A Synergism Between Yoghurt Bacteria and Yeasts and the Effect of Their Association Upon the Viability of the Bacteria

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The yeasts that are found in yoghurt, leben and dahi are generally regarded as contaminants in that, as a rule, they result in unpleasant odors, yeasty taste, or bitter taste.

Little is known about the nature of these yeasts and their action upon the chemical constituents and the bacterial flora of yoghurt and the related products. Ram Ayyar (1928) described a yeast found in dahi which fermented dextrose and sucrose, assimilated lactic acid, and peptonized the curd. Dahi lactobacilli grown with this yeast lived for 1 month, whereas, grown alone, the lactobacilli ordinarily died in 7 days. Graham (1943) found that Lactobacillus bulgaricus usually died in 2 to 3 weeks but survived many months when grown with different yeasts. Five of these yeasts did not utilize lactic acid, whereas other yeasts which assimilated lactate did not extend the life of L. bulgaricus.

The author obtained samples of yeast-contaminated yoghurt which, although several months old, contained viable organisms of L. bulgaricus and Streptococcus thermophilus. As a result of this observation, a number of yeasts were isolated from various samples of yoghurt with the purpose of investigating the effect of these yeasts upon the viability of the above bacteria.

MATERIALS AND METHODS

Twenty-seven yeasts were isolated from 35 samples of commercial yoghurt, most of which were obtained from small producers and restaurants in Wilmington (Delaware), Philadelphia and New York City. These yeasts were found to fall into three types and are referred to throughout this paper as y1, y2, and y3. They were isolated by streaking yoghurt on Sabouraud's agar and incubating at 25 C. The ability to sporulate was tested by the first four methods described by Wickerham (1951). The lactic acid and citric acid assimilation tests were carried out according to Wickerham and Burton (1948) and the vitamin deficiency as suggested by Wickerham (1950).

The L. bulgaricus and S. thermophilus cultures were obtained from samples of yoghurt and from the American Type Culture Collection (ATCC). Both organisms were isolated by the double plate technique as recommended by McLaughlin (1946). The lactobacilli and streptococci used are designated as L1, L2, L3, L4, and S1, S2, S3, S4, respectively, L4 being ATCC 9224 and S4 ATCC 7952.

Series of tubes with equal quantities of plain milk, milk with 5 per cent dextrose, and curdled milk containing 0.65 per cent lactic acid were inoculated with each one of the three types of yeasts and incubated at 37 C for 48 hours. The cultures were kept at room temperature and at regular intervals tubes from each series were titrated with $\frac{N}{50}$ NaOH and their pH was determined by the Beckman pH meter.

Bottles containing 50 ml of milk were inoculated in duplicate with each one of the three types of yeasts in combination with Lactobacillus L4 and Streptococcus S4. Both organisms were grown singly to serve as controls. After incubation, one set of cultures was kept at room temperature (20 to 25 C), and the other in the refrigerator (1 to 2 C). In all cultures, the total acidity and the pH were measured at regular intervals.

Tubes of litmus milk were inoculated in duplicate with each one of the four strains of lactobacilli and streptococci in combination with each one of the three yeasts. After incubation, the cultures were removed to room temperature and 48 hours later one set was placed in the refrigerator. Two more similar sets of cultures which, after incubation, were covered with a layer of paraffin oil were prepared and treated in the same way. All cultures were examined at regular intervals for viability by subculturing one loopful in litmus milk, heating it at 62 C for 10 minutes to eliminate the yeast, and examining for growth after 48 hours' incubation.

RESULTS AND DISCUSSION

Table 1 shows the characteristics according to which the isolated yeasts were classified. Their common properties were inability to ferment lactose and assimilate lactic acid, absence of pellicle, failure to sporulate, and a comparatively high growth temperature. The latter may indicate adaptation of these yeasts to the fermentation temperature of yoghurt. On the basis

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of the characteristics referred to by Henrici (1941) for the classification of the asporogenous yeasts it was found that the three types of the isolated yeasts belong to the genus Torulopsis. One of the types, y2, was identified as Torulopsis molischiana according to the key and species description published by Lodder and Kreger-van Rij (1952).

All yeasts had the ability to peptonize milk coagulated by lactic acid, whereas no change was produced in plain milk. This indicates that these yeasts possess proteolytic enzymes which become active under the acid conditions brought about by the lactic acid. The same peptonizing effect was displayed by the y1 and y2 types of yeasts on hydrochloric acid and citric acid milk curds. However, the y3 yeasts, although they grew in these curds, failed to cause proteolysis. As indicated in table 2, the peptonization of the lactic acid curds was followed by gradual decreases in total acidity and pH.

The same type of peptonization took place when the yeasts were grown at room temperature with S. thermophilus or L. bulgaricus, or both. This indicates again that lactic acid plays a significant part in the proteolytic action. Litmus milk cultures of L. bulgaricus and

### Table 1. Differentiation of the isolated yeasts

<table>
<thead>
<tr>
<th>Yeasts</th>
<th>Morphology</th>
<th>Growth Temp.</th>
<th>Fermentation</th>
<th>Assimilation</th>
<th>Vitamin Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shape</td>
<td>42°C</td>
<td>45°C</td>
<td>Dextrose</td>
<td>Galactose</td>
</tr>
<tr>
<td>y1</td>
<td>Long-oval</td>
<td>+</td>
<td>+</td>
<td>AG</td>
<td>A</td>
</tr>
<tr>
<td>y2</td>
<td>Elongated</td>
<td>+</td>
<td>+</td>
<td>A</td>
<td>—</td>
</tr>
<tr>
<td>y3</td>
<td>Oval to round</td>
<td>+</td>
<td>—</td>
<td>AG</td>
<td>A</td>
</tr>
</tbody>
</table>

A = acid; AG = acid and gas.

### Table 2. Decrease of acidity in cultures of yeasts grown in acidified milk

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Initial</th>
<th>After 60 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA</td>
<td>pH</td>
</tr>
<tr>
<td>y1</td>
<td>0.12</td>
<td>6.4</td>
</tr>
<tr>
<td>y2</td>
<td>0.10</td>
<td>6.8</td>
</tr>
</tbody>
</table>

*1 = milk + yeast; 2 = milk + dextrose + yeast; 3 = milk + lactic acid + yeast.

† TA = total acidity expressed in percentage of lactic acid.

### Table 3. Acidity changes in milk cultures of Streptococcus thermophilus and Lactobacillus bulgaricus grown in association with the yeasts at room temperature

<table>
<thead>
<tr>
<th>Streptococcus</th>
<th>Initial</th>
<th>After 80 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA*</td>
<td>pH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.74</td>
<td>4.2</td>
</tr>
<tr>
<td>S + y1</td>
<td>0.76</td>
<td>4.1</td>
</tr>
<tr>
<td>S + y2</td>
<td>0.63</td>
<td>4.3</td>
</tr>
<tr>
<td>S + y3</td>
<td>0.75</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* TA = total acidity.

### Table 4. Extension of viability of Streptococcus thermophilus and Lactobacillus bulgaricus strains when grown with the yeasts

<table>
<thead>
<tr>
<th>Streptococcus</th>
<th>Litmus Milk</th>
<th>Lactobacillus</th>
<th>Litmus Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>22-34*</td>
<td>55-70</td>
<td>25-40</td>
</tr>
<tr>
<td>S + y1</td>
<td>110-150</td>
<td>230-260</td>
<td>20-35</td>
</tr>
<tr>
<td>S + y2</td>
<td>110-150</td>
<td>240</td>
<td>20-35</td>
</tr>
<tr>
<td>S + y3</td>
<td>85-140</td>
<td>200-240</td>
<td>15-30</td>
</tr>
</tbody>
</table>

* Figures indicate minimum and maximum viability in days observed with four isolates of streptococci or lactobacilli.
S. thermophilus grown with yeasts of the three types showed the usual acid coagulation reaction. However, upon standing at room temperature the cultures exhibited complete reduction followed by gradual peptonization which began with the formation of a purple ring on the surface and ended, after 7 to 8 weeks, in complete proteolysis. This synergistic proteolysis was followed by a proportionate decrease in acidity as shown in table 3. The same cultures, when kept in the refrigerator, showed no appreciable signs of peptonization and practically no change in acidity even after 3 months.

In order to define the nature of the decrease in acidity, determinations of lactates were carried out at regular intervals in cultures of bacteria grown with and without yeasts. The method used (Heinemann, 1940) consisted of dehydrating the separated lactates to acetaldehyde, which then was determined colorimetrically through its reaction with veratrole. The results of these tests showed that in the case of cultures containing y1 and y2 yeasts, the lactic acid content was equivalent to the initial total acidity and it remained constant throughout the experiment. In the case of the y3 yeasts the lactates decreased concurrently with the acidity and totally disappeared at the end of the sixth week. This showed that the y3 yeasts which did not assimilate lactic acid in the special medium did so when grown in milk and in association with the lactic acid bacteria. Determinations of ammoniacal nitrogen in y1 and y2 cultures showed that at the last stages of peptonization, ammonia will account for from 70 to 75 per cent of the lactic acid present. The remaining lactic acid can probably be accounted for as calcium lactate which is formed by the liberation of calcium from the casein breakdown.

Table 4 shows that, with one exception, the bacteria lived appreciably longer when associated with the yeasts than when grown alone; their viability was extended by four to five times at room temperature and by seven to eight times under refrigeration. The exception occurred in the paraffin oil cultures kept at room temperature in which the viability of the bacteria did not improve. It is probable that the permanent anaerobic conditions created by the continuous growth of the bacteria under the cover of the paraffin oil had interfered with the normal growth of the yeasts and, as a result, with their protective action. This view is supported by the fact that, although the yeasts were present, the litmus remained reduced in the cultures for months without the slightest sign of peptonization of the milk.

It has been generally believed that the early death of S. thermophilus and L. bulgaricus cultures is due to the toxic effect of the accumulated lactic acid and that elimination of this acid contributes to the maintenance of their viability. This view appears to be confirmed by the finding that the viability of these bacteria was increased as a result of their association with yeasts which, through synergism, either neutralized or assimilated lactic acid. The additional fact, however, that the viability of the same cultures was equally improved by refrigeration, during which lactic acid remained unchanged, leads to the broader conclusion that the extension of viability of the bacteria may have been induced also, or exclusively, by factors other than the elimination of lactic acid.

Acknowledgment

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Summary

A number of nonlactic fermenting yeasts belonging to the genus Torulopsis were isolated from yoghurt.

These yeasts had the ability to peptonize milk only in the form of acid curd. Growth of the yeasts in milk with strains of Streptococcus thermophilus and Lactobacillus bulgaricus resulted in synergistic peptonization followed by gradual decrease in acidity.

It was found that the decrease in acidity, depending on the type of yeasts, was brought about either through assimilation of lactic acid or through its neutralization by the by-products of proteolysis.

As a result of the associated growth of the yeasts with the bacteria the viability of S. thermophilus and L. bulgaricus strains was maintained in milk for 5 to 8 months. It is suggested that such a procedure as described in this study may be used for the maintenance of cultures of the above bacteria.

References


Heinemann, B. 1940 A rapid colorimetric method for the determination of lactic acid in milk. J. Dairy Sci., 23, 960-972.


