BUTYRIBACTERIUM, A NEW GENUS OF GRAM-POSITIVE, NON-SPORULATING ANAEROBIC BACTERIA OF INTESTINAL ORIGIN

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The classification of the gram-positive, non-sporulating, anaerobic bacteria of intestinal origin has been a matter of much difficulty. Originally these bacteria were placed by Castellani and Chalmers (1919) in the genus *Bacteroides* along with a number of gram-negative, anaerobic organisms. Since this was obviously unsatisfactory, Eggerth (1935), Prévot (1938), and Weiss and Rettger (1938) decided to separate the gram-positive and gram-negative forms into different genera. The genus *Bacteroides* was redefined[2] (Weiss and Rettger, 1937; Bergey, *et al.*, 1939) to include only gram-negative organisms, while the gram-positive types were placed (King and Rettger, 1942) in the genus *Lactobacillus* on the basis of their morphological, general physiological and cultural similarities to other bacteria of this genus. It was recognized that the gram-positive bacteria under consideration differ from typical lactobacilli in their relations to oxygen. But since various types intermediate between the obligately and facultatively anaerobic species were known to exist, this difference did not appear to require a separation of the two groups.

In one respect, however, the evidence indicating a close relationship between the gram-positive anaerobes of intestinal origin and typical lactobacilli was incomplete. The former organisms had never been shown, with a few exceptions, to carry out a typical lactic acid fermentation. It is true that superficial evidence, obtained merely by the observation of acid and gas production in cultures, indicated a fermentation of the homo- or hetero-fermentative types; large amounts of acid were formed and little or no visible gas could be seen. But the actual fermentation products had not been identified. It therefore seemed desirable to study the fermentations of some representative strains.

Through the coöperation of Professor L. F. Rettger, a number of strains isolated by Dr. K. H. Lewis and Dr. J. W. King were obtained and examined with respect to the volatile acids formed during growth on a medium containing glucose as the fermentable substrate. The strains were found to fall into two groups. The first group contains eight strains, mostly belonging to Lewis and

[1] This investigation was started by the senior author while working as a Guggenheim Fellow in the laboratory of Professor L. F. Rettger, Department of Bacteriology, Yale University.

[2] Prévot (1938) has presented a much more complicated and arbitrary classification of this group that involves a number of families and genera defined largely by morphological characters. Since Prévot's classification disregards the metabolic activities that form the basis of the present paper, it need not be further considered here.

Rettger's (1940) A1 and A3 groups, which produce either very little volatile acid of any kind or only acetic acid, identified by the Duclaux distillation method. The second group includes five strains, mostly belonging to Lewis and Rettger's group A2, which form large quantities of volatile acid, consisting of both acetic acid and one or more higher fatty acids. All strains of the second group are also distinguished by their ability to ferment lactate very readily.

Because the nature of their fermentation products is important for the classification of anaerobic bacteria, the identity of the fatty acids has been established with considerable care. For this purpose six liters of a medium containing approximately 1% sodium lactate, 0.3% Difco yeast extract, 3 vol. % yeast autolysate, 0.05% $(NH_4)_2SO_4$, 0.2% K_2HPO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.01% $Na_2S \cdot 9H_2O$, pH 7.4, was inoculated with strain 32 of Lewis and Rettger (1940) and incubated at 37°C. for one week. The volatile acids were separated from the fermented medium by steam distillation, converted to the sodium salts and concentrated to small volume, acidified with sulfuric acid and extracted with ether, the extract being dried over anhydrous sodium sulfate. The ether was removed and the fermentation acids were subjected to a fractional vacuum distillation which showed them to consist of two main components, acetic and butyric acids, plus a small amount of a higher acid, probably caproic.

The acetic acid fraction consisted of 8.1 ml. which distilled between 55.0° and 66.5°C. at 98 mm. pressure. The Duclaux distillation values obtained with this fraction agreed with those for pure acetic acid to within one per cent. A second fraction of 19.1 ml. distilled at 76.5–77.0°C. at 18 mm. pressure. The acid equivalent weight of this fraction was 88.9 (butyric acid, 88.0) and the refractive index was 1.3973 (butyric acid, 1.3991). The Duclaux distillation values agreed within the limits of experimental error with those for pure butyric acid. After most of the butyric acid had been distilled off, a small residue of a higher boiling acid was left in the distillation flask. This fraction was shown to contain an acid more volatile than valeric in the Duclaux distillation, tentatively identified as caproic acid.

No evidence was obtained for the presence of propionic acid, although the distillation method used could detect as little as 0.3 ml. propionic acid in the presence of a large excess of acetic and butyric acids.

Quantitative data on the products of lactate fermentation by strain 32 are given in table 1. The yields of volatile acids are calculated from Duclaux distillation data on the assumption that the only acids present are acetic, butyric and caproic. Less complete data indicate that the other four strains carry out a similar type of lactate fermentation.

It can be seen that these bacteria carry out a new and rather remarkable type of "butyric acid" fermentation. The distinctive features of this fermentation are the absence of any appreciable amount of molecular hydrogen (no visible gas), the large yield of volatile acids, and, especially, the small yield of carbon dioxide. Most butyric-acid-forming clostridia produce at least 100 mM carbon dioxide per 100 mM of triose fermented as compared with only 40 mM produced by strain 32.

These results throw a new light on the classification of this group of gram-positive, non-sporulating anaerobes. In spite of their morphological and cultural similarities with the rod-shaped lactic acid bacteria, they can no longer be placed, even tentatively, in the genus *Lactobacillus*. Their abilities to ferment lactate and to produce butyric acid distinguish them sharply from the lactobacilli. Since there is no other existing genus in which these bacteria can logically be placed, it seems desirable to create a new genus for them. We propose the name *Butyribacterium* as being descriptive of both their metabolic and morphological characteristics. The new genus may be defined as follows:

Non-sporulating, non-motile, gram-positive, straight or slightly bent rods. Anaerobic or microaerophilic. Chemo-heterotrophic, fermenting carbohydrates and lactic acid with formation of acetic and butyric acids and carbon dioxide as the main products. Generally catalase-negative, but sometimes weakly catalase-positive.

TABLE 1

Fermentation of lactate by strain 32

(Yields are given in mM/100 mM lactate fermented)

PRODUCT	YIELD
Carbon dioxide	40.1
Acetic acid	43.7
Butyric acid	28.6
Caproic acid	7.1
Carbon recovery (%)	94.7
Redox index	0.98

The genus *Butyribacterium* evidently belongs with the genera *Propionibacterium* and *Lactobacillus* in the tribe Lactobacillae of the family Lactobacteriaceae (Bergey *et al.*, 1939).

The type species is *Butyribacterium rettgeri nov. spec.*, named in honor of Professor L. F. Rettger who has contributed so much to our knowledge of this group of bacteria. This species includes several strains erroneously placed in the species *Lactobacillus bifidus* type II (Weiss and Rettger, 1938). Buday's (1898) *Bacillus cadaveris butyricus* may belong in the same genus but it obviously differs from our type species because of its larger size, its conspicuous chain formation and arborescent colonies, and its vigorous gas production.

The type culture of *B. rettgeri* is strain 32 of Lewis and Rettger (1940). This and at least two of the other four strains belonging to the genus were isolated from the intestinal contents of white rats. One strain is of human origin.

A description of *B. rettgeri*, modified from Lewis and Rettger's (1940) description of their group A2 (which includes strain 32), follows.

Morphology (fig. 1). Straight or slightly bent, non-capsulated, non-motile rods, 0.7 x 2–3 μ, occurring singly, in pairs, and short chains; branched cells not observed but swollen and clubbed cells occasionally formed. Gram-positive.

Cultural characters. Glucose-cysteine agar colonies: circular, entire or finely irregular margin, translucent often with opaque center, greyish-white with yellowish tinge, convex when small, later umbonate, glistening, smooth, finely granular. Colonies develop slowly but after seven days attain a diameter up to 1.5 mm.

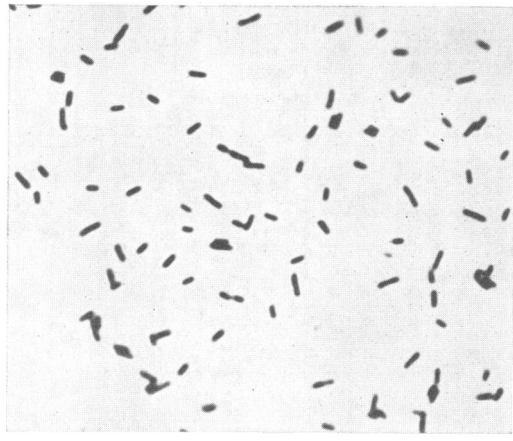

FIG. 1. *B. rettgeri*, STRAIN 32. GRAM STAIN OF CELLS FROM A TRYPTONE YEAST-EXTRACT LACTATE AGAR PLATE INCUBATED ANAEROBICALLY FOR FOUR DAYS AT 37°C. ×1500

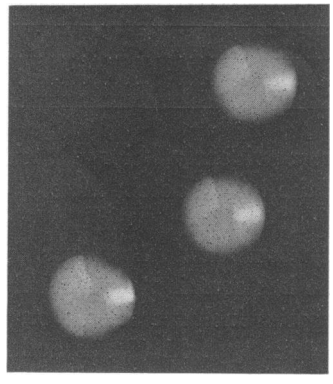

FIG. 2. FOUR-DAY OLD COLONIES OF STRAIN 32 ON A TRYPTONE YEAST-EXTRACT LACTATE MEDIUM. ×7.5

The light and dark sectors are artefacts caused by the lighting

Tryptone yeast-extract lactate agar colonies (fig. 2): similar to above except that they attain a larger size (2 mm. in 4 days at 37°C.) and are pulvinate rather than umbonate in cross section.

Glucose-cysteine gelatin: growth but no liquefaction in 30 days.

Glucose-cysteine broth: abundant turbidity and sediment; no surface growth.

Stab cultures in King and Rettger's (1942) medium: heavy growth in two days: gas production often causes slight splitting of the agar.

Temperature relations. Minimum growth temperature 15°C., optimum 37°C., maximum 40–45°C. Survives 30 minutes at 60°C. under the conditions used by Lewis and Rettger (1940).

Fermentative activity. Glucose and maltose fermented with formation chiefly of acetic and butyric acids and carbon dioxide; sometimes a little visible gas. Lactate fermented readily without visible gas. Arabinose, xylose, lactose, sucrose, trehalose, rhamnose, mannitol, sorbitol, dulcitol, and glycerol not fermented.

Anaerobic.

Non-proteolytic.

Indole and hydrogen sulfide not formed.

Catalase negative.

Source: isolated from intestinal contents of a white rat.

Our study of other species of the genus *Butyribacterium* is being continued and the results will be published later.

SUMMARY

1. A new genus, *Butyribacterium*, is proposed for gram-positive, non-sporulating, non-motile, anaerobic or microaerophilic rod-shaped bacteria that ferment carbohydrates and lactate, forming acetic and butyric acids and carbon dioxide as the main products.

2. The type species, *B. rettgeri nov. spec.*, is described.

3. The butyric acid fermentation carried out by this organism differs from similar fermentations due to anaerobic spore formers by the absence of hydrogen, the larger yield of volatile acids and the smaller yield of carbon dioxide.

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