farciminis*, *L. bavaricus*, *L. homohiochii*, *L. reuteri*, and *L. sakei* were amine-positive and tyramine is quantitatively the most important BA produced (Montel *et al*. 1999; Bover-Cid *et al*. 2001). Many authors (Silla Santos 1998; Montel *et al*. 1999; Bover-Cid *et al*. 2001) did not observe histidine decarboxylase activity in lactobacilli isolated from sausage (Suzzi and Gardini 2003). In contrast, this enzyme was present in many *Lactobacillus* spp. and in *L. sakei* isolated from fish (Dapkevicius *et al*. 2000), *Lactobacillu*s spp. from sausages (Suzzi and Gardini 2003), *L. bulgaricus* (Bover-Cid and Holzapfel 1999), and in a strain of *L. acidophilus* (Bover-Cid and Holzapfel 1999). The ability to produce histamine has been found in some *Leuconostoc* (Dapkevicius *et al*. 2000) and in *Oenococcus oeni* (Lonvaud-Funel 2001). In wine more than 20 amines have been identified and their total concentration has been reported to range from a few mg/ l to about 50 mg/l depending on the quality of the wine (Moreno-Arribas *et al*. 2000; Lonvaud-Funel 2001; Moreno-Arribas *et al*. 2003; Landete *et al*. 2005a, 2005b). The variability of the amine contents in wine could be explained on the basis of differences in the winemaking process, time and storage conditions, raw material quality, and possible microbial contamination during winery operations (Lonvaud-Funel 2001). The types and levels of BA in beers are affected mainly by raw materials, brewing techniques and hygienic conditions (Izquierdo-Pulido *et al*. 1994; Romero *et al*. 2003). Agmatine, tyramine and putrescine are usually the prevailing amines in beer while histamine, β-phenylethylamine, tryptamine, cadaverine, spermine and spermidine were detected at relatively low levels (in general <2 mg/l). The physiological role of amine formation by microorganisms has not yet been completely elucidated. The capacity to decarboxylate amino acids is generally considered a strain-dependent characteristic rather than a species property. Some studies suggest new, interesting hypotheses on the physiological role of amine in microorganisms (Schiller *et al*. 2000; Tkachenko *et al*. 2001). In *E. coli*, the expression of *oxyR*, the gene that protects this bacterium against oxidative stress, was enhanced by physiological concentrations of the biogenic amine putrescine. Moreover, putrescine was shown to produce a protective effect if the DNA is damaged by reactive oxygen species (Tkachenko *et al*. 2001). Cells of *E. coli* grown in M9 minimal medium and subjected to a hyperosmotic shock by addition of 0.5 M NaCl started immediately to excrete putrescine, elevating its concentration in the supernatant from 0 to 25 μM within 30 min, suggesting that putrescine may be involved in osmotic stress tolerance in *E. coli*. Therefore, bacteria which possess amino acid decarboxylase activity, could overcome or reduce the effects of factors such as oxygen and NaCl, that induce stress responses in the cells, with the production of BA.

Polyamines may act as endogenous modulators of outer membrane permeability, possibly as a part of an adaptative response to acidic conditions (Schiller *et al*. 2000; Tkachenko *et al*. 2001).

Several authors reviewed the occurrence of BA in specific foods. LAB producing histamine, putrescine, cadaverine and tyramine with histamine the most important BA reaching levels higher than 300 mg l^{-1} have been described in wine (Lonvaud-Funel 2001; Guerrini *et al*. 2002; Moreno-Arribas *et al*. 2003; Landete *et al*. 2005a), and cheese (Stratton *et al*. 1991), while tyramine, putrescine and cadaverine with tyramine the most important BA reaching levels higher than 500 mg kg^{-1}, have been rewied elwhere (Suzzi and Gardini 2003). Moreover, methods for the detection of BA in food have recently been summarised (Marcobal *et al*. 2006).

This review will summarise the recent investigations on the molecular bases of amine production in fermented beverages such as wine and the development of molecular tools for the early detection of BA. Moreover, we will try to point out those open questions that can be further investigated.

TARGETS

Histidine decarboxylase

Histamine and tyramine are the most studied BA due to their toxicological effect. Most of the intoxications produced by BA are related to histamine, because this amine may lead a dilation of blood vessels, capillaries and arteries, causing headaches, hypotension, gastrointestinal distress and edemas. Histamine is normally present at low levels in the human body and participates in diverse key functions, including vascular permeability, neurotransmission, and the allergic response (Taylor 1986). The concentration of histamine in blood may increase substantially after ingestion of foods containing high doses of histamine. Substances inhibiting histamine metabolism, such as ethanol present in alcohols, may augment this increase. Fermented foods are frequently contaminated by histamine that is generated by microorganisms with histidine decarboxylase activity. The histamine in wine is of particular interest because it has been reported that the presence of alcohol and other amines promotes its effects by inhibiting the human detoxification systems (Chu and Bejdanes 1981). Yeast, acetic acid bacteria, and LAB are possible histamine producers, although the latter are reported to be the principal bacteria responsible for histamine production in wine (le Jeune *et al*. 1995; Lonvaud-Funel 2001; Landete *et al*. 2005a). The bacterial population in wine is a complex mixture of different species of LAB (*Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Oenococcus*), with *O. oeni* as the predominant species in wine during and after malolactic fermentation. Although there are previous works about the ability of wine LAB to produce histamine, their conclusions are contradictory. For instance, Lonvaud-Funel and Joyeux (1994), le Jeune *et al*. (1995), Coton *et al*. (1998a) and Guerrini *et al*. (2002) found that histamine decarboxylase activity is present in a high proportion of wine strains. In contrast, Moreno-Arribas *et al*. (2003) found the frequency of this characteristic to be very low. As histamine concentration increases after malolactic fermentation in some cases, many authors have focused the screening of histamine production on *O. oeni* (Coton *et al*. 1998a; Guerrini *et al*. 2002). Landete *et al*. (2005a) demons-

trated that, of the LAB present in wine, *Lactobacillus hilgardii* and *Pediococcus parvulus* are those mainly responsible for the high histamine concentrations in wine. In recent years, it has been reported that some *O. oeni* strains are responsible for histamine accumulation in wine (le Jeune *et al.* 1995; Guerrini *et al.* 2002). However, although the proportion of *O. oeni* histamine producers is very high, this species produces histamine at low levels (Guerrini *et al.* 2002).

Histamine is produced by enzymic decarboxylation of the histidine present in foods. There are two distinct classes of histidine decarboxylases: that of eukaryotic and Gram-negative bacteria, which requires pyridoxal phosphate as a cofactor, and that of Gram-positive bacteria, which uses a covalently bound pyruvoyl moiety as a prosthetic group (Recsei *et al.* 1983; Coton *et al.* 1998b). The gene encoding histidine decarboxylase (*hdcA*) has been identified in different Gram-positive micro-organisms, such as *C. perfringens* (van Poelje and Snell 1990), *O. oeni* (Coton *et al.* 1998b) and *Lactobacillus* 30a (Vanderslice *et al.* 1986). With the exception of *C. perfringens*, this gene is part of an operon which includes a second gene, *hdcB*. Although different functions such as regulation or transport have been suggested for the latter gene (Coton *et al.* 1998), its true function is still unknown. Recently, it has been reported that the *hdc* gene is subject to different regulations. The expression of *hdc* is mediated by the bacterial growth phase. The expression was observed to be highest in the exponential growth phase because histidine decarboxylation generates metabolic energy (Molenaar *et al.* 1993), and maximum energy is consumed during growth and the period of greatest cell division. Histidine (1 or 2 g l^{-1}) induces the expression of the *hdc* gene and histamine (2 g l^{-1}) causes a decrease in the expression of this gene. Therefore, the presence of histidine in wine should have a double effect on histamine production because it is both the substrate of HDC and it induces the expression of *hdc* (Landete *et al.* 2006). Temperature and pH had an effect on the activity of HDC but not on *hdc* expression. Tartaric acid and L-lactic acid, and SO_2 had neither an effect on enzyme synthesis nor on its activity. This behaviour was observed for all the studied microorganisms. Thus, the conditions that normally occur during malolactic fermentation and later on, could favor histamine production (Landete *et al.* 2006). SO_2 could prevent bacterial growth, but does not diminish the HDC enzyme activity.

The *hdc* gene may also be delivered by mobile elements. In *Lactobacillus hilgardii* isolated from wine the ability to form histamine is easily lost depending on culture conditions (Lucas *et al.* 2006). Loss of HDC activity corresponded to the loss of a large plasmid named pHDC. The *hdcA* locus on the plasmid was identified and shown to be part of a four gene cluster most likely involved in the histamine-producing pathway. One of the gene products is an integral membrane protein that was shown to catalyze the exchange of histidine and histamine (Lucas *et al.* 2006).

Tyrosine decarboxylase, Tyramine and β-phenylethylamine

Tyrosine decarboxylase (TDC) converts tyrosine to tyramine and its purification and characterization has been reported for LAB (Connil *et al.* 2002; Lucas and Lonvaud-Funel 2002; Coton *et al.* 2004). Tyramine is a strong vasoconstrictor normally metabolized in the intestine by monoamine oxidase (MAO) (Joosten 1988). Although the intake of tyramine is in general of no risk for consumers, in the case of hereditary deficient MAO patients or of patients treated with inhibitors of MAO (MAOIs), the consumption of this amine can be problematic (Caston *et al.* 2002). Therefore knowledge of tyramine content in food is of major importance in avoiding such problems in patients.

LAB vary in their ability to form tyramine. Several strains of *Leuconostoc mesenteroides* isolated from wine (Coton *et al.* 2002; Landete *et al.* 2005), *E. faecalis* and *Enterococcus faecium* identified in fermented sausages (Suzzi and Gardini 2003) are able to produce tyramine. Among *Lactobacillus* genera, strains of *Lactobacillus curvatus*, *L. brevis*, *L. paracasei* and *L. sakei* produce tyramine (Coton *et al.* 2002; Landete *et al.* 2005a, 2005b). Most of the tyramine produced in fermented sausages is probably due to *L. curvatus* (Suzzi and Gardini 2003) while *L. brevis* and *L. hilgardii* appear to be the main tyramine-producer in wine (Lonvaud-Funel 2001; Landete *et al.* 2005a).

Lactobacillus plantarum is frequently isolated from red wine undergoing malolactic fermentation (MFL) and sterilized with sulphite (Spano *et al.* 2004, 2006, 2007a) and it usually contributes to the production of undesirable products such as histamine and precursors of ethyl carbammate (Lonvaud-Funel 1998; Spano *et al.* 2004). Recently, we found some *L. plantarum* strains isolated from wine able to to decarboxylase tyrosine suggesting that *L. plantarum* may be a tyramine producer in wine. However, this ability is apparently rare and confined only to those strains harbouring the tyrosine decarboxylase (*tdc*) gene (Spano *et al.* 2007b).

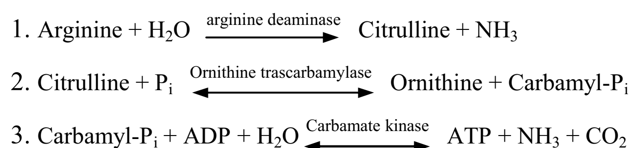
In the last few years, the *tdc* gene has been identified in several LAB and primers for the detection of tyramine-producing LAB have been developed (Coton *et al.* 2002, 2004; Fernandez *et al.* 2004; Coton and Coton 2005; Marcobal *et al.* 2006). The genes coding for bacterial tyrosine decarboxylases have been identified in LAB such as *Enterococcus faecalis*, *Lactobacillus brevis*, and *Lactococcus lactis* (Coton *et al.* 2002, 2004; Coton and Coton 2005; Marcobal *et al.* 2006). In *L. brevis* and *L. lactis*, the gene was contained in an operon containing four genes. The *tyrDC* gene was preceded by a gene homologous to tyrosyl-tRNA synthetases and followed by two genes coding for secondary transporters, a putative tyrosine transporter (*tyrP*) and a putative Na^+/H^+ antiporter. The combination of the tyrosine decarboxylase and tyrosine transporter genes could code for a proton motive pathway converting tyrosine into tyramine provided that the transporter gene codes for an electrogenic precursor-product. It has recently been demonstrated that the transporter catalyzes tyrosine-tyramine exchange with high efficiency and that a net positive charge is translocated across the membrane during exchange (Lucas *et al.* 2006).

β-phenylethylamine production has been described during food fermentations and has been suggested to be formed as a result of the activity of tyrosine decarboxylating bacteria towards this structurally related amino acid (Santos *et al.* 2003; Marcobal *et al.* 2006). However, of all the LAB harbouring the *tdc* gene, L-phenylalanine decarboxylation has been verified only for *L. brevis* and *E. faecalis* (Bover-Cid *et al.* 2001; Moreno-Arribas and Lonvaud-Funel 2001; Beutling and Walter 2002; Suzzi and Gardini 2003). Recently, a gene from *E. faecium* RM58 coding for a protein which is almost identitical to *E. faecalis* tyrosine decarboxylase has been expressed in *Escherichia coli*, resulting in L-phenylalanine and L-tyrosine decarboxylase activities (Marcobal *et al.* 2006). These results suggest that tyrosine decarboxylase activity may be involved on the formation of both tyramine and β-phenylethylamine and LAB should be considered as potential β-phenylethylamine-producing bacteria in fermented foods and beverages.

Putrescine and agmatine

Putrescine is one of the most common BA found in fermented beverages such as wine (Soufleros *et al.* 1998). Agmatine in wine has been found in smaller amounts than putrescine (Glória *et al.* 1998). However, the potentialities of the microorganisms to produce agmatine from arginine, one of the major amino acid found in wine, is a subject of interest because arginine enhances its concentration in wine during the fermentation process. Arena and Manca de Nadra (2001) and Arena *et al.* (2002) reported that *Lactobacillus hilgardii* X1B is able to produce putrescine and agmatine from arginine and ornithine. They also reported that putrescine could be formed from agmatine through a pathway that does not involve amino acid decarboxylase. Mangani *et al.*

(2005) reported that putrescine was produced by *O. oeni* strains in wine not only from ornithine but also from arginine. In this case, putrescine may originate from strains possessing the complete enzyme system to convert arginine to putrescine or by a metabiotic association, with an exchange of ornithine, between strains capable of metabolizing arginine to ornithine but unable to produce putrescine and strains capable of producing putrescine from ornithine but unable to degrade arginine (Mangani *et al.* 2005). Putrescine production by this metabiotic association occurred once the malolactic fermentation was completed, whereas conversion of ornithine to putrescine by a single culture of the ornithine decarboxylating strain concurred with the degradation of malic acid (Mangani *et al.* 2005). The synthesis of putrescine in *E. coli* involves either decarboxylation of (i) arginine by arginine decarboxylase to produce agmatine, agmatine is then hydrolyzed by agmatine ureohydrolase; or (ii) decarboxylation of by ornithine decarboxylase (ODC). Amino acids are naturally present in grapes. Arginine is the most abundant amino acid found in wines and its concentration decreases significantly during malolactic fermentation. However, ornithine is one of the less abundant amino acids in wine, and during malolactic fermentation an increase in the concentration of ornithine, a precursor of putrescine, is noted (Mangani *et al.* 2005). This situation can be explained by the fact that an alternative pathway of synthesis exists for putrescine, resulting from arginine. Arginine is quantitatively one of the most important amino acids in grape musts and wine (Sponholz *et al.* 1991). Although arginine is predominantly metabolized by yeasts during vinification, considerable amounts may still be present in wine at the end of alcoholic fermentation (Liu and Pilone 1998). Therefore, arginine is generally available for metabolism by wine LAB during malolactic fermentation, which is a secondary fermentation following alcoholic fermentation. The complete degradation of arginine by LAB occurs via the arginine deiminase (ADI) pathway, leading to the production of ammonia, ornithine and CO_2 and involves three enzymatic reactions:



1. $\text{Arginine} + H_2O \xrightarrow{\text{arginine deaminase}} \text{Citrulline} + NH_3$

2. $\text{Citrulline} + P_i \xleftrightarrow{\text{Ornithine trascarbamylase}} \text{Ornithine} + \text{Carbamyl-}P_i$

3. $\text{Carbamyl-}P_i + ADP + H_2O \xleftrightarrow{\text{Carbamate kinase}} ATP + NH_3 + CO_2$

During degradation of arginine, ornithine is excreted. According to recent studies, *O. oeni* posesses the *odc* gene (Marcobal *et al.* 2004) while strains of *L. plantarum* are able to produce ornithine and ammonia from arginine through the ADI pathway (Spano *et al.* 2004). Moreover, *L. plantarum* strains able to degrade arginine are also putrescine producers (Spano *et al.* 2007, unpublished results). Therefore, it should be stated that arginine may be at the origin of ornithine and putrescine. As result of the metabolism of wine LAB possessing ODC activity, arginine could be converted to ornithine by the ADI pathway, and ornithine could be metabolised into putrescine by the action of the ornithine decarboxylase (**Fig. 1**). Recently, the nucleotide sequence of a 17.2-kb chromosomal DNA fragment containing the *odc* gene has been determined in the putrescine producer *O. oeni* RM83. This DNA fragment contains 13 open reading frames, including genes coding for five transposases and two phage proteins (Marcobal *et al.* 2006). The origin of the ability to produce putrescine in *O. oeni* is probably the first evidence of a horizontal gene transfer involved in BA production (Marcobal *et al.* 2006).

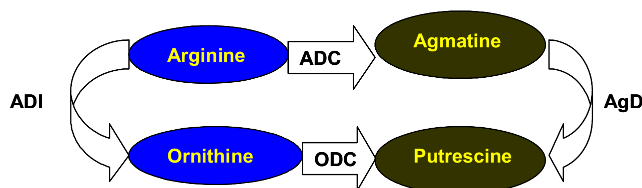


Fig. 1 Role of arginine in the metabolism of agmatine and putrescine. Arginine may be transformed into agmatine by the arginine decarboxylase (ADC) enzyme. Furthermore, agmatine may be metabolize to putrescine by the agmatine deiminase (AgD). However, putrescine may also be produced from arginine trough the arginine deaminase pathway (ADI). Arginine is first metabolized to ornithine and ornithine is then transformed into putrescine by the ornithine decarboxylase (ODC).

TOOLS

Early detection of BA-producing bacteria is essential in the food industry in order to avoid the risk of amine formation. The capability of BA formation seems to be strain dependent rather than species specific. Several methods to detect the production of BA by microorganisms have been developed, from simple methods as paper chromatography or spectrofluorimetric determination to more sophisticate techniques, such as automated systems for detection of microbial metabolic activities or automated conductance measurements (see Marcobal *et al.* 2006 for review).

However, detection of BA-producing bacteria by conventional culture techniques is often tedious and unreliable. Several studies describing loss of ability to produce BA in LAB after prolonged storage or cultivation of isolated strains in synthetic media have been reported. For instance, the instability of HDC cells of *L. hilgardii* is easily explained by the loss of pHDC, which depends greatly on bacterial culture conditions (Lucas *et al.* 2005). The increase of the population of HDC cells in a poor and acidic medium may be attributed to a growth advantage of HDC cells, given that histidine decarboxylation and the exchange of histidine and histamine could provide metabolic energy and help the organism to better survive in acidic environments (Lucas *et al.* 2005). The advantage would be lost in rich medium with a mild pH. Moreover, it was reported that HDC cells of *O. oeni* isolated from wine rapidly lost the capacity to form histamine when they were grown in a synthetic medium (Lonvaud-Funel and Joyeux 1994). This finding highlights the importance of growth conditions on the ability of LAB to produce BA.

The improvement of fast, reliable and culture-independent molecular tools, usually based on polymerase chain reaction (PCR) techniques but also on a proteomic approach (Pessione *et al.* 2005), is an interesting alternative. Since the BA are produced by the decarboxylation of a precursor amino acid by the enzymatic action of an amino acid decarboxylase, it is possible to develop molecular detection methods targeting the genes encoding amino acid decarboxylase enzymes.

Polymerase chain reaction (PCR) and quantitative real-time PCR (qPCR)

PCR has become an important method for the rapid, sensitive, and specific detection of targeted genes. Several oligonucleotide primers have been described to detect amino acid decarboxylases encoding genes by PCR (**Table 2**). Since the designed oligonucleotides are based on amino acid regions conserved in specific amino acid decarboxylases, this implies that these primers would allow the detection of all of them. Two enzyme groups of bacterial histidine decarboxylases have been found: the pyruvoil-dependent and the pyridoxal phosphate-dependent. Pyruvoil-dependent histidine decarboxylases are present in Gram-positive bacteria. Based on this group of histidine decarboxylases several oligonucleotides have been designed (le Jeune *et al.* 1995; Coton *et al.* 2005; Landete *et al.* 2005a; Costantini *et al.* 2006). Similarly, several oligonucleotides for the detection of genes encoding tyrosine decarboxylases have been designed (Lucas and Lonvaud-Funel 2002; Coton *et al.* 2004; Fernández *et al.* 2004; Muñoz *et al.* 2004; Costantini *et al.* 2006). Ornithine decarboxylases, as well as lysine decarboxylases, be-

Table 2 Oligonucleotides used in PCR assays for the detection of genes coding for amino acid decarboxylases.

Gene	Oligonucleotides	Amplicon size (bp)	Reference
hdc	106+107	534	de las Rivas *et al.* 2005
	CL1mod + JV17H5	450	Landete *et al.* 2005
	HDC3-HDC4	435	Coton and Coton 2005
	HS1-F+HS1-R	372	Munõz *et al.* 2004
	HS2-F+HS2-R	531	Munõz *et al.* 2004
	PHDC1 + PHDC2	497	Costantini *et al.* 2006
tdc	P1-rev + P1-for	924	Lucas and Lonvaud-Funel 2002; Costantini *et al.* 2006
	TD2 + TD5	1.100	Coton *et al.* 2004
	TDC1 + TDC2	720	Fernandez *et al.* 2004
	TDC-F + TDC-R	825	Munõz *et al.* 2004
	Pt3 + Pt4	560	Costantini *et al.* 2006
odc	3 + 16	1.146	Marcobal *et al.* 2005; Costantini *et al.* 2006
	PUT1-F + PUT1-R	1.440	Munõz *et al.* 2004
	PUTI2-F + PUTI2-R	624	Munõz *et al.* 2004
	AODC1 + AODC2	1.500	Costantini *et al.* 2006
ldc	CAD1-F + CAD1-R	1.098	Munõz *et al.* 2004
	CAD2-F + CAD2-R	1.185	Munõz *et al.* 2004

hdc, hystidine decarboxylase gene; *tdc*, tyrosine decarboxylase gene; *odc*, ornithine decarboxylase gene; *ldc*, lisine decarboxylase gene. Adapted from Marcobal *et al.* (2006).

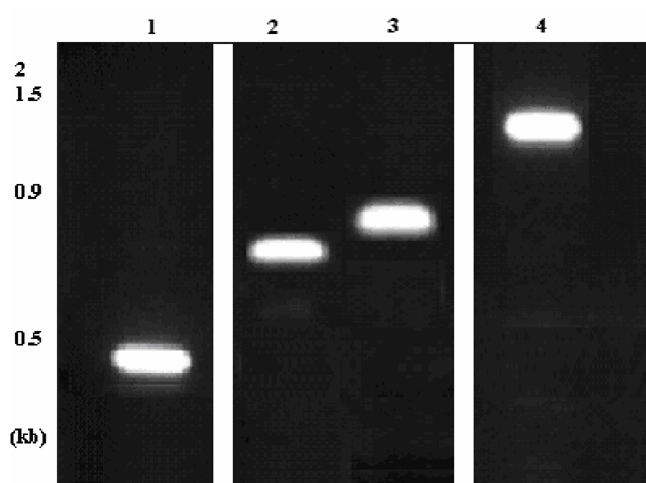


Fig. 2 PCR detection of wine lactic acid bacteria producing histamine, tyramine and putrescine. DNAs extracted from histamine and putrescine producers *Lactobacillus plantarum* strains and tyramine producers *Lactobacillus brevis* ATCC 367. Lane 1, a 372 bp DNA fragment from the histidine decarboxylase gene from wine *Lactobacillus plantarum* amplified with primers HIS1-F and HIS1-R. Lane 2 and 3, a 824 bp and 924 bp DNA fragment from the tyrosine decarboxylase gene of *Lactobacillus brevis* ATCC 367 using oligonucleotides P1-rev/P2-for or TDC-F/TDC-R respectively. Lane 4, a 1440 bp DNA fragment from the ornithine decarboxylase gene from wine *Lactobacillus plantarum* using oligonucleotides PUT-F/PUT-R. The molecular size of a 1 kb standard (Promega) was included on the left.

long to two different groups and specific oligonuclotides have been designed for all of them (Muñoz *et al.* 2004; Marcobal *et al.* 2005; Costantini *et al.* 2006). **Fig. 2** shows some amino acid decarboxylase genes amplified from BA-producer strains.

Recently, a multiplex PCR assay for the simultaneous detection of LAB strains, which potentially produce histamine, tyramine, and putrescine on fermented foods has been developed (Coton and Coton 2005; Marcobal *et al.* 2005). These primers were based on sequences from histidine, tyrosine, and ornithine decarboxylases from LAB. Under the optimized conditions, the assay yielded a 367-bp DNA fragment from histidine decarboxylases, a 924-bp fragment from tyrosine decarboxylases, and a 1,446-bp fragment from ornithine decarboxylases. This assay is useful for the detection of both amine-producing bacteria in control collection strains and in a LAB collection (Marcobal *et al.* 2005).

Although sensitive and specific under optimized conditions, conventional PCR has one drawback – the need to analyze the data by traditional end-point analysis. Real-time quantitative PCR (qPCR) is a powerful alternative. This would allow continuous monitoring of the PCR amplification process (Wittwer *et al.* 1997) and, under appropriate conditions, quantification of the template. In addition, qPCR offers significant advantages over other molecular methods in terms of the speed by which assays are performed and the ability to quantify the target microbial population. Real-time qPCR has been successfully used to detect pathogenic microorganisms in food or food-related samples (McKillip *et al.* 2004; Spano *et al.* 2005), and also to quantify bacterial genera and species in food (Furet *et al.* 2004; Pinzani *et al.* 2004). Recently a real-time qPCR method for the direct detection and quantification of histamine-producing LAB in food has been developed (Fernandez *et al.* 2006). The proposed method was optimized to quantify the presence of histamine-producing microorganisms in cheeses, both during the cheese-making process and in the final product. The *hdcA* gene was chosen as a target for detecting and quantifying the histamine-producing bacteria (Fernandez *et al.* 2006). The results reported show that the procedure is a rapid (total processing time <2 h), specific and highly sensitive technique for detecting potential BA producing strains (Fernandez *et al.* 2006). Therefore, a real time PCR approach may substitute the traditional qualitative PCR in order not only to detect but also to quantify BA-producing bacteria.

A reverse transcription qPCR (RT-qPCR) has also been developed for targeting histamine producing bacteria during wine MFL fermentation in wine. Using this approach and primers Hdc1 and Hdc2 (Fernandez *et al.* 2006), the authors were able to detect, *in vivo*, histamine producing bacteria and to analyze the effect of SO_2 and ethanol on the expression of the *hdc* gene (Spano *et al.* 2007 unpublished results).

TRIUMPHS

The improvement of fast, reliable and culture-independent molecular tools, usually based on PCR approaches has recently allowed a fast and accurate detection of BA-producing bacteria in fermented beverages. Oligonucleotides based on genes coding for amino acid decarboxylase enzymes have been developed. Actually, using several target genes, we are able to identify all of the LAB involved in BA production in a given sample.

However, it should be stated that molecular tools may also be limited. DNA is the preferred target for BA identification although targeting mRNA for a specific BA rather then DNA is probably a more suitable tool. In fact DNA may still be detectable when cells might have disappeared due to food process management or abiotic stresses encountered (e.g., high ethanol concentration, low pH), while de-

tection of mRNA reflects the presence of effectively viable microorganisms. Detection of genes involved in amino acid decarboxylation is usually performed previously to bacteria isolation. However, this method allows the detection of only those bacteria able to grow on definite media. Therefore, a molecular approach performed directly on food samples is preferred. The main problem in dealing with *in vivo* PCR-based techniques is the recovery of nucleic acids (DNA and RNA) which are suitable and pure enough for subsequent PCR experiments. Samples analyzed are usually full of PCR-enzymes inhibitors that may make difficult an amplification process. The development of appropriate nucleic acids preparation will also allow the detection of those bacteria able to produce BA but in uncultivable state (VNC).

BA may be oxidized by the action of amino oxidase (AO). The potential role of microorganisms involved in food fermentations with AO activity has been investigated with the aim of preventing or reducing the accumulation of BA in foods. (Leuschner *et al*. 1998) tested *in vitro* the potential amine degradation carried out by many species of bacteria isolated from foods and, in particular, by strains belonging to the genera *Lactobacillus*, *Pediococcus* and *Micrococcus*. Tyramine oxidase activity of several microbial strains was strictly dependent on pH, temperature and NaCl, as well as glucose. Moreover, this enzyme was characterrised by a higher potential activity under aerobic conditions. Temperature has also an important effect on histamine degradation. The highest degradation rate of this amine was observed at 37°C, but at 22 and 15°C, degradation was still considerable (Dapkevicius *et al*. 2000).

Many LABs are usually used as starter cultures in several fermented foods and beverages. In general, the choice of starter cultures is fundamental to guarantee the quality of final products. For this reason, the inability to form BA, the capability to tolerate stresses that are common during food processing and storage and to possess amine oxidase activity, should be relevant criteria to be taken into consideration in the selection of starter cultures for the management of fermented food and beverages.

REFERENCES

Arena ME, Manca de Nadra MC (2001) Biogenic amine production by lactic acid bacteria. *Journal of Applied Microbiology* **90**, 158-162

Arena ME, Manca de Nadra MC, Muñoz R (2002) The arginine deiminase pathway in the wine lactic acid bacterium *Lactobacillus hilgardii X1B*: structural and functional study of the *arcABC* genes. *Gene* **301**, 61-66

Beutling DM, Walter D (2002) 2-Phenylethylamine formation by enterococci *in vitro*. *European Food Research Technology* **215**, 240-242

Bover-Cid S, Holzapfel WH (1999) Improved screening procedure for biogenic amine production by lactic acid bacteria. *International Journal of Food Microbiology* **59**, 391-396

Caston JC, Eaton CL, Gheorghui BP, Ware LL (2002) Tyramine-induced hypertensive episodes and panic attacks in hereditary deficient monoamine oxidase patients: case reports. *Journal of the Society of Clinical and Medical Association* **98**, 187-192

Connil N, Le Breton Y, Dousset X, Auffray Y, Rince A, Prevost H (2002) Identification of the *Enterococcus faecalis* tyrosine decarboxylase operon involved in tyramine production. *Applied and Environmental Microbiology* **68**, 3537-3544

Costantini A, Cersosimo M, del Prete V, García-Moruno E (2006) Production of biogenic amines by lactic acid bacteria: screening by PCR, thin-layer chromatography, and high-performance liquid chromatography of strains isolated from wine and must. *Journal of Food Protection* **69**, 391-396

Coton E, Rollan G, Bertrand A, Lonvaud-Funel A (1998a) Histamine-producing lactic acid bacteria in wines: early detection, frequency and distribution. *American Journal of Enology and Viticulture* **49**, 199-204

Coton E, Rollan GC, Lonvaud-Funel A (1998b) Histidine decarboxylase of *Leuconostoc oenos* 9204: purification, kinetic properties, cloning and nucleotide sequence of the *hdc* gene. *Journal of Applied Microbiology* **84**, 143-151

Coton M, Coton E, Lucas P, Lonvaud-Funel A (2004) Identification of the gene encoding a putative tyrosine decarboxylase of *Carnobacterium divergens* 508. Development of molecular tools for the detection of tyramine-producing bacteria. *Food Microbiology* **21**, 125-130

Coton E, Coton M (2005) Multiplex PCR for colony direct detec-tion of Gram-positive histamine- and tyramine-producing bacteria. *Journal of Microbiological Methods* **63**, 296-304

Chu CH, Bejdanes LF (1981) Effect of diamines, polyamines and tuna fish extracts on the binding of histamine to mucin *in vitro*. *Journal of Food Science* **47**, 79-88

Dapkevicius MLNE, Nout MJR, Rombouts FM, Houben JH, Wymenga W (2000) Biogenic amine formation and degradation by potential fish silage starter microorganisms. *International Journal of Food Microbiology* **57**, 107-114

Fernandez M, Linares DM, Alvarez MA (2004) Sequencing of the tyrosine decarboxylase cluster of *Lactococcus lactis* IPLA 655 and the development of a PCR method for detecting tyrosine decarboxylating lactic acid bacteria. *Journal of Food Protection* **67**, 2521-2529

Fernandez M, del Rýo B, Linares DM, Martýn MC, Alvarez MA (2006) Real-time polymerase chain reaction for quantitative detection of histamine-producing bacteria: use in cheese production. *Journal of Dairy Science* **89**, 3763-3769

Furet JP, Quenee P, Tailliez P (2004) Molecular quantification of lactic acid bacteria in fermented milk products using real-time quantitative PCR. *International Journal of Food Microbiology* **97**, 197-207

Glória MB, Watson BT, Simon-Sarkadi L, Daeschel MA (1998) A survey of biogenic amines in Oregon Pinot noir and Cabernet sauvignon wines. *American Journal of Enology and Viticulture* **49**, 279-281

Guerrini S, Mangani S, Granchi L, Vincenzini M (2002) Biogenic amine production by *Oenococcus oeni*. *Current Microbiology* **44**, 374-378

Izquierdo-Pulido M, Mariné-Font A, Vidal-Carou MC (1994) Biogenic amines formation during malting and brewing. *Journal of Food Science* **59**, 1104-1107

Joosten HMJ, van Boeckel MAJS (1988) Conditions allowing the formation of biogenic amines in cheese: A study of the kinetics of histamine formation in an infected Gouda cheese. *Netherlands Milk and Dairy Journal* **41**, 3-24

Landete JM, Ferrer S, Pardo I (2005a) Which lactic acid bacteria are responsible for histamine production in wine? *Journal of Applied Microbiology* **99**, 580-586

Landete JM, Ferrer S, Polo L, Pardo I (2005b) Biogenic amines in wines from three Spanish regions. *Journal of Agricultural and Food Chemistry* **53**, 1119-1124

le Jeune C, Lonvaud-Funel A, ten Brink B, Hofstra H, van der Vossen JMBM (1995) Development of a detection system for histidine decarboxylating lactic acid bacteria on DNA probes, PCR and activity test. *Journal of Applied Microbiology* **78**, 316-326

Liu S-Q, Pilone GJ (1998) A review: Arginine metabolism in wine lactic acid bacteria and its practical significance. *Journal of Applied Microbiology* **84**, 315-327

Lonvaud-Funel A, Joyeux A (1994) Histamine production by wine lactic acid bacteria: Isolation of a histamine producing strain of *Leuconostoc oenos*. *Journal of Applied Bacteriology* **77**, 401-407

Lonvaud-Funel A (2001) Biogenic amines in wines: role of lactic acid bacteria. *FEMS Microbiology Letters* **199**, 9-13

Lucas P, Lonvaud-Funel A (2002) Purification and partial gene sequence of the tyrosine decarboxylase of *Lactobacillus brevis* IOEB 9809. *FEMS Microbiology Letters* **211**, 85-89

Lucas PM, Wolken WAM, Claisse O, Lolkema JS, Lonvaud-Funel A (2005) Histamine-producing pathway encoded on an unstable plasmid in *Lactobacillus hilgardii* 0006. *Applied and Environmental Microbiology* **71**, 1417-1424

Maijala R, Nurmi F, Fischer A (1995) Influence of processing temperature on the formation of biogenic amines in dry sausages. *Meat Science* **39**, 19-22

McKillip JL, Drake M (2004) Real-time nucleic acid-based detection methods for pathogenic bacteria in food. *Journal of Food Protection* **67**, 823-832

Mangani S, Guerrini S, Granchi L, Vincenzini M (2005) Putrescine accumulation in wine: role of *Oenococcus oeni*. *Current Microbiology* **51**, 6-10

Marcobal A, de las Rivas B, Moreno-Arribas MV, Muñoz R (2004) Identification of the ornithine decarboxylase gene in the putrescine-producer *Oenococcus oeni* BIFI-83. *FEMS Microbiology Letters* **239**, 213-220

Marcobal A, de la Rivas B, Muñoz R (2006a) First genetic characterization of a bacterial β-phenylethylamine biosynthetic enzyme in *Enterococcus faecium* RM58. *FEMS Microbiology Letters* **258**, 144-149

Marcobal A, de las Rivas B, Muñoz R (2006b) Methods for the detection of bacteria producing biogenic amines on foods: A survey. *Journal of Consumer Protection and Food Safety* **1**, 187-196

Marcobal Á, de las Rivas B, Moreno-Arribas MV, Muñoz R (2006c) Evidence for horizontal gene transfer as origin of putrescine production in *Oenococcus oeni* RM83. *Applied and Environmental Microbiology* **72**, 7954-7958

Martuscelli M, Crudele MA, Gardini F, Suzzi G (2000) Biogenic amine formation and oxidation by *Staphylococcus xylosus* strains from artisanal fermented sausages. *Letters in Applied Microbiology* **31**, 228-232

Molenaar D, Bosscher JS, ten Brink B, Driessen JM, Konings WN (1993) Generation of a proton motive force by histidine decarboxylation and electrogenichistidine/histamine antiport in *Lactobacillus buchneri*. *Journal of Bacteriology* **175**, 2864-2870

Montel MC, Masson F, Talon R (1999) Comparison of biogenic amine content in traditional and industrial French dry sausages. *Sciences des Aliments* **19**, 247-254

Moreno-Arribas, MV, Torlois S, Joyeaux A, Bertrand A, Lonvaud-Funel A (2000) Isolation, properties and behaviour of tyramine-producing lactic acid bacteria from wine. *Journal of Applied Microbiology* **88**, 584-593

Moreno-Arribas V, Lonvaud-Funel A (2001) Purification and characterization of tyrosine decarboxylase activity of *Lactobacillus brevis* IOEB 9809. *FEMS Microbiology Letters* **195**, 103-107

Moreno-Arribas MV, Polo MC, Jorganes F, Muñoz R (2003) Screening of biogenic amine production by lactic acid bacteria isolated from grape must and wine. *International Journal of Food Microbiology* **84**, 117-123

Muñoz R, de las Rivas B, Marcobal A, Carrascosa AV (2004) Simultaneous detection of bacteria producing biogenic amines by PCR. Spanish patent application 200402314

Pessione E, Mazzoli R, Giuffrida MG, Lamberti C, Garcia-Moruno E, Barello C, Conti A, Giunta C (2005) A proteomic approach to studying biogenic amine producing lactic acid bacteria, *Proteomics* **5**, 687-698

Pinzani P, Bonciani L, Pazzagli M, Orlando C, Guerrini S, Granchi L (2004) Rapid detection of *Oenococcus oeni* in wine by real-time quantitative PCR. *Letters in Applied Microbiology* **38**, 118-121

Recsei PA, Moore WM, Snell EE (1983) Pyruvol-dependent histidine decarboxylases from *Clostridium perfringens* and *Lactobacillus buchneri*. *Journal of Biological Chemistry* **258**, 439-444

Romero R, **Bagur MG**, **Sanchez-Vinas M**, **Gazquez D** (2003) The influence of brewing process on the formation of biogenic amines in beers. *Annals of Bioanalytical Chemistry* **2**, 162-167

Santos WC, Souza MR, Cerqueira MMOP, Glória MBA (2003) Bioactive amines formation in milk by *Lactococcus* in the presence or not of rennet and NaCl at 20 and 32°C. *Food Chemistry* **81**, 595-606

Sattler J, **Hesterberg R**, **Lorenz W**, **Schmidt U**, **Crombach M**, **Stahlknecht CD** (1985) Inhibition of human and canine diamine oxidase by drugs used in an intensive care unit: relevance for clinical side effects? *Agents Actions* **16**, 91-94

Schiller D, Kruse D, Kneifel H, Kramer R, Burkovski A (2000) Polyamine transport and role of *potE* in response to osmotic stress in *Escherichia coli*. *Journal of Bacteriology* **182**, 6247-6249

Silla Santos MH (1996) Biogenic amines: their importance in foods. *International Journal Food Microbiology* **29**, 213-231

Spano G, Chieppa G, Beneduce L, Massa S (2004) Expression analysis of putative *arc*A, *arc*B and *arc*C genes partially cloned from *Lactobacillus plantarum* isolated from wine. *Journal of Applied Microbiology* **96**, 185-190

Spano G, Beneduce L, Terzi V, Stanca AM, Massa S (2005) Real-Time PCR for the detection of *Escherichia coli* O157:H7 in dairy and cattle wastewater. *Letters in Applied Microbiology* **40**, 164-169

Spano G, Vernile A, Beneduce L, Tarantino D, de Palma L, Massa S (2006) Characterization of wine *Lactobacillus plantarum* by PCR-DGGE and RAPD-PCR analysis and identification of *Lactobacillus plantarum* strains able to degrade arginine. *World Journal of Microbiology and Biotechnology* **22**, 769-773

Spano G, Lonvaud-Funel A, Claisse O, Massa S (2007a) *In vivo* PCR-DGGE analysis of *Lactobacillus plantarum* and *Oenococcus oeni* populations in red wine. *Current Microbiology* **54**, 9-13

Spano G, Arena ME, Fiocco D, Manca de Nadra MC, Pardo I (2007b) Characterization of a *Lactobacillus plantarum* strain able to produce tyramine and partially cloning of a putative tyrosine decarboxylase gene. *Current Microbiology*, in press

Sponholz WR, Kurbel H, Dittrich HH (1991) Beitraege zur bildung von ethylcarbamat im wein. *Wein-Wissenschaft* **46**, 11-17

Stratton JE, Hutkins RW, Taylor SL (1991) Biogenic amine in cheese and other fermented foods. *Journal of Food Protection* **54**, 460-470

Soufleros E, Barrios ML, Bertrand A (1998) Correlation between the content of biogenic amines and other wine compounds. *American Journal of Enology and Viticulture* **49**, 266-269

Suzzi G, Gardini F (2003) Biogenic amines in dry fermented sausages: a review. *International Journal of Food Microbiology* **88**, 41-54

Taylor SL (1983) Histamine poisoning associated with fish, cheese and other foods. FAO/WHO monograph CX/PH 83/11

ten Brink B, Damink C, Joosten HMLJ, Huis in't Veld JHJ (1990) Occurrence and formation of biologically amines in food. *International Journal of Food Microbiology* **11**, 73-84

Tkachenko AG, Nesterova LY, Pshenichnov K (2001) Role of putrescine in the regulation of the expression of the oxidative stress defence genes of *Escherichia coli*. *Microbiology* **70**, 133-137

van Poejle PD, Snell EE (1990) Cloning, sequencing, expression, and site-directed mutagenesis of the gene from *Clostridium prefringens* encoding pyruvoyl-dependent histidine decarboxylase. *Biochemistry* **29**, 132-139

Vanderslice P, Copeland WC, Robertus JD (1986) Cloning and nucleotide sequence of wild type and a mutant histidine decarboxylase from *Lactobacillus* 30A. *Journal of Biological Chemistry* **261**, 15186-15191

Wittwer CT, Herrmann MG, Moss AA, Rasmussen RP (1997) Continuous fluorescence monitoring of rapid cycle DNA amplification. *Biotechniques* **22**, 130-131

Wolken AM, Lucas PM, Lonvaud-Funel A, Lolkema JS (2006) The mechanism of the tyrosine transporter tyrP supports a proton motive tyrosine decarboxylation pathway in *Lactobacillus brevis*. *Journal of Bacteriology* **188**, 2198-2206