Tyramine Formation by *Pediococcus* spp. during Beer Fermentation

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ABSTRACT

The role of *Pediococcus* spp. in the production of tyramine was studied. Strains of these microorganisms were isolated from industrial beer fermentations where high tyramine formation occurred. It has been verified that *Pediococcus* spp. are able to form tyramine during beer fermentation, and the quantity of tyramine produced depends on the degree of contamination. Thus, CFU of *Pediococcus* spp. ranging from 4 × 10⁵ to 1 × 10⁶ CFU/ml led to low tyramine formation (<5 mg/liter). Tyramine production between 5 and 15 mg/liter was related to *Pediococcus* spp. counts from 1 × 10⁶ to 5 × 10⁶ CFU/ml, while counts of these microorganisms above 5 × 10⁶ CFU/ml led to tyramine levels ranging from 15 to 20 mg/liter. Prolonged storage in culture media of the isolated strains of *Pediococcus* spp. as well as isolation of these microorganisms from beer and transfer to media caused them to lose their ability to produce tyramine. Determination of tyramine levels in beer was found to be a reliable indicator of the degree of contamination by *Pediococcus* spp. during beer fermentation.

Key words: Biogenic amines, tyramine, beer, lactic acid bacteria, *Pediococcus* spp.

Production of beer fulfills the three factors that govern the formation of amines postulated by Rice et al. (17). Free precursor amino acids are available, microorganisms with amino acid-decarboxylating activity can occur, and favorable conditions for the growth of the microorganisms can be found. High levels of histamine, tyramine, and cadaverine in beers may indicate defective food-manufacturing processes related to poor sanitary conditions (7, 10, 12). Cerutti et al. (3) suggested that it is possible to obtain beers with low amine levels by using high-quality raw materials and applying suitable technologies. In recent work (11), only tyramine, among other amines such as histamine, cadaverine, and putrescine, was subject to wide fluctuations in beers of the same type and produced by particular breweries irrespective of whether they were produced by the same company. Relatively high levels of this amine were specific to certain breweries.

Lactic acid bacteria are often related to the amine buildup in beers (7, 12, 23). The reduction of pH and dissolved oxygen that occurs during beer fermentation results in a medium that can support only a limited number of microorganisms, among them lactic acid bacteria. Contamination by those bacteria may occur during fermentation through the brewer’s yeast being contaminated by microorganisms. Recently, we found that no significant changes in the amine contents were observed during beer fermentation, except for tyramine (12). Tyramine formation showed great variability and was linked to the presence of lactic acid bacteria, which were identified as species of *Pediococcus*. We are not aware of any previous research where the microorganisms related to tyramine formation during brewing were *Pediococcus* spp. These microorganisms are usually recognized as amine producers in wine, cheese, and fermented sausages (10, 18, 22). The relationship of species of *Lactobacillus* to amine formation in beer has been studied more extensively. In a laboratory study, Zee et al. (23) found that *Lactobacillus brevis* was responsible for tyramine and putrescine production in wort inoculated with those microorganisms and fermented with a top-fermenting yeast. Donhauser et al. (7) also found that species of *Lactobacillus* (i.e.,
L. brevissimilis, L. frigidus, and L. brevis) led to an increase in the total content of amines in contaminated beer.

The present study was undertaken to demonstrate the role of Pediococcus spp. in the production of tyramine, with special emphasis on beer fermentation. A principal objective was to establish the levels of tyramine that could be expected when a particular degree of contamination by Pediococcus spp. was present during beer fermentation. Our data could establish the role of tyramine in beer as an indicator of bacteria contamination during brewing. Finally, data are provided on the effect of the Pediococcus spp. cultivation in culture media on their tyramine-producing activity.

**MATERIALS AND METHODS**

Samples

A Spanish brewery collaborated with us and provided all the samples for this study. The beer was a lager, Pilsner, and a bottom-fermenting brewer's yeast (Saccharomyces cerevisiae var. uvarum) was used for all fermentations.

The fermentations described below were conducted using glass fermenters (3-liter capacity, 150 cm high) recommended by the European Brewery Convention (EBC) (8) and the fermentation temperature (11°C) and time (7 days) were the same as those of the industrial processes. Fermentation was started by adding a yeast slurry, calculated to obtain a final count of 20 × 10^6 cells per ml of wort, to 3 liters of wort, and then the mixture was transferred to a glass fermenter. Wort (13° Plato) was obtained at the industrial plant and the ingredients employed were malt, hops, and adjunct cereals (rice and corn). Wort was always taken just after the boiling step. Fermentation samples were drawn using a sterile hypodermic syringe through a rubber stopper located 50 cm from the top of the fermenter. Glass fermenters were thoroughly cleaned and disinfected every time a new fermentation was started. Samples were collected in sterile flasks and divided into two fractions. One fraction was used for immediate microbial analysis; the other was centrifuged at 4,000 rpm at 4°C for 30 min, and the supernatant was stored at −20°C for tyramine analysis.

**Experimental designs**

A. Fermentations inoculated with strains of Pediococcus spp. maintained in skim milk medium for 1 year. Six mixtures of wort and pure-culture yeast ready to start fermentation were each inoculated with a strain of Pediococcus spp. An inoculum of Pediococcus spp. was suspended in quarter-strength Ringer solution and added to the mixture of wort and yeast. The strains of Pediococcus spp. were previously isolated from samples of industrial fermentations, where a substantial tyramine formation occurred, in NBB medium (Nachweiss Medium für bierchadliche Bakterien, Döhler, Darmstadt, Germany) (2, 5) and maintained in NBB broth for 2 to 3 days at 28°C. Cultures were then centrifuged as above and the cells collected were resuspended in quarter-strength Ringer solution. The suspension was centrifuged and the cells were collected and resuspended in skim milk medium (Difco Laboratories, Detroit, MI) supplemented with 1% glucose, 0.3% yeast extract, and 1% calcium carbonate. Strains under these conditions could be kept for up to 3 months at −20°C. All strains isolated were identified according to procedures described by Garvie (9).

B. Fermentations inoculated with Pediococcus spp. maintained in NBB medium. Two parallel fermentations were carried out in 12 independent batches. Each batch involved a fermentation control (Pediococcus spp.-free), and a fermentation inoculated with a strain of Pediococcus spp. A total of 12 strains of Pediococcus spp. were tested. All strains were isolated from samples coming from industrial plant fermentation in which substantial tyramine formation was detected. Strains were isolated for pure cultures in NBB medium and maintained on soft NBB agar (NBB agar and NBB broth, 1:1) by stabbing for 2 to 3 days at 28°C. Then the isolated strains were kept at 4°C up to 1 month.

C. Fermentations carried out with pitching yeasts obtained from the brewery. Four parallel fermentations were carried out in six independent batches. One fermentation was a control, constituted of only wort and pure-culture yeast. Yeast coming from the industrial plant where contamination by Pediococcus spp. was detected was used to carry out the other three fermentations. To obtain different concentrations of Pediococcus spp. cells, this yeast from the brewery was mixed with pure-culture yeast in different proportions, such as 10:90 (yeast: pure-culture yeast), 25:75, or 50:50. A fermentation with only yeast from the brewery was also included.

D. Study of the loss of the ability to produce tyramine by Pediococcus spp. as a consequence of transfer back and forth between fermentation samples and culture medium. Three different protocols, described below, were designed. In all three, Pediococcus spp. were added to initial concentrations of ca. 1 × 10^5 CFU/ml.

**FIGURE 1.** Flow diagrams of the different protocols used to contaminate small-scale beer fermentations with Pediococcus spp.
fermentations followed. Protocol A involved minimal handling, since the strain was transferred to a culture medium only once.

Protocol B (Fig. 1, B) was initiated as in protocol A, but once the fermentation finished, a colony of *Pediococcus* spp. was isolated from a sample and cultured in NBB medium for 10 days. Then *Pediococcus* spp. was added to a new mixture of wort and pure-culture yeast ready to begin fermentation. The protocol was also repeated six times. Protocol B also allowed us to study how the successive transfer in culture media could affect the tyramine-producing activity.

Protocol C (Fig. 1, C) involved maintaining the isolated strain on NBB medium, from which the successive fermentations were contaminated.

**Microbial analysis**

*Pediococcus* spp. colonies were counted on NBB agar plates. One hundred microliters of a sample or of a dilution in quarter-strength Ringer solution was spread on NBB agar plates and incubated at 28°C for up to 14 days under anaerobic conditions. Duplicate plate counts were obtained throughout. A rigorous control was carried out in all the fermentations to verify that no contamination by other microorganisms had occurred.

**Analytical determinations**

Tyramine determination was carried out according to a spectrofluorometric method proposed by Rivas-Gonzalo et al. (19).

Each analysis was always carried out in duplicate. All reagents were of analytical grade. Tyramine free-base standard was purchased from Sigma Chemical Co. (St. Louis, MO).

**Statistical analysis**

Nonlinear regression was calculated to assess the relationship between tyramine levels and *Pediococcus* spp. counts. Analyses were performed by means of the SPSS Statistical Software Package version 6.0.1 (SPSS Inc., Chicago, IL).

**RESULTS AND DISCUSSION**

It has been reported that microorganisms involved in biogenic amine formation in wine, once isolated from the alcoholic beverage and maintained in culture media, could fail to produce amines due to a loss of amino acid-decarboxylating activity (1, 15). A preliminary experiment was conducted to evaluate whether strains of *Pediococcus* spp. isolated from fermentations in which substantial tyramine formation occurred and maintained on skim milk medium for one year retained their tyramine-producing activity during beer fermentation (experimental design A). Although a viable population of *Pediococcus* spp. remained constant throughout fermentation, and at the same initial levels, no tyramine was produced in any of the fermentations. Therefore, it seemed that the strains of *Pediococcus* spp. isolated from beer and kept in skim milk medium for a long period lost their tyrosine-decarboxylating activity.

To determine whether the long storage period was the cause of the lack of tyramine production, new fermentations were performed after inoculation with *Pediococcus* spp., but now strains recently isolated were used (experimental design B). The objective was to reduce the time from isolation to inoculation. The results obtained are summarized in Table 1.

**TABLE 1. Tyramine formation during beer small-scale fermentations inoculated with Pediococcus spp.**

<table>
<thead>
<tr>
<th>Fermentation</th>
<th>Tyramine* (mg/l)</th>
<th>Pediococcus spp. (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>A</td>
<td>3.6</td>
<td>5 × 10^3</td>
</tr>
<tr>
<td>B</td>
<td>2.2</td>
<td>6 × 10^3</td>
</tr>
<tr>
<td>C*</td>
<td>nf</td>
<td>1 × 10^4</td>
</tr>
<tr>
<td>D</td>
<td>7.3</td>
<td>5 × 10^4</td>
</tr>
<tr>
<td>E</td>
<td>nf</td>
<td>9 × 10^4</td>
</tr>
<tr>
<td>F*</td>
<td>nf</td>
<td>1 × 10^4</td>
</tr>
<tr>
<td>G</td>
<td>20.4</td>
<td>3 × 10^5</td>
</tr>
<tr>
<td>H</td>
<td>16.0</td>
<td>3 × 10^5</td>
</tr>
<tr>
<td>I*</td>
<td>nf</td>
<td>2 × 10^5</td>
</tr>
<tr>
<td>J</td>
<td>nf</td>
<td>3 × 10^5</td>
</tr>
<tr>
<td>K</td>
<td>nf</td>
<td>3 × 10^5</td>
</tr>
<tr>
<td>L*</td>
<td>nf</td>
<td>3 × 10^5</td>
</tr>
</tbody>
</table>

*a Total formation calculated as final minus initial tyramine concentration.

*b Fermentations marked with an asterisk: no survival of the strain.

c nf, no formation.

C, F, I, and L. The *Pediococcus* spp. counts in those processes rapidly declined by the second or third day of fermentation. Among the fermentations where *Pediococcus* spp. remained viable and constant, tyramine formation occurred in several fermentations and the quantity formed varied according to the degree of contamination. Thus, a high level of tyramine formation was observed in two processes (G and H) inoculated with up to 2 × 10^5 CFU/ml of *Pediococcus* spp. cells, while lower counts of those microorganisms (ca. 6 × 10^3 CFU/ml) produced much lower tyramine quantities (i.e., A and B). In control fermentations (only pure-culture yeast and wort) carried out in parallel, no tyramine formation was detected.

In contrast, no tyramine formation was found in fermentations E, J, and K, although *Pediococcus* spp. counts were occasionally similar to fermentations where tyramine was produced. Apparently, some strains of *Pediococcus* spp. failed to produce tyramine because these particular strains of *Pediococcus* spp. could not tolerate the medium changes, such as from beer to culture medium. Changes of culture media can provoke the loss of the tyrosine-decarboxylating activity from microorganisms, as suggested by Lonvaud-Funel and Joyeux (15). Furthermore, the different strains of *Pediococcus* spp. used in the individual fermentations may have differed in their ability to form tyramine.

An optimal protocol to contaminate beer fermentations was designed. The protocol involved the use of yeasts coming from industrial plants in which contamination by *Pediococcus* spp. was detected. Thus, the protocol allowed us to follow beer fermentations in which the counts of *Pediococcus* spp. could be controlled to a certain degree and no isolations of the microorganisms were involved.

First, we observed that the viable population of *Pediococcus* spp. remained constant in all of the fermentations. Therefore, the minimal handling of *Pediococcus* spp. seems a good choice to prevent a sudden loss of viability, as observed before. Furthermore, we demonstrated that different...
levels of *Pediococcus* spp. cells could lead to the formation of different quantities of tyramine (Table 2). Tyramine production increased as the level of *Pediococcus* spp. contamination in the yeast increased. No tyramine formation occurred in those control fermentations where only pure-culture yeast and wort were employed. Correlation between tyramine contents and *Pediococcus* spp. counts was assessed by regression analysis. The coefficient of correlation was calculated between tyramine formation and maximum *Pediococcus* spp. counts observed in each fermentation. The exponential model (Fig. 2) showed a high correlation coefficient (\( r = 0.9861, P < 0.001, n = 21 \)) with a coefficient of determination (\( r^2 \)) of 97.2%. Therefore, lower or higher tyramine formation could be explained by the presence of *Pediococcus* spp. during beer fermentation. As observed in Fig. 2, the correlation between tyramine formation and *Pediococcus* spp. counts fit an exponential model. A minimal count of *Pediococcus* spp. would be required to produce detectable amounts of tyramine.

Thus, *Pediococcus* spp. counts ranging from \( 4 \times 10^3 \) to \( 1 \times 10^4 \) CFU/ml led to a low tyramine formation (<5 mg/liter). Tyramine production between 5 and 15 mg/liter was related to *Pediococcus* spp. counts from \( 1 \times 10^4 \) to \( 5 \times 10^4 \) CFU/ml, while *Pediococcus* spp. counts above \( 5 \times 10^4 \) CFU/ml led to tyramine levels ranging from 15 to 20 mg/liter. Those results confirmed the results previously obtained by us \((12)\) at the industrial plant.

Therefore, the usefulness of the tyramine determination levels in beers as a reliable indicator of the degree of contamination by *Pediococcus* spp. during fermentation observed at industrial scale has also been confirmed in controlled contaminated fermentations. Our results are in agreement with those of Cerutti et al. \((4)\), who pointed out that beers with low tyramine contents can be produced if hygienic conditions during brewing are maintained. In a previous work, we confirmed the effectiveness of the acid phosphoric washing of the pitching yeast to reduce tyramine formation, since it eliminated *Pediococcus* spp. and other microorganisms that may eventually spoil the yeast \((12)\).

Although they did not state figures, Cerutti et al. \((4)\) recommended that no more than “some” concentration of tyramine should be present in beers to avoid any risk for the consumer. Tailor et al. \((20)\) reported that values above 10 mg/liter of tyramine in beers were unsafe for patients on MAOI. Since the contribution of the raw materials and of the mashing to the final tyramine levels in beer is usually low \((3, 14, 23)\), we calculated that counts of *Pediococcus* spp. cells around \( 3 \times 10^4 \) CFU/ml during fermentation would produce tyramine concentrations above 10 mg/l in the end-product, at least in the type of beer studied.

Finally, work was undertaken to ascertain how strains of *Pediococcus* spp. lost their ability to produce tyramine as a

### TABLE 2. Tyramine formation and *Pediococcus* spp. counts during beer fermentations with yeast contaminated with *Pediococcus* spp.

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Control</th>
<th>10:90</th>
<th>25:75</th>
<th>50:50</th>
<th>100:0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TYR PED</td>
<td>TYR PED</td>
<td>TYR PED</td>
<td>TYR PED</td>
<td>TYR PED</td>
</tr>
<tr>
<td>1</td>
<td>nf</td>
<td>nd</td>
<td>4.2</td>
<td>5 \times 10^3</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>nf</td>
<td>nd</td>
<td>---</td>
<td>---</td>
<td>5.1</td>
</tr>
<tr>
<td>3</td>
<td>nf</td>
<td>nd</td>
<td>---</td>
<td>---</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>nf</td>
<td>nd</td>
<td>---</td>
<td>---</td>
<td>7.9</td>
</tr>
<tr>
<td>5</td>
<td>nf</td>
<td>nd</td>
<td>3.1</td>
<td>6 \times 10^3</td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td>nf</td>
<td>nd</td>
<td>4.0</td>
<td>5 \times 10^3</td>
<td>---</td>
</tr>
</tbody>
</table>

\( a \) Tyramine formation calculated as final minus initial tyramine concentration.

\( b \) Control: only pure-culture yeast.

\( c \) Four parallel fermentations were carried out in each batch.

\( d \) No formation, nf; not detected, nd; dash indicates not determined.

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**FIGURE 2. Relationship between *Pediococcus* spp. counts (log CFU/ml) and tyramine formation (mg/liter) during beer fermentation.**
TABLE 3. Changes in tyramine formation and cell concentration of Pediococcus spp. during six successive fermentations following different protocols for contaminating small-scale beer fermentations

<table>
<thead>
<tr>
<th>Fermentation</th>
<th>Tyramine (mg/liter)</th>
<th>Pediococcus spp. (maximum CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>8.0</td>
<td>$1 \times 10^4$</td>
</tr>
<tr>
<td>2nd</td>
<td>0.5</td>
<td>$2 \times 10^4$</td>
</tr>
<tr>
<td>3rd</td>
<td>0.8</td>
<td>$4 \times 10^4$</td>
</tr>
<tr>
<td>4th</td>
<td>nf</td>
<td>$8 \times 10^2$</td>
</tr>
<tr>
<td>5th</td>
<td>nf</td>
<td>$2 \times 10^2$</td>
</tr>
<tr>
<td>6th</td>
<td>nf</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

a Tyramine formation calculated as final minus initial tyramine concentration.

b Six successive fermentations were carried out in each protocol.

c No formation, nf; dash indicates not determined.

A consequence of the changes in the culture medium. For that purpose, strains of Pediococcus spp. were maintained following three different protocols, which differed in how the strains were handled. Tyramine formation and the maximum Pediococcus spp. counts found in each fermentation for the three protocols are shown in Table 3.

Results showed that Pediococcus spp. lost its tyramine-producing activity throughout the successive fermentations in all protocols. Pediococcus spp. under protocol A showed a rapid, constant decline in its viability among the consecutive fermentations. Tyramine-producing activity also declined sharply at the second fermentation, even though Pediococcus spp. counts were still above $1 \times 10^4$ CFU/ml. Strains in protocol B remained viable throughout all the successive fermentations; however, from fermentation one to two a 47% decrease in the production of tyramine was noted and from fermentation two to three a 79% reduction. In protocol C, even though Pediococcus spp. remained viable, the strain completely lost its ability to produce tyramine by the third fermentation. Therefore, it seemed that the cultivation of strains in culture media could lead to failures in producing tyramine during beer fermentations, confirming data reported by other authors (1, 15).

In conclusion, Pediococcus spp. appeared to be able to produce tyramine during beer fermentation, and the quantity of the amine formed seemed to depend on the number of Pediococcus spp. cells present. We could also conclude that Pediococcus spp. might lose its tyramine-producing activity as a consequence of the successive isolations from beer to medium.

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