The Relationships of Bacteria Within the Family Bacteroidaceae
as shown by Numerical Taxonomy

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SUMMARY
A comparison, by numerical taxonomy methods, was made of 72 named or
freshly isolated strains of Gram-negative anaerobes which were considered
to belong within the family Bacteroidaceae. In the first analysis 57 strains
were compared. Representative strains were then selected and compared
with additional named strains in a second analysis. Four phena were
identified: (1) strains of Sphaerophorus, Fusobacterium and Bacteroides
melaninogenicus, (2) Bacteroides, (3) unnamed poultry isolates, (4) a second
group of unnamed poultry isolates. The most useful differential tests were
found to be: (a) cell morphology, (b) the terminal pH value in glucose broth
and the production of formic, acetic, propionic or butyric acids, (c) the pro-
duction of propionic acid from threonine, (d) growth stimulation by bile, (e)
the effect of various inhibitors.

INTRODUCTION
Difficulties in the identification and classification of the anaerobic non-sporing
Gram-negative bacteria were first encountered in studies of the intestinal flora of
chickens, when a group of organisms was isolated which bore no resemblance to any
named strains (Barnes & Goldberg, 1962; Goldberg, Barnes & Charles, 1964). It was
decided to use the methods of numerical taxonomy in an attempt to assign these
organisms to a particular genus and at the same time to eliminate some of the con-
fusion which exists within the family Bacteroidaceae. At present it is difficult even to
attach particular characteristics to named organisms since several different classifica-
tions are in use (Prévet, 1938; Bergey's Manual 1957; Beerens, Castel & Fievez,
1962), and, owing to the difficulties of maintaining these organisms as freeze-dried
cultures, there has been little interchange of isolates between workers of different
countries.

The tests used in this investigation were based on the more important characters
described in Bergey's Manual (7th ed. 1957) together with those tests which were
considered important by Beerens, Schaffner, Guillaume & Castel (1963). It was hoped
to obtain further information on the relationships of various named strains to each
other, and also to select key tests for identification purposes, by using strains from as
many sources as possible in a combination of tests.
METHODS

Sources of organisms. The origin of the strains used in the two computer analyses is given in Table 1.

Media and cultural conditions. Details of the media used and conditions of culture have already been described by Goldberg et al. (1964) and Barnes, Impey & Goldberg (1966). The incubation temperature was 37° unless otherwise stated.

Table 1. The origin of the strains used in the two computer analyses

<table>
<thead>
<tr>
<th>Source</th>
<th>Computer analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>From the National Collection of Type Cultures (NCTC)</td>
<td></td>
</tr>
<tr>
<td><em>Bacteroides necrophorus</em> 7155</td>
<td>1</td>
</tr>
<tr>
<td><em>B. fragilis</em> 9343</td>
<td>1 and 2</td>
</tr>
<tr>
<td><em>B. melaninogenicus</em> 9337</td>
<td>2</td>
</tr>
<tr>
<td>From the American Type Culture Collection (ATCC)</td>
<td></td>
</tr>
<tr>
<td>From Dr H. Beerens (Institut Pasteur, Lille)</td>
<td></td>
</tr>
<tr>
<td><em>Sphaerophorus necrophorus</em> Fievez N117, N167, N252, <em>Fusiformis fusiformis</em> 389</td>
<td>2</td>
</tr>
<tr>
<td>From Dr T. Mitsuoka (Animal Physiology Laboratory, The Institute of Physical and Chemical Research, Tokyo, Japan): isolated from chicken caeca</td>
<td></td>
</tr>
<tr>
<td>Strains AIII-45, AU23-33, CH32-17, CH32-20</td>
<td>1</td>
</tr>
<tr>
<td>CH36-9, N5-43, N209-12, N210-25, N212-47</td>
<td></td>
</tr>
<tr>
<td>Strain AU21-27</td>
<td></td>
</tr>
<tr>
<td>From Dr S. M. Finegold (Dept. of Medicine, Wadsworth Veterans Hospital, Los Angeles, California)</td>
<td></td>
</tr>
<tr>
<td><em>Bacteroides melaninogenicus</em> B477, B536, B537</td>
<td>2</td>
</tr>
<tr>
<td>From Dr R. J. Gibbons (Forsyth Dental Centre, Boston)</td>
<td></td>
</tr>
<tr>
<td><em>Fusobacterium polymorphum</em>, <em>Fusobacterium JV5</em></td>
<td>2</td>
</tr>
<tr>
<td>Isolated by the authors</td>
<td></td>
</tr>
<tr>
<td>From human faeces—strains 9/1 and 12/1</td>
<td>1 and 2</td>
</tr>
<tr>
<td>From chicken caeca—strains belonging to Groups 1, 2, 3 and 4 of Barnes &amp; Goldberg (1965)</td>
<td>1 and 2</td>
</tr>
<tr>
<td>Group 1, strain EBF59/96P</td>
<td></td>
</tr>
<tr>
<td>Group 2, strains EBF59/91P, 95P</td>
<td>1</td>
</tr>
<tr>
<td>Group 3, strains EBF61/31B, 66, 67, 68 and 69</td>
<td>1</td>
</tr>
<tr>
<td>Group 4, strain EBF61/60B</td>
<td>1 and 2</td>
</tr>
<tr>
<td>Group 4, strains EBF59/78, 85, 92P, 100, EBF60/26, EBF61/30, 63</td>
<td>1</td>
</tr>
<tr>
<td>Group 4, strains EBF58/74, EBF59/72, EBF61/42, 56, 61</td>
<td>1 and 2</td>
</tr>
<tr>
<td>From duck caeca—strains EBD1/1B, 3B and 4A</td>
<td>2</td>
</tr>
<tr>
<td>From turkey carcass—strains EBT2/61 and 68</td>
<td>2</td>
</tr>
</tbody>
</table>

For *Bacteroides melaninogenicus* strains all the media were supplemented with menadione 0·5 μg/ml. and laked blood 5% (v/v) which was prepared by repeated freezing and thawing.

Morphology. Examinations for Gram reaction, size, shape and appearance, were carried out using 18–20 hr. cultures in Reinforced Clostridial Medium (RCM) of (Hirsch & Grinstein, 1954). Colonies on RCM agar (Hirsch & Grinstein, 1954) or blood agar (Beerens et al. 1963) were examined after 2 or 3 days incubation in a 90% hydrogen and 10% carbon dioxide atmosphere.
Lechithinase. Lechithinase production was determined using the method of Fievez (1963). The egg-yolk plates were examined daily up to 5 days.

<table>
<thead>
<tr>
<th>Table 2. Characters examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
</tr>
<tr>
<td>Morphology</td>
</tr>
<tr>
<td>Gram reaction</td>
</tr>
<tr>
<td>Shape of cells</td>
</tr>
<tr>
<td>Arrangement</td>
</tr>
<tr>
<td>Length</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Diameter</td>
</tr>
<tr>
<td>Colonies on RCM agar</td>
</tr>
<tr>
<td>Size</td>
</tr>
<tr>
<td>Edge</td>
</tr>
<tr>
<td>Surface</td>
</tr>
<tr>
<td>Colour</td>
</tr>
<tr>
<td>Appearance</td>
</tr>
<tr>
<td>Colonies on blood agar</td>
</tr>
<tr>
<td>Haemolysis</td>
</tr>
<tr>
<td>Growth in RCM broth</td>
</tr>
<tr>
<td>20 °C</td>
</tr>
<tr>
<td>45 °C</td>
</tr>
<tr>
<td>Turbidity</td>
</tr>
<tr>
<td>Gas production</td>
</tr>
<tr>
<td>Glucose fermentation</td>
</tr>
<tr>
<td>Terminal pH</td>
</tr>
<tr>
<td>Volatile fatty acids produced</td>
</tr>
<tr>
<td>Formic, acetic, proplionic or butyric acids</td>
</tr>
<tr>
<td>Fermentation of:</td>
</tr>
<tr>
<td>Sucrose, cellobiose, galactose, arabinose, xylose, mannitol, inositol, salicin, and glycerol</td>
</tr>
<tr>
<td>Cysteine milk</td>
</tr>
<tr>
<td>Lechithinase production</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
</tr>
<tr>
<td>Indole production</td>
</tr>
<tr>
<td>Hydrogen sulphide production</td>
</tr>
<tr>
<td>Propionic acid from threonine</td>
</tr>
<tr>
<td>Growth stimulation by 10% bile</td>
</tr>
<tr>
<td>Growth in the presence of:</td>
</tr>
<tr>
<td>Brilliant green 1/100,000, penicillin 10 μg/ml., neomycin 25 μg/ml., polymyxin B 10 μg/ml., and chloro- tetracycline 10 μg/ml.</td>
</tr>
<tr>
<td>Growth stimulation by menadione</td>
</tr>
</tbody>
</table>

Biochemical tests. For carbohydrate fermentation, H₂S production, indole, etc., additions were made to the basal medium of Beerens (1953–54) as described by Goldberg et al. (1964).

Detection of volatile fatty acids. The volatile fatty acids produced from the fermentation of glucose were determined by the method of Guillaume, Beerens & Osteux (1956) as modified by Charles & Barrett (1963).

Bile Stimulation. Stimulation of growth was determined by adding 10% bile to the Basal Glucose Phosphate (BGP) medium of Barnes & Goldberg (1962).

Effect of inhibitors. Growth in the presence of brilliant green 1/100,000 (v/v),
penicillin 10 μg./ml., neomycin 25 μg./ml., polymyxin B 10 μg./ml., or chlortetracycline 10 μg./ml. was tested by adding the required concentration of each inhibitor to RCM broth.

Theonine test. The production of propionic acid from threonine was detected following the method of Beerens (personal communication). This test was later modified by Beerens \& Tahon-Caste1 (1965). The strains were examined using both techniques.

Characters. The organisms were examined for the characters given in Table 2.

Computer analysis. Both the computer analyses were carried out by Mr J. C. Gower (Rothamstead Experimental Station, Harpenden, Hertfordshire) using the similarity coefficient defined by Gower (1967) and a single linkage sorting programme (Sneath, 1957). The coefficient of similarity used allows for the inclusion of quantitative and multivalue qualitative characters as well as dichotomies for which negative matches do not contribute to the similarity coefficient. A detailed discussion of the choice of coding has been given by Thornley (1967).

In both analyses four tests were recorded as dichotomies, these were the production of formic, acetic, propionic or butyric acids, whilst hydrogen sulphide production was scored quantitatively (Table 2). All of the remaining tests were recorded as alternatives where equal weighting was given to the positive or negative answer. In the first analysis 83 and in the second 86 features were analysed.

RESULTS

Preliminary computer analysis. In the original computer analysis the main aim was to try and relate the chicken isolates to named strains. As they were intestinal in origin it was considered probable that they would relate more closely to Bacteroides than to the fusobacteria. It was also evident that morphologically they bore little resemblance to Fusobacterium species. Hence most of the named strains used for comparative purposes were either Bacteroides (Ristella in some classifications) or strains designated Eggerthella (Beerens et al. 1963). Only two strains of fusobacteria were included.

The 57 strains tested are listed in Table 1, whilst the characters analysed are given in Table 2. All the characters were used with the exception of haemolysis, lecithinase production and growth stimulation by menadione (as Bacteroides melaninogenicus strains were omitted from the first analysis). All of the strains were obligate anaerobes and inhibited by 10 μg./ml. chlortetracycline, none fermented glycerol or hydrolysed gelatin. These properties were therefore omitted from the analysis.

The similarity matrix is shown in Fig. 1. Five phena were defined which contained 50 of the strains examined.

1) Eggerthella clostridiformis. The four strains received from Beerens were identical with Bacteroides necrophorus strain NCTC 7155. Although at the time of analysis these strains were not considered to form spores, it was subsequently shown that all of these five strains produced spores after prolonged incubation on RCM agar (but not on the VL agar of Beerens). They were therefore excluded from further consideration.

2) Bacteroides. This phenon included the ATCC strains, those of Beerens and Bacteroides fragilis strain NCTC 9343, together with 8 of the chicken isolates from Mitsuoka. Sphaerophorus necrophorus ATCC 12290 also had a high similarity to the Bacteroides strains.
(3) *Sphaerophorus*. The strain *S. varius* ATCC 8501 was closely related to three strains obtained from Beerens. One of the chicken isolates 59/96 also grouped with these strains although having a much lower similarity value.

(4) *Chicken isolates*. Group 4. Fourteen of the strains previously designated group 4 (Barnes & Goldberg, 1965) had a low similarity value to either *Bacteroides* or *Sphaerophorus*. A full description of these organisms was given by Goldberg, Barnes & Charles (1964) and the computer results justify their caution in not assigning the organisms to a particular genus.

(5) *Chicken isolates*. Group 3. A further group of 6 chicken isolates had the lowest similarity to all the other groups. They were originally designated group 3 by Barnes & Goldberg (1965). They have since been shown to be Gram-positive in very young cultures and their position within the family Bacteroidaceae is thus in doubt.

The only two fusobacteria, *Fusobacterium polymorphum* and *F. biacutus*, showed little similarity to each other. *Fusobacterium biacutus* has since been shown to produce spores (Dr H. Beerens, personal communication) and should therefore be eliminated from the family Bacteroidaceae.

**Further analysis of representative strains**

The results from the first analysis were sufficiently encouraging that the computer analysis was repeated to include additional named strains. In particular, three further strains of *Sphaerophorus necrophorus* were included as the strains originally obtained from Dr Beerens were atypical in certain characters. Two further strains of *Fusobacterium* were also tested but it was difficult to obtain representative strains of these organisms as they tend not to be maintained in stock culture collections. Four strains of *Bacteroides melaninogenicus* were added, the requirement for menadione being scored as a separate character. Representative chicken isolates were included, together with a few freshly isolated strains from ducks and turkeys.

Two further tests were used which had been considered important in the differentiation of the pathogenic strains of *Sphaerophorus* (Fievez, 1963). These were lecithinase production and haemolysis.

In all 40 strains were analysed; of these 25 strains had been used in the first analysis. The additional 15 strains are listed above. The similarity matrix is shown in Fig. 2. It is evident that the additional strains and the extra tests used widened the differences between the main groups of organisms shown in Fig. 1. Four phena were now evident:

1. Containing *Sphaerophorus*, *Fusobacterium* and *Bacteroides melaninogenicus* together with 3 poultry isolates.
2. *Bacteroides*, together with the representative chicken isolate of Mitsuoka.
3. The chicken isolates belonging to Group 4 (Barnes & Goldberg, 1965).
4. The most representative chicken isolate belonging to Group 3 (Barnes & Goldberg, 1965) together with freshly isolated turkey and duck strains.

The family relationships are shown in the dendrogram Fig. 3, whilst the characteristics of the organisms are given in Tables 3 and 4.

In considering these results the discussion will be confined mainly to representative strains obtained from the Type Culture Collections and other workers. The poultry isolates will be discussed in detail elsewhere (Barnes & Impey, to be published).

**Phenon 1: Sphaerophorus, Fusobacterium and Bacteroides melaninogenicus**. The main characters which were common to *Sphaerophorus* and *Fusobacterium* and which
Fig. 2. Similarity matrix of 40 strains, 25 of which were also used in the first analysis.
differentiated these organisms from *Bacteroides* were the production of propionic acid from threonine, the high terminal pH in glucose broth, the production of butyric acid in glucose broth and the failure to produce acid from most of the other carbohydrates tested. All the strains tested grew in the presence of brilliant green 1/100,000.

The strains of *Sphaerophorus* differed morphologically from those of *Fusobacterium*. They also produced copious gas, and generally produced propionic acid as well as acetic and butyric acids from glucose. They were inhibited by polymyxin 10 µg/ml. They produced lecithinase and grew at 20°.

The *Fusobacterium* spp. were characterized by forming long rods or filaments with
Table 3. Some of the more important differential characters within the family Bacteroidaceae

<table>
<thead>
<tr>
<th>Description of cells</th>
<th>Terminal pH</th>
<th>Volatile fatty acids produced</th>
<th>Propionic acid from formic acid</th>
<th>Butyric acid</th>
<th>Clostridial gas production</th>
<th>Stimulability by bile</th>
<th>Stimulability by menadione</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4·6 to 5·5</td>
<td>5·6 to 6·2</td>
<td>Formic</td>
<td>Acetic</td>
<td>Propionic</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sphaerophorus spp.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(3)†</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>S. necrophorus,</strong> Fievez strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Fusobacterium spp.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>B. melaninogenicus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>+</td>
<td>+</td>
<td>- (3)</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Bacteroides spp.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>+</td>
<td>-</td>
<td>- (7)</td>
<td>- (8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chicken isolates, group 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>- (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Chicken isolates, group 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>+</td>
<td>- (3)</td>
<td>+</td>
<td>(3)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Strains EBD 1/1 B, 4 A, EBF 59/96 P and B. symbiosis ATCC 12829 have not been included in this Table.
† Parentheses denote number of strains with that particular reaction.
### Table 4. Other characters of the anaerobic non-sporing Gram-negative rods

<table>
<thead>
<tr>
<th></th>
<th>No. of strains*</th>
<th>β-haemolysis</th>
<th>Leucinase</th>
<th>Indole</th>
<th>H$_2$S†</th>
<th>Growth at:</th>
<th>Behaviour in cysteine milk*</th>
<th>Growth in presence of:</th>
<th>Acid from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$20^\circ$</td>
<td>$45^\circ$</td>
<td>Brilliant Green 1/100,000</td>
<td>Polymyxin 10 μg/ml.</td>
</tr>
<tr>
<td><em>Sphaerophorus</em> spp. 4</td>
<td>4</td>
<td>-</td>
<td>+</td>
<td>(3)§</td>
<td>+ + +</td>
<td>+</td>
<td>- (3)</td>
<td>NC</td>
<td>+</td>
</tr>
<tr>
<td><em>S. necrophorus</em> Fievez strains</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ +</td>
<td>+</td>
<td>+ (2)</td>
<td>NC</td>
<td>+</td>
</tr>
<tr>
<td><em>Fusobacterium</em> spp. 4</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>+ + +</td>
<td>+</td>
<td>- (3)</td>
<td>+</td>
<td>- (3)</td>
<td>(3) +</td>
</tr>
<tr>
<td><em>B. melaninogenicus</em> Bacteroides spp.</td>
<td>4</td>
<td>N.T.</td>
<td>N.T.</td>
<td>+</td>
<td>+ (2)</td>
<td>+</td>
<td>-</td>
<td>A + C (4)</td>
<td>- (11) +</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<td>A + C (4)</td>
<td>- (9)</td>
<td>(9) +</td>
</tr>
<tr>
<td><em>Chicken isolates</em> Group 4</td>
<td>5</td>
<td>+</td>
<td>(3)</td>
<td>-</td>
<td>+ + +</td>
<td>+ (3)</td>
<td>A + C (4)</td>
<td>- (3)</td>
<td>+ + +</td>
</tr>
<tr>
<td>Group 3</td>
<td>4</td>
<td>+ (3)</td>
<td>- (3)</td>
<td>-</td>
<td>+ + +</td>
<td>NC</td>
<td>A + C (4)</td>
<td>- (3)</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

* Strains EBD 1/1 b, 4A, EBF 59/96p and *B. symbiosis* ATCC 12829 have not been included in this Table.
† H$_2$S: +++, strongly positive; +, positive; -, negative.
‡ NC, no change; A, acid; A + C, acid and clot.
§ Parentheses denote number of strains with that particular reaction.
∥ N.T., not tested.
pointed ends; they were non-haemolytic; did not produce lecithinase; did not produce propionic acid from glucose.

The strains of *Bacteroides melaninogenicus* were related more closely to the *Sphaerophorus* strains than to *Bacteroides*. Their resemblance was based partly on their morphology, the production of formic, acetic and butyric acids in glucose broths and their failure to ferment sucrose, cellobiose, arabinose, xylose, mannitol, inositol and salicin. They differed from *Sphaerophorus* and *Fusobacterium* in their failure to produce propionic acid from threonine, inhibition by brilliant green 1/100,000, and growth in the presence of polymyxin 10 µg./ml. In these latter three characters they resembled *Bacteroides*.

**Phenon 2: Bacteroides.** Morphologically the *Bacteroides* strains could not be differentiated from *Sphaerophorus*. The group was differentiated by failure to produce propionic acid from threonine, fermentation of glucose with a low terminal pH (4.6–5.5), the failure to produce butyric acid. The organisms grew in the presence of polymyxin 10 µg./ml and generally fermented a wider range of carbohydrates. Many but not all of the strains were stimulated by bile.

**Phenon 3: Chicken isolates (group 4).** These organisms were characterised by their large size and shape, the production of acetic and propionic acids in glucose broth and the fermentation of a wide range of carbohydrates, in particular mannitol. They showed a low similarity both to *Sphaerophorus* and *Bacteroides*.

**Phenon 4: Chicken isolates (group 3).** These organisms showed the least similarity to all other groups. In 6 hr broth cultures the organisms had a Gram-positive reaction but tended to be negative in 24 hr cultures. The organisms were very pleomorphic, their shapes ranging from coccoidal forms to long rods. Of the carbohydrates tested only glucose was fermented. Formic acid was produced by all the strains together with trace amounts of acetic and butyric acids with some of the strains.

**DISCUSSION**

There have been several detailed studies recently on individual genera within the family Bacteroidaceae, e.g. *Fusobacterium* (Baird-Parker, 1960), *Sphaerophorus* (Fievez, 1963), *Bacteroides* (Beerens et al. 1963), and the tests used above were based on those found to be important for each of these genera. The results obtained justify the division of the family into a number of groups but these groups can only be assigned to specific genera after agreement has been reached on the correct nomenclature.

The properties of the *Fusobacterium* strains agreed closely with those described by Baird-Parker (1960), whilst the *Sphaerophorus necrophorus* strains (Fievez N 117, N 167, N 252) conformed with the description of the species given by Fievez (1963), but differed in several characteristics from the other four *Sphaerophorus* strains tested (Table 4). The close relationship between *Fusobacterium* and *Sphaerophorus* which was shown in this analysis is supported by Sebald (1962), who found that the DNA base ratios were similar for the organisms within the two genera and suggested that they should be included together in one genus.

The separation of the *Bacteroides* strains into a separate group from *Fusobacterium* and *Sphaerophorus* is also supported by the DNA base ratio analyses of Sebald (1962) who showed that the G+C ratio was > 41 % as compared with 27–34 % for the other two genera. Beerens et al. (1963) suggested that many of the species described within the
genus *Bacteroides* (or *Ristella*) should be grouped together within one species *Eggerthella convexa*. This analysis confirmed the difficulty of differentiating between the various species but certain tests such as those used by Reinhold (1964) to differentiate *B. convexus* from *B. thetaiotaomicron* were not included.

It is evident that a number of type-culture collection strains have been incorrectly named—in particular the strain *Bacteroides necrophorus* NCTC 7155 which is identical with *Eggerthella clostridiformis* (Beerens) and does not resemble the other *Sphaerophorus* strains. The strain *S. necrophorus* ATCC 12290 is almost certainly a *Bacteroides* strain. *B. symbiosis* ATCC 12829 shows little resemblance to the other *Bacteroides* strains and needs further investigation.

There was no difficulty in recognizing the strains of *Bacteroides melaninogenicus* because of their typical black colonies and requirements for menadione; however, the taxonomic position of this organism within the family needs careful consideration, It was assigned by Beerens et al. (1962) to a separate genus, and support for this suggestion comes from its low similarity to the other *Bacteroides* strains.

One of the main purposes of the analysis was to determine which tests were most useful for identification purposes. Perhaps the most difficult problem was initially to determine which organisms might come within the family Bacteroidaceae, which is defined as containing Gram-negative anaerobic non-sporing rods. Strains were included in the first analysis which were subsequently shown to produce spores on particular media. Included amongst these was *Fusiformis biacutus* which has always been considered a non-sporing organism. Similarly, the *Eggerthella clostridiformis* strains of Beerens formed spores. The whole relationship of the Gram-negative sporing organisms to the Bacteroidaceae needs further investigation. The problem of obtaining a reliable Gram reaction has again been demonstrated. In these analyses the group 3 chicken isolates were included initially as Gram-negative bacteria but were subsequently found to be Gram-positive. However, they had a low similarity to all the other organisms tested, indicating that they also differed in many other properties.

As can be seen from Table 3, the morphological differentiation of the *Sphaerophorus* strains from *Bacteroides* was difficult, but *Fusobacterium* and the two groups of chicken isolates all had a characteristic morphology. Most of the traditional tests (Table 4) such as indole production, hydrogen sulphide production and the fermentation of various carbohydrates were too variable within the groups to be used to separate them. Amongst the differential tests considered to be most useful are those shown in Table 3. These were: (1) the terminal pH in glucose broth and the types of volatile fatty acids produced, i.e. formic, acetic, propionic or butyric acids, (2) the production of propionic acid from threonine, (3) growth stimulation by bile. Other useful tests shown in Table 4 included the effect of various inhibitors, in particular polymyxin 10 μg./ml.

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REFERENCES


GOWER, J. C. (1967). A general coefficient of similarity and some of its properties. (Submitted to *Biometrics*.)


