THE FERMENTATION OF ACETYL-METHYL-CARBINOL BY THE ESCHERICHIA-AEROBACTER GROUP AND ITS SIGNIFICANCE IN THE VOGES-PROSKAUER REACTION

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The ability of certain members of the Aerobacter genus to ferment acetyl-methyl-carbinol is indicated by their transitory Voges-Proskauer reactions. These strains produce acetyl-methyl-carbinol from glucose and, consequently, yield positive Voges-Proskauer reactions after incubation for from one to three days in Clark and Lubs' medium (1915). When incubation is continued for longer periods, however, the reactions become negative. Paine (1927), and Williams and Morrow (1928) showed that these bacteria "destroyed" the acetyl-methyl-carbinol which was present in filtrates of young cultures. In their experiments, the sterile filtrates were inoculated with the cultures to be tested and, after various periods of incubation, the presence or absence of acetyl-methyl-carbinol was determined by the Voges-Proskauer method. A negative reaction was considered as evidence that the acetyl-methyl-carbinol had been "destroyed." Supplementary experiments showed that the negative reactions were not due to an exhaustion of peptone. Williams and Morrow concluded, therefore, that "it seems probable that the compound serves as a source of carbon."

Although the work just cited showed that acetyl-methyl-carbinol is "destroyed" by certain members of the Escherichia-

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Aerobacter group, it was not clear whether the bacteria fermented it or reduced it to 2,3-butylene glycol.

In view of the importance given to the Voges-Proskauer reaction in the separation of the Escherichia and Aerobacter genera, and because of the relationship between the content of acetyl-methyl-carbinol and the flavor of certain foods, it seemed to be important to determine directly the ability of Escherichia-Aerobacter strains to ferment acetyl-methyl-carbinol and to utilize this compound as the sole source of carbon. The present paper gives the results of such an investigation.

MATERIALS AND METHODS

In all, 175 cultures, representing all of the common species of the Escherichia-Aerobacter genera, were employed. Of these strains, 130 had been isolated recently from water or from human feces and urine. The others were selected from the laboratory collection. The characteristics of each culture were determined in detail to permit exact taxonomic allocations to be made.

Fermentation tests were carried out in a liquid medium made of peptone, 0.5 per cent; meat extract, 0.3 per cent and acetyl-methyl-carbinol (Lucidol or Eastman), 1 per cent, and adjusted to pH 6.8 or 7.0. Either brom cresol purple or brom thymol blue was added to serve as an indicator. The ability of the bacteria to utilize acetyl-methyl-carbinol, when present in the medium as the sole source of carbon, was determined in a synthetic medium composed of Na(NH₄)HPO₄ + 4H₂O, 1.5 grams; KH₂PO₄, 1 gram; MgSO₄, 0.2 gram; CH₃·CO·CHOH·CH₃, 2 grams and distilled water, 1000 cc. This medium is a modification of Koser’s (1924) citrate medium. In certain experiments the amount of acetyl-methyl-carbinol in the synthetic medium was varied so that it contained 0.5, 1, or 4 grams. The pH of this medium was also 6.8 or 7.0. The media were dispensed in chemically clean culture tubes. Most lots of media were sterilized by filtration through a Berkefeld N candle, but some were sterilized in the autoclave at a pressure of 15 pounds for 20 minutes. Inoculations were made with a needle from broth cultures that were from 12 to 18 hours old. Incubation was at
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37°C. The results of the fermentation tests in nutrient broth were recorded daily for 14 days. The synthetic medium was examined daily for 6 days to detect growth. Every strain which grew in the synthetic medium was carried through at least 2 serial passages to determine its continued ability to utilize acetyl-methyl-carbinol as a sole source of carbon. At the completion of the incubation period, the reaction of the synthetic medium was determined by the addition of a few drops of brom-thymol blue.

RESULTS

Acetyl-methyl-carbinol was fermented by 62 per cent of the strains of Aerobacter oxytocum and by 50 per cent of the strains of Aerobacter aerogenes, but not by any of the strains of Aerobacter cloacae, Aerobacter levans, or by any of the members of either the Escherichia genus or the Escherichia-Aerobacter "intermediate" group. There was never any indication of gas production. Every strain belonging to the Aerobacter genus, with the exception of two, which fermented acetyl-methyl-carbinol in nutrient broth also grew and produced acid in the synthetic medium, and gave a negative Voges-Proskauer reaction in Clark and Lubs' medium, when incubation was continued for from 3 to 5 days. The results of the various tests are summarized in table 1.

Usually, fermentation in nutrient broth was evident within from 2 to 4 days, but with some cultures it could not be detected before from 5 to 8 days. In general, growth occurred in the synthetic medium within 24 hours, but with the strains which exhibited delayed fermentation in nutrient broth it was not evident until the second or third day. The rapidity of fermentation in broth and the growth in the synthetic medium paralleled the rate of destruction of acetyl-methyl-carbinol during continued incubation in Clark and Lubs' medium.

The media which were sterilized in the autoclave and those sterilized by filtration yielded identical results.

In general, the variations in the content of acetyl-methyl-carbinol in the synthetic medium did not affect either the rapidity or the amount of bacterial growth. With a few strains,
however, there was better growth in the media which contained either 0.2 or 0.4 per cent of acetyl-methyl-carbinol than in those which contained either 0.05 or 0.1 per cent.

Bromeresol purple and bromthymol blue served equally well to indicate fermentation.

No correlation was found between the ability to ferment acetyl-methyl-carbinol and any other cultural characteristic.

**TABLE 1**
*Relationship between the fermentation of acetyl-methyl-carbinol and the Voges-Proskauer reaction*

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>NUMBER OF STRAINS</th>
<th>FERMENTATION OF ACETYL-METHYL-CARBINOL</th>
<th>VOGES-PROSKAUSER REACTION (CLARK AND LUBS' MEDIUM) AFTER INCUBATION FOR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Nutrient broth</td>
<td>Synthetic medium</td>
</tr>
<tr>
<td><em>Aerobacter aerogenes</em></td>
<td>15</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Aerobacter oxytocum</em></td>
<td>20</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Aerobacter cloacae</em></td>
<td>22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Aerobacter levan</em></td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia group</em></td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia-Aerobacter &quot;intermediate&quot; group</em></td>
<td>35</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The results show that within the *Escherichia-Aerobacter* group the ability to ferment acetyl-methyl-carbinol is limited to certain strains of *Aerobacter aerogenes* and *Aerobacter oxytocum*, and that only these particular members of the *Aerobacter* genus yield negative Voges-Proskauer reactions in Clark and Lubs' medium when the cultures are incubated for periods exceeding 3 to 5 days. This finding does not interfere with the usefulness of the Voges-Proskauer reaction as a means of separating members of the *Escherichia* and *Aerobacter* genera. It simply necessitates the use of cultures which are less than 3 days old. This is a shorter period of incubation than is usually recommended for
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Routine tests, for example, a period of 4 days is recommended in Standard Methods of Water Analysis (A. P. H. A., 1933). When O'Meara's modification of the Voges-Proskauer test and either Clark and Lubs' medium or Difco V-P medium are employed, almost every member of the Aerobacter genus will yield a positive reaction after incubation for from 12 to 48 hours (Tittsler, 1933; and Levine et al., 1934). Accordingly, cultures to be tested for the production of acetyl-methyl-carbinol should not be more than 2 days old.

All strains of Aerobacter aerogenes and Aerobacter oxytocum produce acetyl-methyl-carbinol from glucose and certain other carbohydrates, but only certain strains are able to ferment it. Obviously, therefore, the intermediate metabolism of all strains is not the same. These bacteria also differ from the citric-acid-fermenting streptococci which reduce acetyl-methyl-carbinol to 2,3-butylene glycol (Hammer et al., 1935). Furthermore, Werkman (1930) found that various cultures of the Aerobacter genus produce acetyl-methyl-carbinol from 2,3-butylene glycol. There is evidence which suggests that these various activities are related to differences in oxidation-reduction potentials.

On the basis of this study it cannot be stated whether or not some members of the Aerobacter genus will ferment the acetyl-methyl-carbinol in certain foods such as butter, bread, coffee and honey, or in tobacco and beer. It is probable, however, that they may under suitable environmental conditions. If they do, the desirable aroma and flavor of many foods will be affected adversely, because these characteristics are dependent upon diacetyl, an oxidation product of acetyl-methyl-carbinol (van Niel, Kluyver and Derx, 1929; Schmalfuss and Barthmeyer, 1929; Visser't Hooft and de Leeuw, 1935; and Hammer, 1935).

SUMMARY

1. The ability to ferment acetyl-methyl-carbinol has been determined for 175 strains of the Escherichia-Aerobacter group.

2. Approximately one-half of the strains of Aerobacter aerogenes and Aerobacter oxytocum fermented this substance, but it was not attacked by any of the strains of Aerobacter cloacae, Aer-
bacter levans, or by any of the members of either the Escherichia genus or the Escherichia-Aerobacter "intermediate" group.

3. Every strain which fermented acetyl-methyl-carbinol also grew and produced acid in a synthetic medium which contained acetyl-methyl-carbinol as the sole source of carbon. These strains also gave negative Voges-Proskauer reactions in Clark and Lubs' medium, when the cultures were incubated for periods exceeding from 3 to 5 days.

4. Cultures of the Escherichia-Aerobacter group to be tested for the production of acetyl-methyl-carbinol should not be more than 2 days old.

5. The relation of acetyl-methyl-carbinol to the flavor of certain foods is discussed.

REFERENCES


