FERMENTATION OF RAFFINOSE BY SHIGELLAЕ

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The purpose of this paper is to report the results of studies on the fermentation of raffinose by relatively large numbers of cultures that belonged to the four Shigella subgroups and to the Escherichia coli group. The studies reported comprised a portion of an extensive reinvestigation of the biochemical reactions given by shigellae, including evaluations of the usefulness of several newer tests and methods in taxonomy. The results of the more comprehensive studies will be published elsewhere.

A total of 1263 Shigella cultures were tested in 0.5 percent raffinose broth containing Andrade's indicator. The strains employed were submitted to the laboratory during the last several years, but the majority were recently isolated. After inoculation the raffinose broth tubes were incubated at 37°C and observed daily for two weeks, after which they were examined at intervals of 3 days until a total of 30 days had elapsed.

The results of the tests are recorded in table 1. Cultures that belonged to the Shigella dysenteriae and Shigella boydii serotypes, including several provisional serotypes, did not

1 A mimeographed outline of biochemical methods is available upon request.
ferment raffinose, whereas the majority of *Shigella flexneri*, and all of *Shigella sonnei* strains utilized this substrate. Of 646 *S. flexneri* cultures tested, approximately 59 per cent fermented raffinose (20 per cent within 1 day, 39 per cent delayed) and 41 per cent did not. Only cultures of the mannitol negative variety of *S. flexneri* 4a and *S. flexneri* 6 (all biotypes) consistently failed to produce acid from this test substance. These two types constituted about 27 per cent of the 646 *S. flexneri* cultures tested. Of the remaining 475 *S. flexneri* strains, approximately 81 per cent utilized raffinose (27 per cent within 1 day, 54 per cent slowly) and 19 per cent did not.

Several *S. sonnei* strains were studied to determine whether their fermentation of raffinose was mutative. The cultures were inoculated onto agar plates and after incubation, 15 to 20 colonies from each plate were subcultured to individual tubes of raffinose broth. The progeny of each of the isolated colonies from the *S. sonnei* cultures studied produced acid from raffinose in approximately the same length of time. For example, the progeny from 18 of 19 colonies from one *S. sonnei* culture fermented this substance after 7 days' incubation while the nineteenth tube became acid after 8 days' incubation. The results of these limited studies indicated that fermentation of raffinose by *S. sonnei* strains may not be mutative in character. However, many additional *S. sonnei* cultures should be examined before definite conclusions are drawn. Also, *S. flexneri* strains should be studied in this regard.

In addition to the above-mentioned *Shigella* cultures, 344 *E. coli* strains were tested for raffinose fermentation. Seventy-six of these were aerogenic, motile biotypes and 268 were of the anaerogenic nonmotile biotypes commonly referred to as members of the Alkalescens-Dispar (A-D) group. Of the total *E. coli* cultures studied 58, or 17 per cent, fermented raffinose. Twenty-eight per cent of the 76 aerogenic, motile *E. coli* types and 14 per cent of the A-D group strains produced acid from raffinose. It was of interest to note that cultures belonging to A-D 0 antigen groups 1 (107 strains) and 2 (89 strains) failed to ferment raffinose.

The *Shigella* group or genus is differentiated from other Enterobacteriaceae by means of their biochemical reactions
and the group is divided into four subgroups or species by means of a combination of the biochemical and serological characteristics of the types contained in each subgroup. The results of the studies reported indicated that raffinose was of little value in distinguishing members of the Shigella group from anaerogenic, nonmotile \textit{E. coli} cultures in general. However, the use of raffinose may be of assistance in the differentiation of A-D 01 and 02 strains from the majority of \textit{S. flexneri} cultures, since strains belonging to these commonly occurring A-D 0 antigen groups did not utilize raffinose while the majority of \textit{S. flexneri} cultures did so. Of more importance was the fact that within the Shigella group itself, raffinose was of considerable value in the biochemical differentiation of members of subgroups B (\textit{S. flexneri}) and C (\textit{S. boydii}), in the delineation or characterization of the four Shigella subgroups or species, and in the determination of the subgroup to which a culture may belong.

**SUMMARY**

Twelve hundred sixty-three cultures that belonged to the four Shigella subgroups or species and 344 \textit{Escherichia coli} strains were tested for their ability to ferment raffinose. Members of subgroups A (\textit{Shigella dysenteriae}) and C (\textit{Shigella boydii}) did not utilize this substance whereas the majority of subgroup B (\textit{Shigella flexneri}) and all of the subgroup D (\textit{Shigella sonnei}) cultures did so. The majority of the \textit{E. coli} strains tested failed to produce acid from raffinose.

The results indicated that the use of raffinose as a test substrate was of particular value in the biochemical differentiation of strains belonging to the \textit{S. flexneri} and \textit{S. boydii} subgroups and in the delineation or characterization of the four Shigella subgroups or species.
Table I. Fermentation of Kaffinose by Shigellae

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Tested 1 day</th>
<th>Positive Delayed</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. dysenteriae</td>
<td>42</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. flexneri</td>
<td>26</td>
<td>4</td>
<td>21 (3 to 5 days)</td>
</tr>
<tr>
<td>S. boydii</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. sonnei</td>
<td>135</td>
<td>1</td>
<td>134 (2 to 10 days, majority in 5 days)</td>
</tr>
</tbody>
</table>

* Includes all known biotypes

(+) Mannitol positive variety
(-) Mannitol negative variety