CURRENT MICROBIOLOGY Vol. 44 (2002), pp. 374–378
DOI: 10.1007/s00284-001-0021-9

Current Microbiology
An International Journal

Biogenic Amine Production by *Oenococcus oeni*

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Received: 2 August 2001 / Accepted: 28 August 2001

Abstract. The biogenic amine-producing capability of several *Oenococcus oeni* strains, originally isolated from different Italian wines, was determined. The amine-producing capability was quali-quantitatively variable among the strains: out of the 44 strains investigated under optimal growth conditions, more than 60% were able to produce histamine, at concentrations ranging from 1.0 to 33 mg/L, and about 16% showed the additional capability to form both putrescine and cadaverine, to different extents and variable relative proportions. The amine-producing behavior of the strains was confirmed under stress culture conditions, while performing malolactic fermentation. In wine, one randomly chosen strain was very effective in forming putrescine from ornithine. The formation of putrescine from arginine by some strains has been also demonstrated. Consequently, *O. oeni* can really and significantly contribute to the overall biogenic amine content of wines. Practical consequences of these findings are discussed.

Biogenic amines (BAs) are organic basic compounds that occur in different kinds of food or beverages, such as fishery products, cheese, dry sausages, wine, beer, and other fermented products. BAs are mainly generated by decarboxylation of the precursor amino acids through substrate-specific enzymes of the microorganisms present in the food [23]. Several toxicological problems resulting from the ingestion of food containing relatively high levels of BAs are due to the physiological activity of BAs on the human metabolism. Indeed, histamine and tyramine, the most studied BAs, are responsible for toxicological effects owing to their well-known vasoactive and psychoactive properties [4]. In wine, it has often been reported that BA concentration increases after malolactic fermentation, red wines usually being richer in amines than white wines [18]. Most studies on the BA presence in wine concerned histamine and several lactic acid bacteria belonging to the *Lactobacillus*, *Leuconostoc*, and *Pediococcus* genera that really proved capable of histamine formation [12], *Pediococcus* strains being usually considered among the major ones responsible for histamine accumulation in wine [1]. However, Lonvaud-Funel and Joyeux [13] demonstrated that also *Oenococcus oeni*, the bacterial species most commonly found in wines and most frequently associated with malolactic fermentation (MLF), might play a role in the production of histamine in wine. Even if this BA is present in fermented foods, in vegetable and meat products at higher concentrations than in wine, alcohol and the presence of other BAs such as 1-methylhistamine, methylamine, ethylamine, tryptamine, 2-phenylethylamine, tyramine, putrescine, cadaverine, and spermidine may increase the toxicity of histamine and exceed the limits for sensitive people [8, 10, 20]. In this connection, putrescine, the amine generally present at the highest concentration in wines [21], is known as the most effective potentiator of the histamine toxicity to humans [22]. Moreover, certain amines like putrescine and cadaverine are potential precursors of carcinogenic nitrosamines [4]. Hence, the presence of BAs is becoming of health concern also for wine consumers, as can be inferred by the renewed interest in studies on BA occurrence and formation in wine [6, 11, 12, 16, 21]. However, in spite of this need for healthier products, no information is available on the capability of *O. oeni* to produce BAs other than histamine as well as on the frequency of this capability among wild strains of the species. The aim of this study was to investigate this matter by testing, besides the type and a reference strain, 42 *O. oeni* strains isolated from different Italian wines.

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Table 1. Frequency and distribution of biogenic amine-producing capability among *Oenococcus oeni* strains under optimal growth conditions

DAB Code	Grapevine cultivar	Italian region	Type of wine	Positive/Total
AG	Aglianico	Basilicata	red	3/7
BM	Sangiovese	Tuscany	red	3/5
BR	Nebbiolo	Piedmont	red	4/4
CA	Cannonau	Sardinia	red	3/3
CD	Chardonnay	Puglia	white	3/5
CH	Sangiovese	Tuscany	red	2/8
NB	Fiano	Campania	white	1/2
NV	Nero d'Avola	Sicily	red	3/4
VR	Vermentino	Sardinia	white	4/4

Materials and Methods

Organisms and culture conditions. In total, 44 strains of *Oenococcus oeni* were used: 42 strains were obtained from the culture collection of the Department of Agricultural Biotechnology (DAB, University of Florence, Italy), the type strain NCDO 1674 from the National Collection of Dairy Organisms (Reading, UK), and the reference strain DSM 20257 from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (GmbH, Germany). The strains were grown at 30°C in MRS broth (Difco) supplemented with 0.2% (wt/vol) tomato juice broth (Difco), at pH 4.8 under microaerophilic conditions. Once the stationary phase was reached, a portion of the cultures was centrifuged (3500 *g* for 20 min) and supernatants were utilized for biogenic amine determinations. Each pellet was resuspended in 15 ml of the test medium with 12.0% (vol/vol) ethanol, as described by Bastianini et al. [3]. After 24 h of incubation at 30°C, the BA content of the spent media was determined. Experiments in wine were performed according to the same procedure. The wine was obtained from Sangiovese grapes fermented by a commercial strain of *Saccharomyces cerevisiae*; after completion of the alcoholic fermentation, the wine was filtered by using the Sartocon II Crossflow System (Sartorius) equipped with a 0.2-μm pore-size module. After filtration, the wine composition was as follows: ethanol, 11.0% (vol/vol); malic acid, 1.8 g/L; citric acid, 0.5 g/L; FAN (free α-amino nitrogen), 3 mg/L; total SO_2, 35 mg/L; with a pH of 3.3.

All experimental cultures were carried out in duplicate, and the average values are reported.

Analytical determinations. Viable cell counts were carried out by pour plate seeding in MRS agar. Ethanol concentration in wine was determined by liquid chromatography, according to Schneider et al. [19]; total SO_2 was determined as described by Ough and Amerine [17]; citric acid, malic acid, and ammonia were determined enzymatically with kits from Boehringer-Mannheim; FAN analysis was performed according to Crowell et al. [7]. Biogenic amines were determined by RP-HPLC of their dansyl derivatives, according to Marcé et al. [14]. A Beckman System Gold™ Liquid Chromatograph (Beckman Instruments, Inc.), equipped with a fluorimetric detector (Jasco 821-FP), was used. BA separation was performed on a Beckman Ultrasphere ODS 5 μm column (250 × 4.6 mm i.d.), according to Lasekan and Lasekan [11].

All analytical data are the means of two separate determinations.

Results

The incidence of BA producers among the assayed strains of *O. oeni*, divided according to their origin, is shown in Table 1; the amounts of individual BAs produced by the positive strains after their growth under optimal culture conditions are reported in Table 2. Strains capable of producing at least one BA accounted for 61% of the investigated strains and were found among the isolates from all types of wine. All the positive strains exhibited histidine-producing capability and, among these, seven strains showed the additional property of forming both putrescine and cadaverine. Accumulation of individual BAs in spent culture media was

Table 2. Growth yield and putrescine (PUT), cadaverine (CAD), and histamine (HIS) production by *Oenococcus oeni* strains after growth under optimal culture conditions (nd, no detection)

Strain	Growth yield (g/L)	PUT (mg/L)	CAD (mg/L)	HIS (mg/L)
AG1/2	1.60	nd	nd	17.3
AG2/21	1.41	nd	nd	2.9
AG2/25	1.24	nd	nd	5.8
BM33/97	1.06	nd	nd	5.3
BM34/97	1.06	nd	nd	3.0
BM52/97	1.03	nd	nd	4.5
BR13/97	0.88	12.9	23.2	2.7
BR14/97	1.20	17.6	43.6	12.7
BR15/97	1.11	13.5	28.9	2.5
BR16/97	1.19	9.9	21.0	4.3
CA7/1	0.69	53.6	14.8	4.9
CA7/2	1.34	nd	nd	14.2
CA7/3	0.84	116	34.2	3.0
CD3/1	1.21	146	30.0	1.0
CD3/3	1.14	nd	nd	1.3
CD4/1	1.13	nd	nd	32.8
CH23/4	1.59	nd	nd	1.1
CH23/5	1.25	nd	nd	6.4
NB6/26	1.42	nd	nd	6.2
NV13/2	0.58	nd	nd	1.0
NV13/7	1.35	nd	nd	7.2
NV13/8	1.34	nd	nd	16.8
VR10/2	1.22	nd	nd	1.1
VR10/3	1.14	nd	nd	17.0
VR10/4	1.22	nd	nd	2.7
VR10/5	1.22	nd	nd	14.7
NCDO1674	1.26	nd	nd	16.3

Table 3. Putrescine (PUT), cadaverine (CAD), histamine (HIS), and spermidine (SPD) production by *Oenococcus oeni* strains in the test medium at pH 3.2 with added ethanol (nd, no detection)

Strain	PUT (mg/L)	CAD (mg/L)	HIS (mg/L)	SPD (mg/L)
AG1/2	nd	nd	4.9	1.3
AG2/21	nd	nd	4.6	1.6
BM17/93	nd	nd	nd	nd
BM33/97	nd	nd	1.0	1.7
BR13/97	17.3	21.1	6.3	1.7
BR14/97	17.6	15.4	16.7	1.0
BR15/97	13.9	20.6	5.6	nd
BR16/97	13.8	18.4	2.4	1.1
CA7/1	26.8	8.6	5.3	1.1
CA7/2	nd	nd	3.6	nd
CD3/1	28.5	6.5	2.1	1.4
CD4/1	nd	nd	27.2	1.4
CH23/4	nd	nd	1.0	nd
VR10/3	nd	nd	2.5	2.0

quite variable with the strains, putrescine concentration ranging from 9.9 to 146 mg/L, cadaverine from 14.8 to 43.6 mg/L, and histamine from 1.0 to 32.8 mg/L. As concerns histamine production, nine strains produced less than 3 mg/L (low producers), eight strains produced more than 10 mg/L (high producers), and the other strains showed an intermediate behavior. All the strains that produced putrescine and cadaverine were low producers of histamine, except strain BR14/97, but most of the low-histamine producers did not show any putrescine- or cadaverine-producing capability. In this connection, it is worth mentioning that these two amines were always jointly produced.

No significant production of tyramine, spermine, spermidine, or 2-phenylethylamine was observed, and no relation between bacterial growth yields and BA concentrations in spent media was found. The BA-producing behavior of 13 positive strains and one none-producer (strain BM17/93) was further studied in the malate-containing test medium at pH 3.2 with added ethanol to confirm the BA-producing capability of the strains while performing malolactic fermentation under stress conditions. All the strains, inoculated at high cell concentrations (about 10^8 CFU/ml) into the medium, were able to carry out a complete MLF within 24 h of incubation and confirmed their ability or inability to produce the same BAs as those observed under optimal growth conditions (Table 3). Moreover, ten BA-producer strains showed the additional capability to produce low amounts of spermidine (from 1.0 to 2.0 mg/L). As shown by the data of Table 3, the differences found among the strains in the amount of BAs produced under optimal growth conditions (Table 2) in most cases persisted under stress conditions. Indeed, strains CD4/1 and CD3/1, which yielded the highest concentrations of histamine and putrescine, respectively, after their growth in standard culture medium, maintained their behavior as high producers while performing MLF in the test medium at low pH and in the presence of ethanol; the same happened with strains that produced higher amounts of putrescine than cadaverine or vice versa.

Table 4. Putrescine (PUT, mg/L) and ammonia production by *Oenococcus oeni* strains in arginine (250 mg/L) supplemented wine (nd, no detection; +, ammonia produced; −, no ammonia)

Strain	PUT	NH_3
BM17/93	nd	+
BR13/97	nd	−
BR14/97	nd	−
BR15/97	36.2	+
BR16/97	nd	−
CA7/1	11.9	+
CD3/1	12.9	+
CH23/4	nd	+

To determine whether and to what extent putrescine and cadaverine productions are affected by precursor concentration, two strains, one randomly chosen BA-producer (strain BR14/97) and one none-producer (strain BM17/93), were inoculated at high cell densities (about 10^8 CFU/ml) into a wine containing malate and supplemented with different concentrations of ornithine (0.05, 0.85, and 2.36 mM) or lysine (0.05, 0.19, and 0.88 mM). After 24 h of incubation, once MLF was completed by both strains, BA analysis confirmed the inability of the strain BM17/93 to produce putrescine and cadaverine, independently of the precursor concentration; the BA producer strain BR14/97 released amounts of putrescine and cadaverine increasing with the concentrations of ornithine and lysine, respectively. However, a significant difference was observed between these two BA productions: putrescine concentration was almost equimolecular with ornithine added, whereas cadaverine increased to a lesser extent (from 0.03 to 0.06 mM).

The high efficiency of putrescine production from ornithine by strain BR14/97 suggested assay of the BA-producing behavior of some strains (all the putrescine positive and two putrescine negative) in the same wine but supplemented with arginine, since this amino acid is usually present at high concentrations in grape musts and can be metabolized to ornithine and ammonia by some *O. oeni* strains via the arginine deiminase pathway [9, 15]. The results, reported in Table 4, demonstrated that, among the six putrescine producers tested, only three strains were able to degrade arginine and to form putrescine. The strain CH23/4, chosen as representative of

histamine-positive but putrescine-negative strains, confirmed its ability to degrade arginine, previously shown [9], and its inability to produce putrescine, as well as the strain BM17/93. Hence, the two catabolic properties, arginine degradation and ornithine decarboxylation, are not necessarily co-existing in putrescine producer *O. oeni* strains.

Discussion

Formation of biogenic amines is a diffuse but variable metabolic property of *O. oeni*, as demonstrated by the quali-quantitatively highly variable capability to produce individual BAs found among the 44 strains here investigated. Most of these strains, originally isolated from wines of different oenological areas, exhibited histamine-producing capability, indicating that this metabolic feature is not rare within the species, as already suggested by other authors who found, among several *O. oeni* strains, a high frequency of bacteria carrying the histidine decarboxylase gene [6]. The amounts of histamine produced under the same experimental conditions by the strains, both after their growth in standard culture medium and after MLF in an ethanol-containing medium at low pH, were quite variable, ranging from 1.0 to about 30 mg/L, and demonstrate that some strains of *O. oeni*, bacterial species generally considered safe, can really represent a potential hazard for histamine production in wine. Moreover, a significant number of the histamine-positive strains showed the additional capability to produce both putrescine and cadaverine, to different extents and variable relative proportions. This ascertained capability of some strains to simultaneously produce different amines could explain the findings of Coton et al. [6] on the presence of amines other than histamine, and putrescine in particular, in wines from which histidine decarboxylase-positive strains of *O. oeni* were isolated. The putrescine-producing capability shown by some *O. oeni* strains appears of particular interest for the importance of its practical consequences. Indeed, putrescine, besides being the most effective potentiator of the histamine toxicity to humans [20], usually is the most abundant amine in BA-containing wines and, at concentrations of 15–20 and 20–30 mg/L in white and red wines, respectively, is reported to cause a significant decrease in the sensorial quality of wine [2].

Putrescine accumulation in wine requires the presence of both putrescine producer strains and precursor amino acid, namely ornithine. The concentration of this amino acid in wine is generally low, but its value may increase, like that of the other amino acids, as a consequence of both technological and biological factors, e.g., extended contact with lees and microbial proteolysis. On the basis of the results here described, *O. oeni* can really and significantly contribute to the overall BA content of wines, and to putrescine accumulation in particular. Indeed, one randomly chosen strain was very effective in forming putrescine from ornithine, other strains also being able to form putrescine from arginine. In this respect, a microbial combination of arginine deiminase-positive with ornithine decarboxylase-positive strains in the same wine, as for instance might occur with a mixed population of the strains CH23/4 or BM17/93 and BR14/97, could enhance the production of putrescine by metabiosis, a situation where two or more microorganisms have an exchange of metabolites, which could result in depreciation of both health and sensorial wine qualities.

As a concluding remark, none of the investigated strains was found able to produce tyramine, suggesting a low diffusion of this metabolic property within the *O. oeni* species, as already emphasized by Moreno-Arribas et al. and Choudhury et al. [5, 16].

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