Comparison of the Sphingolipid Content of Rumen
Bacteroides Species

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Ten strains of Bacteroides ruminicola were found to contain phosphosphingolipids. Four strains of Bacteroides amylophilus and one strain each of Bacteroides succinogenes and Bacteroides sp. were devoid of phosphosphingolipids.

D. Abraham (Ph.D. thesis, Univ. of Maryland, College Park, 1965) investigated the lipids isolated from a mixed population of rumen bacteria and found an unusual sphingolipid containing ethanolamine. Sphingolipids were later isolated from Bacteroides ruminicola strain 23 grown on a chemically defined lipiddo-free medium (J. E. Kunsmann, Jr., I. Katz, and M. Keeney, Abstr. Amer. Chem. Soc. 152nd Meet., 1966). Further analysis of six strains of B. ruminicola confirmed the presence of phosphosphingolipids in this species (7). Exhaustive studies by White and co-workers (8, 10, 12, 13) have shown the presence of high concentrations of phosphosphingolipids in Bacteroides melaninogenicus. Otherwise, sphingolipids are exceedingly rare in bacteria (1).

Ten strains of B. ruminicola, four strains of Bacteroides amylophilus, and one strain each of Bacteroides succinogenes and Bacteroides sp. were supplied from the culture collection maintained by the Division of Microbiology and Veterinary Medicine, University of Wyoming. Anaerobic culture maintenance, media, harvesting procedures, and lipid extraction methods have been previously described (6). The washed total lipids extracted from stationary-phase cells were fractionated on silicic acid columns (9) as described by Kunsmann (7). The phospholipid fraction isolated from the silicic acid columns was assayed for phosphorus as described by Bartlett (2) and modified by Marinetti (9). Lipid containing approximately 300 μg of phosphorus was then subjected to the hydrolysis procedure of LaBach and White (8) to deacylate the diacyl phosphoglycerides. The mild alkaline methanolysis included the acid hydrolysis step of Wells and Dittner (11), which eliminates the presence of plasmalogens. The intact phosphosphingolipids were washed and separated from the resulting fatty acids on a 1-g silicic acid column (7). The purified phosphosphingolipids were analyzed for phosphorus as described above and the percentage of phosphosphingolipid in the total phospholipid fraction was calculated. Table 1 lists the phosphosphingolipid content by species. All strains of B. ruminicola, the most abundant Bacteroides species found in the rumen, contained phosphosphingolipids. The results confirm a previous report indicating that B. ruminicola contains phosphosphingolipids.

| Species            | Strain | Phosphosphingolipid (%) |
|--------------------|--------|--------------------------|
| Bacteroides ruminicola* | 23    | 37.1 (3.0)              |
|                    | B18    | 36.6 (4.1)              |
|                    | B127   | 51.6 (4.4)              |
|                    | GA20   | 56.1 (4.5)              |
|                    | 742-1  | 70.1 (6.5)              |
|                    | GA33   | 56.5 (5.1)              |
|                    | 118B   | 45.5 (1.1)              |
|                    | B4     | 45.3 (2.5)              |
|                    | GA103  | 37.9 (0.1)              |
|                    | 44     | 53.9 (1.7)              |
| B. amylophilus      | 78     | 0                        |
|                    | 1020   | 0                        |
|                    | 1413   | 0                        |
|                    | 70     | 0                        |
| B. succinogenes     | S-85   | Trace*                  |
| Bacteroides sp.     | B107   | 0                        |

* Expressed as percentage of total lipid phosphate.

* Strains 23, B18, B127, GA20, and B-742-1 are strains of B. ruminicola subsp. ruminicola. Strains GA33, 118B, B4, and GA103 are isolates of B. ruminicola subsp. brevis. The taxonomic position of strain 44 is uncertain. The organism appears nutritionally similar to B. ruminicola (3).

* Average of two determinations. Values in parentheses are standard deviations.

* Less than 4% lipid phosphate left intact.
tains large quantities of phosphosphingolipids (7), but the extremely high values (90%) previously reported for phosphosphingolipids in B. ruminicola GA33 could not be duplicated. Among the currently recognized predominant rumen Bacteroides species, phosphosphingolipids appear restricted to isolates of B. ruminicola, since they could not be detected in isolates representing other predominant rumen Bacteroides species (Table 1). Additional studies have shown that, except for B. ruminicola, sphingolipids are rare among ruminal bacterial, since they are apparently absent from Butyryvibrio fibrisolvens (6), Selenomonas ruminantium, Megasphaera (Peptostreptococcus) eldenii, Ruminococcus flavefaciens, and R. albus. These studies also indicate that the ability or inability of rumen bacteria to form phosphosphingolipids is not appreciably influenced by growth media or conditions. The presence or absence of phosphosphingolipids in rumen bacteria is thus apparently a function of the species (J. E. Kunsman, Ph.D. thesis, Univ. of Maryland, College Park, 1966). In the present study, small amounts of sphingolipids (9.7%) were detected in strain B85, an isolate originally designated as Bacteroides sp. (4) but now known to be Fusobacterium necrophorum. F. necrophorum is seldom isolated as a predominant rumen bacterium (4) and is usually obtained from other environments (5). It would be interesting to determine whether F. necrophorum and Bacteroides isolates obtained from other habitats contain phosphosphingolipids.

The taxonomic position of strain 44 is uncertain; however, its comparatively high phosphosphingolipid content (53.6%) combined with its nutritional characteristics (3) suggests its inclusion in the species B. ruminicola. The sphingolipids in B. ruminicola and F. necrophorum are presumably located in cytoplasmic membrane, as they apparently are in B. melaninogenicus (10). Demonstration of these unusual components in two Bacteroides species and F. necrophorum stimulates interest in studies concerning the structure and function of the membranes of these organisms.

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