A Research Note

Histamine and Tyramine Production by a Lactobacillus Strain Subjected to External pH Decrease

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ABSTRACT

The preservative effect of fermentation is based on the pH decrease during processing. However, most studies concerning the influence of pH on the formation of biogenic amines have been made in broths with different initial pH values. A histamine- and tyramine-positive Lactobacillus strain isolated from dry sausage was added at an initial level of 3.9-4.4 log_{10} CFU/ml and incubated on a shaker at room temperature (20-23°C) for 6 days. The pH was decreased in histidine- or tyrosine-fortified MRS broth by adding glucono-delta-lactone (GDL) or lactic acid during the incubation. The external acidification decreased the growth and the production of histamine and tyramine by the strain. GDL was more effective as a preventative than lactic acid. According to these results, a rapid pH decrease resulting in decreased growth of amine-positive lactic acid bacteria in the beginning of fermentation may be a means of preventing the formation of high levels of amines in foods.

Amines in foods are produced mainly by the breakdown of amino acids due to the action of decarboxylases of microbial origin. Histamine is a heterocyclic amine which is often associated with so-called scombroid food poisoning. Tyramine is an aromatic amine elevating blood pressure and eliciting headaches. These biogenic amines may represent a food poisoning hazard, increased by potentiating factors such as amine oxidase-inhibiting drugs, alcohol, other food amines, and gastrointestinal diseases.

Biogenic amines are often found in fermented foods such as cheese, dry sausage, and red wine. Over 60 years ago, Koessler et al. (12) proposed that biogenic amine formation is a protective mechanism of bacteria against acidic environments. Many later reports have supported this theory (e.g., 9,10). The effect of pH on the formation of biogenic amines has mainly been studied by adjusting the initial pH value of the broth to different levels but not by decreasing the pH during incubation (e.g., 3,5,6,10,22). However, during fermentation the pH decreases gradually. Therefore, it is important to ascertain how pH decrease, and not only the initial pH, affects the formation of amines. In our previous studies, an accelerated pH decrease caused by GDL decreased the levels of histamine formed in minced meat, dry sausages, and fortified MRS broth (14,16). Glucono-delta-lactone hydrolyzes spontaneously in water to gluconic acid, causing a decrease in pH, and is sometimes used in the meat industry for dry sausage manufacture.

The purpose of this work was to study the effect of external pH decrease on the formation of histamine and tyramine in fortified MRS broth. The effect of GDL was also compared with an equivalent pH decrease achieved by adding lactic acid.

MATERIALS AND METHODS

MRS broth (Oxoid CM 259), L-tyrosine (Fluka 93830), L-histidine-monohydrochloride (Merck 4350), lactic acid (BDH 10138), and glucono-delta-lactone (GDL, Finnsugar Bioproducts) were used in the study. de Man, Rogosa and Sharpe agar with sorbic acid (MRS-S) was prepared from MRS agar of LabM (LAB93) and sorbic acid (Fluka 85510) according to the Pharmacopoeia of Culture Media for Food Microbiology (1).

The histamine and tyramine producing Lactobacillus strain G106 (930 and 920 ppm, respectively), previously isolated from a dry sausage containing 108 ppm histamine and 190 ppm tyramine, was used in the study (13). It was a catalase-negative, homofermentative gram-positive rod growing at 15°C but not at 45°C and hence, conforming with the Orla-Jensens group "Streptobacterium" (11). It fermented only galactose, glucose, fructose, mannose, N-acetyl glucosamine, and lactose and was esculine positive in API50CHL system. It produced arginine, did not grow at pH 3.9, and the final pH in MRS broth was 3.7 with the methods reported in a previous study (15). The strain was stored at -75°C until used. Before each trial, it was adapted by incubating in basic broth (MRS-H or MRS-T, see Table 1) at 30°C for 24 h.

One milliliter of preadapted culture (dilution 10^2) was added to 500 ml of each of the broths studied (A through D) (Table 1). This gave initial cell counts at the beginning of incubation of log_{10} 4.4 CFU/ml (histidine-containing broths) and 3.9 CFU/ml (tyrosine-containing broths). The bottles of media (A-D) were incubated on a shaker at room temperature (22-23°C) for 6 days. The pH values of the broths B and C were decreased by adding acidified basic broth through intravenous infusion set during the first 9 h of incubation. The volume of acidified broth added was
TABLE 1. The different broths used in the study.

<table>
<thead>
<tr>
<th>Code</th>
<th>Broth</th>
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<tbody>
<tr>
<td>A</td>
<td>Basic broth = MRS + 2% histidine or tyrosine (MRS-H or MRS-T) (pH 5.7)</td>
</tr>
<tr>
<td>B</td>
<td>Basic broth (pH decreased by adding the basic broth acidified by 1% lactic acid)</td>
</tr>
<tr>
<td>C</td>
<td>Basic broth (pH decreased by adding the basic broth acidified by 3% lactic acid)</td>
</tr>
<tr>
<td>D</td>
<td>Basic broth + 1% GDL immediately before the incubation</td>
</tr>
</tbody>
</table>

removed from the basic broth to give the same final volume in every bottle (A-D).

Broth A served as a negative control for external pH decrease. During the first 9 h, the pH in broth B was decreased by adding basic broth acidified by 1% lactic acid at approximately the same rate as GDL decreased the pH in broth D (basic broth + 1% GDL). The pH of broth C was decreased more rapidly than that of broth B by adding basic broth acidified by 3% lactic acid.

During incubation, pH was monitored with a WTW microprocessor pH Meter (pH 537) using a WTW electrode type E56 (three replicate samples). For the enumeration of lactic acid bacteria, duplicate samples were serially diluted with a diluent processor pH Meter using a WTW electrode type E56 (three replicate samples). For the enumeration of lactic acid bacteria, duplicate samples were serially diluted with a diluent containing 0.1% peptone and 0.85% NaCl in sterile deionized water and serial dilutions were cultivated on MRS-S plates at 22°C for 5 days anaerobically. Histamine and tyramine were detected from duplicate samples at 24 h, 48 h and 6 days of incubation by a high-pressure liquid chromatography method as described by Eerola et al. (8). The detection limit was 1 ppm.

RESULTS AND DISCUSSION

During the growth of Lactobacillus strain G106, the pH decreased relatively slowly in basic broths. However, the lowest pH values attained during the incubation reached 4.2 (MRS-H) and 3.9 (MRS-T). The pH of externally acidified broths (B-D) decreased more rapidly during the first day of incubation, as was intended (Fig. 1 and 2). The growth of strain G106 was clearly decreased by external acidification, especially by GDL and in tyrosine-fortified broths (Fig. 3 and 4). The external pH decrease increased the lag time and decreased the growth rate of the strain. GDL was more effective as a preventative than lactic acid. The growth of strain G106 was decreased more in tyrosine-fortified broth than in histidine-fortified. This was probably due to the lower pH achieved during the first 9 h of incubation (4.7-4.9 in histidine- and 4.4-4.5 in tyrosine-fortified broths).

Histamine and tyramine were formed during the late logarithmic phase of growth (Fig. 5 and 6). Lower levels of amines were formed during 48 h of incubation, in keeping with the decrease in growth caused by the external acidification methods. After 6 days of incubation, the differences in histidine-fortified broths were rather small both in histamine levels and cell numbers. However, in the tyrosine-fortified broths, a clear difference could still be seen after 6 days of incubation. In MRS-T + GDL, hardly any growth was observed, and therefore, no tyramine production occurred.

According to these results, accelerated pH decrease in the beginning of fermentation, either by lactic acid or by
Glucono-delta-lactone prevented the growth of strain G106 more effectively than lactic acid. This could be seen even when the pH was decreased more rapidly by lactic acid than by GDL (e.g., broth C). This may explain the results of our previous experiments in which lower levels of histamine were detected in sausages fermented by starter culture with GDL addition (16). The more preventative effect of GDL on the growth and amine production of strain G106 is difficult to explain. One possible reason is that lactic acid is a natural product of LAB, and therefore, these bacteria are more adapted to protect themselves against its effects.

More histamine and tyramine were produced during incubation when the pH was not externally acidified. Therefore, it would be important to study the effects of different factors such as salt concentration and temperature on the formation of biogenic amines in the fermentation environment. In a fermentor, the pH decreases and not only the initial pH can be monitored and controlled. This would provide information which could be better applied to actual food fermentation processes.

During dry sausage fermentation, the pH decrease is one of the main factors controlling product quality. It appears that means of achieving a rapid pH decrease could also decrease the ability of amine-positive nonstarter LAB to grow and produce amines. These methods include the choice of a good starter culture as well as suitable amounts of food additives (especially sugars), a well-designed processing program, etc. In this in vitro study no competition of other bacteria existed. However, it is probable that with a good amine-negative competitor as starter culture the growth of these kind of amine-positive LAB could largely be prevented. As sausages are often fermented for several weeks, it is also important that the starter culture used can compete with nonstarter LAB during the later phase of ripening. In this way the product quality is maintained and the levels of biogenic amines can be reduced. It is naturally also important to choose good quality raw materials in order to minimize the numbers of possible amine-forming bacteria and proteolytic activity.

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REFERENCES


