THE FERMENTATION OF ALPHA-METHYL-D-GLUCOSIDE BY MEMBERS OF THE COLI-AREOGENES GROUP

STEWART A. KOSER AND FELIX SAUNDERS

Departments of Hygiene and Bacteriology and of Physiological Chemistry, the University of Chicago

Received for publication, January 11, 1932

In the course of a study of the fermentation of certain substituted sugars by bacteria, it was found that alpha-methyl-d-glucoside was fermented with the production of acid and gas by several strains of Bacterium aerogenes but not by fecal Bacterium coli. Since the separation of these members of the coli-aerogenes group has assumed some interest, it was decided to extend this observation and to test a larger number of cultures.

For this purpose advantage was taken of a collection of Bacterium aerogenes and other related types which were isolated originally from soil and then kept as laboratory cultures for several years. In this collection of soil cultures were representatives both of the aerogenes section and of the soil colon cultures which are methyl-red positive, Voges-Proskauer negative and citrate positive (Koser, 1924). To complete the list of colon group cultures subjected to the test, a number of strains were isolated from normal fecal specimens of man and several of the lower animals: monkey, sheep, rabbit and guinea pig.

The entire series of coli-aerogenes cultures, including the freshly isolated fecal strains and the older soil strains, was then tested a number of times in broth containing alpha-methyl-d-glucoside. Finally, all cultures were subjected to the other tests recommended for differentiation of the members of this group of bacteria. Thus, the fermentation of the glucoside was compared with the utilization of citrate, the fermentation of cellobiose, and the results of the methyl-red and Voges-Proskauer tests.

267
Alpha-methyl-d-glucoside differs from glucose in the substitution of a methyl radical for the hydrogen of the hydroxyl group attached to the number 1 carbon atom, as shown below. Two separate lots of the glucoside were used in our tests. One lot was secured from the Eastman Kodak Company, the other was prepared by one of us. Both lots gave identical results. Our preparation of the glucoside was accomplished by the usual method of refluxing glucose with methyl alcohol containing hydrogen chloride under anhydrous conditions (Patterson and Robertson, 1929). It was recrystallized until it no longer gave any reduction with Fehling’s solution. The melting point was 168°C. (uncorrected).

For fermentation tests, the glucoside was added in 0.5 or 1.0 per cent concentration to ordinary nutrient broth, pH 7.0. Brom-thymol-blue, or in some instances brom-cresol-purple, was added as indicator and the broth tubed with inner inverted fermentation vials. Sterilization was accomplished either by filtration or in the autoclave. In the earlier tests, when it was not known whether the glucoside could withstand autoclave sterilization, Seitz filters were used. A 10 per cent solution of the sugar in distilled water was filtered and then added aseptically to tubes of nutrient broth containing indicator. Later it was found that the results were the same after the usual period of autoclave sterilization. An incubation temperature of 30°C. was employed
FERMENTATION OF ALPHA-METHYL-D-GLUCOSIDE

for most of the tests, though in one instance 37°C. was used with similar results.

In table 1 fermentation of the methyl glucoside is correlated with source of the cultures and with the other differential tests. Among the 103 fecal cultures, 3 were atypical in that they produced visible turbidity in the synthetic citrate medium and fermented cellobiose. The same 3 cultures fermented the glucoside. All of the other fecal cultures were consistently negative. Most of the 28 aerogenes types from soil presented a decided contrast to the fecal coli cultures. Twenty-three fermented the glucoside promptly, 4 produced slow fermentation and 1 gave a negative result. In the case of the methyl red +, citrate + types from soil (the "intermediate" strains) no uniform result was secured. Of a total of 25 strains, 5 produced prompt fermentation, 10 slow fermentation and the remaining 10 were completely negative throughout an incubation period of three weeks. It is evident that in the case of the so-called "intermediate" types, the fermentation of alpha-methyl-glucoside failed to show a correlation with source of the cultures. In separating the aerogenes

<table>
<thead>
<tr>
<th>SOURCE OF CULTURES</th>
<th>NUMBER OF CULTURES</th>
<th>METHYL-Rd RED TEST</th>
<th>VOGUE-PROSKAUER TEST</th>
<th>UTILIZATION OF CITRATE</th>
<th>FERMENTATION OF CELLOBIOSE</th>
<th>FERMENTATION OF ALPHA-METHYL-D-GLUCOSIDE</th>
<th>SECTION OF CULTURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feces (human, animal)</td>
<td>103</td>
<td>+ (103)</td>
<td>- (103)</td>
<td>- (100)</td>
<td>+ (3)</td>
<td>- (99)</td>
<td>+ (4)</td>
</tr>
<tr>
<td>Soil</td>
<td>28</td>
<td>- (27)</td>
<td>+ (28)*</td>
<td>+ (28)</td>
<td>+ prompt (28)</td>
<td>+ prompt (23)</td>
<td>+ 4-6 days (4)</td>
</tr>
<tr>
<td>Soil</td>
<td>25</td>
<td>+ (25)</td>
<td>- (25)</td>
<td>+ (25)</td>
<td>+ prompt (15)</td>
<td>+ prompt (5)</td>
<td>+ 3-4 days (6)</td>
</tr>
</tbody>
</table>

* One of the soil cultures was both methyl-red positive and Vogee-Proskauer positive; otherwise an apparently typical aerogenes.

The number of cultures giving a positive or negative test is shown by the figures in parenthesis. Both acid and gas were produced as a result of fermentation of the alpha-methyl-d-glucoside and of cellobiose.
section from the typical fecal coli, however, the alpha-methyl-glucoside gave a satisfactory differentiation.

In other experiments it was found that the substitution of nutrient agar for broth as a base for the fermentation tests afforded just as reliable a separation of coli and aerogenes types. Gas production was sufficient to tear the agar medium and acid could be shown by the use of a suitable indicator. In some further tests it appeared that the glucoside was fairly stable. A batch of the glucoside broth was prepared, tubed, and sterilized in the autoclave at 15 pounds pressure for twenty minutes. Half of the lot was inoculated immediately with the entire series of coli-aerogenes cultures, while the other half was allowed to stand at room temperature for a month before inoculation. This was then inoculated with the same series of cultures and observed closely for any differences in fermentation, especially by the coli strains. No difference could be observed. Evidently glucoside broth may be kept for a considerable time after autoclave sterilization without detracting from the accuracy of subsequent fermentation tests.

Several preliminary experiments were made to determine the nature of the acids formed as a result of the glucoside fermentation by Bacterium aerogenes. In this case it seemed desirable to simplify the culture medium as far as possible and the synthetic citrate medium (Koser, 1924) was used, with the substitution of 1 per cent of glucoside for the citrate as the only source of carbon. In such a medium the aerogenes cultures developed more slowly than in nutrient broth, often requiring two to three days at 30°C. for the production of visible turbidity and acid. A representative Bacterium aerogenes culture was inoculated into 100 cc. amounts of the synthetic glucoside medium and subjected to chemical analysis after several different periods of incubation at 30°C.

Several cultures were distilled according to the Duclaux method, both with and without preliminary acidification. In no case could any steam-volatile acid be detected in the distillate. Other cultures were acidified and then extracted repeatedly with ether. No acid could be detected in the residue after the ether had been evaporated and qualitative tests for lactic acid were
FERMENTATION OF ALPHA-METHYL-D-GLUCOSIDE negative. Qualitative tests for acetic and tartaric acids were also negative. It is therefore concluded tentatively that the principal final metabolic product of Bacterium aerogenes in the synthetic glucoside medium is carbon dioxide.

SUMMARY
1. Typical Bacterium coli of fecal origin is unable to ferment alpha-methyl-d-glucoside.
2. Members of the aerogenes section usually ferment alpha-methyl-d-glucoside promptly with the production of acid and gas, though a few aerogenes strains produce a slow fermentation or give a negative result.
3. The fermentation of alpha-methyl-d-glucoside serves to separate the aerogenes section from fecal Bacterium coli. It is not so useful, however, for separation of the methyl-red positive soil types from the methyl-red positive fecal Bacterium coli. Both citrate and cellobiose are more reliable for this purpose.
4. Alpha-methyl-d-glucoside appears to be fairly stable. Nutrient broth containing 0.5 or 1.0 per cent of the glucoside can be sterilized in the autoclave and allowed to stand at room temperature for a month before inoculation without interfering with the results of the test.

REFERENCES