A LACTOSE FERMENTING YEAST PRODUCING FOAMY CREAM

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Large amounts of cream are lost during the hot summer months from an undesirable fermentation known as "foamy cream." This loss is due chiefly to the mechanical action of the fermentation, rather than to the effect of undesirable odors and flavors, for often one-third to one-half of the cream is lost from the can through foaming while in transit. The fermentation is best identified by this characteristic foaming action and by the yeasty or fruity odor imparted to the cream.

EXAMINATION OF CREAM

Numerous samples of foamy cream collected from different parts of Kansas by the State Dairy Commissioner have been sent to the bacteriological laboratory for analysis. The microbial flora as revealed by direct microscopic examination and by plate cultures proved to be similar for all samples. The cream was plated on whey and lactose agar respectively. The latter medium was acidified with 1 cc. of a 1 per cent tartaric acid solution per tube of medium.

Yeast cells were very prominent, as well as rod shaped bacteria, which appeared as short or long filaments and showed numerous granules on staining. The latter organism upon isolation proved to be a member of the B. bulgaricus group.

All cream showing such a flora caused foaminess when placed in raw or sterile cream; and the inoculated cream always exhibited a microbial flora identical with that of the original product.
The predominating type proved upon isolation to be a lactose fermenting yeast. Its ability to produce foamy cream was easily demonstrated by placing it in raw or sterile cream. The characteristic action of the organism is noted in figure 1.

PREVIOUS WORK ON LACTOSE FERMENTING YEASTS

In this country, very few investigators have reported the presence of yeasts of this type. An abnormal fermentation in Swiss cheese, as reported by Russell and Hastings (1905) was due to a lactose fermenting yeast. Harrison (1902) observed yeasts capable of fermenting lactose in large numbers in American cheese and milk. He attributed a bitter fermentation in milk and dairy products to their presence.
The majority of lactose fermenting yeasts have been isolated and studied by European investigators. Grotenfelt (1889) described such a yeast isolated from milk and named it *Saccharomyces acidi-lactici*. Beijerinck (1889) isolated a lactose yeast from kefir grains, and one from Edam cheese. The former is known as *Saccharomyces kephyr* and the latter as *Saccharomyces tyrocola*.

While Beijerinck states that both of these yeasts were *Saccharomyces*, other investigators who have studied them assert that both are incapable of sporulating and hence should be classed as Torulae. A non-spore producing yeast was isolated from Grana cheese by Bochicchio (1894) and named *Lactomyces inflans-caseigrana*. A typical lactose fermenting *Saccharomyces* obtained from Emmenthaler cheese, is described by Freudenreich and Jensen (1897). Jensen (1902) also noted two species in butter which were true yeasts. Maze (1903) studied ten different torula from soft cheese. One fermented lactose only, while the others fermented glucose, levulose, maltose and sucrose. Duclaux (1900) describes three lactose fermenting yeasts, isolated by Kayser, Adametz and himself. All are non-spore producing yeasts and capable of fermenting glucose, lactose, sucrose, galactose, invert sugar and maltose, slightly. All are apparently different species however. Another typical *Saccharomyces*, *Saccharomyces fragilis*, was isolated from kefir, by Jorgensen (1911).

According to Lafar (1911) the yeasts of Jorgensen, Freudenreich and Jensen, and Jensen and Maze, are the only lactose fermenting yeasts which can definitely be reported as *Saccharomyces*.

Yeasts capable of fermenting lactose, according to the present classification are grouped as either true or false yeasts. If true yeasts, they sporulate and hence are considered as belonging to Hansen’s fifth subgroup of *Saccharomyces*, if false yeasts, they are non-sporulating varieties, or Torulae. Of the lactose fermenting yeasts studied, representatives of both genera are known. The differentiating factor is sporulation and the observations of different investigators, upon this point, for the same yeasts do not agree. Hansen states that the torula may be only a tempo-
rary stage of development of yeasts. He has demonstrated that, at the least, spore production is not a stable factor, for he has been able to produce an asporogenic race of Saccharomyces by varying the condition of cultivation. This would lead one to doubt the usefulness of attempting to use sporulation in yeasts as of much diagnostic value.

MORPHOLOGICAL, CULTURAL AND BIOCHEMICAL CHARACTERISTICS

The yeast causing "foamy cream" is oval to elliptical in shape, averaging 5 microns in length and 2 microns in breadth.

Typical spores were not demonstrated by cultivation upon gypsum blocks or potato at temperatures of 25°C. and 35°C. According to the present means of classifying yeasts, the organism is therefore a false yeast or torula. The average colony varies from 2 to 3 mm. in diameter; but more minute forms are frequently noted. In appearance the colonies are spherical with smooth edges, having a raised smooth glistening surface. Plate cultures of the organism emit a yeasty or fruity odor. Upon lactose agar it grows moderately, exhibiting a raised, smooth, and dull to glistening growth. It produces a slight cloudiness in sugar broth; no pellicle, acid reaction in litmus milk; and fails to liquefy gelatin. In milk about 0.3 per cent acid is produced. Gas production was demonstrated in glucose, lactose, sucrose, levulose, galactose, maltose and bile lactose; although the action in galactose, maltose and bile lactose was slow and feeble.

The relation of temperature to the rate of fermentation of the organism is represented in table 1. Litmus milk fermentation tubes were inoculated with 0.1 cc. broth culture of the yeast and incubated at 18°C., 25°C. and 37°C., respectively. The results are self explanatory and easily account for the predominance of foamy cream during hot weather. The highest temperature studied, 37°C., is not necessarily the optimum temperature for the development of the organism, but lack of incubation facilities prevented a more exact study of this point.
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TABLE 1
The relation of temperature to fermentation

<table>
<thead>
<tr>
<th>TEMPERATURE OF INCUBATION</th>
<th>DAYS OF INCUBATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>deg. C.</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>No change</td>
</tr>
<tr>
<td>25</td>
<td>Slight acid, gas slight</td>
</tr>
<tr>
<td>37</td>
<td>Acid, gas 100 per cent</td>
</tr>
</tbody>
</table>

*The percentage of gas refers to the amount formed in the closed arm of a Smith fermentation tube.

POWERS OF RESISTANCE

 Thermal death point

Twenty-four hour lactose broth cultures were thoroughly shaken and equal portions placed in sterile capillary pipettes of uniform capacity and thickness of glass. Both ends were sealed by heat and the sealed tubes were then placed in water baths of constant temperatures for ten minutes. At the end of ten minutes the tubes were removed from the water bath and cooled by placing in cold water. The pipettes were opened aseptically and tubes of litmus milk were inoculated with the contents and incubated at 37° for four days. No growth was observed in cultures made from these tubes after exposure to a temperature of 55°C. or higher. The thermal death point therefore lies between 50° and 55°C.

Resistance toward chemicals

The yeast’s resistance toward various chemicals was determined by using a twenty-four-hour lactose broth culture of the organism. This was thoroughly mixed to break up all clumps and masses. Three standardized platinum loops were used for
inoculating the tubes of disinfectants and for transferring the organisms from the disinfectants to the culture media. Sterile litmus milk was used as the culture medium.

The disinfectants employed were: washing soda, calcium hypochlorite, lime, boric acid, cresol and carbolic acid. The vitality of the exposed cultures was determined by incubating

<table>
<thead>
<tr>
<th>DISINFECTANT</th>
<th>STRENGTH OF DISINFECTANT</th>
<th>MINUTES EXPOSED TO DISINFECTANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washing soda</td>
<td>1</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Washing soda</td>
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<td>+ + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Calcium hypochlorite</td>
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</tr>
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<td>Calcium hypochlorite</td>
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<tr>
<td>Lime</td>
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</tr>
<tr>
<td>Lime</td>
<td>5</td>
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</tr>
<tr>
<td>Boric acid</td>
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<tr>
<td>Cresol</td>
<td>2</td>
<td>+ + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Carbolic acid</td>
<td>5</td>
<td>+ + + + + + + + + + + + + + + + +</td>
</tr>
</tbody>
</table>

Growth +; no growth -.

The results are noted in table 2.

The results in table 2 indicate that washing soda exhibits little disinfectant action on the yeast. Exposure for two hours to both 1 and 2 per cent solutions failed to kill the organism, while a 5 per cent solution prevented their growth in seventy-five minutes. Calcium hypochlorite prevents the development of the yeast in 2 and 5 per cent solutions within one minute, while a 1 per cent solution acts similarly in three minutes.
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Lime possesses disinfectant properties in 1, 2 and 5 per cent solutions with three minutes' exposure.
Boric acid exhibits no killing powers in strengths of 1, 2 and 5 per cent after two hours.
Cresol in 0.5 per cent solution fails to destroy the yeast in two hours while 1 per cent kills in three minutes and 2 per cent in one minute.
Carbolic acid in 5 per cent solution is likewise efficient within one minute.

Resistance toward desiccation

Data showing the ability of the yeast to withstand desiccation are somewhat limited. However, the results obtained demonstrate it to be very resistant to drying. Soil and alfalfa stems were placed in sterile containers and inoculated with milk cultures of the yeast. The absence of yeasts in the soil and alfalfa used was assured before inoculation. The substances were examined at frequent intervals to note the effect of desiccation. The yeasts were observed after eighty-six days in large numbers in both substances. No further analysis of the materials was made.

CONCLUSIONS

1. A lactose-fermenting yeast is the essential organism in the abnormal fermentation of cream, known as "foamy cream."
2. Raw or sterile cream inoculated with a pure culture of the yeast shows typical foaming characteristics.
3. The optimum temperature for growth is near 37°C. This accounts for the prevalence of foamy cream during hot weather only.
4. The thermal death point of the yeast is near 55°C. for ten minutes.
5. The yeast offers slight resistance toward efficient disinfectants.
6. The organism is quite resistant towards desiccation.
REFERENCES


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