MiniReview

Biogenic amines in wines: role of lactic acid bacteria

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Abstract

Biogenic amines have undesirable physiological effects when absorbed at too high a concentration. Several kinds of food and beverages contain biogenic amines. Lactic acid bacteria can decarboxylate amino acids. Since winemaking involves the growth of lactic acid bacteria for malolactic fermentation, biogenic amines may occur. However, not all bacterial strains carry these activities. In the same wine-producing area, some wines may contain very low amounts of biogenic amines while others may have relatively large quantities. It is now possible to detect the presence of undesirable histamine-producing strains by PCR test or DNA probe based on the presence of the gene encoding histidine decarboxylase. Other strains have the ornithine and/or tyrosine decarboxylase. When biogenic amine-producing strains are present, the winemaker is encouraged to inoculate selected malolactic starters to replace the indigenous microflora. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Biogenic amine; Lactic acid bacterium; Malolactic fermentation

1. Introduction

Amines have an important metabolic role in living cells. Polyamines are essential for growth; other amines like histamine and tyramine are involved in nervous system functions and the control of blood pressure. Biogenic amines are undesirable in all foods and beverages because if absorbed at too high a concentration, they may induce headaches, respiratory distress, heart palpitation, hyper- or hypotension, and several allergenic disorders [1]. Histamine is the most toxic and its effect can be potentiated by other amines [2]. But human sensitivity varies with the individual detoxifying activities of human body. For example, biogenic amines, especially histamine, are fixed by mucin in the intestinal mucosa. If their concentration is too high, some are absorbed [3]. Moreover some enzymes involved in biogenic amine metabolism, such as histamine methyltransferase, are specific; others such as monoaminoxidase and diaminoxidases are less specific. However these enzymes are inhibited by several types of drugs, by ethanol and even by other food amines, lowering the efficiency of detoxification [4–6]. Therefore when considering the toxic effects of biogenic amines, the quantity of food, the concentration of total biogenic amines, and the consumption of ethanol and drugs must also be taken into account [7]. It is conceivable that the simultaneous consumption of fermented foods and beverages causes disorders, even if each separate product might not be considered as hazardous.

Biogenic amines are produced by lactic acid bacteria during the process of fermentation of foods and beverages by amino acid decarboxylation, for example in cheese, sausage, fermented vegetables and wine. Many bacterial genera are able to decarboxylate amino acids. This reaction is thought to favor growth and survival in acidic media, since it induces an increase in pH. In wine, several amino acids can be decarboxylated; as a result histamine, tyramine, putrescine, cadaverin and phenylethylamine are usually found, the first three being the most frequent. Most amino acid decarboxylases are pyridoxal-5′-phosphate (PLP)-dependent, just like various enzymes involved in amino acid transformation [8]. As regards histidine decarboxylase (HDC), while mammalian HDCs are stimulated by PLP, bacterial enzymes from *Clostridium perfringens*, *Lactobacillus* 30A, *Micrococcus* sp. and from *Oenococcus oeni* are PLP-independent. HDCs of Gram positive bacteria already studied belong to a group of pyruvoyl-dependent enzymes, where a pyruvate residue

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linked to the protein is involved in the decarboxylative mechanisms [7]. Activation of proHDC by hydrolysis into α and β subunits between two serine residues generates the pyruvoyl group at the extremity of β subunit [7].

The demand of consumers for better and healthier foods has led to a renewed interest in studies on biogenic amines. Several analytical methods have been proposed. Presently, the precise and reliable high performance liquid chromatography is used for several foods and especially for wine analysis [9]. Moreover, the microbiology of fermented foods and beverages, which in the past was limited to the natural development of indigenous microflora, is now more controlled, and technologically more advanced through the use of selected starters.

Malolactic fermentation is required after alcoholic fermentation for nearly all red wines and most white wines. Its principal result is deacidification by malic acid decarboxylation and complexification of sensory quality by secondary bacterial metabolism [10]. Wine lactic acid bacteria activities have been studied for more than 20 years but research has mainly focused on malic acid degradation by *O. oeni*, the predominant species [11]. However, other lactic acid bacteria species develop, and other substrates are metabolized, which induce the favorable sensory changes required during vinification, or possibly undesirable reactions [10]. Our greater knowledge of wine lactic acid bacteria flora and metabolisms is now providing laboratory and practical cellar tools for a better control of wine quality.

2. Presence of biogenic amines in wines

Histamine, tyramine and putrescine are the major biogenic amines in wines. According to the principal component analysis performed by Soufleros et al. [9], their concentration is low after alcoholic fermentation, and increases in most wines during malolactic fermentation to a very variable extent. Other amines such as methylamine, ethylamine, phenylethylamine, isoamylamine and diaminopentane (cadaverin) already present in grape must are produced and degraded during vinification. Compiling many results, namely those published [13–17], we are forced to conclude that there is no general rule for the evolution and presence of biogenic amines in wines. Curiously, some wines contain most biogenic amines, while others contain none [18]. A principal component analysis was conducted on 135 wines from a variety of wine types produced in different regions and 27 variables (19 amino acids and biogenic amines) were determined [9]. It was clearly shown that in some producing areas, biogenic amines are found in higher levels than in others. This is related in part to the type of winemaking and whether it involves malolactic fermentation or not.

As biogenic amines are produced by decarboxylation of amino acids, this supposes that the bacteria in wine have all the enzymatic equipment necessary for the reactions (decarboxylase and transport system), and that amino acids are present in sufficient amounts. The abundance of amines is strictly related to the microflora but also to the amino acid composition of the wine after alcoholic fermentation. The latter results from the composition of the grape must, which itself depends on the grape variety and vine nutrition [9] on the one hand, and on yeast metabolism on the other. As lactic acid bacteria develop in wine after yeasts, the yeasts have already changed the composition of the initial grape must in nitrogen compounds by using some amino acids and secreting others during alcoholic fermentation (i.e. yeast cells in autolysis phase). Moreover, if wines are maintained in contact with yeast lees, lactic acid bacteria find more peptides and free amino acids to hydrolyze and decarboxylate. This explains the higher level of amines in some wines which are produced with an extended lees contact. The other reason is that the decarboxylating capacity of bacteria is very variable [18] according to strain. In this respect, pH is the most important factor determining not only the biological activity of bacteria in wine but also their variety. The higher the pH, the more complex the bacterial microflora, because pH acts as a selective factor of microorganisms in wine. At high pH, biogenic amines are always produced in high amounts [19]. This is a consequence of an easier total growth, and of the greater bacterial diversity. White wines which are generally more acidic contain lower biogenic amine concentrations than red wines [13].

After malolactic fermentation, wine is sulfited in order to eliminate yeasts and bacteria which are no longer desirable. This would normally prevent any changes in composition due to microorganisms. However, several compounds vary in level and this is the case with biogenic amines. In Burgundy wines, histamine, tyramine and putrescine have been shown to increase in Chardonnay and Pinot noir during malolactic fermentation, and also during ageing [13]. Histamine, tyramine and diaminobutane content continuously increases. In their study, Gerbaux and Monamy showed that the more active phase was between the fourth and eighth month after malolactic fermentation. It is obvious that sulfur dioxide (SO_2) does not completely stop all the biochemical reactions triggered by bacteria. Due to high pH, a situation which is becoming more and more frequent, SO_2 is less active and it is accentuated in red wines due to its combination to polyphenols. Viable but non-cultivable forms of lactic acid bacteria have also been suspected in wines, and possibly retain some biological activities in order to survive. Amino acid decarboxylation is an example [20].

3. Histamine production by lactic acid bacteria in wines

Of the biogenic amines, histamine is most frequently found in wines. The first extensive studies were undertaken

some 25 years ago and there has been considerable controversy regarding the role of lactic acid bacteria. The first research showed that among European, American or South African wines, biogenic amines were more frequent and abundant in red wines than in white wines [12,14,15]. While some authors attributed the increase in levels of histamine to malolactic fermentation [15,16], other authors did not connect the two phenomena [14,17].

For a long time and even today, enologists have considered that only *Pediococcus* strains were responsible for histamine [16]. This genus is always represented in wine microflora, in addition to *Lactobacillus*, *Leuconostoc* and *Oenococcus*, but usually at a low proportion. In winemaking, usually the species *O. oeni* dominates during malolactic fermentation and selection for it is more effective at a lower pH. In brief, enologists differentiated bad lactic acid bacteria, among them histamine-producing *Pediococcus* sp., from good bacteria *O. oeni*. However, the situation is not so simple.

When the entire bacterial flora is harvested by centrifugation from wines containing biogenic amines just after malolactic fermentation, it can induce amino acid decarboxylation after inoculation into sterile wine. The isolation of strains from such wines showed definitely that some strains of *O. oeni* could decarboxylate histidine. Analyses of such samples showed that the difference in percentage of histidine-decarboxylating *O. oeni* strains among the total population might explain the difference in the final concentrations of histamine [19].

For a given strain the production of histamine is enhanced in the poorest growth conditions, i.e. lacking fermentable substrates such as sugar and malic acid. This suggests that histidine decarboxylation can be used as an additional mechanism for energy generation in cells which are deprived of other substrates [21]. This also explains why the histamine concentration increases, even after malolactic fermentation, when most energy sources have been metabolized. The phenomenon is enhanced if maceration, or storage with yeast lees, is prolonged, since more substrate (histidine) is available from yeast autolysis.

Research on strain *O. oeni* IOEB 9204, one of the histamine-producing strains isolated from a wine [19], led to characterization of the HDC activity. It is an allosteric enzyme, with a high degree of cooperativity, so that when the histidine concentration increases, binding to the active site is favored. At the optimal pH, 4.8, it follows Michaelis–Menten kinetics, while it is sigmoidal at other pHs [22]. Histamine is a competitive inhibitor. Citric and L-lactic acids also have inhibitory effects on HDC activity for both whole cells and cell-free extract. However citric acid is fermented by bacteria during malolactic fermentation, while L-lactic acid accumulates in wine as a result of malic acid decarboxylation. However 2 g l^{-1} of L-lactic acid inhibits only 22% the activity.

HDC of *O. oeni* was purified to homogeneity. Denaturing gel electrophoresis showed that it is composed of two subunits of about 28 000 (α chain) and 11 000 (β chain). Comparison of the amino acid and the nucleotide sequences of hdcA gene of *Lactobacillus* 30A, *Lactobacillus buchneri* and *C. perfringens* showed conserved regions and several primer pairs were chosen for PCR reactions using *O. oeni* DNA as template. After verification of its sequence, an amplicon was used as DNA probe which hybridized only with histidine-decarboxylating strains [23] whatever their species or origin (beer, cheese, wine etc…). The entire *O. oeni* gene sequence encodes a putative single polypeptide of 315 amino acids corresponding to the pro-enzyme chain π by analogy to the other pyruvoyl-dependent HDC. The molecular masses of the α and β subunits, deduced from the *O. oeni* sequence, are 25 380 and 8848. The identity of the chain π amino acid sequence is 80% with *Lactobacillus* 30A. The homology is very high for the β chain, 94% with *L. buchneri* and 83% with *Lactobacillus* 30A [22]. The hexameric structure [αβ]$_6$ would be the same for *O. oeni* and the other *Lactobacillus* species.

Such results have been applied for the detection of histamine-producing strains in wine. The specific hdc probe can be used for colony hybridization, which gives the percentage of the histidine-decarboxylating population in the total bacterial microflora. To determine the frequency of undesirable bacteria in wines, a direct PCR test was designed to rapidly detect even low proportions of such bacteria. Moreover, it avoids the 10–12 days needed for colony development [18]. Two hundred and fifty samples, taken from tanks representing 118 different wines covering different wine-producing areas, were tested. Histidine-decarboxylating bacteria were found in all the areas, and about half of the tested wines (49%) contained such strains. DNA/DNA hybridization using the hdc DNA probe proved that on average 80% of the total population was potentially able to produce histamine. All clones isolated among the 'hdc' positives were identified as *O. oeni*. At the same time, it was established that wines where the PCR test was negative did not contain histamine, and most often no other biogenic amines, while 'positive' wines contained not only histamine but also tyramine and putrescine [18].

4. Practical applications

The use of molecular tools for early and rapid detection of undesirable bacteria is one of the most important developments in wine microbiology today. Studies on histamine have shown that the frequency of undesirable strains is higher than previously thought, that their presence is not predictable and finally, that the activity is not restricted to the species level but indeed characterizes strain.

HDC specifically decarboxylates histidine. This implies that other bacteria have tyrosine decarboxylase activity or ornithine decarboxylase activity, or that some strains carry all of them. To date, strains with only ornithine decarbox-

ylase activity (*O. oeni* IOEB 8419) (unpublished results) and tyrosine decarboxylase activity have been isolated. The tyramine-producing strains isolated from wines were identified as *Lactobacillus brevis* and *Lactobacillus hilgardii* [23]. Among them, the best producers of tyramine also produce phenylethylamine. Presently, rapid detection of tyramine-producing strains is not possible. Basic research on tyrosine decarboxylase activity and its corresponding gene is therefore necessary.

In some cellars, wine, whatever the vintage, always contains biogenic amines, while in others this is never the case. In the latters, absence of amino acid-decarboxylating bacteria is probably the reason, as was shown with histamine. Wine lactic acid bacteria originate from the natural microflora of the grape berries and are present on wine cellar equipment. They are spontaneously selected during the winemaking process, but they can be overgrown and eliminated by addition of commercial malolactic starters. Interest in these concentrated lyophilized cultures is relatively new but increasing. After decades of inconsistent and unpredictable results, efficient ready to use preparations are now commercially available. *O. oeni* strains are selected for their enological properties, including absence of amino acid decarboxylases. In cellars where biogenic amines are usually high, repeated experiments showed that in inoculated wines, biogenic amine concentrations were very low, while uninoculated control wines contained all the usual amines (unpublished data). It may be supposed that starters can either completely eliminate the indigenous bacteria, or that they can degrade biogenic amines which might be produced by the undesirable strains. The second hypothesis should be evaluated. Indeed, a study on histidine- and tyrosine-degrading microorganisms suggests that those which are able to metabolize biogenic amines are unable to decarboxylate histidine, tyrosine, phenylalanine, lysine or ornithine [24].

5. Conclusion

At present, there are no regulations regarding the level of biogenic amines in wines. However, it may happen (and already has) that wines are not accepted on some markets because they contain too high levels of biogenic amines. Moreover, it is normal that winemakers should feel concerned by this problem and take it into account in order to produce high quality wines. In the recent years, we have verified that biogenic amines are produced by lactic acid bacteria and have found that *O. oeni* strains can be involved for histamine. Hybridization with specific DNA probes, for HDC detection and *O. oeni* species, showed that they are very widespread in some cellars, and rare in others. The amino acid-decarboxylating activity is strain specific, and it may be that within the different species of *Lactobacillus*, *Pediococcus* and *Leuconostoc*, all the decarboxylating activities are randomly distributed. The ecology of amino acid-decarboxylating wine lactic acid bacteria should be studied.

To meet consumer demand, wines are produced that are less acidic than in the past. Grape maturity is prolonged as far as possible to increase the extractability of phenolic compounds and the concentration of aroma precursors. Therefore, total acidity is lower and pH higher. Since pH is the most effective factor for bacterial selection, this has a side effect. Indeed, as pH increases, the number and variety of the microbial population increase. It is not rare that malolactic fermentation is conducted by a very diverse bacterial population, instead of *O. oeni* alone, in wines which frequently have a pH above 3.5 after alcoholic fermentation. Thus, it is not surprising that a wide variety of lactic acid bacteria survive and grow in wine, thereby increasing the risk of the development of undesirable strains. In addition, at higher pH, SO_2 added at the end of malolactic fermentation is less efficient. Relatively high lactic acid bacteria populations are often encountered several months after vinification. Even if they are not growing, such latent populations survive and are still metabolically active. For example, they still produce histamine. Moreover, several decarboxylation pathways have been shown to provide energy to lactic acid bacteria [25], so amino acid-decarboxylating strains might survive longer than those that do not decarboxylate.

Since we have become aware of this situation, it is now necessary to understand the actions of lactic acid bacteria present in wines. Fortunately rapid, sensitive and specific tools can be expected from the results of molecular studies. As shown above for histamine, PCR primers and DNA probes can be designed from the knowledge of genes which determine the synthesis of the decarboxylating enzyme. As tyramine and putrescine are considered as the other important amines besides histamine, the conditions of their production must be understood in the physiological and genetic terms. Moreover, malolactic starters are now more efficient and can be inoculated directly in wines. Their use will probably become more frequent in the future. Indeed, the absence of amino acid decarboxylase activity is now included in the selection criteria for the industrial preparation of malolactic starters.

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