A Research Note

Nisin Sensitivity of Lactic Acid Bacteria Isolated from Cured and Fermented Meat Products

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ABSTRACT

Thirty strains of lactic acid bacteria from different meat sources (bologna, summer sausage, thuringer sausage, chicken loaf and bacon) were tested for nisin sensitivity. The maximum concentration of nisin permitting growth for 20 strains was 50 IU/ml. Lactobacilli classified as atypical were sensitive to <5 IU nisin/ml. These strains could not be induced to increase resistance by five transfers to media with increased nisin concentrations. The ten strains with the higher resistance to nisin were checked for nisinase activity. One strain, *Lactobacillus brevis*, showed weak nisinase activity and the rest were negative.

Nisin is an antimicrobial agent produced by certain strains of *Streptococcus lactis*. It is a polypeptide with a molecular weight of about 7000. The antimicrobial activity of this compound is limited to gram-positive organisms (8). Because of its low toxicity, use of nisin as a food preservative has attracted a number of researchers to look for potential applications. The greatest potential now appears to be in the area of dairy technology (8). The use of nisin for meat preservation has not found much success. There are problems of low solubility and heat sensitivity at neutral pH values and possible binding to meat proteins. These problems have limited its application in controlling *Clostridium botulinum* (12,14). Such problems also apply to the control of lactic acid bacteria (LAB), the principal organisms found in vacuum-packaged processed meats (13). The literature is replete in the area of nisin sensitivity of meat LAB because most of the work has been directed towards the streptococci and dairy starter cultures (4,15). Although Hurst (8) and others have indicated that LAB show sensitivity to nisin, the extent of this sensitivity among LAB has not been clearly identified. A further problem has been reports of the enzyme nisinase which inactivates the polypeptide and destroys its activity. Infrequent reports in the literature have shown that certain LAB may possess nisinase (1,4,10). Unfortunately, these reports do not indicate if nisinase is a common property among LAB.

This research was designated to gather information about the range of sensitivity of LAB isolated from commercial meat products and to determine if nisinase is common in such isolates. This information would lead to understanding why nisin is ineffectual in meat systems.

MATERIALS AND METHODS

Isolation and identification of lactic acid bacteria from meat products

Thirty strains of lactic acid bacteria were isolated from commercial cured meat products (bologna, summer sausage, thuringer sausage, chicken loaf and bacon) as described by Dodds and Collins-Thompson (6).

Preparation of nisin solutions

A pure nisin preparation with an activity of $4.0 \times 10^7$ IU/g was obtained from Aplin and Barret Ltd. (Towbridge, England). An international unit has been described as the activity exhibited by 1.0 μg of a standard batch of commercial nisin. A stock solution containing 25000 IU/ml in 0.02 N HCl was prepared weekly. The pH was adjusted to pH 3.0 and the solution was autoclaved (15 min at 121°C) and stored at 4°C.

Nisin assay

Nisin concentrations were determined by the plate diffusion method of Tramer and Fowler (16) using *Micrococcus flavus* (supplied by K. Rayman, Health Protection Branch, Ottawa, Ontario) as the assay organism. Some modifications were done to this method to increase the sensitivity of the test (3).

Growth studies

The tolerance of LAB to nisin was determined in test tubes containing 8 ml of MRS broth (Oxoid). Nisin was added from the stock solution to give concentrations of 5, 10, 25, 50, 100, 200 and 500 IU/ml of broth. The tubes were inoculated with 1 ml of an 18-h culture of the selected LAB strain adjusted to contain ca. $10^5$ cells/ml. Inoculated tubes without nisin and uninoculated tubes containing nisin were included as controls. Sterilized distilled water (1 ml) was added to controls. The tubes were incubated aerobically at 30°C (New Brunswick Environmental Shaker 924). At timed intervals over a 24-h period, growth was monitored by determining the optical density at 650...
When necessary, the cultures were diluted in MRS broth. All these analyses were done in triplicate.

**Determination of residual nisin**

After incubation for 24 h, cultures were centrifuged for 15 min at 5000 × g for 30 min (Sorval RT 6000 refrigerated centrifuge). The culture supernatant fluid was separated from the pellets which were resuspended in 1 ml of 0.02 N HCl solution and kept at 4°C until needed for the nisin assay. The culture supernatant fluid was filter-sterilized (Nalgene 0.45 μm) and dialyzed for 24 h against phosphate buffer, pH 7.0. Dialysis was necessary to avoid interference in the assay from other antimicrobial agents produced by some LAB.

Membrane tubing (Spectrapor) with a molecular weight cutoff of ca. 3500 was used for the dialysis of the culture supernatant fluid. Before use, the tubing was filled with sterile distilled water and exposed to a UV lamp for 2 h. The sterile distilled water was then replaced by the supernatant fluid which was dialyzed against phosphate buffer, pH 7.0, for 24 h at 4°C. The phosphate buffer was changed every 6 h.

**Measurement of nisinase activity**

Strains of LAB that were capable of growing in the presence of 25 to 200 IU nisin/ml were selected for this study. Strain selection was based on choosing organisms with sufficient growth in the presence of nisin, thus allowing the possible production of nisinase. These isolates were grown aerobically at 30°C (see growth studies) for 18 h in MRS broth (Difco) in the presence of 50 IU nisin/ml. The initial inoculum level used was 10⁶/ml. After growth, the cells were centrifuged for 15 min at 5000 × g at 4°C to obtain the supernatant fluid and cell pellet. Residual nisin levels were determined in both pellets and dialyzed supernatant fluids by the method above.

Controls were set up using uninoculated flasks plus nisin and inoculated flasks with no nisin. All supernatant fluids that showed greater losses of nisin than the control were filter-sterilized and nisin was added to make up 50 IU/ml. After 12 h, the residual nisin was assayed on the supernatant fluid to determine possible losses as a result of nisinase activity. For a control, the cell-free supernatant fluid was autoclaved. Losses of nisin as a result of autoclaving were determined before the addition of nisin to make up to 50 IU/ml for the different supernatant fluids. The supernatant fluids were then incubated at 30°C for 12 h and a nisin determination was done to determine the percentage nisin recovered.

**RESULTS AND DISCUSSION**

The results depicted in Table 1 represent the range of nisin sensitivity of thirty LAB isolates. Most strains were sensitive to 25 IU nisin/ml or less. The exceptions were strains of Lactobacillus plantarum, Lactobacillus lactis and Lactobacillus viridescens. One strain of Lactobacillus brevis was able to grow in 200 IU nisin/ml (Table 1). The most sensitive strains to nisin were those classified as "atypical" lactobacilli. This group was difficult to classify within the current taxonomic schemes (13). They were isolated from different commercial vacuum-packaged bacon samples and differed from recognized strains of LAB in their patterns of sugar fermentations (2). The atypical lactobacilli examined in this study resembled the "atypical streptobacteria" described by Cavett (5) and Mol et al. (11). Shahani (15) found that among the various *Streptococcus* species he studied, nisin sensitivity varied considerably. The minimum level of nisin required to inhibit milk coagulation by *Streptococcus cremoris* was 2 Reading units (equivalent to IU/ml). The most resistant strain studied by Shahani was *Streptococcus thermophilus* (100 Reading units nisin/ml).

Attempts to increase the resistance of the atypical lactobacilli to nisin by transferring through five cycles in MRS broth in the presence of increasing concentration of nisin (5, 10, 20 and 25) IU/ml were not successful. The resistance of these strains to nisin remained below 10 IU/ml. Carlson and Bauer (4) were able to induce *Bacillus subtilis* and *Staphylococcus aureus* to grow in media containing from 2.5 to 2000 IU nisin/ml after 17 transfers. They were also able to reverse the resistance to nisin after seven passages through nisin-free medium. The difference between our results and those of Carlson and Bauer may be linked to the presence of nisinase enzymes. Both *Bacillus* species and *S. aureus* have been shown to produce a nisinase enzyme (4,9). Nisin depleting LAB as a result of nisinase activity has been reported (1,4,7).

Of the ten isolates studied, only one species of *L. brevis* showed evidence of nisinase activity. This activity, however, was weak (Table 2). This result shows that nisinase activity is not a common characteristic with the isolates used in this study. It also shows that nisinase is not a common feature among meat LAB, hence ruling out this enzyme as a possible mechanism for nisin depletion in meats. This conclusion supports the contention that binding or chemical depletion is the major cause of nisin losses in meats (14).

**ACKNOWLEDGMENT**

This work was supported by the Ontario Ministry of Agriculture and Foods.

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate No.</th>
<th>Max. concentration (IU/ml) permitting growth in 24 h a</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. lactis</em></td>
<td>1</td>
<td>50 b</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td><em>L. brevis</em></td>
<td>4</td>
<td>200</td>
</tr>
<tr>
<td><em>L. brevis</em></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td><em>L. viridescens</em></td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td><em>L. mesenteroides</em></td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Atypical lactobacilli (5 strains; 2)</td>
<td>&lt;5</td>
<td></td>
</tr>
</tbody>
</table>

aGrown aerobically in MRS broth at 30°C.

bArithmetic means of three determinations.

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**TABLE 1. Nisin sensitivity of selected lactic acid bacteria isolated from cured meats.**
TABLE 2. Nisinase activity of strains of lactic acid bacteria from cured meat resistant to 50 IU nisin/ml.

<table>
<thead>
<tr>
<th>Stain</th>
<th>Fraction A</th>
<th>Fraction B</th>
<th>Fraction C</th>
<th>Fraction D</th>
<th>Fraction E</th>
<th>Nisin depleted (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. plantarum</em> (Strain 2)</td>
<td>14</td>
<td>0</td>
<td>35</td>
<td>14</td>
<td>14</td>
<td>72</td>
</tr>
<tr>
<td><em>L. brevis</em> (Strain 4)</td>
<td>8</td>
<td>8</td>
<td>36</td>
<td>13</td>
<td>9</td>
<td>84</td>
</tr>
<tr>
<td><em>L. lactis</em> (Strain 1)</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>70</td>
</tr>
</tbody>
</table>

*Arithmetic means of three determinations.

a,b,A, supernatant fluid after growth in MRS broth at 30°C for 24 h with 50 IU nisin/ml.
B, cell pellet from A.
C, uninoculated broth plus 50 IU nisin/ml in MRS broth at 30°C for 24 h.
D, supernatant fluid before autoclaving and reincubated at 30°C for 24 h with 50 IU nisin/ml.
E, supernatant fluid after autoclaving and reincubated at 30°C for 24 h with 50 IU nisin/ml.

REFERENCES