Does *Oenococcus oeni* produce histamine?

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23 Abstract

24

25 The presence of histamine in wine and other fermented foods may pose a toxicological
26 risk for consumers. Production of histamine by *Oenococcus oeni*, which is the main
27 agent of malolactic fermentation in wine and thus very important for wine industry, has
28 been extensively analysed with contradictory results. If histamine production by *O. oeni*
29 strains is a widespread trait, enological practices will be affected and the use of non-
30 producing commercial *O. oeni* starters should be strongly recommended to avoid
31 histamine production during winemaking. However, a review of published data showed
32 that most evidence strongly supports the view that histamine production is not a
33 characteristic of *O. oeni* strains. We therefore propose the adoption of common
34 analytical methods and the introduction of publicly-available validated histamine-
35 producing *O. oeni* reference strains as a common positive control in assays to resolve
36 this important issue.

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52 1. Introduction

53 Biogenic amines occur in different kinds of foods, such as cheese, fish products,
54 beer, and wine (Smit et al., 2008). They are of concern in relation to food spoilage,
55 safety, and intolerance and their content in foods should be as low as possible. At high
56 concentrations, biogenic amines are risk factors for food intoxication, while moderate
57 levels may lead to food intolerance. They are undesirable in all foods and beverages as
58 they can induce headaches, respiratory distress, hyper/hypotension and several allergic
59 disorders (Silla, 1996). Of biogenic amines, histamine is the most dangerous due to the
60 toxological effects derived from its vasoactive and psychoactive properties (Halász et al.,
61 1994). Sensitive persons suffer from numerous undesirable reactions after intake of
62 histamine-containing foods (Bodmer et al., 1999). To minimize its toxological effects, it

is suggested that histamine concentration should not exceed 2 mg/l in alcoholic beverages (ten Brink et al., 1990).

Histamine is produced by enzymatic decarboxylation of the amino acid histidine (Landete et al., 2008). It is assumed that most histamine in wine is produced by histidine decarboxylases from lactic acid bacteria (LAB) during malolactic fermentation (MLF) (Davis et al., 1985; Gerbaux et al., 1997; Lonvaud-Funel, 2001), a biochemical process that occurs in wine after alcoholic fermentation.

Oenococcus oeni is the dominant species during the MLF of wines (Davis et al., 1986; Van Vuuren and Dicks, 1993) and, consequently, many authors have considered it to be the main organism that produces histamine in wine (Coton et al., 1998a; Landete et al., 2005a; López et al., 2009; Lucas et al., 2008a). However, this conclusion may not be the only explanation for histamine production in wine. Other species of LAB occur during wine production and have been found in the grape must, during MLF and after MLF. These are generally species of *Lactobacillus* (eg. *L. hilgardii*) and *Pediococcus* which are also known to produce histamine (Costantini et al., 2006; Landete et al., 2005a; Landete et al., 2007a; Lucas et al., 2005). In addition, BA producing species of *Enterobacteriaceae* have recently been reported in wine grape musts (Pinto et al., 2011).

As discussed here, there is controversy regarding whether or not *O. oeni* is the principal bacterial species implicated in histamine production in wine (Tables 1 and 2), and the issue therefore requires clarification in order to develop strategies for managing and controlling histamine production in wine.

There are two mechanisms by which wine MLF can occur: i) natural fermentation due to the growth of indigenous strains, mainly *O. oeni*, which is the best adapted to the harsh environmental wine conditions; or ii) guided fermentation after

inoculation with starter cultures mainly of selected *O. oeni* strains (du Toit, 2011; Krieger et al., 1993). Consequently, as it has been posited that most *O. oeni* strains are histamine-producers, there is a need for reliable data to support this. If it is accepted that most *O. oeni* strains are histamine-producers, then prevention should be primarily based on the use of selected non-histamine producing starters to carry out MLF. On the other hand, if *O. oeni* strains are not able to decarboxylate histidine and to produce histamine, prevention should focus not on the use of selected strains but on control of winemaking conditions to eliminate any possible histamine-producing contaminant bacteria and to promote *O. oeni* growth, with either commercial starter or indigenous strains, if spontaneous MLF is accomplished. In this case, special attention should be paid to several factors, such as: i) pH, since *O. oeni* is the most acid-tolerant species of enological microorganism and *O. oeni* populations tend to dominate at lower pH values whereas pH values above pH 3.4 favour the growth of *Lactobacillus* and *Pediococcus* spp.; ii) concentration of SO₂, to inhibit growth of undesirable bacteria after MLF; iii) time of contact with the lees, which can be a source of nutrients for undesirable bacterial growth in wine at the end of MLF; and iv) control of commercial yeast starters, which can be a vehicle for contaminating histamine-producer bacteria (Costantini et al, 2009).

Some of the discrepancies among reports on histamine production by *O. oeni* in the literature might be explained by the difficulties of determining histamine in cultures or complex media such as wine (Del Prete et al., 2009; Ough et al., 1987) and/or by the molecular methods (Marcobal et al., 2006) used to assess histamine production by *O. oeni* strains.

As this issue has important safety and economical implications, after an exhaustive review of the available published data and an evaluation of the

methodological procedures used, we have concluded that there are no solid data to support histamine production by *O. oeni* strains.

2. Histamine content in wine

As a consequence of the growth of interest in consumer protection, the presence of histamine in wine has been extensively studied for many years, and particularly over the last 10. The first reference to the presence of histamine in wines was made by Tarantola (1954). Later on, several studies from Germany, France and US reported concentrations in wine ranging from 0.1 mg/l to 21 mg/l (Lafon-Lafourcade, 1975; Marquardt, 1963; Ough, 1971; Quevauvillier and Maziere, 1969). Similar studies carried out in Switzerland for white and red wines found quite low levels of histamine, averaging 1.1 mg/l and 3.5 mg/l respectively, with maximum levels rarely exceeding 10 mg/l (Mayer and Pause, 1971). The maximum histamine content in high quality Portuguese wines was 1.7 mg/l (Mafra et al., 1999); white and red wines from Rioja (Spain) had average levels of 0.84 and 8.72 mg/l respectively (Vazquez-Lasa et al., 1998); and Cabernet Sauvignon and Pinot noir wines from Oregon (USA) gave average values of 2.71 and 7.2 mg/l respectively, with a maximum level of 24 mg/l (Gloria et al., 1998).

Similarly low levels of histamine in wine have recently been reported. For instance, in Chinese red wines histamine content varied between 0 and 10.51 mg/l, with 86% of wines containing between 0 and 5.0 mg/l (Zhijun et al., 2007). The average histamine level found in Spanish red wines was 2.97 mg/l, with a maximum of 15.7 mg/l (Izquierdo-Cañas et al., 2009). A mean of 8.5 mg/l and a maximum concentration of up to 27 mg/l of histamine were recently found in Austrian wines (Konakovsky et al., 2011).

All these data indicate that most wines had low histamine contents, rarely exceeding 5 mg/l, with only a few wines showing levels over 10 mg/l. These results have two significant implications: 1) histamine content in wines does not differ as a function of their geographic origin; and 2) recent oenological practices with greater use of commercial starters have not reduced histamine content in wines.

3. Methods for the characterization of histamine producer bacteria

Methods for determining histamine production by a bacterial strain may be grouped into two types: methods involving cultures of the microorganism and determination of the histamine produced in the medium, and molecular methods based on PCR detection of the bacterial *hdc* gene.

Methods to determine histamine production by bacterial growth in a differential medium containing a pH indicator have been reported. These methods are based on a change in the colour of the medium when histamine, an alkaline compound, is produced. They are an easy, cheap and rapid solution for preliminary selection of strains displaying histidine decarboxylase activity (Choudhury et al., 1990; Leitao et al., 2000; Rosi et al., 2008). However, several authors have reported false-positive reactions due to the formation of other alkaline compounds, and also false negative responses as a result of the fermentative activity of some bacteria that produce acid along with histamine (Bover-Cid and Holzapfel, 1999; Marcobal et al., 2006). In addition, fastidious microorganisms, such as some strains of *O. oeni*, may not grow well in some of these media. All these data indicate that such methods are not accurate enough for the characterization of histamine producing bacteria.

Various different methods have been proposed to avoid false-positive or negative results in screening procedures. These entail the culturing of bacteria in a

synthetic growth medium, to which the amino acid precursor histidine is added to enhance the results, and subsequent determination of the histamine production in cell-free supernatants by chromatographic detection methods such as ion-exchange chromatography, thin-layer chromatography (TLC), or HPLC (Costantini et al., 2006; Garai et al., 2007; Garcia-Moruno et al., 2005; Gardini et al., 2005; Moreno-Arribas et al., 2003; Ruiz et al., 2010). These are the most reliable methods for detecting histamine production by a bacterial strain, but more attention needs to be paid to the amount of the amino acid precursor histidine that is added considering the detection limit of the chromatographic method used to determine the histamine produced.

Recently, a practical and reliable TLC method for the characterization of biogenic amine producing bacteria was adopted by the OIV (International Organisation of Vine and Wine). With this method (International oenological Codex, COEI-2-AMIBIO: 2010) histamine production can readily be detected by the addition of 5g/l of histidine to the culture medium since the detection limit for histamine is 1g/l.

HPLC is a more sensitive technique, but again, for accurate detection of histamine by HPLC it is necessary to consider the sensitivity of the method used. Various different HPLC methods with different detection limits have been proposed for histamine determination (Costantini et al., 2006; Coton et al., 1998a; Mafra et al., 1999; Soufleros et al., 2007). And in these cases attention must be paid to ensure that the concentration of precursor histidine added to the medium is appropriate for the detection limit of the method used. Furthermore, for HPLC histamine detection the presence of potentially interference substances in the medium must also be taken into account. For instance, HPLC detection of amines involves derivatization with different reagents, such as dansyl chloride or α -phthalaldehyde, and these procedures could produce

interference from substances possessing the same retention time as the histamine-
derivate.

More recently, molecular methods, mainly based on the PCR, have been
reported for the detection of histamine-producing bacteria by amplifying genes that
encode histidine decarboxylase enzymes (see Landete et al., 2007b and Marcobal et al.,
2006 for reviews). Molecular methods are fast and culture-independent; however, false-
positive and false-negative results can also occur. False-positive results may be due to
contamination with a previous amplicon, to the presence of a mixture of bacterial
strains, or to unspecific amplifications. False-negative results are mainly due to the
presence of inhibitors in the PCR reaction. Also, special attention should be paid to
correct selection of primers. And again, it is important to remember that when
determining the possible production of histamine by amplification of the *hdc* gene, the
presence of the gene does not necessarily mean that it is expressed under wine
conditions.

Published data about *O. oeni* strains as histamine producing or non-histamine
producing are contradictory. When contradictory results are being presented to the
community, the first step should be to completely rule out analytical errors. We have
therefore extensively reviewed the published results on the question of whether or not
O. oeni produces histamine.

4. Studies reporting histamine production by *O. oeni*

Reports describing histamine production by *O. oeni* strains are summarized in
Table 1. Histamine production by *O. oeni* strains from bacterial cultures has been
reported after chromatographic detection in synthetic media (Del Campo et al., 2000;

Guerrini et al., 2002) or in wines (Lonvaud-Funel and Joyeux, 1994; López et al., 2009).

Del Campo et al. (2000) examined *O. oeni* strains isolated during the cidermaking process for their ability to produce histamine in media supplemented with L-histidine (10 g/l). Samples taken from growing cultures were centrifuged and the cell-free supernatant collected and analysed by a method based on the formation of a fluorescent complex with o-phthaldialdehyde. Results showed that 58% of the strains were weak histamine producers (< 2 mg/l), and the remaining 42% produced 2 mg/l, which the authors considered high. However, this histamine production is extremely low (5000-fold lower than expected) when compared to the precursor histidine concentration present in the media (10 g/l), suggesting a possible misinterpretation in the identification of chromatographic peaks identified as histamine.

Guerrini et al. (2002) determined the histamine-producing ability of 44 *O. oeni* strains originally isolated from different Italian wines, which were grown in MRS broth and analyzed by RP-HPLC. More than 60% of the strains exhibited histamine-producing ability. However, in this study histidine precursor was not added to the medium and the authors did not report the histidine content found in it.

In fact, in this article, *O. oeni* DSM 20252 type strain (ATCC 23279, NCDO 1674, CECT 4100) was described as histamine producing, when most studies have reported that it is a non-producer (Choudhury et al., 1990; Coton et al., 1998a; Marcobal et al., 2005; Moreno-Arribas et al., 2003).

López et al. (2009) reported that when spontaneous MLF occurred, a high level of histamine production (9.27 mg/l) was determined by HPLC, and that only *O. oeni* strains were isolated from the wine studied, suggesting that they were the responsible for histamine production. However, this report did not take into account that other

bacteria and LAB may also be present in wine; for example biogenic amine producing enterobacteria (which could not be isolated from the growth media recommended for LAB) have been also found in musts (Pinto et al., 2011).

HPLC analyses of wines from three Spanish regions showed that 58% of the wines contained histamine-producing *O. oeni* strains (Landete et al., 2005b). However, as in the other studies cited (Del Campo et al., 2000; Guerrini et al., 2002), *O. oeni* strains also produced low histamine concentrations (from 11–34 mg/l in synthetic media and 0.5–11 mg/l in wine) in media containing 20 g/l of histidine precursor, and no explanation was given for it.

Low levels of histamine production have also been reported by *O. oeni* strains belonging to a strain collection from the University of Firenze (Rosi et al., 2008). Eight out of the 26 strains analyzed were histamine producers (2.6–5.6 mg/l) in a medium containing 20 g/l of the corresponding amino acid precursor, but again no explanation was offered for such a low histamine concentration considering the high concentration of amino acid precursor present in the medium.

Molecular methods for the characterization of *O. oeni* histamine producers were initially based on PCR amplification of *O. oeni* IOEB 9204 fragments to determine the partial sequence of the histidine decarboxylase encoding gene (Le Jeune et al., 1995). Later on, the complete gene and the flanking nucleotide sequences were located, identified, and cloned (Coton et al., 1998b). However, functional expression of the histidine decarboxylase encoding gene has not yet been performed

In 2008, a 3,800 nucleotide sequence of the *hdc* region of *O. oeni* IOEB 9294 was deposited in the databases (Gen Bank accession DQ132887). Additionally, sequences of *hdc* regions from other LAB were also deposited: *Lactobacillus sakei* LTH 2076 (3,796 nucleotides, DQ132888), *Lactobacillus hilgardii* IOEB 0006 (5,732

nucleotides, AY651779), *Tetragenococcus muriaticus* LMG 18498 (3,780 nucleotides, DQ132889), *Lactobacillus buchneri* DSM 5987 (3,808 nucleotides, DQ132890), and *Lactobacillus* 30a strain (2,867 nucleotides, DQ132891). Detailed analysis of these regions showed that the approximately 3.8 kb overlapping sequence was 99% identical among all these bacteria, except for *L. buchneri* and *Lactobacillus* 30a. Consequently, there is also a high degree of identity among the deduced amino acid sequence of the histidine decarboxylases encoded by the *hdc* genes on these bacteria (Figure 1). The *O. oeni* histidine decarboxylase is 99% identical to its counterpart protein in *L. sakei* and *L. hilgardii*, 98% to that of *T. muriaticus* protein, 97% to the one from *L. buchneri*, and 80% to the *Lactobacillus* 30a protein. Lucas et al. (2008b) suggested that this high identity could be evidence of horizontal transfer of the histamine-producing pathway in LAB (accession DQ132887).

However, although the *hdc* region from *O. oeni* IOEB 9294 is available, additional *O. oeni* genomic data indicate that this region is not a common trait among *O. oeni* strains. For instance, *in silico* analysis of the complete genome of the *O. oeni* PSU-1 strain revealed no sign of the *hdc* gene cluster found in the *O. oeni* IOEB 9294 strain (Mills et al., 2005). Moreover, no *hdc* gene has been located on the available sequence from the strain *O. oeni* ATCC BAA-1163, formerly known as IOB 8413 (NZ_AAUV000000000) (Beltramo et al., 2006).

In addition, some shortcomings of the molecular method were found (Landete et al. 2005a) when it was shown that 56% of the *O. oeni* strains that were positive for histamine production by HPLC were not amplified with the primers originally described by Le Jeune et al. (1995). However, these authors achieved proper amplification in all *O. oeni* histamine-producer strains tested after further modification of the sense primer.

5. Studies reporting no production of histamine by *O.oeni*

Studies reporting lack of histamine production by *O. oeni* strains are summarized in Table 2. Inability to produce histamine by *O. oeni* strains has been demonstrated by chromatographic detection in bacterial cultures and in wines. Weiller and Radler (1976) tested four *O. oeni* strains by ion exchange chromatography in a culture medium containing histidine, and none was able to decarboxylate histidine. Likewise, when synthetic media or wine were inoculated with several *O. oeni* strains (PSU-1, ML34, OENO) to induce MLF, no significant levels of histamine were found after HPLC analysis (Ough et al, 1987). Similar results were obtained when 88 *O. oeni* strains were screened for their ability to produce biogenic amines (Straub et al., 1995). In this study, resting cells from the 88 *O. oeni* strains were incubated in buffer supplemented with L-histidine (100 mg/l) and their supernatants were analyzed by HPLC to show that none of the tested strain produced histamine.

Cilliers and Van Wyk (1985) prepared wines which underwent malolactic fermentation by two *O. oeni* strains, *O. oeni* PSU-1 and *O. oeni* France, and showed by gas-liquid chromatography that none of them produced histamine. Later on, Leitao et al. (2000) tested histidine decarboxylase activity in 220 *O. oeni* isolates by differential Niven media and liquid chromatography. They showed that only 6 isolates (2.7%) presented activity in Niven differential media, but no significant levels of histamine were detected by liquid chromatography, even after malolactic fermentation with these strains. In another study, Merlot wines were inoculated with active dry malolactic bacteria *O. oeni* DSM 7008 (Viniflora OENOS) or *O. oeni* DSM 12923 (Manfroi et al., 2009), and again no histamine production was detected in the wines by RP-HPLC, irrespective of the *O. oeni* starter culture used.

When the differential method proposed by Bover-Cid and Holzapfel (1999) was used to test the ability of eleven *O. oeni* strains isolated from Trentino Alto Adige (Northern Italy) red wines to produce BA *in vitro*, it was found that none was able to decarboxylate histidine to produce histamine (Gardini et al., 2005). The same procedure was used to examine the occurrence of histamine producing bacteria among 10 *O. oeni* strains isolated from natural Spanish ciders (Garai et al., 2007). Again, no histamine production was observed in cultures of *O. oeni* grown in decarboxylase media, and these results were later confirmed by HPLC analysis.

Early studies (Choudhury et al., 1990) carried on the type strain of *O. oeni* (DSM 20252, ATCC 23279, NCDO 1674, or CECT 4100) using a differential method and an amino acid analyzer, concluded that this strain is not a histamine producer. This was confirmed when the strain was tested in a synthetic medium containing the precursor amino acid histidine followed by HPLC detection (Coton et al., 1998a; Moreno-Arribas et al., 2003).

Moreno-Arribas et al. (2003) studied histamine production by *O. oeni* strains isolated from Spanish grape must and wine. The assay was based on a preliminary test using a differential decarboxylase medium (Maijala et al 1993) containing 5 g/L of histidine, and the results were further confirmed by RP-HPLC. Again, no potential to form histamine was observed in any the *O. oeni* strains assayed including the *O. oeni* DSM 20252 type strain or the four commercial malolactic starters (Uvaferm ALPHA, Uvaferm MLD, Viniflora OENOS, and Viniflora CH35) tested.

A combination of molecular and chemical methods has also been used to demonstrate the inability of *O. oeni* strains to produce histamine. For example, when the *O. oeni* strains previously reported to be non-histamine-producing when analysed by RP-HPLC (Moreno-Arribas et al., 2003) were assayed by PCR, no amplification was

achieved (Marcobal et al. 2005). Attempts to detect the histidine decarboxylase gene by PCR in a study to select autochthonous *O. oeni* strains were also negative (Ruiz et al., 2010). Finally, the combination of the three alternative methods (TLC, HPLC and PCR) used to detect histamine producing *O. oeni* strains in a bacterial wine collection recently also failed to detect any positive strain (Costantini et al. 2006).

Differential growth assay, HPLC analysis of the amines produced and PCR detection of decarboxylase genes were used on sixty *O. oeni* strains isolated from Greek wines. Again, none of the detection methods used showed that any of the strains analysed produced histamine (Pramateftaki et al., 2012).

6. Considerations about contradictory published data

As summarized in Table 1 and 2, the various methods described above, or combinations of them, have produced contradictory data as to whether or not *O. oeni* strains are histamine producers.

For example, since there is no common, publicly-available *O. oeni* histamine producing strain, a possible candidate might be the *O. oeni* DSM 20252 type strain (ATCC 23279, NCDO 1674, CECT 4100). Even though for some authors this strain is a high histamine producer (Guerrini et al., 2002), for others it is a non-producer (Choudhury et al., 1990; Moreno-Arribas et al., 2003). The latter findings have been corroborated by failure to obtain any PCR amplification of the *hdc* gene in this strain (Marcobal et al., 2005). Similar results, confirming that *O. oeni* DSM 20252 did not produce amines, were reported using a synthetic medium containing a precursor amino acid followed by biogenic amine detection by HPLC (Coton et al., 1998a).

As a possible explanation for some of these discrepancies it has been suggested that the *hdc* gene is located on a large and possibly unstable plasmid of around 100 kb

(Lucas et al., 2008a). In almost all (97%) of the 264 wine samples tested in this study the *hdc* gene was amplified by qRT-PCR, and all the strains isolated in wines were identified as *O. Oeni*. This finding prompted the authors to the assumption that there is a risk of histamine production in almost all wines and that histamine-producing strains of *O. oeni* are frequently presented in wine during malolactic fermentation but may lose this capacity in laboratory subcultures. However, the study has a flaw in that the qRT-PCR procedure was set up using, instead of a *O. oeni* positive histamine-producing strain, *Lactobacillus hilgardii* IOEB 0006, on which the histamine-producing pathway is indeed encoded on an unstable plasmid (Lucas et al., 2005). In any case, it has not been shown whether this instability is actually a result of the loss of the plasmid carrying the *hdc* gene.

The availability of *hdc* gene sequences from different LAB has demonstrated a high degree of homology (up to 99%) among them, which has been suggested to be an evidence of horizontal transfer of the histamine-producing pathway in LAB (accession DQ132887; Lucas et al., 2008b). However, there is also possible that this high degree of identity have led to bacteria misidentification as a consequence of culture contamination, for example between *O. oeni* and *L. hilgardii* strains.

All these contradictory data highlight the need for a common control *O. oeni* histamine producing strain, since as far as we know there has been no solid confirmation that *O. oeni* is the bacterium responsible for the presence of histamine in wines. Because of the absence of a publicly-available histamine-producing *O. oeni* strain, several authors have used the *Lactobacillus* 30a strain (ATCC 33222) as a positive control for histamine production (Costantini et al., 2006; Moreno-Arribas et al., 2003; Izquierdo Cañas et al., 2009). This strain has been extensively characterized (Landete et al., 2008) and shows 80% sequence identity with its equivalent *O. oeni*

IOEB 9204 (Figure 1). However, when *Lactobacillus* 30a (Moreno-Arribas et al., 2003) or *Lactobacillus hilgardii* ISE 5211 (Costantini et al., 2006) were grown in a synthetic medium containing 5 g/l of histidine, the histamine levels found in the culture supernatant were 1.3 g/l and 3.5 g/l respectively. On the other hand, growth in similar media of *O. oeni*, with a histidine decarboxylase 99% identical to the equivalent protein from *Lactobacillus hilgardii* (Figure 1) produced insignificant amounts of histamine (1–40 mg/l) (Del Campo et al., 2000; Landete et al., 2005a; Landete et al., 2007a; Rosi et al., 2008). Thus, even though *O. oeni*, *Lactobacillus* 30a and *L. hilgardii* decarboxylase enzyme are almost identical, in similar conditions *O. oeni* strains produced histamine concentrations up to 1000 times lower. Moreover, in a study reporting the expression of *hdc* gene, Landete et al (2006) found that the effects of histidine on *hdc* expression and HDC activity in *L. hilgardii* 464, *P. parvulus* P270, and *O. oeni* 4042 were similar, because a similar proportional response was induced in the three strains studied with **either histidine, histamine, or pyridoxal 5'-phosphate**, and the regulation levels (expression and activity) were similar in all of them.

All these data strongly suggest that analytical errors may be affecting the published results, as it is difficult to explain such a low production (mg/l) when there is a high concentration of histidine (g/l) in the culture medium. In this respect we refer back to the papers of Ough et al. (1987) and Del Prete et al. (2009), as discussed earlier, to the effect that these results could be due to a misidentification between small chromatographic peaks. Some studies on the characterization of histamine-producing strains are based exclusively on HPLC results of culture media containing amounts of histamine so small that possible errors in the identification of the chromatographic peak or failure to separate out interfering components cannot be ruled out. In addition, the lack of *O. oeni* strains for use as positive controls makes it difficult to compare different

laboratory results. There is ample documentation of occasional high levels of histamine in wine but in the absence of *O. oeni* other contaminant bacteria may be responsible for during and after MLF. In this regard, Buteau et al. 1984 suggested that red wines contain higher histamine levels from contamination of yeast and bacterial preparations with non malolactic bacteria that may have histidine decarboxylase enzymes. Recent TLC and HPLC analyses have shown that some commercial yeast preparations used as starters in winemaking were contaminated with histamine-producing bacteria (Costantini et al. 2009) identified as *Lactobacillus rossiae*, in which the presence of the *hdc* gene was demonstrated by PCR. Recently, Pinto et al. (2011) reported *Enterobacteria* capable of producing biogenic amines in must. These findings are very important in that they demonstrate possible ways in which potentially histamine-producing contaminant bacteria may occur in wine.

7. Conclusions

This review addresses a long-standing question about the involvement of *O. oeni* in histamine production in wines. All studies up until 1994 suggested that *O. oeni* was not the bacterial species responsible for histamine production. Later on, the first histamine-producing *O. oeni* strain was identified thanks to the use of molecular detection methods. However, histamine production by *O. oeni* strains needs to be reconsidered for a number of reasons: i) the absence of common reference histamine-producer *O. oeni* strains; ii) the pitfalls of some of the detection methods used, including molecular ones; iii) the very low concentration of histamine reportedly produced by *O. oeni* strains; and iv) the reporting of contradictory data for the same strain or method.

The authors therefore urge that a collaborative international study be organized covering as many countries, institutions and qualified research groups as possible, and possibly sponsored by international organizations like the OIV (International Organisation of Vine and Wine), to unify the most appropriate methods and, more importantly, to exchange common reference strains for validation. To that end, it is suggested that histamine-producing *O. oeni* strains that are able to retain histamine production after laboratory procedures be identified for use as common control strains for all enological and microbiological laboratories.

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649

Figure captions

Figure 1. Comparison of histidine decarboxylase deduced protein sequences from *O. oeni* IOEB 9204 (OOE) (DQ132887), *Lactobacillus sakei* LTH 2076 (LSA) (accession DQ132888), *Lactobacillus hilgardii* IOEB 0006 (LHI) (accession AY651779), *Tetragenococcus muriaticus* LMG 18498 (TMU) (accession DQ132889), *Lactobacillus buchneri* DSM 5987 (LBU) (accession DQ132890) and from the *Lactobacillus* 30a strain (L30) (accession DQ132891). Clustal W program was used to compare predicted sequences. Residues that are identical (*), conserved (:) or semi-conserved (.) in all the sequences of the alignment are also indicated. Dashed represents gaps introduced to maximize similarities.

Table 1. Studies reporting histamine production by *O.oeni*

Year	Description	Detection method	No. ^a	Histamine production	Reference
1998	* Identification of the gene encoding the <i>O. oeni</i> IOEB 9204 histidine decarboxylase, and purification and characterization of the protein	CO ₂ release	1 (IOEB 9204)	ND	Coton et al., 1998b
	* Wines from Southwest France contained histamine-producing <i>O.oeni</i> strains	Indirect deduction from data of histamine in wine DM, HPLC	58/118 wines contain histamine	<i>O.oeni</i> have been isolated in these wines but not tested for histamine production	Coton et al., 1998a
2000	* Isolation of decarboxylase positive strains from Portuguese wines which were unable to produce significant levels of histamine	HPLC	6/220 strains	Strains were positive in DM, but no significant histamine levels were detected by HPLC	Leitao et al., 2000
	* <i>O. oeni</i> strains isolated from Spanish ciders were low histamine producers	HPLC	5/12 strains	2 mg/l in medium containing 10 g/l histidine	Del Campo et al., 2000
2002	* <i>O. oeni</i> strains isolated from Italian wines were histamine producers	HPLC	27/44 strains	1 to 30 mg/l in MRS broth	Guerrini et al., 2002
2005	* Wines from three Spanish regions contained low histamine-producer <i>O. oeni</i> strains	DM, HPLC	7/12 wines	11–34 mg/l in medium containing 20 g/l, or 0.5–11 mg/l in wine	Landete et al., 2005b
	* <i>O. oeni</i> strains were low histamine producer	DM, enzymatic, HPLC, PCR	1/32 strains by DM 24/32 strains by enzymatic, HPLC and PCR	1/32 strains produced 100 mg/l and 23/32 strains produced < 48 mg/l in media containing 20 g/l histidine.	Landete et al., 2005a
2006	* Study of the <i>hdc</i> expression in <i>O. oeni</i> 4024	Molecular methods	1 (<i>O. oeni</i> 4024)	ND	Landete et al., 2006
2007	* <i>O. oeni</i> strains were histamine producers	DM, HPLC, PCR	33/39 strains	22.3±17.7 mg/l in DM containing 2 g/l histidine; and 4.2±3.2 mg/l in wine containing 500 mg/l histidine	Landete et al., 2007a
2008	* High frequency of histamine-producing bacteria in wine samples from the Bordeaux area.	Indirect deduction from data of histamine in wine PCR	256/264 wines contain histamine	<i>O.oeni</i> have been isolated in these wines but not tested for histamine production	Lucas et al., 2008a
	* Two PCR-positive strains were found in a several-hundred <i>O. oeni</i> strain collection.		2/several hundreds strains	ND	Lucas et al., 2008a
	* <i>O. oeni</i> strains were low histamine producers	DM, HPLC	0/26 strains by DM 8/26 strains by HPLC	2.6–5.6 mg/l in a media containing 20 g/l histidine	Rosi et al., 2008
	* 3,800 nucleotides of the HDC region from <i>O. oeni</i> IOEB 9204 were deposited in a nucleotide database.	Molecular methods	1 (IOEB 9204)	ND	Accession DQ132887
2009	* One histamine-producer strain isolated from Tempranillo wines.	DM, HPLC, PCR	1/90 strains	9.24 mg/l in wine	Izquierdo-Cañas et al., 2009
	* Isolation of <i>O. oeni</i> strains from wines having high histamine content	Indirect deduction from data of histamine in wine	ND	wines contain histamine; <i>O.oeni</i> strains have been isolated in these wines, but not tested for histamine production	López et al., 2009

^aNumber of positive strains or wines vs. total analyzed; ND: no determined; DM: Differential medium

1 Table 2. Studies reporting no production of histamine by *O.oeni*

Year	Description	Detection method	No. ^a	Reference
1976	* <i>O. oeni</i> does not decarboxylate histidine	IEC	4 strains	Weiller & Radler, 1976
1985	* <i>O.oeni</i> strains did not produce histamine on wines	GLC	2 strains (PSU-1, France)	Cilliers & Wyk et al., 1985
1987	* <i>O. oeni</i> strains inoculated in synthetic media did not produce histamine	HPLC	3 strains (PSU-1, ML34, OENO)	Ough et al, 1987
1990	* <i>O. oeni</i> DSM 20252 does not produce histamine in DM	DM, amino acid analyzer	1 strain (DSM 20252)	Choudhury et al., 1990
1994	* The commercial starter <i>O. oeni</i> Viniflora Oenos does not produce histamine	HPLC	1 strain (OENOS)	Lonvaud-Funel & Joyeux, 1994
1995	* None of the German <i>O. oeni</i> strains analyzed were histamine producer	HPLC	88 strains	Straub et al., 1995
2003	* <i>O. oeni</i> strains isolated from Spanish wines and four commercial starters did not produce histamine	DM, HPLC	42 strains	Moreno-Arribas et al., 2003
2005	* <i>O. oeni</i> strains isolated from Italian wines were not histamine producers	DM	11 strains	Gardini et al., 2005
	* The <i>O. oeni</i> PSU-1 genome does not posses the <i>hdc</i> gene	Molecular methods	1 strain (PSU-1)	Mills et al., 2005
	* <i>O. oeni</i> strains isolated from Spanish wines and four commercial starters did not produce histamine	PCR	42 strains	Marcobal et al., 2005
2006	* The <i>hdc</i> gene was not expressed in the <i>O. oeni</i> ATCC BAA-1163 (IOB 8413) strain	Molecular methods	1 strain (IOB 8413)	Beltramo et al., 2006
	* <i>O. oeni</i> strains isolated from wine were not histamine producer	TLC, HPLC, PCR	92 strains	Costantini et al., 2006
2007	* <i>O. oeni</i> strains isolated from Spanish ciders were not histamine producer	DM, HPLC	10 strains	Garai et al., 2007
2009	* <i>O. oeni</i> DSM 7008 and DSM 12923 did not produce histamine on wines	HPLC	2 strains	Manfroi et al., 2009
	* <i>O. oeni</i> commercial starters did not produce histamine	TLC, HPLC, PCR	16 strains	Costantini et al., 2009
2010	* <i>O. oeni</i> strains were not histamine producers	DM, PCR	8 strains	Ruiz et al., 2010
2012	* <i>O. oeni</i> strains isolated from Greek wines were not histamine producers	DM, HPLC, PCR	60 strains	Pramateftaki et al., 2012

^aNumber of strains analyzed; IEC, Ion exchange chromatography; GLC, Gas liquid chromatography; DM, Differential medium; TLC, Thin-layer chromatography.

Figure 1

OOE	MSEFDKKLNTLGVDRISVSPYKKWSRGYLEPGNVGNGYVSGLKVDAGVIDKTDDMILDGI	60
LSA	MSEFDKKLNTLGVDRISVSPYKKWSRGYLEPGNVGNGYVSGIKVDAGVVDKTDDMILDGI	60
LHI	MSEFDKKLNTLGVDRISVSPYKKWSRGYLEPGNVGNGYVSGLKVDAGVVDKTDDMILDGI	60
TMU	MSEFDKKLNTLGVDRISVSPYKKWSRGYLEPGNVGNGYVSGLKVDAGVVDKTDDMILDGI	60
LBU	MSEFDKKLNTLGVDRISVSPYKKWSRGYMEPGNIGNGYVSGLKVDAGVVDKTDDMILDGI	60
L30	MSELDAKLNKLGVDRIAISPYKQWTRGYMEPGNIGNGYVTGLKVDAGVRDKSDDDVLDGI	60
	:* ***.**.:****.:***:****:*****.:*:***** **:* * :****	
OOE	VSYDRAETKNAYIGQINMTTASSFSGVG GTVLGYDILRNPEVDKAKPLFTEKQWDGSELP	120
LSA	VSYDRAETKNAYIGQINMTTASSFSGVG GTVLGYDILRNPEVDKAKPLFTEKQWDGSELP	120
LHI	VSYDRAETKNAYIGQINMTTASSFSGVG GTVLGYDILRNPEVDKAKPLFTEKQWDGSELP	120
TMU	VSYDRAETKNAYIGQINMTTASSFSGVG GTVLGYDILRNPEVDKAKPLFTEKQWDGSELP	120
LBU	VSYDRAETKNAYIGQINMTTASSFSGVG GTVLGYDILRNPEVDKAKPLFTEKQWDGSELP	120
L30	VSYDRAETKNAYIGQINMTTASSFTGVQGRVIGYDILRSPEVDKAKPLFTETQWDGSELP	120
	*****.*****.:** * *:*****.*****.*****.*****	
OOE	IYDAKPLQDTLVEYFGTKDDMRHYPAPGAFVCCANKGVTAERPKNADAMKPGQGYGVWSA	180
LSA	IYDAKPLQDTLVEYFGTKDDMRHYPAPGAFVCCANKGVTAERPKNADAMKPGQGYGVWSA	180
LHI	IYDAKPLQDTLVEYFGTKDDMRHYPAPGAFVCCANKGVTAERPKNADAMKPGQGYGVWSA	180
TMU	IYDAKPLQDNLVEYFGTKDDMRHYPAPGAFVCCANKGVTAERPKNADAMKPGQGYGVWSA	180
LBU	IYDAKPLQDTLVEYFGTKDDMRHYPAPGAFVCCANKGVTAERPKNDEDMKPGQGYGVWSA	180
L30	IYDAKPLQDALVEYFGTEQDRRHYPAPGSFIVCANKGVTAERPKNADAMKPGQGYGVWSA	180
	***** *****.:* *****.:*: ***** ***** *****	
OOE	IAISFAKDPTKYSSMYVEDAGVWETPNEDELIEYLKGRRNAMAKSIAACGENTAGENGGA	240
LSA	IAISFAKDPTKYSSMYVEDAGVWETPNEDELIEYLKGRRNAMAKSIAACGENTAGENGGA	240
LHI	IAISFAKDPTKYSSMYVEDAGVWETPNEDELIEYLKGRRNAMAKSIAACGENTAGENGGA	240
TMU	IAISFAKDPTKYASMYVEDAGVWETPNEDELIEYLKGRRNAMAKSIAACGEHTAGENGGA	240
LBU	IAISFAKDPTKYSSMYVEDAGVWETPNEDELIEYLKGRRNAMAKSIAACGENTAGENGGA	240
L30	IAISFAKDPTKDSSMFVEDAGVWETPNEDELLEYLEGRRKAMAKSIAECGQDAH-----A	235
	***** .*:*****.*****.:***:***** **:.: *	
OOE	VFTSSWIGFAHAMMKPGQVGNAITVAPYIAMPVDSIPGGSILTPD TMDIMQNLTMPPEWL	300
LSA	VFTSSWIGFAHAMMKPGQVGNAITVAPYIAMPVDSIPGGSILTPD TMDIMQNLTMPPEWL	300
LHI	VFTSSWIGFAHAMMKPGQVGNAITVAPYIAMPVDSIPGGSILTPD TMDIMQNLTMPPEWL	300
TMU	VFTSSWIGFAHAMMKPGQVGNAITVAPYIAMPVDSIPGGSILTPD TMDIMQNLTMPPEWL	300
LBU	VFTSSWIGFAHAMMKPGQVGNAITVAPYIAMPVDSIPGGSILTPD TMDIMQNLTMPPEWL	300
L30	SFESSWIGFAYTMMEPGQIGNAITVAPYVSLPIDSIPGGSILTPDKMEIMENLTMPPEWL	295
	* *****.:*:***:*****.:*:*****.*****.:**:*:*****	
OOE	DKMGYQPLTKGGNINY	316
LSA	DKMGYQSLTKGGNINY	316
LHI	DKMGYQSLTKGGNINY	316
TMU	DKMGYQSLTKGGNINY	316
LBU	DKMGYQSLTKDGNIKY	316
L30	EKMGYKSLSANNALKY	311
	:****.:*:. . ::*	