Growth Factor Requirements of Ruminal Cellulolytic Bacteria Isolated from Microbial Populations Supplied Diets With or Without Rapidly Fermentable Carbohydrate

L. L. SLYTER and J. M. WEAVER

Animal Science Research Division, U.S. Department of Agriculture, Beltsville, Maryland 20705

Received for publication 21 July 1971

The predominant cellulolytic ruminal bacteria isolated from microbial populations supplied diets containing cellulose as an energy source and essentially devoid of amino acids or rapidly fermentable carbohydrates were shown to require branched-chain acid(s) for growth.

In ruminants consuming natural forage, ruminal cellulolytic bacteria require branched-chain fatty acids for growth (1, 2, 3, 8), and these acids appear to be formed mainly by degradation and deamination of branched-chain amino acids from dietary protein by some of the noncellulolytic bacteria and ciliate protozoa (6, 7, 9). However, diets essentially devoid of amino acids have been shown to support large numbers of cellulolytic bacteria which require branched-chain acids for growth and the rumen was shown to contain these acids (20). This suggested that branched-chain acids secreted as protein into the rumen by the host animal or biosynthesized mainly from carbohydrate by noncellulolytic microorganisms are sources of the branched-chain fatty acids. The cellulolytic bacteria previously isolated (20) were from steers fed diets which contained 12.8% starch (19), and readily fermentable carbohydrates, such as starch, have been suggested to modify the ruminal population to satisfy the strict growth requirements of cellulolytic microorganisms (10).

To test the role of readily fermentable carbohydrates as precursors for growth factors, we isolated 65 strains of ruminal cellulolytic bacteria from $10^4$ dilutions of steer or in vitro fermentor contents and determined their nutritional requirements (Table 1) with methods previously described (20). The cellulolytic bacteria from samples in which wood pulp was the sole carbohydrate source (19) were of similar type, were present in greater numbers (18), and had nutritional requirements similar to cellulolytic bacteria obtained from steers fed starch and wood pulp (18) or starch, glucose, and wood pulp (17). These results suggest that animal diets containing readily fermentable carbohydrate(s) are not needed to maintain large numbers of cellulolytic bacterial with branched-chain fatty acid requirement.

In the present study, four Ruminococcus strains isolated from a protozoal-free in vitro culture, supplied with 17.7 g of a biuret-wood pulp diet (19) twice daily for 2 weeks, required branched-chain fatty acids for growth. This indicates that branched-chain acids can be synthesized directly by other bacteria or that branched-chain amino acids are synthesized and then degraded by other bacteria to form the fatty acids (13, 15).

The conditions required for optimal growth of 27 strains of ruminococci and 4 strains of Bacteroides succinogenes isolated from the steers in the present study are similar to those for strains of ruminococci (1, 2, 8) and B. succinogenes (3, 8) isolated from ruminants fed natural diets. Of the ruminococci, nine required branched-chain acids, nine were stimulated by branched-chain acids, and nine required or were stimulated by clarified rumen fluid, whereas none was shown to require both branched- and straight-chain acids. As expected (3), all four B. succinogenes strains required both branched- and straight-chain fatty acids. The growth factor(s) present in clarified rumen fluid, which was required for the growth of 29% of the ruminococci and 37% of unidentified bacteria, was not identified. However, the unidentified bacteria which grew in clarified rumen fluid were not tested in the straight- plus branched-chain medium and might require a combination of straight- and branched-chain fatty acids.
Table 1. Nutritional requirements of ruminal cellulolytic bacteria isolated from steers fed different carbohydrate and nitrogen sources

<table>
<thead>
<tr>
<th>Growth factora</th>
<th>Percentage of strains requiring growth factorb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wood pulp</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
</tr>
<tr>
<td>Clarified rumen fluid</td>
<td>30.8</td>
</tr>
<tr>
<td>Branched-chain fatty acids</td>
<td>61.5</td>
</tr>
<tr>
<td>Casitone</td>
<td>7.7</td>
</tr>
</tbody>
</table>

a Basal medium contained cellobiose, Casitone (vitamin-free), minerals, hemin, cysteine, sulfite, and B vitamins as previously indicated (20). Clarified rumen fluid growth factor requirement is indicated when optimal growth occurs in basal medium plus rumen fluid but not in basal medium or basal medium supplemented with branched-chain acids or straight-chain acids. A branched-chain fatty acid requirement is indicated if the strain grows in basal medium supplemented with branched-chain fatty acids and with clarified rumen fluid but does not grow in the basal medium or basal medium supplemented with straight-chain fatty acids. In preparing media with fatty acids, the basal medium was supplemented with acids in one-tenth the amount previously described (20). In addition, a branched-plus straight-chain fatty acid medium was prepared which contained the following acids (millimoles/100 ml of medium): isobutyric, 0.013; 2-methyl-butyric, 0.013; isovaleric, 0.013; acetic, 0.8; propionic, 0.13; n-butyric, 0.065; and caproic, 0.006.

b Percentages in columns, left to right, are based on the study of 13, 16, 14, 13, 6, and 3 strains, respectively. Within these groups were six, five, seven, six, two, and one strains of ruminococci, respectively, and zero, one, zero, one, one, and one strain of Bacteroides succinogenes, respectively. The ruminococci and B. succinogenes, but not the unknown cellulolytic strains, were tested for a combined branched- and straight-chain fatty acid requirement for growth. For this reason, the four Bacteroides strains which required the branched- plus the straight-chain fatty acids are listed with those which require clarified rumen fluid. None of the ruminococci required the combined acids for growth.

The nutritional data from this study, although indicating that branched-chain fatty acids are formed by a mixed rumen population fed a diet devoid of amino acids and rapidly fermentable carbohydrates, should not be interpreted to suggest that dietary fatty acid(s) addition could not be stimulatory to animal production. Branched-chain fatty acids have been reported to increase total microbial synthesis (11, 16) and nitrogen retention in ruminants (17), although a beneficial effect has not always been attained (5, 14, 17).

Literature Cited

17. Oljen, R. R., L. L. Slyter, E. E. Williams, Jr., and D. L.

