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ARTICLE

# Biogenic Amine Production by Contaminating Bacteria Found in Starter Preparations Used in Winemaking

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The aim of this work was to investigate if contaminating microorganisms, eventually present in bacteria and yeast preparations used as commercial starters in winemaking, have the ability to produce the biogenic amines histamine, putrescine and tyramine. Thirty commercial starters (14 yeasts *Saccharomyces cerevisiae* and 16 bacteria *Oenococcus oeni*) were cultured in synthetic broth and analyzed by TLC to detect amine production. *Oenococcus oeni* commercial preparations did not contain contaminants, but some yeast preparations resulted contaminated with amine-producing bacteria. Bacterial contaminants were isolated and analyzed for their ability to produce biogenic amines using HPLC and TLC. Decarboxylase genes were identified using PCR analysis followed by sequencing. Fermentations were performed in grape juice with two yeast commercial preparations containing bacterial contaminants to check if the potential biogenic amine production could happen also during winemaking. It was found that this production is possible; in particular, in the conditions used in this work, tyramine production was detected. Therefore, the results of this study have significance in relation to the risk of biogenic amines in wine. Moreover a novel species of *Lactobacillus* was found to be able to produce histamine.

**KEYWORDS: Biogenic amines; bacteria; commercial starter contamination; PCR; TLC; HPLC**

## INTRODUCTION

Biogenic amines (BAs) are undesirable compounds that occur in a wide variety of food products, including fish and meat, as well as in fermented and spoiled foodstuffs (*1*).

The consumption of foods or beverages containing large amounts of BAs can cause problems for consumers (*2*) and can lead to different degrees of intolerance. Tyramine and histamine can evoke unwanted symptoms, such as nausea, vomiting, migraine, hypertension and headache (*3*). Putrescine and cadaverine can increase the negative effect of other amines by interfering with the enzymes that metabolize them (*4*). These compounds can be formed and degraded as a result of normal metabolic activity in animals, plants and microorganisms, and they originate mainly from the decarboxylation of amino acids (*5*). In fermented beverages, such as beer and wine, the reaction is catalyzed by amino acid decarboxylase enzymes produced by microorganisms (*6*). The presence of BAs and the origin of these compounds in wine is well documented (*7*). With regard to yeasts, Caruso et al. (*8*) tested 50 wine yeasts belonging to different species for their capacity to produce BAs in wine, and they found that there are some *Saccharomyces cerevisiae* strains which can produce significant levels of agmatine and according to Soufleros et al. (*7*) some amines present in wine are the result of yeast fermentative activity.

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Regarding bacteria, a wide literature is available on the formation of BAs by lactic acid bacteria. Several research groups support the view that BAs are formed in winemaking mainly during malolactic fermentation due to the decarboxylation of free amino acids (*7*, *9*, *10*). As reported by Halàsz et al. (*6*) and Bover-Cid et al. (*11*), the formation of BAs may be due to spoilage bacteria before, during or after food processing, and it is associated with food hygiene and technology.

In general, wine quality is closely related to the diversity and composition of the microbial species that develop during the fermentative process (*12*). The growth of each wine microorganism species generates many secondary metabolites, which may contribute to the aroma and flavor characteristics of wine but can also supply precursors for unwanted amine biosynthesis by malolactic or other contaminant bacteria, and they are determinants of wine quality (*13*, *14*).

Several qualitative and quantitative methods to detect BA production by microorganisms have been described. Thin-layer chromatography (TLC) was used for the determination of these compounds in foods in some studies, and Garcia-Moruno et al. (*15*) developed a TLC qualitative method to detect these compounds in bacterial cultures. Modern analytical techniques, such as high-pressure liquid chromatography (HPLC), allow the acquisition of reliable quantitative data and a better separation/resolution of individual amines (*16*). In addition, the development of molecular biology techniques has allowed the detection of potential amine-producing microorganisms. The polymerase chain reaction (PCR) offers the advantages of speed, sensitivity

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and simplicity, and specific detection of amino acid decarboxylase genes. Moreover, these molecular methods are able to detect potential BA formation risk in food before that the amine is produced: several research groups applied PCR technique to determinate the lactic acid bacteria producing BAs (*10*, *17*−*24*).

In this work we analyzed commercial cultures of yeasts and bacteria used as starters in alcoholic and malolactic fermentation, respectively, both in lab media and in grape juice fermentation assays. Commercial pure cultures are normally checked by manufacturers regarding BA production, but in this study we tested the presence of bacterial contamination in the commercial preparations and the potential ability of these bacterial contaminants to produce histamine, tyramine and putrescine by PCR, TLC and HPLC methods. The detection of microorganisms exhibiting decarboxylase activity is important for monitoring the risk of food contamination.

## MATERIALS AND METHODS

**Microorganisms.** The microorganisms used in this study are commercial selected strains of bacteria and yeasts used in oenological practice as starter cultures for malolactic and alcoholic fermentation. For commercial reasons their names are not indicated; they are listed in **Table 1** with arbitrary alphabetical letters. These strains were used under aseptic conditions to avoid external contamination. Ten samples (1 g each) were analyzed for every batch starter.

*Lactobacillus* sp. 30a (ATCC 33222), which is able to produce histamine and putrescine (*25*, *26*), and *Lactobacillus brevis* CECT 5354 (ATCC 367), which is able to produce tyramine (*27*), were used as positive controls.

**Growth Conditions.** *Oenococcus oeni* commercial strains were grown in medium for *Leuconostoc oenos* broth (MLO, Scharlau Chemie SA) at 30 °C for three days. Solid medium used for isolation of bacteria was MRS agar (2%).

*Lactobacillus* strains were grown at 30 °C in de Man Rogosa Sharpe (MRS, Merck, Germany) broth, pH 6.

1 g of dry yeast strains *Saccharomyces cerevisiae* was rehydrated in 10 mL of 5% sucrose at 40 °C for 30 min; then 0.1 mL of this preparation was grown at 25 °C in YEPG (1% yeast extract, 1% peptone, 2% glucose) for two days. Solid medium for yeast was YEPG agar (2%).

**Qualitative Detection of Amine Formation by TLC.** The method of Garcia-Moruno et al. (*15*) was used for TLC assays. *O. oeni* commercial preparations were inoculated in MRS broth (pH 4.8) at 30 °C, and commercial yeast preparations were grown at 25 °C in YEPG broth. The broths were supplemented with BA precursor amino acids, such as histidine (5 mg/mL), ornithine (5 mg/mL), and tyrosine (5 mg/mL). Samples were taken at 3, 6, 9, and 12 days of growth. Amines were converted to their fluorescent dansyl derivatives as follows: 1 mL of 250 mM $Na_2HPO_4$, 0.1 mL of 4 M NaOH, and 2 mL of dansyl chloride solution (5 mg/mL in acetone) were added to 1 mL of the sample. The mixture was vortex mixed and incubated at 55 °C for 1 h in the dark. The amines were fractionated on silica gel plates (silica gel 60 F254s, Merck) with a solution of chloroform/triethylamine (4:1) as the mobile phase. The spots were visualized under UV light.

**Isolation and Identification of Lactic Acid Bacteria.** The presence of contaminating lactic acid bacteria in yeast commercial cultures was checked by plating rehydrated yeast on MRS agar containing 0.1% cycloheximide to inhibit the growth of yeasts. The plates were incubated at 30 °C. Single colonies were isolated by random picking and grown in liquid MRS medium at 30 °C. Isolates were identified using 16S rDNA ARDRA (amplified rDNA restriction analysis) according to Rodas et al. (*29*) and 16S rDNA sequencing. The presence of decarboxylation genes was detected by PCR.

The bacterial contamination of *O. oeni* commercial starters was not investigate since no amine production was found in the preliminary TLC and HPLC assays.

**Detection of Decarboxylase Genes in Lactic Acid Bacteria.** *DNA Extraction*. DNA was extracted according to Arena et al. (*28*) as follows: 2 mL of bacterial culture in exponential phase were harvested by centrifugation (15 min, 13400*g*). The washed pellet was treated with lysozyme (10 mg/mL) for 30 min at 37 °C, and protoplasts were lysed with 20% SDS. One volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added, and the mix was centrifuged for 15 min at 13400*g*. The upper phase was collected and precipitated with 99% ethanol. The dry pellet was resuspended in TE (10 mM Tris-HCl pH 7.5, 1 mM EDTA), and the DNA was stored at −20 °C.

**Table 1.** Commercial Preparations Used in This Study with the Relative Amines Produced

| starter | TLC | HPLC |
|---|---|---|
| | *Saccharomyces cerevisiae* | |
| A | tyramine | tyramine |
| B | —[a] | — |
| C | tyramine | tyramine |
| D | tyramine; histamine | tyramine; histamine |
| E | — | — |
| F | — | — |
| G | — | — |
| H | — | — |
| I | — | — |
| L | — | — |
| M | — | — |
| N | — | — |
| P | — | — |
| Q | tyramine; histamine | tyramine; histamine |
| | *Oenococcus oeni* | |
| Oe 1 | — | — |
| Oe 2 | — | — |
| Oe 3 | — | — |
| Oe 4 | — | — |
| Oe 5 | — | — |
| Oe 6 | — | — |
| Oe 7 | — | — |
| Oe 8 | — | — |
| Oe 9 | — | — |
| Oe 10 | — | — |
| Oe 11 | — | — |
| Oe 12 | — | — |
| Oe 13 | — | — |
| Oe 14 | — | — |
| Oe 15 | — | — |
| Oe 16 | — | — |

[a] (−) not detected.

*PCR*. PCR was used to detect the presence of the decarboxylase genes histidine decarboxylase (*hdc*), tyrosine decarboxylase (*tdc*) and ornithine decarboxylase (*odc*) in bacterial cultures. The assays were performed as both single and multiplex PCR. Reactions were performed using a MyCycler instrument (BIORAD) in a 20 $\mu$L volume containing 10 mM Tris−HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphate, 1 U of *Taq* DNA polymerase (Invitrogen), along with 0.4 mM of the primers (MWG, Ebersberg, Germany) for the *tdc* (Pt3−Pt4) and *hdc* genes (PHDC1−PHDC2) described by Costantini et al. (*24*). To detect the *odc* gene were used the primer oligo 16 described by Marcobal et al. (*21*) and the primer AODC1 (*24*); both primers were added to the mix at the concentration of 1 mM. PCR conditions were the following: 94 °C for 1 min; 52 °C for 1 min and 72 °C for 2 min repeated for 30 cycles; the same conditions were used for multiplex assays. The PCR products were separated on agarose gel in 0.5 × TAE (Tris−acetate/EDTA), stained with ethidium bromide and visualized under a UV lamp (GelDoc 2000, Biorad).

*DNA Sequencing*. The 16S rDNA gene was amplified by PCR using primers described by Marchesi et al. (*30*). DNA was amplified in 50 $\mu$L volumes containing 100 ng of template DNA, 200 $\mu$M dNTPs, 0.4 $\mu$M primers, 2.5 U of *Taq* DNA polymerase (Invitrogen) and 2 × polymerase buffer. DNA was amplified in 30 cycles (denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min). The PCR product was sequenced by MWG (Germany).

Sequencing was used also to confirm that the amplification products, obtained with assays for decarboxylase gene detection, corresponded to the genes studied; in this case, the PCR reaction was performed with the primers for those genes.

Sequence similarity searches were performed using the BLASTN and BLASTX algorithms of the BLAST (Basic Local Alignment Search Tool) sequence analysis tool (http://www.ncbi.nlm.nih.gov/BLAST/) from the National Center for Biotechnology Information databases to determine the closest known relatives of the sequences obtained.

**Quantitative Detection of Amine Production by HPLC.** The BAs tyramine, histamine, and putrescine and their precursor amino acids were determined by HPLC according to Costantini et al. (*24*). Analysis was done with a Hewlett-Packard I model 1100 (Hewlett-Packard, Palo Alto, CA) with fluorimetric and diode array detectors. Briefly, samples were subjected to automatic precolumn derivatization using *o*-phthaldialdehyde with 2-mercaptoethanol (OPA Reagent, Agilent Technologies, Palo Alto, CA). All separations were performed on an Alltima C18 column (200 mm × 4.6 mm, 5 mm thick film; Alltech, Deerfield, IL) with a matching guard cartridge of the same type (7.5 mm × 4.6 mm). Duplicate samples were filtered through a 0.2 mm pore-size filter (Schleicher & Schuell, Keene, NH) before injection onto the column. Two eluents were used as mobile phases: eluent A (1.224 g of sodium acetate trihydrate, 500 mL of water, 0.09 mL of triethylamine, and 1.5 mL of tetrahydrofuran) and eluent B (1.088 g of sodium acetate trihydrate, 100 mL of water, 200 mL of acetonitrile, and 200 mL of methanol). A 65 min gradient program commenced with an initial concentration of 10% eluent B at a flow rate of 0.450 mL/min and terminated with 100% eluent B at a flow rate of 0.700 mL/min. At the end of the analysis, there was a postrun period of 15 min during which the flow rate and concentration of eluent B returned to the initial conditions. The fluorescence wavelengths were 340 nm for excitation and 450 nm for emission. Quantification of the BAs was performed by using an internal standard of 15 mg/L norvaline. The BA standard was made in 75% methanol and contained 20 mg/L each of histamine, putrescine, and tyramine (all purchased from Sigma). The amino acid standard was made in 75% methanol and contained 20 mg/L of histidine and tyrosine.

**Test of Two Yeast Commercial Preparations for Their Ability To Produce Biogenic Amines under Alcoholic Fermentation of Grape Juice.** The two *Saccharomyces cerevisiae* commercial starters A and D were selected for the fermentation trials, to determine the real production of BAs in wine. Fermentations were carried out in a 300 mL flask with 200 mL of pasteurized grape juice (Biotta AG, Tagerwilen, Switzerland). The juice was obtained by red grapes, and it had pH 3.2 and 172 g/L of sugars. Trials were conducted in duplicate.

Dry yeast strains were rehydrated in a 5% sucrose solution at 40 °C for 30 min. Yeasts were pregrown on synthetic medium YEPG overnight, $5 \times 10^6$ cell/mL were inoculated in grape juice, and a negative control without inoculum was made. After inoculation, flasks were incubated at 20 °C. Fermentations were followed by weight loss due to $CO_2$ production every day. When $CO_2$ evolution stopped, the fermentation was considered completed. At the end of fermentation, BAs content was evaluated by HPLC and microbiological assays were performed plating the resulting wine to detect the presence of bacteria.

## RESULTS

**Analysis of Commercial Bacterial Starters.** Despite the importance of malolactic fermentation, its occurrence is both highly unpredictable and difficult to control (*31*). Consequently, techniques have been sought that will facilitate the efficient and complete conversion of L-malic acid to L-lactic acid in wine, and the use of selected malolactic bacteria is becoming a common practice.

Sixteen commercial *O. oeni* starters were opened under aseptic conditions and inoculated directly into MRS broth containing the BAs amino acid precursors histidine, tyrosine, and ornithine to investigate their ability to produce BAs. Analysis by TLC showed that no detectable quantities of putrescine, histamine and tyramine were observed; these results are reported in **Table 1**. The inability to produce amines is also a characteristic that the producing firms look after, and these results for the selected *O. oeni* were expected. If bacterial contaminants able to produce BAs were present, the presence of these compounds could be detected by TLC. Obviously with this method the presence in commercial preparations of bacterial contaminants that do not produce BAs cannot be excluded.

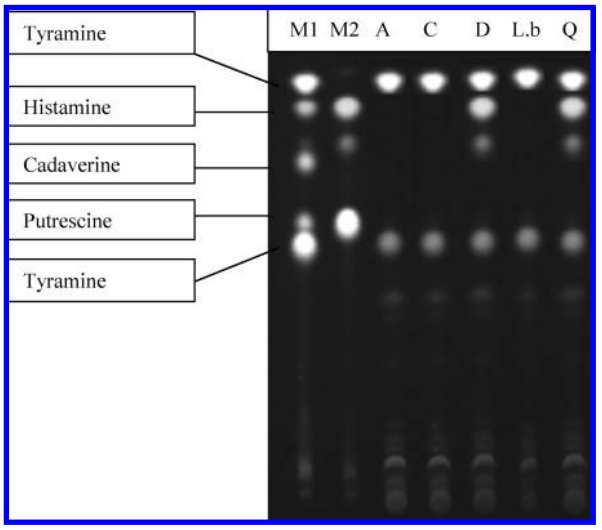


**Figure 1.** TLC detection of biogenic amines produced by commercial yeast preparations. Supernatants from the culture broth were dansylated and separated on precoated silica gel plates (silica gel 60 F254s). M1: standard amines: tyramine (two spots), histamine, cadaverine, putrescine. M2: standard histamine and putrescine. Samples A, C, D, Q: commercial yeast preparations. L.b: positive control *L. brevis* Tyr+.

**Analysis of Bacterial Contaminants of Active Commercial Dry Yeast Starters.** Fourteen active dry yeast cultures were tested. The packages were opened under aseptic conditions and yeasts grown in YEPG with amino acid precursors. A 1 mL sample was taken and analyzed by TLC. **Figure 1** shows the TLC separation. Surprisingly, the results indicated that four preparations were able to produce amines: A and C can produce tyramine; starters D and Q produced tyramine and histamine as reported in **Table 1**. Therefore, further analysis was done to determine if the yeasts had this ability or whether the amine production was due to a bacterial contamination.

Rehydrated yeasts were plated on YEPGA with 0.1% ampicillin to prevent bacterial growth. After 48 h single colonies were picked and inoculated into YEPG broth containing the amino acid precursors histidine, ornithine and tyrosine. Samples were taken after 9 days and analyzed by TLC. None of the pure yeast cultures were able to produce the amines tested (data not shown). These results agree with those reported by Landete et al. (*32*), who did not observe BA production by yeasts in synthetic medium or in wine during alcoholic fermentation.

As indicated in Materials and Methods section, 1 mL samples of rehydrated and diluted yeasts were plated on MRS agar containing 0.1% cycloheximide to prevent the growth of yeast and isolate possible bacterial contaminants. Bacterial colonies were tested by TLC and HPLC for their ability to produce amines. The results indicated that commercial yeast starters A, C, D and Q were contaminated with bacteria able to produce BAs. For these positive colonies, the identification of decarboxylase genes was performed using PCR analysis. **Figure 2** shows the electrophoresis gel of these positive bacteria, obtained by a multiplex PCR assay where different genes have been amplified: 16S gene with primers 63f and 1387r (*30*); *hdc* and *tdc* genes respectively amplified with primers PHDC1−PHDC2 and Pt3−Pt4 (*24*). The result of this analysis showed that ten bacterial

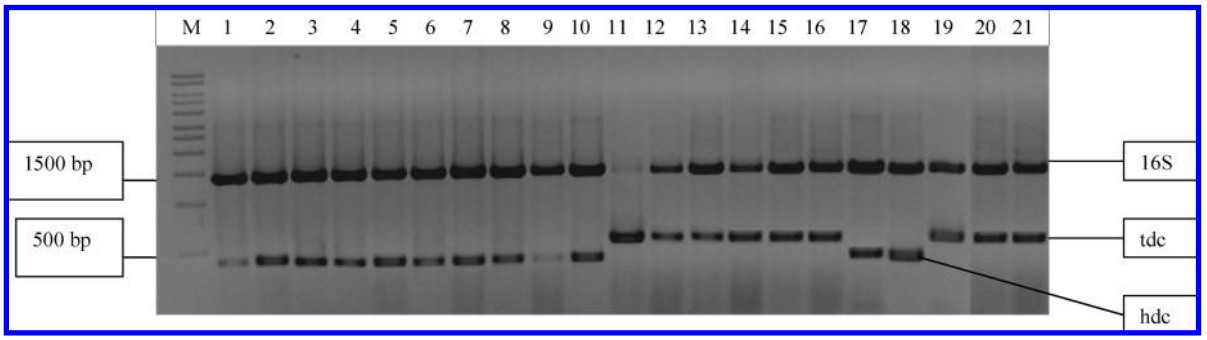


**Figure 2.** Multiplex PCR amplifying 16S, *tdc* and *hdc* genes. M: molecular marker (1 kb, Sigma). Lanes 1−9: *L. buchneri*, isolated from started Q, which amplifies *hdc* gene. Lanes 11−16, 19−21: *L. brevis* which amplifies *tdc* gene. Lanes 17−18: presumptive *L. rossiae* isolated from starter D, which amplifies *hdc* gene. Lane 10: positive control *Lactobacillus* 30a which amplifyies *hdc* gene.

colonies isolated from commercial preparations A, C, D and Q contained *tdc* gene. Moreover *hdc* gene was amplified in nine colonies isolated from starter Q and two colonies isolated from starter D. These bacteria have been identified by 16S sequencing; the colonies tdc+ belong to the species *L. brevis* (99% identity with 16S rRNA of *L. brevis* ATCC 367). The nine colonies hdc+ isolated from starter Q were *Lactobacillus parabuchneri* (99% identity with *L. parabuchneri* 16S rRNA partial sequence, type strain: DSM 5707). It is known that *L. brevis* can produce tyramine (*27*) and *L. buchneri* can produce histamine (*33*): Sumner et al. (*34*) isolated a strain of *L. buchneri* from a sample of cheese implicated in an outbreak of histamine poisoning and showed it to be a high-level histamine producer. The other two colonies carrying *hdc* gene isolated from starter D showed an identity degree of 95% with *Lactobacillus rossiae* type strain (CS1, ATCC BAA-822, DSM 15814, JCM 16176) 16S rRNA gene, partial sequence. Moreover further examination should be done to confirm the exact affiliation to this species. The *tdc* gene partial sequence of a *L. brevis* isolated from preparation C, analyzed in BLAST, had a 99% identity with *tdc* gene of *L. brevis* strain CECT3810. The TDC protein fragment is 95% identical to the corresponding fragment from *L. brevis* IOEB9809 TDC.

The *hdc* gene partial sequence of a presumptive *L. rossiae*, isolated from preparation D, after BLAST analysis resulted to have a 78% identity with *Lactobacillus reuteri* DSM 20016 histidine decarboxylase gene; the analysis showed also a 75% identity with *Lactobacillus* sp. 30a *hdc* gene. The HDC protein fragment showed an 86% identity to HDC from Lactobacillus 30a and an 83% identity to the equivalent proteins from *O. oeni*, *L. hilgardii* and *L. buchneri*.

**Nucleotide Sequence Accession Numbers.** The two strains of presumptive *L. rossiae* isolated in this study have been inserted in the collection of the CRA-Centro di Ricerca per l'Enologia, and they are indicated as ISE 5263 and ISE 5264. The DNA sequences of 16S and *hdc* gene have been deposited in the EMBL/GenBank database with the following accession numbers: FJ791057, FJ791058, FJ794698, FJ794699.

**Test of Two Yeast Commercial Preparations for Their Ability To Produce Biogenic Amines under Alcoholic Fermentation of Grape Juice.** Two of the yeast starters A and D, that resulted spoiled with bacteria able to produce amines (A can produce tyramine and D can produce tyramine and histamine), were tested in fermentation to evaluate if the contamination could represent a problem for a practical point of view. Yeasts were rehydrated and grown in YEPG overnight at 25 °C and $5 \times 10^6$ cell/mL were inoculated in grape juice. The fermentation rate was very similar among the two strains. The resulting wines were analyzed by HPLC to investigate the presence of BAs. The results, relative to the quantities of the amines produced and the corresponding precursor amino acids, are reported in **Table 2**. It can be observed that tyramine has been produced in samples A and D and is not present in the negative control. This result demonstrates that the bacterial contaminants are able to produce amines not only in lab medium but also in wine. This fact is also demonstrated by tyrosine (precursor amino acid of tyramine) reduction.

**Table 2.** Amino Acid and Biogenic Amine Content Results by HPLC Analysis at the End of Fermentation with Starters A and D, Compared with Negative Control[a]

| | histidine | tyrosine | histamine | tyramine | putrescine |
|---|---|---|---|---|---|
| negative control | 6.74 ± 0.56 | 6.48 ± 0.36 | nd[b] | nd | nd |
| starter D | nd | 1.34 ± 0.40 | nd | 2.69 ± 0.43 | nd |
| starter A | nd | 1.83 ± 0.29 | nd | 2.90 ± 0.25 | nd |

[a] Values represent the average of two replications ± standard deviations. Concentrations are reported in mg/L [b] nd: not detected.

Comparing histidine (precursor of histamine) content before and after alcoholic fermentation, it can be seen that histidine disappeared, however no histamine production was detected in wine obtained with starter D.

Samples taken at the end of wine alcoholic fermentation were plated on MRS agar with 10% cycloheximide to inhibit yeast development and bacteria growth was observed. Twenty four colonies were randomly selected and tested for the presence of decarboxylase genes with PCR assays; as shown in **Figure 3** 23 colonies amplified the *tdc* gene. This result can explain the production of tyramine found at the end of fermentation. These colonies have been identified, and they resulted *L. brevis*. No colonies of the presumptive *L. rossiae* have been found; probably this species is not able to growth in acidic environment, or maybe the alcohol content is too high for this species to survive: further studies should be done.

## DISCUSSION

In this work, the presence of contaminating microorganisms in commercial bacteria and yeast preparations was investigated, and they were tested for their ability to produce histamine, putrescine and tyramine. Regarding the commercial strains, none of them were able to produce BAs. These results were expected since for the starter manufacturers the absence of amino acid decarboxylase activity is now included in the selection criteria for the industrial preparation of starters. However commercial preparations in a dry active form can be a substrate for bacterial contamination. O'Brien et al. (*35*) reported the presence of *Enterococcus*, coliforms and *Escherichia coli* in the manufacturing process of commercial yeast. Manufacturers of starters inform about the risk of contamination during storage, and they recommend to

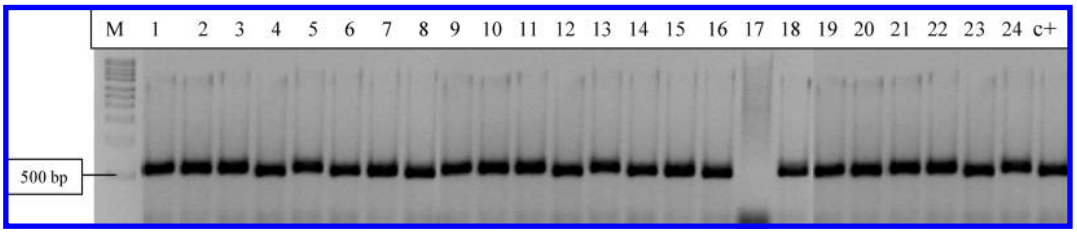


**Figure 3.** *tdc* gene amplification with primers Pt3−Pt4 of the colonies isolated at the end of fermentations with two contaminated starters A, D. M: molecular marker 1 Kb (Sigma). Lanes 1−24: colonies isolated. C+: positive control *L. brevis* ATCC367 *tdc*+.

open the packages just before use; but the results of the present study indicate that commercial yeast starters can be contaminated with lactic acid bacteria in unopened packages. The problem arises during production of these starters, where it is very difficult to maintain sterile conditions, and the results obtained in the present study confirm the presence of contamination.

It is known that commercial yeast starter preparations contain lactic acid bacteria contaminants (*36*), but an important problem can occur if these bacteria are able to produce BAs during alcoholic fermentation; no previous study has taken this factor into account. This work demonstrated that the risk of BA production exists, and it depends on the contaminating bacteria species and that *L. brevis* can survive during the fermentation and can produce amines. In fact, in the fermentation performed with the two starters A and D, tyramine was detected at the end of fermentation. Moreover the failure of the presumptive *L. rossiae* to produce histamine is referred to the conditions used in this study. It could be hypothesized that, since this species contains *hdc* gene, it can produce histamine in other conditions of fermentation.

The tyramine content is low, but this amount is dependent on the fermentation conditions used; in other conditions this quantity could be different. The possibility that the presence of BAs in wine is the result of a bacterial contamination has been reported by some authors: Ough et al. (*37*), Arena and Manca de Nadra (*28*), Moreno-Arribas et al. (*20*), Karovičová and Kohajdová (*38*) and Costantini et al. (*24*).

In this work is shown one of the possible ways of entry of contaminating microorganisms able to produce BAs in wine. The results of this study have, therefore, relevant implications on wine quality, especially for microbiological and toxicological aspects.

An interesting finding of the work is a presumptive novel species of *Lactobacillus* able to produce histamine. In the literature there are reports that lactic acid bacteria able to produce histamine belong to the species *L. hilgardii* (*23*), *L. buchneri* (*33*), *L. reuteri* (*39*), *O. oeni* (*17*, *18*) and *L. sakei* (*22*). In this work, two presumptive strains of *L. rossiae* were shown to contain the *hdc* gene; this species was detected in sourdough by Di Cagno et al. (*40*), but no author has described their ability to produce BAs. This property could be strain-dependent, and further study is needed, but Vogeser et al. (*41*) reported this species as a new beer contaminant with a high spoiling potential.

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