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Potential of wine-associated lactic acid bacteria to degrade biogenic amines

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Abstract

Some lactic acid bacteria (LAB) isolated from fermented foods have been proven to degrade biogenic amines through the production of amine oxidase enzymes. Since little is known about this in relation to wine micro-organisms, this work examined the ability of LAB strains (n=85) isolated from wines and other related enological sources, as well as commercial malolactic starter cultures (n=3) and type strains (n=2), to degrade histamine, tyramine and putrescine. The biogenic amine-degrading ability of the strains was evaluated by RP-HPLC in culture media and wine malolactic fermentation laboratory experiments. Although at different extend, 25% of the LAB isolates were able to degrade histamine, 18% tyramine and 18% putrescine, whereas none of the commercial malolactic starter cultures or type strains were able to degrade any of the tested amines. The greatest biogenic amine-degrading ability was exhibited by 9 strains belonging to the *Lactobacillus* and *Pediococcus* groups, and most of them were able to simultaneously degrade at least two of the three studied biogenic amines. Further experiments with one of the strains that showed high biogenic amine-degrading ability (*L. casei* IFI-CA 52) revealed that cell-free extracts maintained this ability in comparison to the cell suspensions at pH 4.6, indicating that amine-degrading enzymes were effectively extracted from the cells and their action was optimal in the degradation of biogenic amines. In addition, it was confirmed that wine components such as ethanol (12%) and polyphenols (75 mg/L), and wine additives such as SO_2 (30 mg/L), reduced the histamine-degrading ability of *L. casei* IFI-CA 52 at pH 4.6 by 80%, 85% and 11%, respectively, in cell suspensions, whereas the reduction was 91%, 67% and 50%, respectively, in cell-free extracts. In spite of this adverse influence of the wine matrix, our results proved the potential of wine-associated LAB as a promising strategy to reduce biogenic amines in wine.

Key words: Biogenic amines, lactic acid bacteria, wine, amine degradation.

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1. Introduction

Biogenic amines are a group of biologically active compounds that are widespread in nature. The term 'amine' is used for basic nitrogenous compounds of low molecular weight that are produced within the normal metabolism of humans, animals, plants and micro-organisms. In foods and beverages, biogenic amines are formed mainly by the decarboxylation of the corresponding precursor amino acids. This reaction is catalysed by substrate-specific enzymes, decarboxylases, of the microbiota of the food or wine environment.

Some biogenic amines such as histamine, tyramine, putrescine and cadaverine are important for their physiological and toxicological effects on the human body. They may exert either psychoactive or vasoactive effects on sensitive humans. Histamine has been found to cause the most frequent food-borne intoxications associated with biogenic amines; it acts as a mediator and is involved in pathophysiological processes such as allergies and inflammations (Gonzaga et al., 2009). Tyramine can evoke nausea, vomiting, migraine, hypertension and headaches (Shalaby, 1996). Putrescine and cadaverine can increase the negative effect of other amines by interfering with detoxification enzymes that metabolize them (Stratton et al., 1991).

To exhibit these harmful effects the amines need to gain access to the bloodstream. But the existence of a fairly efficient detoxification system in the intestinal tract of mammals prevents biogenic amines from reaching the bloodstream (Taylor, 1985), so they usually do not represent any health hazard to individuals. One of the main detoxification systems is composed of two distinct enzymes, monoamine oxidase (MAO) and diamine oxidase (DAO) (Ten Brink et al., 1990). Mono- and diamine oxidases are present in eukaryotes and have also been described for fungi (i.e. *Aspergillus niger*) (Frébort et al., 2000) and bacteria (Voigt and Eitenmiller, 1978; Murooka et al., 1979; Ishizuka et al., 1993; Yamashita et al., 1993). These

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enzymes convert amines into non-toxic products, which are further excreted out of the organism.

The main biogenic amines associated with wine are histamine, tyramine and putrescine (Marcobal et al., 2006; Ferreira and Pinho, 2006; Ancín-Azpilicueta et al., 2008; Smit et al., 2008). Their presence in wine is considered as marker molecules of quality loss, and some European countries even have recommendations for the amount of histamine acceptable in wine which impacts on the import and export of wines to these countries. Most fermented foods, such as cheese, fermented sausages and beer, which are consumed more frequently than wines, have higher biogenic amine content (Stratton et al., 1991; Izquierdo-Pulido et al., 2000; Fernández et al., 2007). However, the presence of alcohol in wine may enhance the activity of amines because it inhibits monoamine oxidase enzymes (Ten Brink et al., 1990).

The origin of biogenic amines in wines is well documented (Lonvaud-Funel, 2001; Constantini et al., 2009). They are generated either as the result of endogenous decarboxylase-positive micro-organisms in grapes or by the growth of contaminating decarboxylase-positive micro-organisms in the wine (Halász et al., 1994). With regards to wine micro-organisms, a large amount of literature is available on the production of biogenic amines. Several research groups support the view that biogenic amines are formed in winemaking mainly by lactic acid bacteria (LAB) due to the decarboxilation of free amino acids (Coton et al., 1998; Lonvaud-Funel and Joyeux, 1994; Moreno-Arribas et al., 2000; Guerrini et al., 2002; Landete et al., 2005; Constantini et al., 2006; Lucas et al., 2008). It has been reported that during wine storage and ageing, biogenic amine (i.e. histamine and tyramine) concentrations undergo few variations, being observed as a slight decrease of these compounds during the ageing process in oak barrels (Jiménez-Moreno et al., 2003). This might be due to the action of amine oxidase enzymes present in the wines (Ancín-Azpilicueta et al., 2008) although this

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hypothesis remains to be demonstrated, and to this date no studies have been reported in the literature concerning the degradation of biogenic amines by wine-associated micro-organisms. However, the biogenic amine-degrading ability has been investigated in species such as *Micrococcus varians* (Leuschner et al., 1998) and *Staphylococcus xylosus* (Martuscelli et al., 2000; Gardini et al., 2002) isolated from sausages, in LAB starters from fish silage (*Lactobacillus curvatus* and *Lactobacillus sakei*) (Enes-Dapkevicius et al., 2000), and in dairy (Voigt and Eitenmiller, 1978) and meat (Fadda et al., 2001) products.

The aim of the present paper was to explore the ability of lactic acid bacteria isolated from wines and other related ecosystems to degrade histamine, tyramine and putrescine, which are considered to be the main biogenic amines present in wines. Initially, the ability of a large number of wine-associated LAB strains to degrade biogenic amines was evaluated in culture media and, for the most active strains, their biogenic amine-degrading ability was confirmed in malolactic fermentation experiments. To gain a deeper insight into the biogenic amine-degrading activity exhibit by LAB, and for one of the most active strains (*L. casei* IFI-CA 52), experiments were conducted to show if cell-free extracts were as effective as the whole cells in the degradation of histamine. Finally, the influence of wine components such as ethanol and polyphenols, and wine additives, such as SO_2, on the histamine-degrading activity of *L. casei* IFI-CA 52, was evaluated in both cell-free extracts and cell suspensions.

2. Materials and Methods

2.1. *Lactic acid bacteria strains, culture media and growth conditions*

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Table 1 shows the species and origin of all the strains used in this study. A total of 85 LAB, including *Oenococcus oeni* (42 strains), *Pediococcus parvulus* (7 strains), *P. pentosaceus* (4 strains), *Lactobacillus plantarum* (6 strains), *L. hilgardii* (9 strains), *L. zeae (*3 strains*), L. casei* (7 strains*), L. paracasei* (5 strains) and *Leuconostoc mesenteroides* (2 strains) were used in this study. These strains belong to the bacterial culture collection of the Institute of Industrial Fermentations (IFI), CSIC, Spain. They were previously isolated in our laboratory from musts and wines (young, wood-aged and biologically aged sherry wines) and from winemaking products (fermentation lees) over an 8-year period and properly identified by 16S rRNA partial gene sequencing as described by Moreno-Arribas and Polo (2008). Three *O. oeni* strains isolated from commercial malolactic starter preparations (Uvaferm ALPHA, Viniflora OENOS and Viniferm Oeno 104) that were kindly provided by Lallemand (Ontario, Canada), Christian Hansen (Hørsholm, Denmark) and Agrovín (Alcázar de San Juan, Ciudad Real, Spain) were also used. Additionally, the positive reference biogenic amine producers *Lactobacillus* 30a – a histamine- (Valler et al., 1982) and putrescine-producing (Guirard and Snell, 1980) strain from the American Type Culture Collection in Manassas, Va. (ATCC 33222) – and *L. brevis* CECT 5354 – a tyramine-producing strain (Moreno-Arribas and Lonvaud-Funel, 1999) from the Colección Española de Cultivos Tipo (CECT) – were also included in this study.

These strains were kept frozen at -70 ºC in a sterilized mixture of culture medium and glycerol (50:50, v/v). MRS culture media (pH 6.2) based on the formula developed by Man et al. (1960) was employed for *Lactobacillus*, *Pediococcus* and *Leuconostoc*. They were cultivated for 24-48 h. The culture media MLO (pH 4.8) developed by Caspritz et al. (1983) was employed for *O. oeni*. These bacteria were cultivated for 3-4 days. Both media were purchased from Pronadisa (Madrid, Spain). All bacteria were incubated at 30 ºC.

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2.2. ***Determination of the ability of lactic acid bacteria to degrade biogenic amines***

The availability of wine LAB strains to degrade the biogenic amines histamine, tyramine and putrescine was tested in a model system similar to that previously described for other LAB by Enes-Dapkevicius et al., (2000). The broth consisted of MRS or MLO added separately of 0.15 g/L of each amine – histamine dihydrochloride, tyramine or 1,4-diaminobutane dihydrochloride or putrescine – and adjusted to pH 5.5. LAB strains were incubated at 30 ºC in this model system in duplicate and on at least two different days. Samples were taken at time 0 and after 48 (LAB non *O. oeni*) -72 (*O. oeni*) hours of incubation.

Additionally, some LAB strains were tested for their potential to degrade histamine, tyramine and putrescine during MLF in a laboratory experiment using a Tempranillo red wine. LAB were cultured and grown on MRS and MLO at 30 ºC and 5 x 10^7 ufc/mL were inoculated into the wine previously enriched with malic acid (2 g/L) and contaminated with histamine (28 mg/L), tyramine (12 mg/L) and putrescine (36 mg/L). The biogenic amines were purchased from (Fluka, Buchs,Switherland). Malolactic fermentation was monitored by the determination of the malic acid concentration of wines using a Malic acid Kit (Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland). Biogenic amine degradation was determined by quantitative RP-HPLC analysis, as indicated below.

2.3. ***Determination of lactic acid bacteria biogenic amine producers***

Strains were subcultured at 30 ºC in MRS broth for *Lactobacillus* sp., *Pediococcus* and *Leuconostoc*, and MLO broth for *O. oeni*, both of which contained 0.1% of the corresponding amino acid precursor (L-histidine monohydrochlorid, tyrosine di-sodium salt and L-ornithine monohydrochloride), pyridoxal-5'-phosphate (Sigma, St Louis, MO, USA) and growing

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factors, previously described in Moreno-Arribas et al., (2003). The pH was adjusted to 5.3 and the medium was autoclaved. The precursor amino acids were purchased from Sigma (St. Louis, MO, USA). The ability of bacterial isolates to produce amines (histamine, tyramine and putrescine) was tested by Multiplex PCR, according to Marcobal et al. (2005) and Constantini et al. (2006), and HPLC.

2.4. ***Influence of wine matrix on the degradation of histamine by L. casei IFI-CA 52 cell-free extracts and whole cells***

Two days worth of cultures of the *L. casei* IFI-CA 52 strain, which reached an optical density at 600 nm (Beckman Coulter, DU 800 spectophotometer, Brea, USA) of 2.0, were recovered by centrifugation (3,000 g for 10 min at 4 ºC) using a 3744R Falcon refrigerated centrifuge (Heraeus Sepatech, Biofuge 22R, Hanau, Germany). The cell pellet was washed twice with 0.05 M sodium phosphate buffer (pH 7.0) and suspended in 5 mL of the same buffer. The bacterial suspension was homogenized and the cells were disrupted using an ultrasonic disintegrator (Branson, Digital Sonifier, Danbury, USA) at 150 W, 10x30 s with 30 s of pause, supplied with a thermostatic bath (4 ºC). The cell-free extract was separated from the bacterial debris by centrifuging at 14,000 g for 15 min at 4 ºC.

For the study of the influence of wine components (ethanol and polyphenols) and wine additives (SO_2) on the biogenic amine-degrading ability of *L. casei* IFI-CA 52, the assay mixture contained: cell-free extracts or whole cells, the substrate (histamine dihydrochloride (Fluka, Buchs, Switherland), 50 mg/L) and the buffer to a 2.0 mL final volume. After overnight incubation at 30 ºC, the reaction was stopped by the addition of 1 mL hydrochloric acid (HCl) 1M, and the histamine-degrading activity was determined by HPLC.

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For the determination of the optimal pH, 10 mM phosphate buffer pH 7.0 or 10 mM sodium acetate buffer pH 4.6 were used. For the study of the influence of wine components and additives on amine degradation, ethanol (Panreac Química S.A.U., Barcelona, Spain) (12%, final concentration), potassium metabisulphite (Panreac Química S.A., Barcelona, Spain) (30 mg/L) and the commercial wine extract Provinols™ (Seppic, France) (75 and 660 mg/L) were used. The concentrations for the wine extract were selected on the basis of the information provided by the manufacturers (100 mg of Provinols™ corresponds to the polyphenol content of one glass of red wine, 150 mL). Stock solutions of wine extract were prepared beforehand, dissolving the powder in distilled water or in the mixture solution. All the results are the means of three experiments.

2.5. ***Analysis of biogenic amines***

Biogenic amines were analyzed by reversed-phase (RP)-HPLC according to the method described by Marcobal et al., (2005). Briefly, a liquid chromatograph consisting of a Waters 600 controller programmable solvent module (Waters, Milford, MA, USA), a WISP 710B autosampler (Waters, Milford, MA, USA) and an HP 1046-A fluorescence detector (Hewlett Packard) were used. Chromatographic data were collected and analyzed with a Millenium 32 system (Waters, Milford, MA, USA). The separations were performed on a Waters Nova-Pak C18 (150 x 3.9 mm i.d., 60 Å, 4 μm) column, with a matching guard cartridge of the same type. Samples were submitted to an automatic precolumn derivatization reaction with *o*-phthaldialdehyde (OPA) prior to injection. Derivatized amines were detected using the fluorescence detector (excitation wavelength of 340 nm, and emission wavelength of 425 nm). Samples were previously filtered through Millipore filters (0.45 μm) and then directly injected in duplicate into the HPLC system. All reagents used were HPLC grade.

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From the HPLC data, the percentage of biogenic amine degradation was calculated as follows:

$$\% \text{ Degradation} = (C_{control}-C_{strain}) / C_{control} * 100$$

where $C_{control}$ is the concentration of the biogenic amine in the control (no strain incubated) and C_{strain} is the concentration in the medium incubated with the strain.

3. Results

3.1. *Availability of wine-associated LAB to degrade biogenic amines in culture media*

Cell cultures of 85 strains representing 9 species of wine LAB (Table 1) were investigated for their potential to degrade/eliminate histamine, tyramine and putrescine, the major biogenic amines present in wines. None of the LAB strains investigated were able to cause a complete disappearance of histamine, tyramine or putrescine under the experimental conditions used. Among the 85 LAB isolates tested, 25% were able to degrade histamine, 18% tyramine and 18% putrescine, although to different extents. Strains showing a percentage of degradation ≥ 10% of any of the biogenic amines studied are shown in Table 2. Results concerning the *O. oeni* strains isolated from commercial malolactic starter preparations, as well as those concerning the control positive biogenic amine producers *Lactobacillus* 30a ATCC 33222 and *L. brevis* CECT 5354, were negative, so these strains are not included in Table 2. For this screening of biogenic amine-degrading activity, it would have been worth testing positive control strains of amine oxidase producers, but unfortunately, there are none commercially available.

All of the selected positive strains were able to degrade at least two of the three biogenic amines tested; seven strains were able to degrade histamine, six of them tyramine, and all of

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them exhibited the ability to degrade putrescine (Table 2). The degradation percentages ranged from 10% for histamine degradation by *P. pentosaceus* IFI-CA 30 to 69% for putrescine degradation by *P. pentosaceus* IFI-CA 86. In general, putrescine was degraded to a greater extent than histamine and tyramine by all the selected strains. On the other hand, the highest potential for biogenic amine degradation among LAB seemed to be for the *Lactobacillus* and *Pediococcus* groups, in particular *L. plantarum* and *P. pentosaceus* species. With regards to *O. oeni*, the main LAB species involved in MLF, out of the 42 isolates tested, only *O. oeni* IFI-CA 32 was able to reduce histamine and putrescine, but with low activity (Table 2). Furthermore, the following five strains simultaneously degraded the three biogenic amines: *P. pentosaceus* IFI-CA 30 and IFI-CA 83, *P. parvulus* IFI-CA 31, *L. plantarum* IFI-CA 54– all of them isolated from red wines – as well as *L. casei* IFI-CA 52, isolated from a sherry wine during its biological aging (Moreno-Arribas and Polo, 2005). This strain exhibited the greatest potential for histamine, tyramine and putrescine degradation (54%, 55% and 65% of degradation, respectively) (Table 2).

3.2. *Biogenic amine production by LAB able to degrade histamine, tyramine or putrescine*

The nine selected strains exhibiting the highest potential to degrade histamine, tyramine and putrescine in culture media (*L. plantarum* IFI-CA 26, *P. pentosaceus* IFI-CA 30, IFI-CA 83 and IFI-CA 86, *P. parvulus* IFI-CA 31, *O. oeni* IFI-CA 32, *L. hilgardii* IFI-CA 41, *L. casei* IFI-CA 52 and *L. plantarum* IFI-CA 54) were also tested for their ability to produce these compounds (histamine, tyramine and putrescine) in MRS and MLO media spiked with the corresponding amino acid precursors (histidine, tyrosine and ornithine, respectively). None of the lactic acid bacteria tested was able to produce any biogenic amines (results not shown). Furthermore, multiplex PCR assays were performed on these nine strains to test for the presence of decarboxylase genes. None of the strains selected amplified the *hdc*, *tdc* or *odc*

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genes (results not shown), suggesting that LAB strains able to degrade biogenic amines do not contribute to histamine, tyramine and putrescine formation in wines.

3.3. *Availability of selected LAB to degrade biogenic amines in wine malolactic fermentation experiments*

The nine selected lactic acid bacteria strains active in culture media were also tested in malolactic fermentation laboratory experiments to evaluate their potential applicability in biogenic amine removal from contaminated wines, which could represent a technological improvement in the resolution of this problem. Table 3 reports the concentrations of amines in wines inoculated with the selected strains in comparison to the control wine (no strain inoculated), after malolactic fermentation. The concentration of histamine, tyramine and putrescine in the contaminated wine (28 mg/L, 12 mg/L and 36 mg/L, respectively) was not altered after malolactic fermentation either for the control wine or for the wines inoculated with *L. plantarum* IFI-CA 26, *P. pentosaceus* IFI-CA 30, IFI-CA 83 and IFI-CA 86, *P. parvulus* IFI-CA 31, *O. oeni* IFI-CA 32, *L. hilgardii* IFI-CA 41 and *L. plantarum* IFI-CA 54. Only *L. casei* IFI-CA 52 was able to significantly degrade histamine (16% of the initial concentration), tyramine (15%) and putrescine (8%) in the contaminated wine, but at lower percentages than in culture media (Table 2). Therefore, these results indicated that the ability of LAB to reduce biogenic amines was negatively affected by the wine matrix.

3.4. *Influence of enological factors on the degradation of histamine by cell suspensions and and cell-free extracts of L. casei IFI-CA 52*

To gain a deeper insight into the amine-degrading activity exhibited by LAB, and for one of the most active strain found in previous assays (*L. casei* IFI-CA 52), new experiments were

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conducted to show whether cell-free extracts were as effective as whole cells in the degradation of biogenic amines. For both cell suspensions and cell-free extracts, the influence of enological conditions (pH, wine components and enological additives) on the biogenic amine-degrading ability of *L. casei* IFI-CA 52 was evaluated. Histamine was used since it is the most controlled biogenic amine in wine trade transactions with certain countries.

The effect of *L. casei* IFI-CA 52 on the degradation of histamine in whole cells and enzymatic crude cell extracts was evaluated in phosphate (pH 7.0) and sodium acetate (pH 4.6) buffer systems. Both pHs (7.0 and 4.6) showed good results for histamine reduction in cell suspensions of *L. casei* IFI-CA 52 (88 and 85 % of degradation, respectively) (Table 4). Additionally, at pH 4.6, the histamine-degrading ability of the cell-free extracts (84%) was similar to that of the whole cells, indicating that amine-degrading enzymes were effectively extracted from the cells and their action optimal on the degradation of histamine. However, at pH 7.0 the biogenic amine-degrading ability of *L. casei* IFI-CA 52 was slightly lower (72%) in the cell-free extracts in comparison to the cell suspensions, indicating that either genes encoded amine-degrading enzymes were not totally activated, or induced amine-degrading were not totally extracted from the whole cells or the action of the solubilized enzymes was not optimal at this pH.

Results also showed that the presence of wine components such as ethanol and polyphenols strongly affected the histamine-degrading ability of *L. casei* IFI-CA 52 at pH 4.6, for both cell suspensions and cell-free extracts (Table 4). The addition of 12% ethanol (the average concentration in wine) modified the histamine-degrading ability of *L. casei* IFI-CA 52 down to 17 and 7%, respectively, for cell suspension and cell-free extracts, which meant a reduction of 80% in the ability of the whole cells and of 91% in that of the cell-free extracts. Therefore, amine-degrading enzymes seemed to be more sensitive to the presence of ethanol than the

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whole cells in terms of their histamine-degrading ability. Wine polyphenols also exhibited an inhibitory effect on the enzyme activity; by adding a concentration of 75 mg/L, only 13 and 28% of the histamine is degraded by whole cells and cell-free extracts, respectively. In the presence of 660 mg/L of Provinols™, only 10% of the histamine was degraded by whole cells and no activity was present in the cell-free extracts. In other words, wine polyphenols (75 and 660 mg/L) seemed to have more effect on the histamine-degrading ability of the whole cells (85 and <100% of reduction, respectively) than on that of the cell-free extracts (67 and 99% of reduction, respectively), indicating that amine-degrading enzymes were less sensitive to the presence of wine polyphenols than the whole cells.

The effect of potassium metabisulphite (SO_2), the additive most employed in winemaking because of its antioxidant and selective antimicrobial properties, was tested at normal concentration (30 mg/L). As observed in table 4, SO_2 reduced the histamine-degrading ability of *L. casei* IFI-CA 52 down to 75 and 42% respectively for cell suspension and cell-free extracts, which meant a reduction of 11% in the ability of the whole cells and of 50% in that of the cell-free extracts, indicating that amine-degrading enzymes were more sensitive to the presence of SO_2 than the whole cells, as was the case with ethanol.

4. Discussion

Knowledge concerning the origin and factors involved in biogenic amine production in wines is well documented, and recently several reviews on this topic have been published (Ferreira and Pinho, 2006; Ancín-Azpilicueta et al., 2008; Smit et al., 2008; Moreno-Arribas et al., 2010). In contrast, there is a lack of studies concerning amine degradation by wine micro-organisms. In this context, this paper reports novel data about the presence of histamine-, tyrosine- and putrescine-degrading enzymatic activities of wine-associated LAB. Of particular

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interest are the results concerning the degradation of putrescine, since no such degrading ability of any food LAB has previously been reported. The isolates tested belong to the principal species of wine LAB and were selected because they came from wine cellars that often suffer from the problem of biogenic amines in their wines (Marcobal et al., 2004; Marcobal et al., 2006, Martín-Álvarez et al., 2006, Moreno-Arribas and Polo, 2008). Therefore, our results confirmed that, within the natural microbiota of lactic acid bacteria present in wines and other related environments, some species and/or strains possessed the potential to degrade biogenic amines. However, this potential for histamine, tyramine and/or putrescine degradation among wine LAB does not appear to be very frequent, since out of the 85 strains examined, only nine displayed noteworthy amine-degrading activity in culture media. Further studies using other LAB species and/or strains may enable more potent amine-degrading enzyme producers to be identified. However, it was observed that positive strains displayed amine-degrading activity against several biogenic amines simultaneously, in accordance with previous works that also reported the presence of either one or two amines oxidases in other food-fermenting micro-organisms, such as *Micrococcus varians* and *Staphylococcus carnosus* (Leuschner et al*.,* 1998).

The fact that active bacteria which were able to significantly reduce the concentration of biogenic amines in the conditions used in the study came not only from young and wood-aged wines but also from fermentation lees, and especially from biologically aged sherry wines (Table 2), suggests that both fermentation lees and 'flor velum' can be interesting ecological niches for the isolation of potential amine-degrading bacteria.

The potential for amine breakdown proved to be a characteristic related to some species of the genera *Lactobacillus* and *Pediococcus*, which was in agreement with previous works that investigated the distribution of histamine and tyramine oxidase activities among food-

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fermenting micro-organisms (Leuschner et al., 1998). In this study, the most potent amine-degrading species detected were *L. plantarum, P. parvulus* and, in particular, *P. pentosaceus* and *L. casei,* in spite of the fact that strains of these last species have never been reported to degrade histamine, tyramine and/or putrescine. In contrast, the results indicate that, within the natural population of *O. oeni* isolated from wines, the presence of enzymatic activities that degrade histamine, tyramine and/or putrescine was low, suggesting that the potential to reduce amine concentrations in wines is rare in *O. oeni* strains. Regarding commercial malolactic starters, they are regarded as safe with respect to biogenic amine production (Moreno-Arribas et al., 2003; Marcobal et al., 2006). However, to date there has not been any report on the potential role of these starters in the elimination/degradation of biogenic amines in wines, in spite of their wide use in winemaking. In our experiments, none of the commercial malolactic starters tested (n=3) showed any histamine, tyramine or putrescine-degrading ability in culture media, leading to the conclusion that no specific role in the removal of biogenic amines could be attributed to them, although further studies, including a higher number of products, should be carried out.

Once amine-degrading activities of some LAB strains were proven, the next goal was to see if these strains might promote the accumulation of these compounds in wine. Therefore, we tested the production of the most important biogenic amines in wines (histamine, tyramine and putrescine) by the selected positive amine-degrading LAB strains. None of the strains were able to produce these biogenic amines as they did not show the decarboxylase activity necessary for the production of these compounds in wine. Therefore, the biogenic amine-degrading ability of the selected LAB did not appear to be associated with an amine-producing ability.

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In order to check their ability to reduce biogenic amines in wine environment strains possessing amine-degrading ability in culture media were also tested in real systems by simulating wine MLF. The *L. casei* IFI-CA 52 strain, displaying high histamine, tyramine and putrescine breakdown in culture media, had a limited effect on these amines during wine MLF, in line with previous works that indicate that the activity *in vitro* of micro-organisms having mono- and diamino-oxidase activities is not quantitatively reproducible *in vivo* (Gardini et al., 2002).

Although no differences in the amine-degrading activity of *L. casei* IFI-CA 52 were found to be affected by pH (4.6 and 7.0), further experiments in the presence of wine components such as ethanol (12%) and polyphenols (75 and 660 mg/L) and wine additives such as SO_2 (30 mg/L) indicated that the wine matrix definitely affected the ability of the strain to degrade histamine, explaining the differences found between the percentage of histamine degradation by *L. casei* IFI-CA 52 in wine (Table 3) and in culture media (Table 2). Although more studies with other LAB species and strains are required to draw final conclusions, these studies suggested that the wine matrix have a strong effect on the ability of amine-degrading enzymes to reduce undesirable biogenic amines in wine.

The fact that there were no differences in the histamine-degrading ability of the cell suspensions of *L. casei* IFI-CA 52 and their corresponding cell-free extracts indicated that amine-degrading enzymes are intracellular and active at a pH close to wine pH. Therefore, a potential application of amine-degrading strains to prevent the accumulation of biogenic amines in wine could be as starters to be inoculated or as enzymatic preparations to be added to the contaminated wines. Moreover, the wine matrix would influence the efficiency of starters and enzymatic preparations in different ways, as this study also showed that ethanol

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and SO_2 have more effect on the activity of solubilized amine oxidase enzymes than on whole cells, whereas wine polyphenols showed the opposite (Table 4).

In conclusion, this paper presents, for the first time to our knowledge, a screening of the biogenic amine-degrading ability of wine-associated LAB. Among the many and diverse strains tested, some of them have been found to be active in the degradation of histamine, tyramine and putrescine in culture media and in wine. Although the amine-degrading ability of the active LAB seemed to be good at a pH close to wine pH, wine components such as ethanol and polyphenols and wine additives such as SO_2 might limit this ability, as has been seen in the case of *L. casei* IFI-CA 52. In spite of this adverse influence of the wine matrix, our results prove the potential to prevent/reduce the accumulation of these amines in the final wine. Further investigations are needed in order to evaluate the applicability of this LAB potential in winemaking.

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Table 1. Lactic acid bacteria used in this study[a].

	Species	Source
Isolated strains		
IFI-CA 2, IFI-CA 3, IFI-CA 4, IFI-CA 5, IFI-CA 6, IFI-CA 8, IFI-CA 10, IFI-CA 32, IFI-CA 45	*Oenococcus oeni*	Fermentation lees
IFI-CA 11, IFI-CA 12, IFI-CA 13, IFI-CA 14, IFI-CA 15, IFI-CA 17, IFI-CA 20, IFI-CA 21, IFI-CA 22, IFI-CA 27, IFI-CA 28, IFI-CA 33, IFI-CA 34, IFI-CA 35, IFI-CA 36, IFI-CA 37, IFI-CA 38, IFI-CA 40, IFI-CA 42, IFI-CA 44, IFI-CA 46, IFI-CA 47, IFI-CA 56, IFI-CA 58, IFI-CA 59	*Oenococcus oeni*	Young wine/grape must
IFI-CA 60, IFI-CA 79, IFI-CA 81, IFI-CA 82, IFI-CA 96, IFI-CA 100, IFI-CA 101, IFI-CA 102	*Oenococcus oeni*	Oak barrel-aged wines
IFI-CA 19, IFI-CA 23, IFI-CA 24, IFI-CA 29, IFI-CA 31, IFI-CA 57, IFI-CA 97	*Pediococcus parvulus*	Young wine/grape must
IFI-CA 30, IFI-CA 83, IFI-CA 85	*Pediococcus pentosaceus*	Oak barrel-aged wines
IFI-CA 86	*Pediococcus pentosaceus*	Biologically aged sherry wines
IFI-CA 7, IFI-CA 54, IFI-CA 78, IFI-CA 80, IFI-CA 92	*Lactobacillus plantarum*	Young wine/grape must
IFI-CA 26	*Lactobacillus plantarum*	Fermentation lees
IFI-CA 16, IFI-CA 25, IFI-CA 49, IFI-CA 53, IFI-CA 79, IFI-CA 95,	*Lactobacillus hilgardii*	Young wine/grape must
IFI-CA 41, IFI-CA 108, IFI-CA 111	*Lactobacillus hilgardii*	Biologically aged sherry wines
IFI-CA 50, IFI-CA 131, IFI-CA 140	*Lactobacillus zeae*	Biologically aged sherry wines
IFI-CA 78, IFI-CA 93	*Lactobacillus casei*	Young wine/grape must
IFI-CA 51, IFI-CA 52, IFI-CA 69, FI-CA 115, IFI-CA 124,	*Lactobacillus casei*	Biologically aged sherry wines
IFI-CA 18, IFI-CA 94	*Lactobacillus paracasei*	Young wine/grape must
IFI-CA 125, IFI-CA 136, IFI-CA 137	*Lactobacillus paracasei*	Biologically aged sherry wines
IFI-CA 141, IFI-CA 156	*Leuconostoc mesenteroides*	Biologically aged sherry wines
Commercial malolactic starters		
Uvaferm ALPHA	*Oenococcus oeni*	Lallemand
Viniflora OENOS,	*Oenococcus oeni*	Christian Hansen
Viniferm Oeno 104	*Oenococcus oeni*	Agrovín
Type strains		
30a (ATCC 33222)	*Lactobacillus* sp.	ATCC
CECT 5354 (ATCC 367)	*Lactobacillus brevis*	CECT

[a] ATCC, American Type Culture Collection; CECT, Colección Española de Cultivos Tipo

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Table 2. Percentage of degradation of the biogenic amines (histamine, tyramine and putrescine) by wine-associated LAB in culture media.

	Degradation (%)[a,b]		
Strains	**Histamine**	**Tyramine**	**Putrescine**
L. casei IFI-CA 52	54	55	65
L. hilgardii IFI-CA 41	n.e.	n.e.	20
L. plantarum IFI-CA 26	33	n.e.	24
L. plantarum IFI-CA 54	23	17	24
O. oeni IFI-CA 32	12	n.e.	16
P. parvulus IFI-CA 31	21	15	53
P. pentosaceus IFI-CA 30	10	12	49
P. pentosaceus IFI-CA 83	19	22	39
P. pentosaceus IFI-CA 86	n.e.	54	69

[a]Activity is expressed as a percentage of control without strain and according to HPLC quantitative biogenic amine results; [b]Mean values (n=3); n.e.: no effect was observed

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Table 3. Biogenic amine content (mg/L) in biogenic amine-contaminated wine after MLF fermentation in the presence of amine-degrading LAB[a].

Strains	Histamine	Tyramine	Putrescine
Control	28.02 ± 0.52	12.00 ± 0.15	36.10 ± 0.25
L. casei IFI-CA 52	23.10 ± 0.12	10.16 ± 0.14	33.36 ± 0.47
L. hilgardii IFI-CA 41	28.49 ± 0.60	12.10 ± 0.52	36.69 ± 0.17
L. plantarum IFI-CA 26	27.12 ± 0.12	12.01 ± 0.20	35.85 ± 0.23
L. plantarum IFI-CA 54	28.41 ± 0.27	11.45 ± 0.47	35.65 ± 0.29
O. oeni IFI-CA 32	28.75 ± 0.21	11.58 ± 0.36	36.56 ± 0.25
P. parvulus IFI-CA 31	28.41 ± 0.28	12.41 ± 0.18	36.58 ± 0.41
P. pentosaceus IFI-CA 30	28.14 ± 0.24	12.10 ± 0.15	36.08 ± 0.44
P. pentosaceus IFI-CA 83	27.19 ± 0.15	12.14 ± 0.32	35.14 ± 0.30
P. pentosaceus IFI-CA 86	28.75 ± 0.25	12.57 ± 0.43	34.23 ± 0.21

[a]Mean values ± standard deviations (n=3);

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Table 4. Histamine degradation (%) of cell suspensions and cell-free extracts of *L. casei* IFI-CA 52 in phosphate (pH 7.0) and sodium acetate (pH 4.6). Influence of ethanol, wine polyphenols and SO_2.

	Histamine degradation (%)[a]	
	Cell suspensions	Cell-free extracts
Phosphate buffer (pH 7.0)	88	72
Sodium acetate buffer (pH 4.6)	85	84
+ ethanol (12%)	17	7
+ wine polyphenols (75 mg/L)	13	28
+ wine polyphenols (660 mg/L)	n.e.	0.12
+ SO_2 (30 g/L)	75	42

[a]Activity is expressed as a percentage of control and according to HPLC quantitative biogenic amine results;
[b]Mean values (n=3).

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Manuscript title: Potential of wine-associated lactic acid bacteria to degrade biogenic amines

Authors: Almudena García-Ruiz, Eva M. González-Rompinelli, Begoña Bartolomé, and M.Victoria Moreno-Arribas*

Highlights:

For the first time to our knowledge, a screening of the biogenic amine-degrading capacity of wine-associated lactic acid bacteria was presented. Although 25% of the isolates were able to degrade histamine, 18% tyramine and 18% putrescine, the greatest biogenic amine-degrading capacity was exhibited by 9 strains belonging to the *Lactobacillus* and *Pediococcus* groups. Amino oxidase activity of strain *L. casei* IFI-CA 52 was effectively extracted from the cells, and their action was optimal in the degradation of biogenic amines at pH conditions close to those found in wine. Wine components such as ethanol and polyphenols, and additives such as SO_2 might limit this ability, as has been seen in the case of *L. casei* IFI-CA 52.

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