THE OCCURRENCE OF A RED PIGMENT PRODUCING ORGANISM IN CORN MASH OF THE ACETONE BUTYL ALCOHOL FERMENTATION

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The formation of a pink or red color on the surface of corn mash from plant cookers is not uncommon. This pigment is found not only in samples of corn mash cooked under plant conditions (240° to 280°F.) but also to a limited degree in mash prepared in the laboratory. A pale pink color is first noted on the surface of mash which has been allowed to stand for two to three days or longer and this pigment later changes to a dark red or reddish brown.

Since the pigment is not commonly found in the mash heated at low temperatures, but in samples heated above 100°C., it was thought at first that air contamination, possibly mold spores, might be the cause. No evidence, however, could be found to support this supposition. As a result of many tests it was found that samples of mash exposed to air contamination rarely developed a pink or red pigment. More conclusive evidence concerning the cause of the pigment was obtained from microscopic examinations of pink corn mash. Samples which were taken directly from the cookers at the end of the cooking cycle and from various parts of the filling line were stored at 37°C. Great care was taken to draw these samples under conditions which prevented outside contamination. At first it was thought that this contamination was due to molds; after

1 This work was supported in part by a grant from the special research fund of the University of Wisconsin. The cultures were obtained through the courtesy of the Commercial Solvents Corporation of Terre Haute, Indiana.
two to six days, however, such mashess do not show the presence of mold threads but instead show numerous rod-shaped cells, with spores.

Presumably pigment formation is a product of the growth of a highly resistant spore-forming bacterium. In the present paper are reported the results of a study of bacteria found in this colored corn mash from the plant cookers. Some of the more important characteristics are described including the resistance of the spores to heat.

PROCEDURE

To obtain pure cultures of the causative organism, five samples of colored mash from different sources were diluted and isolation plates poured. Corn mash and glucose-beef-peptone-agars were used. Transfers were made from well isolated colonies on these two media and these sub-cultures were replated to insure purity. Without exception the types of colonies obtained from the various sources were alike in appearance, namely a thin spreading growth, of cream-colored colonies. The old colonies showed the presence of spores. Although white or cream-colored and in this respect unlike the growth in corn mash, the presence of spores suggested a possible relationship between these colonies and the chromogenic organism of the mash. When sub-cultures were made from these white colonies into tubes of corn mash and allowed to grow for two to three days, it was found that the colonies which were white on the agar medium produced a decided pink color in the corn mash. Because of this difference in color on various media, a series of tests was made in which different sources of carbon were tried. Of the various substances tested, none gave such a deep red pigment as potato or corn mash.

CULTURAL CHARACTERISTICS OF RED PIGMENT PRODUCER

Surface colonies, ten to twelve hours old consist of a group of wavy threads which radiate from the center. These threads become filamentous and continue to develop as the culture grows
older. Because of this peculiar filamentous growth, the young colonies of the red organism may be mistaken for the granulated lactic acid organism (Thaysen, 1921), a contaminant found in fermenting corn mash.

The rate of growth and general appearance of the bacterial mass is quite different from that of B. mesentericus-vulgatus. The results of many cultural tests showed that the general characteristics of this organism are almost identical with those described by Globig as early as 1888. It belongs to the Mesentericus group and has been described by Migula (1900), by Lawrence and Ford (1916), and also by Bergey as Bacillus globigii (1923), and by Lehmann and Neumann (1920), as Bacillus mesentericus-ruber. All the cultural and morphological tests as reported by these various investigators were carried out and with essentially the same results. It is, therefore, considered unnecessary to report all of these tests. There are, however, certain points which deserve special emphasis. Old potato cultures have a distinct odor of cooked ham.

Some of the cultural characteristics of Bacillus globigii (Migula) are given below:

Morphology. Occurs singly or in short chains. Towards Gram stain variable, some cells positive and some negative. Spores formed in old cultures after six to ten days. Actively motile in twenty-four-hour-cultures by means of peritrichous flagella.

Oxygen requirements. Aerobic.

Gelatin stab. Liquefaction. Shows a pale reddish brown color.

Broth. Fragile white scum.

Agar colonies. Growth spreading, filamentous, irregular, lobed, raised, cream colored.

Litmus milk. Slow peptonization and soft curd.

Temperature relation. Abundant growth at 37°C.

Catalase. Positive.

Glucose yeast agar slant. Thin, spreading growth over entire surface of medium. No pigment.

Potato agar. Thin spreading growth. A slight reddish brown color.

Corn meal agar. Similar to potato agar but very slight color.

Potato slope. At first a pink to red growth which rapidly spreads over the surface. After three days at 37°C., a dark reddish brown.
Corn mash. Surface after three days a faint pink and five to ten days later, a beautiful deep pink.

Starch. Hydrolized. After seventy-two hours at 28°C. a 1.5 cm. wide clear zone on starch agar plates.

Pigment production. Bacillus globigii grows rapidly over the surface of nutrient broth, and of agar, with or without various sugars, but fails to form any distinct red pigment. Approximately the same thing is true of cultures grown on media in which the beef-extract-peptone broth is replaced with yeast water. Towards the natural food products this member of the Mesentericus group behaves differently, producing on potato slopes and corn mash, a beautiful pink to red color.

The effect of varying the hydrogen ion concentration of the medium on the production of color was studied. It was found that within a wide range, change in reaction has only a slight effect on chromogenesis.

THE FERMENTATION OF VARIOUS CARBOHYDRATES

The fermentations were made as follows. One per cent of the desired compound, glucose, mannose, sucrose, fructose, maltose, lactose, and mannitol was dissolved in yeast water and this medium sterilized for thirty minutes at 10 pounds pressure. Duplicate flasks of each compound were inoculated with a young culture of B. globigii and incubated at 37°C. It was found that B. globigii attacks all of these carbon compounds. At first it forms a small amount of acid from these substances and this acid is later destroyed. If the culture medium is kept in shallow layers, the destruction of the sugars is rapid.

From the results of studies (Berthelot and Ossart, 1921; Fred, Peterson and Anderson, 1923) carried out with other members of the B. mesentericus group, for example, B. vulgatus it seemed probable that this red form would produce acetone and ethyl alcohol from the sugars. To test this point large cultures of glucose, xylose and corn mash were prepared. After sterilization these media were inoculated with spores of B. globigii, incubated at 37°C. for five days and analyzed. The methods for
measuring the products of fermentation have been described elsewhere (Fred, Peterson and Davenport, 1919; Fred, Peterson and Anderson 1921). Since no attempt was made to carry out the fermentation in a closed system, it is certain that the gaseous products and some of the volatile substances escaped. Within twelve hours after inoculation there was a profuse growth of B. globigii in all of the media.

In table 1 are given the results of the analyses of the glucose and xylose cultures. The corn mash failed to show any appreciable amount of acetone and ethyl alcohol and hence is not included in the table.

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Acetone and ethyl alcohol production from glucose and xylose</th>
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<tbody>
<tr>
<td></td>
<td>Calculated for 100 cc. of culture</td>
</tr>
<tr>
<td></td>
<td>grams</td>
</tr>
<tr>
<td><strong>Glucose:</strong></td>
<td></td>
</tr>
<tr>
<td>At beginning</td>
<td>1.86</td>
</tr>
<tr>
<td>At end</td>
<td>0.29</td>
</tr>
<tr>
<td>Fermented</td>
<td>1.57</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.154</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>0.307</td>
</tr>
<tr>
<td><strong>Xylose:</strong></td>
<td></td>
</tr>
<tr>
<td>At beginning</td>
<td>1.85</td>
</tr>
<tr>
<td>At end</td>
<td>0.79</td>
</tr>
<tr>
<td>Fermented</td>
<td>1.06</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.029</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>0.051</td>
</tr>
</tbody>
</table>

The data are in agreement with an earlier report on aerobic pentose fermenters (Fred, Peterson and Anderson, 1923). Both glucose and xylose are rapidly fermented with the production of small amounts of acetone and ethyl alcohol. If calculated on the percentage of sugar consumed, the acetone equivalent for glucose is 9.8 per cent and for xylose 2.7 per cent. The alcohol is approximately twice as high, 19.5 per cent for glucose and 4.8 per cent for xylose.

**CHANGES IN THE NITROGENOUS COMPOUNDS OF CORN MASH**

Aside from the changes in the carbon compounds, the proteolytic action of B. globigii on corn mash was studied. The methods
outlined in a previous paper (Peterson, Fred and Domogalla) were followed. Unlike the acetone-butyl alcohol organism, it was found that the red pigment producer brings about only a slight hydrolysis of the proteins of the corn. After twenty-four days incubation the percentage of total nitrogen in the form of soluble compounds had risen from 13 to 24.1 per cent.

Resistance of *B. globigii* spores to heat. In the early paper of Globig great emphasis is laid on the heat resistance of the spores of this organism. He found that a temperature of 113° to 116°C. for twenty-five minutes is required to destroy the spores.

The object of the present test was to determine the heat resistance of *B. globigii* spores in a neutral medium and in corn mash. The method employed was similar to that employed by Bigelow and Esty (1920) and their co-workers. A suspension of spores, three weeks old on beef-peptone-agar was prepared. The spores were scraped from the surface of the agar and suspensions prepared in a neutral phosphate solution of pH 7.0. The total number of spores per cubic centimeter of this suspension was 250,000,000. One cubic centimeter portions were pipetted into special glass tubes 0.6 cm. in diameter and then sealed. These sealed tubes were subjected to a temperature of 115°C. for varying lengths of time. In this neutral phosphate solution it was found that the spores are alive after ten minutes at 115°C. but are destroyed after fifteen minutes.

A second test with four week old spores of *B. globigii* was carried out in a corn mash medium. The number of spores added to each tube was much smaller than in the first test, 2,530,000 instead of 250,000,000. The results follow:

<table>
<thead>
<tr>
<th>TIME IN MINUTES</th>
<th>5</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination of spores</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In the corn mash medium the spores of *B. globigii* are killed after thirteen minutes. A similar test with a culture of 220,000,000 spores per cubic centimeter of *B. mesentericus-vulgatus* gave approximately the same results.
Because of the great heat resistance of *B. globigii* it is not surprising that it may be found in mash which has been cooked under pressure. No doubt the occurrence of this organism in the supposed sterilized mash depends to a large degree on the amount of contamination. Mash richly seeded with the spores of this organism is naturally much more difficult to sterilize than mash of the same concentration but low in numbers of spores.

**THE ASSOCIATED GROWTH OF B. GLOBIGII AND B. GRANULOBACTERPECTINOVORUM**

The effect of various aerobic spore forming bacteria, *B. mesentericus-vulgatus*; *B. globigii* and members of the mesentericus group, on the growth of the acetone-butyl-alcohol organism has been studied. Since the mesentericus organisms are aerobic and the *B. granulobacter* anaerobic it is probable that their associated growth will show a symbiotic relationship.

The results of many tests indicate that this association is without injury to the butyl alcohol organism. Flasks of 5 per cent corn mash were divided into two groups. Five flasks received only the acetone butyl alcohol organism while five other flasks received the same organism and also *B. globigii*. After three days incubation at 37°C the amount of solvents was determined.

<table>
<thead>
<tr>
<th>NUMBER</th>
<th>YIELD OF SOLVENTS PER 1000 CC. OF CULTURE</th>
<th>AVERAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>B. granulobacter-pectinovorum</em> alone</td>
<td>16.1</td>
</tr>
<tr>
<td>2</td>
<td><em>B. granulobacter-pectinovorum</em> alone</td>
<td>15.9</td>
</tr>
<tr>
<td>3</td>
<td><em>B. granulobacter-pectinovorum</em> alone</td>
<td>14.3</td>
</tr>
<tr>
<td>4</td>
<td><em>B. granulobacter-pectinovorum</em> alone</td>
<td>14.8</td>
</tr>
<tr>
<td>5</td>
<td><em>B. granulobacter-pectinovorum</em> with <em>B. globigii</em></td>
<td>14.7</td>
</tr>
<tr>
<td>6</td>
<td><em>B. granulobacter-pectinovorum</em> with <em>B. globigii</em></td>
<td>16.0</td>
</tr>
<tr>
<td>7</td>
<td><em>B. granulobacter-pectinovorum</em> with <em>B. globigii</em></td>
<td>14.2</td>
</tr>
<tr>
<td>8</td>
<td><em>B. granulobacter-pectinovorum</em> with <em>B. globigii</em></td>
<td>15.9</td>
</tr>
</tbody>
</table>
The results of the analysis are shown in table 2. The results of all the experiments with the associated growth of these two organisms indicate that the two organisms may live together without any serious injury to the acetone-butyl alcohol production.

CONCLUSIONS

1. The pink or red pigment found on the surface of cooked corn mash may be caused by a spore former of the mesentericus group.

2. This organism is described in the literature under the name of B. mesentericus-ruber and B. globigii.

3. The spores of this organism are very resistant to heat. In a corn mash the thermal death point of the spores is 13 minutes at 115°C.

4. Color production is most noticeable on potato or on corn mash.

5. In a yeast water medium plus glucose or xylose, B. globigii grows rapidly and destroys a large part of these sugars. The non-gaseous neutral products of the fermentation are acetone and ethyl alcohol.

6. B. globigii may be grown in association with B. granulobacter-pectinovorum without any injury to the acetone butyl alcohol organism.

REFERENCES


MIGULA, W. 1900 System der Bakterien, 554.
