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ORIGINAL ARTICLE

Factors affecting the production of putrescine from agmatine by *Lactobacillus hilgardii* X_1B isolated from wine

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Abstract

Aims: To elucidate and characterize the metabolic putrescine synthesis pathway from agmatine by *Lactobacillus hilgardii* X_1B.

Methods and Results: The putrescine formation from agmatine by resting cells (the normal physiological state in wine) of lactic acid bacteria isolated from wine has been determined for the first time. Agmatine deiminase and *N*-carbamoylputrescine hydrolase enzymes, determined by HPLC and LC-Ion Trap Mass Spectrometry, carried out the putrescine synthesis from agmatine. The influence of pH, temperature, organic acids, amino acids, sugars and ethanol on the putrescine formation in wine was determined.

Conclusions: Resting cells of *Lact. hilgardii* X_1B produce putrescine in wine. The putrescine production was carried out from agmatine through the agmatine deiminase system.

Significance and Impact of the Study: These results have significance from two points of view, wine quality and toxicological and microbiological aspects, taking account that putrescine, which origin is still controversial, is quantitatively the main biogenic amine found in wine.

Introduction

The knowledge of biogenic amines production in foods and alcoholic beverages is important from both toxicological and technological aspects, as these compounds may cause food poisoning (Joosten and Northolt 1989). Alcoholic beverages have been observed to be a dietary precipitant of headaches in patients with migraines (Vidal-Carou *et al.* 1990; Peatfield 1995). Furthermore, biogenic amines such as tyramine and diamines such as putrescine and cadaverine have been described as precursors of carcinogenic nitrosamines (ten Brink *et al.* 1990). Moreover, the simultaneous presence in alcoholic beverages of ethanol and polyamines (i.e. putrescine, spermine and spermidine) could strengthen the toxic effect of other biogenic amines. Putrescine and cadaverine, although not toxic in themselves, intensify the adverse effects of histamine and tyramine as they interfere with the enzymes that metabolize them (ten Brink *et al.* 1990; Straub *et al.* 1995).

Putrescine in grapevines has been associated with the potassium deficiencies of the soil (Brodequis *et al.* 1989). Thus, viticultural practices may contribute to the presence of putrescine in wine (Leitão *et al.* 2005). The vinification method may also influence the final amine concentration in wine (Coton *et al.* 1999; Lounvaud-Funel and Joyeux 1994). The presence of biogenic amines in wine has been suggested as an index of poor quality or of bad manufacturing practices (Radler and Fäth 1991; Lehtonen *et al.* 1992). Biogenic amines could be formed by lactic acid bacteria (LAB) during fermentation of alcoholic beverages, and lactobacilli seem to be the main factor affecting amine formation during alcoholic beverages storage (Donhauser *et al.* 1992). Amino acid decarboxylating enzymes have been reported as responsible for biogenic amines formation (Farías *et al.* 1993, 1995; Kalač *et al.* 2002).

Putrescine is the most abundant biogenic amine found in wine (Lethonen *et al.* 1992; Glória *et al.* 1998; Soufleros *et al.* 1998; Landete *et al.* 2005); and this amine could

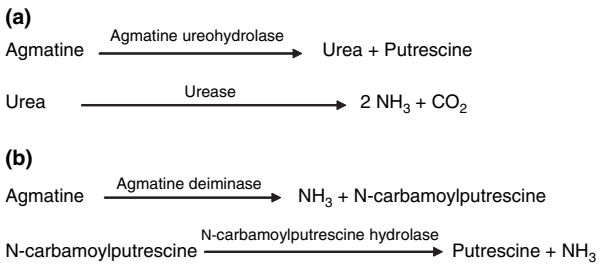
be formed from ornithine, as in *Oenococcus oeni* (Guerrini *et al.* 2002), or from agmatine as in *Bacillus subtilis* (Sekowska *et al.* 1998). Agmatine is the most abundant in beers (40–56%), followed by putrescine (13–42%) (Glória and Izquierdo-Pulido 1999); and agmatine has also been reported in other foods as milk (Bardocz 1993; Lima and Glória 1999). In wines, agmatine has been found mainly in red wines, with a maximum value of about 20 mg l^{-1} (Anli *et al.* 2004; Bauza *et al.* 1995; Bover-Cid *et al.* 2006).

Putrescine is formed by the decarboxylation of either ornithine or arginine into agmatine, which is then directly or indirectly converted into putrescine via carbamoylputrescine. In a previous paper, Arena and Manca de Nadra (2001) reported that *Lact. hilgardii* X_1B is able to produce putrescine from arginine. In this strain, the putrescine production from arginine involves the formation of both agmatine and ornithine. According the metabolites formation from arginine, the data indicate that agmatine could contribute to the putrescine formation.

The decrease in agmatine levels in food could be related to its role as a precursor of polyamines: putrescine, spermidine and spermine, essential growth factors for several micro-organisms (Bardocz 1993; Lima and Glória 1999). However there is no information available on the putrescine formation from agmatine in LAB isolated from beverages.

Different pathways for conversion of agmatine into putrescine have been reported in other micro-organisms: (a) agmatine ureohydrolase and urease pathway for *B. subtilis* (Sekowska *et al.* 1998), or (b) agmatine deiminase and *N*-carbamoylputrescine hydrolase pathway for *Streptococcus faecalis* and *Pseudomonas aeruginosa* (Simon and Stalon 1982; Lu *et al.* 2002) (Scheme 1).

The fermented beverages are complex systems with a wide number of factors influencing the metabolic activities of micro-organisms. A large host of factors has been observed to affect the wine bacterial metabolism, including, temperature, pH, alcohol content, organic acid and sugar concentration and the time of bacterial survival (Buteau *et al.* 1984; Radler and Fäth 1991; Glória *et al.* 1998).



Scheme 1

The aim of this study is to determine the ability of *Lact. hilgardii* X_1B to produce putrescine from agmatine, and to establish the influence of the different physicochemical factors in this conversion.

Material and methods

Micro-organism

Lactobacillus hilgardii X_1B, was isolated and identified from an Argentinean wine (Strasser de Saad and Manca de Nadra 1987), and its identity was confirmed according to the FISH method (Blasco *et al.* 2003).

Culture conditions

Agmatine degradation was determined in the basal medium (BM) containing in g l^{-1}: 2, tryptone (Cultimed E-08110, Panreac, Spain); 2, yeast extract (Pronadisa, 170200, Spain); 1, glucose (Panreac 131341, Spain); 0·02, K_2HPO_4; 0·03, KH_2PO_4; 0·02, $MnSO_4$; 0·03, $MgCl{\cdot}6H_2O$ and 12% tomato juice that exerted a stimulatory effect on the growth of LAB (Babu *et al.* 1992; Dicks *et al.* 1995; Arena and Manca de Nadra 2002).

The medium was adjusted to pH 6·0, optimal for growth of *Lact. hilgardii* X_1B, with 1 mmol l^{-1} KOH before sterilization in autoclave at 121°C for 20 min. Sterile-filtered agmatine was added as part of BM to 1 g l^{-1}. Biogenic amine standards (agmatine and putrescine) were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Amine formation by resting cells

Lactobacillus hilgardii X_1B was grown statically at 28°C in the BM. Cultures were harvested after an incubation of 24 h. Cells were washed with sterile sodium phosphate buffer 0·2 mol l^{-1} pH 6·0. Centrifuged cells were resuspended in the same buffer to OD_{600nm} = 0·80.

The reaction mixture to determine the putrescine formation contained in a final volume of 1·5 ml: 0·5-ml sodium phosphate buffer pH 6·0 (0·2 mol l^{-1}), 0·5 ml L-agmatine solution (3 g l^{-1}) adjusted to pH 6·0; 0·5 ml cells suspension. In different assays, this sodium phosphate buffer (0·2 mol l^{-1}) was added individually with 0, 0·5, 1·0, 5·0, 10 and 20 g l^{-1} of: arginine, citrulline, ornithine, glucose, fructose, or 0, 0·5, 1·0, 5·0 and 10 g l^{-1} of: DL-lactic acid, L-malic acid, DL-malic acid, citric acid, tartaric acid or different ethanol concentrations (0%, 5%, 10%, 12%, 15%, 20% v/v). To check the actual effect of wine on putrescine formation, similar experiments were carried out in a Spanish Tempranillo wine instead of buffer, added with the same compounds at the same concentrations mentioned before, but at the pH of the wine (3·40). Before these

corrections this wine contained in g l^{-1} 0·56 of citric acid, 3·60 of tartaric acid, 0·04 of succinic acid, 1·26 of lactic acid, 4·19 of malic acid, and 0·01 of glucose.

The reaction mixtures were incubated overnight at 28°C in screw-capped tubes unless otherwise specified. To study the influence of pH and temperature, the reaction mixtures were assayed at 0, 4, 24, 28, 37, 40 and 50°C at pH 6·0; and at 28°C the assays were carried out at pH values of 2·5, 3·2, 3·5, 4·0, 4·5, 6·0, 7·0, 8·0 and 10·0.

The samples were then centrifuged at 14 500 ***g*** for 8 min at 4°C and filtered through a regenerated cellulose membrane (0·45 μm pore size). Immediately the biogenic amines were determined in the supernatant.

Biogenic amines determination

Samples were precolumn derivatized with orthophthaldialdehyde (OPA) and injected into the HPLC system (Merck, Darmstadt, Germany) equipped with an L-Intelligent pump (Merck-Hitachi, Tokyo, Japan), AS-2000A Autosampler (Merck-Hitachi), T-6300 column thermostat (at 40°C) and a L-7485 LaChrom fluorescence detector (Merck-Hitachi). An excitation wavelength of 335 nm and an emission wavelength of 450 nm were used. OPA reagent was prepared as follows: 50 mg OPA were dissolved in 2·25 ml of methanol; 0·25 ml of borate buffer 0·4 M (pH 10) and finally 0·05 ml of mercaptoethanol were added. A gradient of solvent A (2·268 g KH_2PO_4 and 14·968 g $Na_2HPO_4{\cdot}12H_2O$ adjusted to pH = 5·8 with H_3PO_4 and filled up with deionized water to 1 l) and solvent B (100% methanol) was applied to a 100 RP-18 column (Merck-Hitachi) (25 cm × 5 μm) as follows: 0–20 min, 40–55% B linear gradient, 1 ml min^{-1}; 20–45 min, 55–85% linear gradient, 1 ml min^{-1}. Quantification: External standard method was used injecting standards using known amounts of biogenic amines.

N-carbamoylputrescine determination

To detect *N*-carbamoylputrescine, it was necessary to analyse HPLC fractions by LC-MS. The *N*-carbamoylputrescine could not be quantified, as this compound is very unstable (there are in fact no standards of it). Fractions from HPLC were collected and directed injection of peaks identified by LC-Ion Trap Mass Spectrometry. The determinations were carried out on a Bruker Esquire 3000 Plus Ion Trap mass spectrometer (Bruker Instruments, Billerica, MA, USA) coupled to an Agilent 1100 ChemStation (Agilent Tecnologies, Waldbronn, Germany). The ionization method was API (Atmospheric Pressure Interfase)-electrospray interface (ESI), in positive mode by using the following ionization source parameters: N_2 nebulizer gas 10 psi; dry gas 5 l min^{-1}, dry temperature 250°C.

Urea determination

Urea was measured by an enzymatic method (kit 1810057, from Wiener, Rosario, Argentina).

Statistical analysis

To validate the method the MINITAB Student test was used. Three replicate determinations were carried out. Relative SD for amine concentrations were ≤9%.

Results

Pathway of agmatine degradation

To establish the pathway involved in the conversion of agmatine to putrescine from *Lact. hilgardii* X_1B, the intermediary compounds formation were studied.

From the results obtained when resting cells were assayed in buffer medium added with agmatine, it was determined that *Lact. hilgardii* X_1B was able to convert agmatine to putrescine without urea formation. Previously, Arena *et al.* (1999) reported that *Lact. hilgardii* X_1B is not able to use urea; this means that the ammonium formation in the putrescine synthesis is not depending on the urease pathway in this organism. *N*-carbamoylputrescine was detected in the media from HPLC fractions by LC-MS, indicating that the conversion of agmatine into putrescine was carried out via agmatine deiminase and *N*-carbamoylputrescine hydrolase.

Kinetics of formation of putrescine from agmatine

Taking into account that some LAB present in wine are able to survive as resting cells after malolactic fermentation during the wine storage and ageing, it is important to determine the ability of these micro-organisms to form

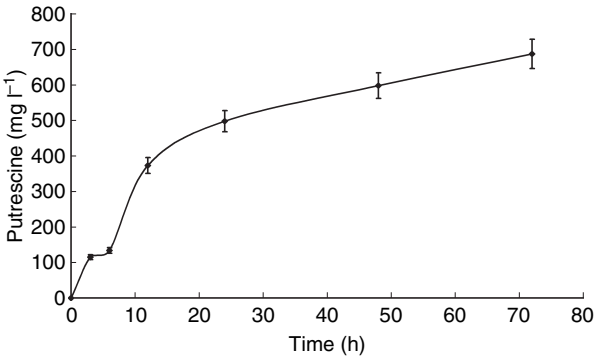


Figure 1 Kinetics of putrescine formation by resting cells of *Lactobacillus hilgardii* X_1B. Incubation 28°C. Means of three replicates, no significant difference ($P \leq 0{\cdot}05$).

putrescine. Figure 1 shows the dynamics of putrescine formation by *Lact. hilgardii* X_1B. Putrescine was produced during 70 h incubation at 28°C. After this time there was not observed a significant increase of this biogenic amine. The utilization of agmatine proceeded immediately after the cell inoculation, and the conversion of agmatine to putrescine was 73% after 10 incubation days (data not show).

Effect of temperature, pH and ethanol on putrescine formation by *Lact. hilgardii* X_1B

Figure 2a shows the effect of the temperature on the putrescine formation by *Lact. hilgardii* X_1B. There was no activity at 0°C and it was noticeable from 4°C. The optimum temperature was 37°C, however above 40°C a noticeable diminution was observed. To exclude a possible thermal decomposition of agmatine, a control test with agmatine without cells was carried out incubating the buffer at 40 and 50°C. In these conditions, the concentration of agmatine remained invariable.

Figure 2b shows the formation of putrescine by *Lact. hilgardii* X_1B at different pH values. Agmatine was degraded in a very limited extent when the pH values were close to 2·5, and the maximum putrescine formation was observed at pH 4·5. The production was maintained to pH 6·0 and a diminution was observed when higher pH values were tested. At pH values normally found in wine, from 3·2 to 3·8, the amount of putrescine formed was 53% and 75% respectively of the maximum amount formed at pH 4·5.

Figure 2c shows the influence of ethanol in the putrescine formation by *Lact. hilgardii* X_1B. The total amount of putrescine formed decreased when ethanol concentration increased. The putrescine formation from agmatine was 23%, 42%, and 82% lower in presence of 10%, 12% and 15% ethanol, respectively.

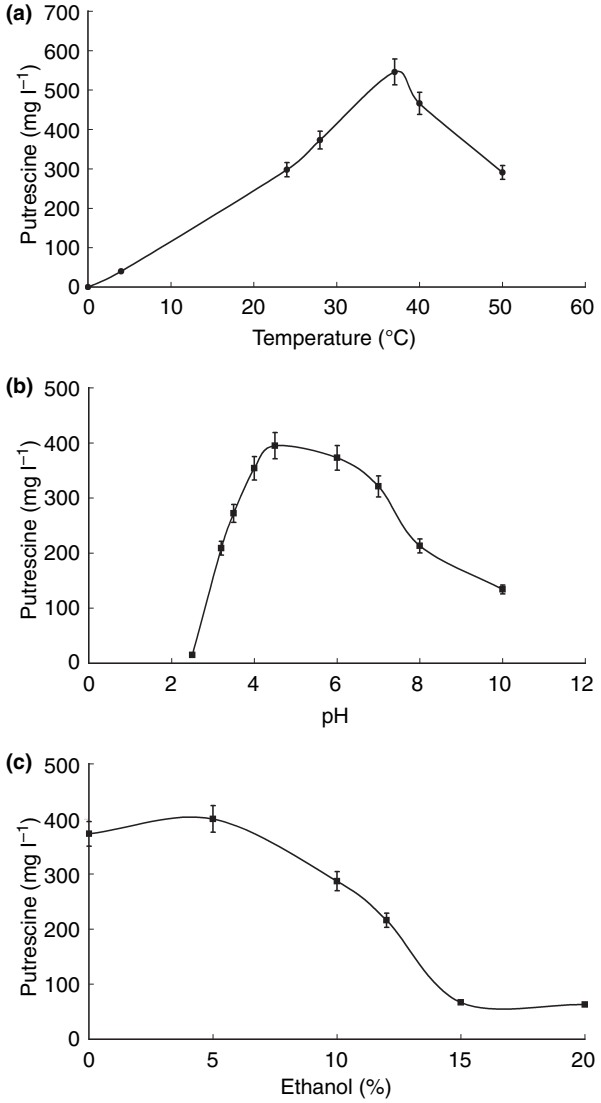


Figure 2 Effect of (a) temperature at pH 6; (b) pH at 28°C; (c) ethanol at 28°C and pH 6 on the putrescine production by resting cells of *Lactobacillus hilgardii* X_1B. Time of incubation 12 h. Means of three replicates, no significant difference ($P \leq 0{\cdot}05$).

Effect of sugars, amino acids and organic acids on putrescine formation by *Lact. hilgardii* X_1B

Figure 3 shows the effect of sugars on putrescine production from *Lact. hilgardii* X_1B. The putrescine formation was inversely proportional to the sugar concentration. Independently of the sugar added to the medium, with a concentration of 1 g l^{-1} the production of putrescine diminishes to 60%. The inhibitory effect on putrescine formation increased as sugars concentrations increased. In presence of 5, 10 and 20 g l^{-1} glucose or fructose the

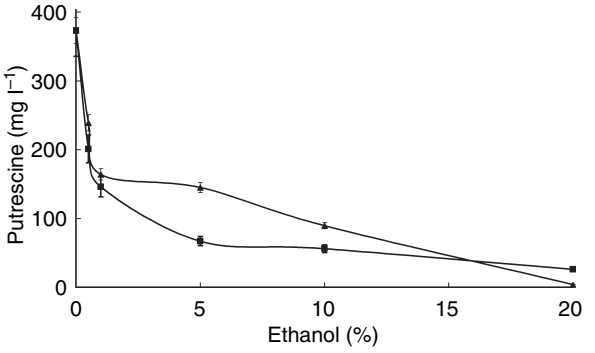


Figure 3 Influence of different sugars concentration on the putrescine formation by *Lactobacillus hilgardii* X_1B in buffer. Reaction mixture added with different glucose (▲) and fructose (■) concentrations, after 12 h incubation at 28°C. Means of three replicates, no significant difference ($P \leq 0{\cdot}05$).

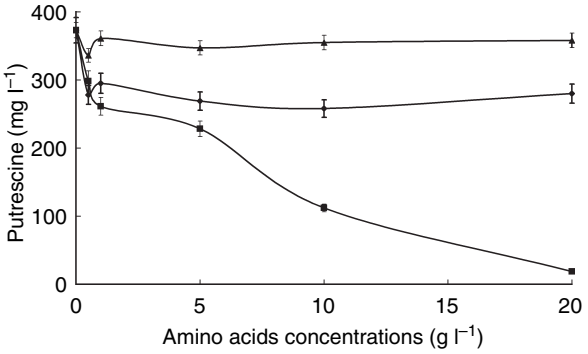


Figure 4 Influence of amino acids concentration on putrescine production by *Lactobacillus hilgardii* X_1B. Reaction mixture added with different amino acids concentrations (0, 0·5, 1, 5, 10, 20 g l^{-1}): arginine (■), citrulline (♦) and ornithine (▲); after 12-h incubation at 30°C. Means of three replicates, no significant difference ($P \leq 0{\cdot}05$).

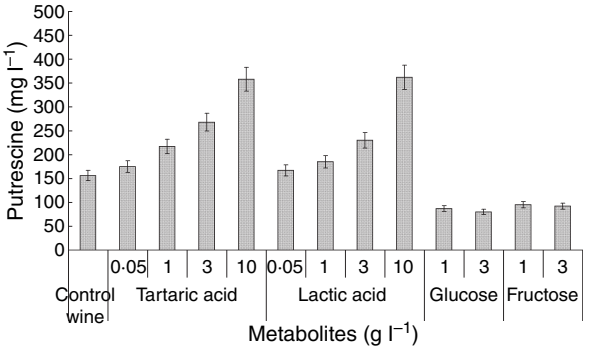


Figure 5 Putrescine production by *Lactobacillus hilgardii* X_1B in wine. Effect of organic acids and sugars. The wine was added with different organic acids and sugars concentrations, after 12-h incubation at 28°C. Means of three replicates, no significant difference ($P \leq 0{\cdot}05$).

inhibitory effect was: 82% and 61%; 85% and 75% and 93% and 99% respectively.

The effect of amino acids involved in the arginine deiminase pathway, was studied (Fig. 4). In presence of 1 g l^{-1} arginine, an inhibition of 30% in putrescine formation from agmatine was observed. When the concentration was 20 g l^{-1} a total inhibition in its production was observed.

When the reaction was carried out in presence of citrulline (0·5–20 g l^{-1}) the formation of putrescine decreased by 27%. The addition of ornithine at concentrations from 0·5 to 20 g l^{-1} did no modify the putrescine production. In all cases, pyridoxal phosphate was omitted from the mixture of reaction to diminish the interference of decarboxylase activity in the putrescine production.

The effect of DL-lactic, L-malic, DL-malic, tartaric and citric acids in the mixture of reaction on the putrescine formation was determined (data not show). The results indicated that only L-lactic and tartaric acids modified the putrescine synthesis, showing a stimulatory effect that linearly correlates with the organic acid concentration in the range of 1 to 10 g l^{-1}; for 0·5 g l^{-1} there was not a noticeable effect. At the highest concentration of L-lactic and tartaric acids, an increase in putrescine formation of 20% and 35% respectively, was observed.

Modifications in putrescine production by the effect of wine

Figure 5 shows the effect of wine and different wine compounds on the conversion of agmatine into putrescine. The amount of putrescine synthesized in the wine was 170 mg l^{-1}; this was near to the expected values regarding the presence of 12·5% of ethanol and a pH value of 3·4.

The wine is a complex medium and the influence of the main metabolites present in this beverage was determined. Glucose, fructose and different organic acids: DL-lactic, L-malic, DL-malic, tartaric and citric acids were assayed. To avoid the pH influence in all experiments in wine, the initial pH value was adjusted to pH 3·40.

The addition of glucose and fructose to the wine produced an important inhibitory effect, higher than that observed in buffer. The presence of 1 g l^{-1} of glucose inhibits 44% and 56% and the presence of 1 g l^{-1} fructose inhibits 38% and 61% the putrescine formation in buffer and wine, respectively. L-malic, DL-malic and citric acids, did not influence the putrescine formation from agmatine in wine (data not show). This agrees with the results in buffer. However, when tartaric or L-lactic acids concentrations were increased in the wine, there was a positive correlation between the amount of the organic acids and the amine synthesis (Fig. 5). The values found in wine varied between 1·2 and 4·8 g l^{-1} for tartaric acid and 0·004 and 4·2 g l^{-1} for L-lactic acid.

Discussion

Lactobacillus hilgardii X_1B is able to degrade agmatine with the formation of *N*-carbamoylputrescine as intermediate and putrescine as final product. *B. subtilis* degrades agmatine by agmatine ureohydrolase to form putrescine and urea (Sekowska *et al.* 1998). *Streptococcus faecalis* (*Enterococcus faecalis*) ATCC 11 700 produces putrescine from agmatine without urea production (Simon and Stalon 1982). Guerrini *et al.* (2002) and Mangani *et al.* (2005) demonstrated in *O. oeni* the synthesis of putrescine from ornithine or from arginine via ornithine, and Marcobal *et al.* (2004) identified the ornithine decarboxylase gene in the putrescine-producer *O. oeni* BIFI-83.

Soufleros *et al.* (1998) reported that it would be desirable to inhibit all bacterial activities as soon as possible after complete malolactic fermentation, and our results agree with this as an important increase in putrescine was observed after 10 days of incubation. With months of ageing and storage, much higher amounts of biogenic amines could be obtained unless preventive actions are taken. Although the highest putrescine levels are obtained for temperature and pH values higher than those usually found in wines, the synthesis level is still quite high for regular storage conditions for temperature and pH.

The influence of ethanol in the biogenic amines formation is not well established (Glória *et al.* 1998). We determined that the amount of putrescine produced at the normally ethanol concentration found in wine (12%) is quantitatively significant. Besides, *Lact. hilgardii* is also found in grape musts and fermenting wines, where the ethanol content is lower and can produce more putrescine. This confirms the importance of LAB removal after MLF accomplishment in wine (Soufleros *et al.* 1998).

Glucose and fructose are the most important sugars present in grapes, musts and wines. The putrescine formation from *Lact. hilgardii* X_1B was inversely proportional to the sugar concentration. Simon and Stalon (1982) reported that in *E. faecalis* glucose inhibits the enzyme responsible for agmatine degradation (experiments made with 4·5 g l^{-1} glucose). In dry wines, where the sugar concentration is less than 4·5 g l^{-1} the putrescine production could be reduced in a 75% approximately. On the other hand, González-Fernández *et al.* (2003) reported that the production of high amounts of putrescine by the natural microbiota in sausages was not avoided when the concentration of sugar was 0·1%.

Arena *et al.* 1999, 2002 and Arena and Manca de Nadra 2005 reported that *Lact. hilgardii* X_1B uses arginine via arginine deiminase system. From arginine, *Lact. hilgardii* X_1B is also able to produce agmatine through arginine decarboxylase enzyme (Arena and Manca de Nadra 2001), and agmatine could be the substrate to produce *N*-carbamoylputrescine through agmatine deiminase enzyme. Adams *et al.* (1992) reported that agmatine or *N*-carbamoylputrescine caused accumulation of putrescine in the grape leaves, whereas arginine did not lead to an increase in putrescine.

The effect of arginine, citrulline and ornithine, related to the synthesis of putrescine in *Lact. hilgardii* X_1B indicates that arginine and citrulline, but not ornithine, inhibit partially the agmatine degradation. The same inhibitory effect of arginine in agmatine utilization was observed in *E. faecalis* growing in a complex medium (Simon and Stalon 1982).

The presence of L-lactic or tartaric acids appears to increase the amount of putrescine produced by *Lact. hilgardii*. During the formation of L-lactic acid in wine by the malolactic fermentation the principal component analysis revealed that the synthesis of putrescine and lactic acid were positively correlated (Soufleros *et al.* 1998). Some LAB may produce lactate from citrate, tartrate, fumarate or mannitol, and lactate is the main end-product of sugar catabolism (Pilone 1975; Radler 1975; Liu *et al.* 1995). Nevertheless, lactate can be catabolysed under aerobic conditions by lactate oxidase or NAD^+-independent LDH in some LAB (Liu 2003), with the production of pyruvate, acetate and CO_2. This may explain tartrate stimulatory effect, either directly or by its conversion in lactate, on the synthesis of putrescine. In wine, the majority of biogenic amines (except putrescine) were negatively correlated with malic and citric acids content (Soufleros *et al.* 1998).

In this paper we determined that agmatine is metabolized to putrescine via agmatine deiminase by a wine strain of *Lact. hilgardii*. We reported the effects on its production of different physico-chemical factors and sugars and organic acids normally present in its natural environmental. As in grape musts there is low ethanol and lactic acid, but high glucose and fructose amounts and an opposite situation is found in wines, the different factors will affect differently the synthesis of putrescine via arginine deiminase in the changing scenario of the winemaking process. Amounts of 15–20 and 20–30 mg l^{-1} of putrescine in white and red wines, respectively, are able to produce an important diminution in sensorial quality (Woller 1993). The results from this work have implications not only for wine quality but also for toxicological and microbiological aspects, taking account that putrescine is quantitatively the main biogenic amine found in wine.

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References

Adams, D.O., Bates, D.J., Adams, D.F. and Franke, K.E. (1992) The effect of agmatine and other precursors on the

accumulation of putrescine in grape leaves. *Am J Enol Vitic* **43**, 239–243.

Anli, R.E., Vural, N., Yilmaz, S. and Vural, Y.H. (2004) The determination of biogenic amines in Turkish red wines. *J Food Compos Anal* **17**, 53–62.

Arena, M.E. and Manca de Nadra, M.C. (2001) Biogenic amine production by *Lactobacillus*. *J Appl Microbiol* **90**, 158–162.

Arena, M.E. and Manca de Nadra, M.C. (2002) Comparative survey in *Lactobacillus plantarum* of the growth and metabolism of arginine and citrulline in different media. *J Agric Food Chem* **50**, 6497–6500.

Arena, M.E. and Manca de Nadra, M.C. (2005) Influence of ethanol and low pH on arginine and citrulline metabolism in lactic acid bacteria from wine. *Res Microbiol* **156**, 858–864.

Arena, M.E., Saguir, F.M. and Manca de Nadra, M.C. (1999) Arginine, citrulline, and ornithine metabolism by lactic acid bacteria from wine. *Int J Food Microbiol* **52**, 155–161.

Arena, M.E., Manca de Nadra, M.C. and Muñoz, R. (2002) The arginine deiminase pathway in the wine lactic acid bacterium *Lactobacillus hilgardii* X_1B: structural and functional study of the *arcABC* genes. *Gene* **301**, 61–66.

Babu, V., Mital, B.K. and Garg, S.K. (1992) Effect of tomato juice addition on the growth and activity of *Lactobacillus acidophilus*. *Int J Food Microbiol* **17**, 67–70.

Bardocz, S. (1993) The role of dietary polyamines. *Eur J Clin Nutr* **47**, 683–690.

Bauza, T., Blaise, A., Daumas, F. and Cabanis, J.C. (1995) Determination of biogenic amines and their precursor amino acids in wines of the Vallée du Rhône by high-performance liquid chromatography with precolumn derivatization and fluorimetric detection. *J Chromatogr* **707**, 373–379.

Blasco, L., Ferrer, S. and Pardo, I. (2003) Development of specific fluorescent oligonucleotide probes for in situ identification of wine lactic acid bacteria. *FEMS Microbiol Lett* **225**, 115–123.

Bover-Cid, S., Iquierdo-Pulido, M., Mariné-Font, A. and Vidal-Carou, M.C. (2006) Biogenic mono-, di- and polyamine contents in Spanish wines and influence of a limited irrigation. *Food Chem* **96**, 43–47.

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

Brodequis, M., Dumery, B. and Bouard, J. (1989) Mise en évidence de polyamines putrescine, cadavérine, norspermidine, spermidine et spermine dans les feuilles et les grapes de *V. vinifera*. *Conn Vigne Vin* **23**, 1–6.

Buteau, C., Duitschaever, C.M. and Ashton, G.C. (1984) High-performance liquid chromatographic detection and quantitation of amines in must and wine. *J Chromatogr* **284**, 201–210.

Coton, E., Torlois, S., Bertrand, A. and Lonvaud-Funel, A. (1999) Biogenic amines and wine lactic acid bacteria. *Bull OIV* **815-816**, 22–34.

Dicks, L.M.T., Dellaglio, F. and Collins, M.D. (1995) Proposal to reclassify *Leuconostoc oenos* as *Oenococcus oeni* (corrig.) gen. nov., comb. nov. *Int J Syst Bacteriol* **45**, 395–397.

Donhauser, S., Wagner, D. and Geiger, E. (1992) Biogenic amines. Importance, intake and assessment. *Brauwelt* **132**, 1272–1280.

Farías, M., Manca de Nadra, M.C., Rollan, G.C. and Strasser de Saad, A.M. (1993) Histidine decarboxylase activity in lactic acid bacteria. *J Int Sci Vigne Vin* **27**, 191–193.

Farías, M., Manca de Nadra, M.C., Rollan, G.C. and Strasser de Saad, A.M. (1995) Histidine decarboxilase production by *Lactobacillus hilgardii*: effect of organic acids. *Curr Microbiol* **31**, 15–18.

Glória, M.B. and Izquierdo-Pulido, M. (1999) Levels and significance of biogenic amines in Brazilian beers. *J Food Comp Anal* **12**, 129–136.

Glória, M.B., Watson, B.T., Simon-Sarkadi, L. and Deschel, M.A. (1998) A survey of biogenic amines in Oregon Pinot Noir and Cabernet Sauvignon wines. *Am J Enol Vitic* **49**, 279–283.

González-Fernández, C., Santos, E.M., Jaime, I. and Rovira, J. (2003) Influence of starter cultures and sugar concentrations on biogenic amines contents in chorizo dry sausage. *Food Microbiol* **20**, 275–284.

Guerrini, S., Mangani, S., Granchi, L. and Vincenzini, M. (2002) Biogenic amine production by *Oenococcus oeni*. *Curr Microbiol* **44**, 374–378.

Joosten, H.M.L.J. and Northolt, M.D. (1989) Detection, growth and amine-producing capacity of lactobacilli in cheese. *Appl Environ Microbiol* **55**, 2356–2359.

Kalač, P., Šavel, J., Křížek, M., Pelikánová, T. and Prokopová, M. (2002) Biogenic amines formation in bottled beer. *Food Chem* **79**, 431–434.

Landete, J.M., Ferrer, S., Polo, L. and Pardo, I. (2005) Biogenic amines in wines from three Spanish regions. *J Agric Food Chem* **53**, 1119–1124.

Leitão, M.C., Marques, A.P. and San Romão, M.V. (2005) A survey of biogenic amines in commercial Portuguese wines. *Food Control* **16**, 199–204.

Lehtonen, P., Saarimen, M., Vesanto, M. and Riekkola, M.L. (1992) Determination of wine amines by HPLC using automated precolumn derivatisation with *o*-phthaldehyde and fluorescence detection. *Z Lebensm Unters Forsch* **194**, 434–437.

Lima, A.S. and Glória, M.B. (1999) Aminas bioactivas en alimentos. *Bulletin, Sociedade Brasileira de Ciência e tecnologia de alimentos* **33**, 70–79.

Liu, S.-Q. (2003) Practical implications of lactate and pyruvate metabolism by lactic acid bacteria in food and beverage fermentations *Int. J Food Microbiol* **83**, 115–131.

Liu, S.-Q., Davis, C.R. and Brooks, J.D. (1995) Growth and metabolism of selected lactic acid bacteria in synthetic wine. *Am J Enol Vitic* **46**, 166–174.

Lounvaud-Funel, A. and Joyeux, A. (1994) Histamine production by wine lactic acid bacteria: isolation of a

histamine-producing strain of *Leuconostoc oenos*. *J Appl Bacteriol* **77**, 401–407.

Lu, C.D., Itoh, Y., Nakada, Y. and Jiang, Y. (2002) Functional analysis and regulation of the divergent *spuABCDEFGH-spuI* operons for polyamines uptake and utilization in *Pseudomonas aeruginosa* PAO1. *J Bacteriol* **184**, 3765–3773.

Maijala, R., Eerola, S., Lievonen, S., Hill, P. and Hirvi, T. (1995) Formation of biogenic amines during ripening of dry sausages as affect by starter cultures and thawing time of raw materials. *J Food Sci* **69**, 1187–1190.

Mangani, S., Guerrini, S., Granchi, L. and Vincenzini, M. (2005) Putrescine accumulation in wine: role of *Oenococcus oeni*. *Curr Microbiol* **51**, 6–10.

Marcobal, A., de las Rivas, B., Moreno-Arribas, M.V. and Muñoz, R. (2004) Identification of the ornithine decarboxylase gene in the putrescine-producer *Oenococcus oeni* BIFI-83. *FEMS Microbiol Lett* **239**, 213–220.

Peatfield, R.C. (1995) Relationships between food, wine, and beer-precipitated migraines headaches. *Headache* **1**, 1167–1168.

Pilone, G.J. (1975) Control of malo-lactic fermentation in table wines by addition of fumaric acid. In *Lactic Acid Bacteria in Beverages and Food* eds. Carr, J.G., Cutting, C.V. and Whiting, G.C. pp. 121–138. London: Academic Press.

Radler, F. (1975) The metabolism of organic acids by lactic acid bacteria. In *Lactic Acid Bacteria in Beverages and Food* eds. Carr, J.G., Cutting, C.V. and Whiting, G.C. pp. 17 London: Academic Press.

Radler, F. and Fäth, K.P. (1991) Histamine and other biogenic amines in wines. In *Proceedings of the International Symposium on Nitrogen in Grapes and Wine* ed. Rants, J.M. pp. 185–195. Davis, CA: American Society for Enology and Viticulture.

Sekowska, A., Berlin, P. and Dancln, A. (1998) Characterization of polyamine synthesis pathway in *Bacillus subtilis* 168. *Mol Microbiol* **29**, 851–858.

Simon, J.P. and Stalon, V. (1982) Enzymes of agmatine degradation and the control of their synthesis in *Streptococcus faecalis*. *J Bacteriol* **152**, 676–681.

Soufleros, E., Barrios, M.L. and Bertrand, A. (1998) Correlation between the content of biogenic amines and other wine compounds. *Int J Food Microbiol* **49**, 266–277.

Strasser de Saad, A.M. and Manca de Nadra, M.C. (1987) Isolation and identification of the lactic acid bacteria from Cafayate (Argentina) wines. *Microbiol Alim Nutr* **5**, 45–49.

Straub, B.W., Kicherer, M., Schilcher, S.M. and Hammes, W.P. (1995) The formation of biogenic amines by fermentation organisms. *Z Lebensm Unters Forsch* **201**, 79–82.

Vidal-Carou, M.C., Ambatle-Espunyes, A., Ulla-Ulla, M.C. and Mariné-Font, A. (1990) Histamine and tyramine in Spanish wines: their formation during the winemaking process. *Int J Food Microbiol* **41**, 160–167.

Woller, R. (1993) Les amines biogènes dans les vins. Groupe d'experts Nutrition et santé. *Office international de la vigne et du vin*. Feuillet Bleu No. 3 2072/020893. Paris: OIV.