THE TAXONOMIC SIGNIFICANCE OF FERMENTATIVE VERSUS OXIDATIVE METABOLISM OF CARBOHYDRATES BY VARIOUS GRAM NEGATIVE BACTERIA

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For many years bacteriologists have observed that some bacteria produce acid from carbohy-
drates only under aerobic conditions while others produce acid both under aerobic and anaerobic
conditions. The significance of these observations does not seem to have been appreciated generally
by taxonomists. Studies of bacterial physiology have made it increasingly evident that the bac-
terial metabolism of carbohydrates may be accom-
plished by two apparently fundamentally
different mechanisms (see for example Porter, 1946; Werkman and Wilson, 1951). By one
mechanism, appropriately called fermentation,
the glucose molecule first is phosphorylated and
then split into two triose molecules which undergo
further changes. This process is independent of
oxygen. By the other mechanism, which we shall
call oxidation, the glucose molecule is not split
into two triose molecules, but the aldehyde group
is oxidized to a carboxyl group forming gluconic
acid. Further oxidation may take place to form
various products such as 2-ketogluconic acid.
Several studies of this mechanism, summarized by
Sebek and Randles (1952), have failed to
detect phosphorylation of the glucose molecule
preliminary to oxidation. In the absence of com-
pounds such as nitrates, the oxidation of carbo-
hydrates is a strictly aerobic process, whereas
fermentation is an anaerobic process.

EXPERIMENTAL METHODS AND RESULTS

The practical distinction between oxidation
and fermentation of carbohydrates rests on the
role played by atmospheric oxygen. The degree
of acidity produced by oxidative metabolism is
usually lower than that produced by fermenta-
tive metabolism. Since most bacteria produce
alkaline substances from peptone, the small
amount of acid which may be produced by oxida-
tive metabolism may be completely neutralized
in the ordinary carbohydrate-peptone media.
These difficulties can be overcome by using a
medium with a relatively high carbohydrate con-
centration, a low peptone concentration, and
aerobic conditions.

The medium which we have adopted to detect
oxidation of carbohydrates and distinguish it
from fermentation has the following composi-
tion: peptone, 0.2 per cent; NaCl, 0.5 per cent;
K₂HPO₄, 0.03 per cent; agar, 0.3 per cent;
bromthymol blue, 0.003 per cent; carbohydrate,
1.0 per cent; pH 7.1.

For the peptone we recommend a pancreatic
digest of casein. Other types of peptone may or
may not be satisfactory. Some bacteria, such as
species of the genus Brucella, seem to grow better
with sodium chloride in the medium. The phos-
phate is added to promote fermentation and to
stabilize the pH. The purpose of the agar is to
prevent convection currents in the medium and
consequent mixing of the acid produced at the
surface with the bulk of the medium. The con-
centration of agar also is optimum for determina-
tion of motility.

The bromthymol blue is dissolved in water
and 0.3 ml of a 1 per cent solution added to each
100 ml of medium. Alcoholic solution of indicator
should not be used because acid may be produced
from the alcohol added. The carbohydrate cannot
be sterilized with the medium because of the
chemical changes which occur. For critical work
and with unstable carbohydrates, sterilization
by filtration is recommended. Practically it seems
that most carbohydrates may be sterilized satisfac-
torily by autoclaving a 10 per cent aqueous
solution. The sterile carbohydrate solution is
added aseptically to the sterile melted base.

The medium is tubed to a depth of about 1 1/2
inches. The oxygen which diffuses into the
medium on storage does not seem to alter ap-
preciably the reactions obtained. Duplicate tubes
of the solidified medium are inoculated by

1 Acknowledgment is made to the Standard
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stabbing. After inoculation one of the pair of tubes is covered with a layer of sterile melted petrolatum to a depth of 1/4 to 3/4 inch.

Several types of reactions may be observed in the medium: fermentative organisms will produce an acid reaction throughout in both tubes.

Oxidative organisms will produce an acid reaction in the open tube only, leaving the petrolatum covered tube unchanged with little or no apparent growth. The acid reaction produced by the oxidative organisms is apparent first at the surface and extends gradually downward into the medium. Where the oxidation is weak or slow, it is usual to observe an initial alkaline reaction at the surface of the open tube. This may persist for a variable length of time, up to several days of incubation, before turning acid and must not be mistaken for a negative reaction. Nonfermenters and nonoxidizers produce no change in the covered tube and only an alkaline reaction in the open tube. Organisms which oxidize glucose but do not ferment it have never been observed to ferment any carbohydrate, and the petrolatum covered tube may be omitted in subsequent tests with other carbohydrates.

### TABLE 1

**Carbohydrate metabolism of some representative types of bacteria**

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>GLUCOSE</th>
<th>LACTOSE</th>
<th>SUCROSE</th>
<th>GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open</td>
<td>Covered</td>
<td>Open</td>
<td>Covered</td>
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<tr>
<td><em>Alcaligenes faecalis</em></td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
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<tr>
<td><em>Bacterium anitratum</em></td>
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<tr>
<td><em>Agrobacterium tumefaciens</em></td>
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<tr>
<td><em>Malleomyces pseudomallei</em></td>
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<td>A</td>
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<tr>
<td><em>Shigella dysenteriae</em></td>
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<tr>
<td><em>Shigella sonnei</em></td>
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<td>A</td>
<td>A</td>
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<tr>
<td><em>Vibrio comma</em></td>
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<td>A</td>
<td>A</td>
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<tr>
<td><em>Salmonella enteritidis</em></td>
<td>AG</td>
<td>AG</td>
<td>AG</td>
<td>AG</td>
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<tr>
<td><em>Escherichia coli</em></td>
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<tr>
<td><em>Aeromonas liquefaciens</em></td>
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<td>AG</td>
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<tr>
<td><em>Aerobacter aerogenes</em></td>
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<td>AG</td>
<td>AG</td>
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</tr>
<tr>
<td><em>Unclassified species</em></td>
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<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td><em>Some paracolon bacilli</em></td>
<td>AG</td>
<td>AG</td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

**Legend:** — means neutral or alkaline reaction; A means acid reaction; AG means acid and gas reaction.

* Typical strains supplied by W. W. Ferguson, Michigan State Department of Health.
† Typical culture supplied by A. C. Hildebrandt, University of Wisconsin.
‡ Typical culture supplied by S. T. Cowan, National Collection of Type Cultures, England.

Our studies show that most gram negative bacteria that metabolize carbohydrates do so either exclusively oxidatively or fermentatively. Either reaction may be so weak or slow that the results are doubtful. Some bacteria apparently both ferment and oxidize carbohydrates. The medium will not demonstrate oxidation of a carbohydrate which is actively fermented by the organism. If the fermentation is weak or slow, oxidative metabolism may be demonstrated. The group of bacteria commonly called paracolon bacteria apparently may oxidize lactose, ferment lactose, or carry out both reactions. With some strains acid
is produced in the open tube fairly promptly, while the petrolatum covered tube very slowly increases in acidity during several weeks of incubation. These organisms may be true lactose oxidizers, and the slowly developing acidity in the covered tube may be due to unknown factors, or they may have a very slight fermentative ability towards lactose. In any case the main action on lactose is oxidative. Others of these bacteria appear to have little or no oxidative action on lactose. The open tube develops an alkaline top with a faint to fairly acid butt, while the covered tube slowly becomes increasingly acid. These are the typical slow lactose fermenters.

In table 1 are recorded the typical reactions of a variety of bacteria. The bacteria were selected to illustrate the various types of reactions which may be obtained on the medium.

DISCUSSION

The fermentative species of gram negative bacteria seem to form a group distinctly different from the nonfermentative species. The fermentative bacteria are typically facultative, while the nonfermentative bacteria are generally strictly aerobic. In our experience growth of a nonfermenter in the absence of air (and substances such as nitrate) is very rare. Indole production by nonfermenters has never been observed by us, and we have tested several hundred strains of various species from various sources. Most fermentative strains reduce nitrates to nitrites but failure to do so is common among the nonfermenters.

While the commonly accepted taxonomic schemes tend to separate the fermenters and the nonfermenters into different genera, there are some exceptions. These exceptions are partly due to failure to distinguish between oxidative and fermentative metabolism of carbohydrates. In Bergey's manual of determinative bacteriology, 6th edition (Breed et al. 1948), several species of fermenters are classified in the genus Pseudomonas, the type species of which is a typical glucose oxidizer. We heartily support the suggestion from several sources that the species of fermentative, polar monotrichous, gram negative rods be placed in a separate genus, namely Aeromonas. In the Manual we also find both fermenters and nonfermenters in the genus Vibrio. The type species, Vibrio comma, is a fermenter, and we suggest that only fermentative organisms be included in the genus. Two species, described in the 6th edition of Bergey's manual as Vibrio perolans and Vibrio cuneatus, were obtained from the American Type Culture Collection. Vibrio perolans was found to be a fairly straight rod with lophotrichous flagella and with no detectable action on carbohydrates. Vibrio cuneatus also was found to be a fairly straight rod with polar multitrichous flagella. The action on carbohydrates was typically oxidative and not fermentative. Neither of the two species seems related to Vibrio comma either physiologically or morphologically, and the body curvature they may have shown at one time is no longer evident.

We have examined several strains of the organism named Bacterium anitratum by Schaub and Hauber (1948). These strains were all typical oxidizers of carbohydrates, such as glucose and lactose, and not fermenters. The failure of the organism to reduce nitrates to nitrites, while sufficiently unusual among the fermenters to justify the epithet "anitratum", is a fairly common characteristic among the nonfermenters.

SUMMARY

The basic difference between oxidative and fermentative metabolism of carbohydrates is discussed. A method is presented which serves to distinguish between the two metabolic patterns. The reactions in the medium of various types of bacteria are given, and the taxonomic significance of these reactions discussed.

REFERENCES


