The Classification of Fusobacteria from the Human Mouth

By A. C. BAIRD-PARKER

*Department of Bacteriology, University of Birmingham**

SUMMARY: 237 strains of fusobacteria were studied and classified by such characters as cell composition, metabolism and morphology. They are shown to be closely related metabolically and in cell composition, but distinguishable by morphology into two species: *Fusobacterium nucleatum* Knorr and *F. polymorphum* Knorr. It is suggested that these organisms are actinomycetes which have lost a number of characters in undergoing adaptation to parasitism.

The allocation of species to the genera *Fusiformis* Hoelling and *Fusobacterium* Knorr has frequently been made without proper regard to the original definitions (*Topley and Wilson's Principles*, 1955; Hoffman, 1957). There has been confusion between the true oral fusobacteria and the superficially similar *Leptotrichia* (Knorr, 1923; Hine & Berry, 1937; Omata & Disraely, 1956), *Bacteroides* (Schwabacher, Lucas & Rimington, 1947) and even with the flagellated micro-organism *Spirillum sputigenum* that apparently belongs to the protozoan genus *Selenomonas* (Jeynes, 1955, 1956). The suggestion by Bøe (1941) that the genus *Fusobacterium* should contain only the primarily oral anaerobes described in this paper is acceptable and in accordance with my own conclusions. It has been the object of this investigation to establish more definite characters for the oral members of the genus *Fusobacterium* in order to distinguish them from other organisms, especially oral parasites, with which they have been confused.

METHODS

Isolation and purification of strains

All strains were freshly isolated from oral material. Saliva and tooth scrapings emulsified in serum were plated out on the *Fusobacterium* medium (F.M.) described by Baird-Parker (1957) to which, after sterilization by autoclaving at 10 lb./sq.in. for 20 min., the following additions were made: 5 % (v/v) Seitz-filtered serum; streptomycin to 20.0 µg./ml.; ethyl violet to a final concentration of 1:15,000. Incubation was carried out at 37° in McIntosh and Fildes jars filled with an atmosphere of 90 % (v/v) hydrogen + 10 % (v/v) carbon dioxide. Plates were examined after 4 days and representatives of the different colonial types subcultured into the above medium, but without dyes or antibiotics and with the agar concentration decreased to 0.2 % (w/v). Isolates were replated on to dye- and antibiotic-free F.M. medium in order to check their purity. Strains were maintained in semisolid agar in bijou bottles and subcultured

* Present address: Unilever Ltd., Colworth House, Sharnbrook, Bedford.

every 2 days for the first week following isolation and thereafter every 4 days. Freeze-dried cultures have survived for periods greater than 18 months without loss of viability.

Morphology

Features of surface colonies were examined with a binocular plate microscope fitted with a 59 mm. objective and $\times 10$ eyepieces. Organisms were stained by Gram's method and by the following modification of the method of Robinow (1945) in order to demonstrate cell and cross-walls: air-dried smears were mordanted with 10 % (w/v) tannic acid for 2 hr., washed with water and stained with 0.5 % (w/v) ethyl violet for 5–10 min. The ability of carbol fuchsin stained organisms to resist decolorization by mineral acids was tested with strengths of conc. sulphuric acid from 0.05 to 1.0 % (v/v) applied for 30 sec. Microscopical examinations were carried out using a N.A. 1.30, 2 mm. apochromatic objective and a $\times 20$ Holos eyepiece. Organisms were also examined in the electron microscope for the presence of flagella and intracellular granules.

Broth cultures (18–24 hr.) were examined for motility by sealing portions of culture fluid in capillary tubes, incubating at 37° for 1 hr. and examining under a $\frac{1}{4}$ th objective (Weinberg, Nativelle & Prévot, 1937). The method of Tittsler & Sandholzer (1936) for the detection of motility in slightly motile organisms was also used.

Physiology

Growth in the presence of different air + carbon dioxide mixtures as well as at different Eh values was investigated by inoculating plates of F.M. medium, containing concentrations of L-cysteine (0.1–2.0 %, w/v) and incubating in McIntosh and Fildes jars filled with gas mixtures prepared by the method of Mullaney (1956). Catalase activity was tested by flooding 3-day agar cultures with a '20 vol.' solution of hydrogen peroxide and examining for the evolution of gas bubbles from the colonies (Society of American Bacteriologists, 1957). The possession of an oxidase enzyme was tested by pouring a 1.0 % (w/v) solution of tetramethyl-*p*-phenylenediamine over the colonies on an agar plate and examining for the development of a purple colour within the colonies (Mackie & McCartney, 1953).

The following methods and media are the result of trial and error to find the best growth conditions and most sensitive methods for investigating the metabolism of fusobacteria.

The reaction of all media was adjusted between pH 7.4 and 7.6 and sterilized at 10 lb./sq.in. for 20 min. unless otherwise stated. Cultures were incubated at 37° in McIntosh and Fildes jars filled with an atmosphere of hydrogen in the case of measurement of end pH values; otherwise a mixture of 10 % (v/v) carbon dioxide + 90 % (v/v) hydrogen was employed. All tests were carried out in $\frac{1}{4}$ oz. screw-capped bijou bottles and all negative results repeated.

Indole production. Medium (% w/v): Oxoid Tryptone, 1.0; Oxoid yeast extract, 0.1; Lab. Lemco, 0.3; glucose, 0.1; L-cysteine HCl, 0.05; disodium hydrogen phosphate, 0.5. Cultures were incubated for 7 days. The indole was extracted by shaking 3 ml. culture fluid with 1 ml. toluene (Isenberg &

Sundheim, 1958). The presence of indole was indicated by the formation of a reddish colour in the organic phase when *c.* 0.5 ml. of Ehrlich's reagent was added by a Pasteur pipette to form a layer at the interface between the culture fluid and toluene layer.

Hydrogen sulphide production. Medium (% w/v): proteose peptone No. 3 (Difco), 1.0; Lab. Lemco, 0.1; Oxoid yeast extract, 0.1; glucose, 0.5; L-cysteine HCl, 0.05; disodium hydrogen phosphate, 0.5. Tests were carried out in sealed bijou bottles containing strips of filter paper impregnated with lead acetate. The decomposition of specific inorganic and organic sulphur-containing compounds was investigated by means of washed suspensions of organisms, using the methods of Olitzki (1954). Tests were performed in 10 by 75 mm. hard glass tubes fitted with rubber bungs to exclude air. Incubation was in a water bath at 37°.

Ammonia production from urea, glycine, arginine, asparagine, serine, phenylalanine and cysteine was tested with washed suspensions of organisms. Tubes were incubated for periods of up to 8 hr., samples withdrawn at intervals and tested with Nessler's reagent. Ammonia from peptone was tested after growth for 14 days in F.M. medium.

Hippurate hydrolysis. Seitz-filtered sodium hippurate was added to the following medium to a final concentration of 1.0% (w/v): proteose peptone no. 3, 1.0; Oxoid yeast extract, 0.1; Lab. Lemco, 0.3; L-cysteine HCl, 0.05; glucose, 0.5; disodium hydrogen phosphate, 0.5. The formation of free benzoic acid was tested by the addition of 1.5 ml. of 50% (v/v) conc. sulphuric acid which caused its precipitation as leaf-like crystals after standing at room temperature for periods of up to 4 hr. (Ayers & Rupp, 1922). The presence of adequate growth was always noted before carrying out the tests; negative results were repeated.

Reduction of nitrate and nitrite. Medium (% w/v): proteose peptone no. 3, 1.0; Lab. Lemco, 0.3; Oxoid yeast extract, 0.1; glucose, 0.1; L-cysteine HCl, 0.05; disodium hydrogen phosphate, 0.5; 0.01, either sodium nitrate or sodium nitrite. Cultures were incubated for 10–14 days at 37°; then to each culture which showed good growth 2 or 3 drops of iodine were added followed by 1 ml. each of the Griess-Ilosvay reagents (Society of American Bacteriologists, 1957). The tests were stood for 30 min. at room temperature and the results noted. False negatives, i.e. reduction of nitrate beyond nitrite to ammonia or nitrogen, were checked by adding powdered zinc to the broths. The production of ammonia from nitrite was tested with suspensions of washed organisms.

The breakdown of gelatin was tested by Frazier's technique (Frazier, 1926); proteolytic activity was tested against coagulated egg-white protein, inspissated horse serum and whole casein. The breakdown of casein was tested using the method described by Hastings (1904).

Lecithinase activity was measured by the method of McClung & Toabe (1947) and haemolysis by plating on F.M. medium containing a 5% (v/v) suspension of horse blood.

Cell and cell-wall analyses were made as described by Davis & Baird-Parker (1959*a*).

End products from the breakdown of carbohydrates

(a) *Acid production.* A medium containing the following constituents gave reasonable growth of fusobacteria in the absence of added carbohydrate and good growth in the presence of fermentable carbohydrate (% w/v): proteose peptone no. 3, 1.0; Lab. Lemco, 0.3; yeast extract, 0.1; L-cysteine HCl, 0.05. All sugars were Seitz-filtered and added to the separately-sterilized basal medium to a final concentration of 1.0% (w/v), or in the case of rare sugars 0.5% (w/v). The complete medium was 'flash-sterilized' by the method of Davis & Rogers (1939). Cultures were incubated for periods up to 14 days; acid production was determined electrometrically or by Johnson's narrow range pH papers. The use of pH indicator in the medium was found to be completely unreliable.

(b) *Gas production.* The formation of soluble and insoluble gases in the fermentation of glucose was investigated by the methods of Gibson & Abd-El-Malek (1946) and of Hayward (1957). F.M. medium containing 5.0% (w/v) glucose was used.

(c) *Acetoin production.* Medium (% w/v): proteose peptone no. 3, 1.0; Oxoid yeast extract, 0.1; Lab. Lemco, 0.3; L-cysteine HCl, 0.05; glucose, 2.0; disodium hydrogen phosphate, 0.5. Sterile glucose solution was added aseptically after heat sterilization of the other constituents. Cultures were incubated for 8 days and acetoin tested for by the method of Barritt (1936).

(d) *Starch hydrolysis.* Medium (% w/v): proteose peptone no. 3, 1.0; Oxoid yeast extract, 0.1; Lab. Lemco, 0.3; L-cysteine HCl, 0.05; glucose 0.5; starch, 0.02; agar, 2.0; modified from Eckford's (1927) medium. Plates were incubated for 21 days and the breakdown of starch detected by flooding with Lugol's iodine.

(e) *Aesculin and arbutin breakdown.* Medium (% w/v): proteose peptone no. 3, 1.0; Lab. Lemco, 0.3; Oxoid yeast extract, 0.1; glucose, 0.1; disodium hydrogen phosphate, 0.5; either aesculin or arbutin, 0.5. Cultures were incubated for 14 days. The presence of a phenolic aglycone was detected by adding 0.5 ml. of a 1.0% (w/v) solution of ferric ammonium citrate and examining for the development of a dark reddish brown colour in the medium and also loss of fluorescence in the case of aesculin (Barnett, Ingram & Swain, 1956).

RESULTS

The 237 strains of fusobacteria isolated from the human mouth could be divided by cell morphology into three groups; a comparison of the features of the groups is given in Table 1. Groups I and II correspond to *Fusobacterium nucleatum* Knorr and *F. polymorphum* Knorr respectively, while group III strains, representing less than 6% of the total isolates, were morphologically intermediate and similar in appearance to *Fusiformis fusiformis* as described by Prévot & Taffanel (1942), although differing from this organism by not fermenting a wide range of carbohydrates. The results of the physiological tests confirm the observations of many workers (e.g. Hine & Berry, 1937; Prévot,

Tardieux, Joubert & de Cadore, 1956) that fusobacteria vary considerably in their ability to metabolize organic and inorganic compounds in classical biochemical tests and that these are of little value in species determination. However, it was possible to establish the following characters for the genus as a whole and to define biochemically distinct types within each species.

Table 1. *Comparison of overall characters of fusobacteria*

Characteristic	Group I	Group II	Group III
Colony form	Variable; medusoid or smooth with lightly veined surface (Pl. 1, fig. 2) or granular with very irregular surface and edge (Pl. 1, fig. 3)	Variable; medusoid or semi-rough with deeply veined surface and undulant edge (Pl. 1, fig. 1). Mucoid variants common	As group II
Organism size	3.0–7.0 μ $\times$ 0.3–0.5 μ	5.0–30 μ $\times$ 0.4–0.7 μ (filaments common)	2.0–10.0 μ $\times$ 0.3–0.5 μ (filaments rare)
Fructose	+	166/168	+
Galactose	51/56	155/168	12/13
Glucose	+	164/168	+
Sucrose	12/56	45/168	7/13
End pH in 1.0 (v/v) glucose broth	5.6–7.0	5.8–7.3	6.0–6.6
Hippurate hydrolysis	38/56	148/168	9/13
H ₂ S from peptone broth	+	163/168	+

Characters common to all groups. Catalase and oxidase negative; ammonia formed from peptone, serine, cysteine and asparagine but not from urea, glycine, arginine or phenylalanine; indole produced and nitrite reduced by all strains; nitrate weakly attacked by most strains; hydrogen sulphide formed from cysteine and in small amounts from thiosulphate but not from methionine, thioglycollate, sulphite, or metabisulphite; gelatin, egg white protein and casein not attacked. No acid from the following carbohydrates: mannose, arabinose, rhamnose, xylose, lactose, maltose, melibiose, trehalose, melezitose, raffinose, dextrin, inulin, glycogen, starch, dulcitol, erythritol, mannitol, sorbitol, aesculin, arbutin, cellobiose, salicin; acetoin not formed from glucose.

$$\text{Fractional figures} = \frac{\text{no. of strains positive}}{\text{no. of strains tested}}.$$

Members of the genus *Fusobacterium* are primarily obligate anaerobic oral parasites requiring carbon dioxide and a rich peptone medium containing a source of fermentable carbohydrate for good growth. They are non-acid-fast, Gram-negative, unbranched, non-motile, slender filaments of various lengths with tapered ends which do not form endospores but contain characteristic intracellular basophilic granules. Neither catalase nor oxidase is produced. Only simple sugars are weakly fermented with the release of principally volatile fatty acids and small amounts of carbon dioxide which can be detected manometrically, but not by sensitive cultural methods. Acetoin is not formed. Amino acids are broken down with the release of hydrogen sulphide, ammonia or indole. The organisms are not proteolytic and do not hydrolyse gelatin. Nitrate is sparingly attacked and nitrite is reduced to ammonia.

Group I (Fusobacterium nucleatum Knorr)

Colonial morphology. Variable, but usually 0.5–1.0 mm. in diameter with a smooth, lightly veined surface and an entire or undulate edge (Pl. 1, fig. 2). Medusoid or mucoid colonies are rare but the irregular, nodulate colony illustrated in Pl. 1, fig. 3, is commonly found.

Cell morphology. Small, non-flagellate organisms 0.3–0.5 μ wide and *c.* 3.0–7.0 μ long, forming occasionally filaments up to 25 μ in length. The organisms divide by constriction giving rise to pointed cells containing one or more basophilic granules.

Metabolic characters. Glucose, fructose and galactose are fermented by most strains with an end pH value generally *c.* pH 6.1–6.3. Sucrose is fermented by some strains. There is no proteolytic or urease activity and gelatin is not hydrolysed. Hydrogen sulphide is formed by the breakdown of L-cysteine, ammonia by deamination of L-cysteine, serine and asparagine, and indole from tryptophan. With the exception of the distinct colonial variant (Pl. 1, fig. 3) sodium hippurate is usually attacked with the release of benzoic acid. Nitrate is weakly attacked by most strains and nitrite invariably reduced to ammonia. A lecithinase is not produced and horse blood is not haemolysed although greening of the blood frequently occurs on exposure of cultures to air.

Three varieties can be distinguished on the basis of fermentation pattern, colonial morphology and the hydrolysis of sodium hippurate (Table 2).

Table 2. *Characteristics of varieties of Fusobacterium nucleatum and F. polymorphum*

Species	Type	No. isolates	Colony form	Glucose	Fructose	Galactose	Sucrose	Hippurate
<i>F. nucleatum</i>	I	13	Pl. 1, fig. 1/fig. 2	+	+	+	+	12/13
	II	26	Pl. 1, fig. 1/fig. 2	+	+	25/26	—	+
	III	17	Pl. 1, fig. 3	+	+	13/17	—	—
<i>F. polymorphum</i>	I	45	Pl. 1, fig. 1	+	+	+	+	41/45
	II	107	Variable	103/107	105/107	96/107	—	+
	III	16	Variable	+	+	14/16	—	—

Fractional figures indicate = $\frac{\text{no. of strains positive}}{\text{no. of strains tested}}$.

Group II (Fusobacterium polymorphum Knorr)

Colonial morphology. Brain-like colonies *c.* 1.0–2.0 mm. diam. with a deeply veined surface and undulate edge (Pl. 1, fig. 1). Mucoid variants common.

Morphology. Pointed, non-flagellate, filamentous organisms, width 0.4–0.7 μ , length *c.* 5.0–30.0 μ (Pl. 1, fig. 6). Intracellular basophilic granules can be readily demonstrated (Pl. 1, fig. 5). Filaments > 200 μ long are commonly encountered in cultures of all ages. The organisms are septate and divide by constriction. Swollen and spirillar filaments characteristic of an L-cycle are found in cultures of recently isolated strains.

Metabolic characters. These are identical with those shown by strains of *Fusobacterium nucleatum* and the group can be similarly subdivided (Table 2).

Group III (Fusiformis fusiformis Prévot & Taffanel?)

The organisms placed in this group are non-motile Gram-negative fusiforms of width $0.3-0.5\mu$ and length $2.0-10.0\mu$, which occasionally form long filaments in old cultures; 13 strains were isolated. Other characters of this group are identical to those exhibited by groups I or II. It seems probable that members of this group should be placed with group II strains on account of similarities in cell composition (see below).

Cell composition

The amino-acid composition of the cell walls of six strains each of groups I and II and one strain of group III was examined. It was very characteristic and consisted mainly of glutamic acid, DL-alanine, lysine, glycine, valine, phenyl-alanine, aspartic acid, 'slow-moving components' and, in four strains of group II and the group III strain, traces of serine. The two 'slow-moving components' were only resolved in Hoare & Work's solvent (Hoare & Work, 1957). One was identical with the 'slow-moving component' described by Cummins & Harris (1958) as muramic acid, the other component had a smaller R_f than muramic acid, but was similar to it and to diaminopimelic acid in forming a greenish yellow colour with ninhydrin. The above pattern of amino acids, which was characteristic of strains isolated from the normal human mouth, was not shown by strains from pathological sources. For example, some strains of *Fusiformis nucleatus* and *F. fusiformis*, supplied by Professor A. R. Prévot, and which had been isolated from abscesses and actinomycotic lesions, were found to contain diaminopimelic acid in place of lysine and only one 'slow-moving component', identical with muramic acid. The other components were identical with those first mentioned.

The sugar composition of organisms of 19 strains of oral fusobacteria consisted of an amino-sugar, ribose and either glucose and/or galactose. The presence of a high proportion of galactose was characteristic of strains of *Fusobacterium nucleatum* (group I) and glucose alone, or with galactose, of *Fusiformis fusiformis* (group III), and *Fusobacterium polymorphum* (group II).

DISCUSSION

The fusiform organisms of the mouth belong to several distinct genera of bacteria and protozoa. Table 3 outlines my classification of these organisms and its relationship to previous classifications. Two of these genera, *Fusobacterium* and *Leptotrichia*, have often been confused; they are probably related in some measure but are entirely distinct. For example, *Fusobacterium plauti-vincenti* (Knorr, 1928) or its most recently introduced synonym, *F. fusiforme* (Veillon & Zuber) Hoffman (Hoffman, 1957) are misnomers as they attribute to the genus *Fusobacterium* an organism which was validly published as the type species of the genus *Leptotrichia* by Trevisan in 1879 (Buchanan, Cowan & Wikén, 1958). The similarities between the two genera are more apparent than real and confusion has almost certainly resulted from their presence in the same situations in the mouth and that they can be isolated under similar

Table 3. *Relationships of mouth organisms which have been classified in either the genus Fusiformis or Fusobacterium*

Baird-Parker's classification	<i>Fusobacterium nucleatum</i> (Knorr)	<i>F. polymorphum</i> (Knorr)	<i>Leptotrichia buccalis</i> (Trevisan)	<i>Selenomonas sputigena</i> (Flügge) von Prowazek	<i>Fusocillus girans</i> (Prévot)	<i>Bacteroides melanogenicum</i> (Oliver and Wherry)
Knorr (1928)	<i>Fusobacterium nucleatum</i>	<i>F. polymorphum</i>	<i>F. plauti-vincenti</i>	<i>Spirillum sputigenum</i> (Miller)	—	—
Varney (1927)	Type I subgroup II and type II	Type I subgroup I and type III	Type IV	—	—	—
Séguin (1928)	—	—	—	<i>Fusobacterium plauti</i>	—	—
Slanetz & Rettger (1933)	Type I	Type II and some Type III	Type IV and some Type III	—	—	—
Spaulding & Rettger (1937)	Group Ia	Group Ib	Group II	—	—	—
Hine & Berry (1937)	<i>Fusiformis nucleatus</i>	<i>F. polymorphus</i>	<i>F. dentium</i>	—	—	—
Bøe (1941)	<i>Fusobacterium plauti-vincenti</i>	<i>F. plauti-vincenti</i>	<i>Leptotrichia buccalis</i>	—	—	—
Prévot (1938, 1940)	<i>Fusiformis fusiformis</i> * (Vincent) Topley & Wilson	<i>F. fusiformis</i> * (Vincent) Topley & Wilson	<i>F. fusiformis</i> * (Vincent) Topley & Wilson	<i>Fusocillus plauti</i>	<i>F. girans</i>	—
Topley & Wilson's Principles (1946)	—	—	<i>Fusiformis fusiformis</i> ?	—	—	<i>Fusiformis melanogenicus</i>
Macdonald (1953)	<i>Fusobacterium nucleatum</i> (Knorr)	<i>F. polymorphum</i> (Knorr)	<i>F. fusiforme</i> (Veillon & Zuber) Hoffman	<i>Spirillum sputigenum</i>	<i>Fusobacterium girans</i>	—
Hoffman (1957)	—	—	—	—	—	—

* Not apparently identical with the organism later described under this name by Prévot & Taffanel (1942).

cultural conditions. The main differences are that *Fusobacterium* is a genus of Gram-negative strictly anaerobic fusiform organisms which divide by constriction giving rise to organisms pointed at both ends, weakly ferment sugars and form indole and ammonia from amino acids, whereas the genus *Leptotrichia* consists of Gram-positive microaerophilic or facultatively anaerobic organisms with well-defined cross-walls; the organisms are lanceolate in *L. buccalis*, and bacillary in *L. dentium*; they ferment a wide range of sugars and do not form indole or ammonia from amino acids (Baird-Parker & Davis, 1958; Davis & Baird-Parker, 1959b).

It is proposed that the genus *Fusobacterium* should be defined in accordance with the characters outlined in this paper and that at the present time only the two species, *F. nucleatum* Knorr and *F. polymorphum* Knorr should be recognized. These resemble one another closely in their metabolic characters and cell composition but can be readily distinguished by their morphology. This distinction is maintained under laboratory conditions. *Fusiformis fusiformis* which is currently defined as a separate species by Prévot and his co-workers is omitted because its identity is uncertain; it is distinct from the organism given this name in *Topley and Wilson's Principles* (1929); see also Berger (1956). It appears to be closely related to *Fusobacterium polymorphum* and should probably be included in this species on account of its similar cell composition. The remaining organisms classified in the genus *Fusiformis* in *Topley and Wilson's Principles* (1955) and in the genus *Fusobacterium* by Hoffman (1957) should, without exception, be placed either in the Bacteroides group or in families and genera distinguished within this group by Prévot (1938). These organisms all differ from the true fusobacteria, either in not possessing a fusiform morphology or in having metabolic characters, such as proteolytic activities, which are not characteristic of the genus. These differences are discussed by Weiss & Rettger (1937); Lewis & Rettger (1940); Lahelle (1947); Jonsen & Thjøtta (1948) and Jackins & Barker (1951). They conclude that oral fusobacteria are distinct from intestinal Bacteroides species. Further evidence for this view is provided by the recent study of Beerens, Guillaume & Petit (1959) who demonstrated that *Fusiformis nucleatus*, *F. polymorphus* and *F. fusiformis* metabolize glucose in a manner which distinguishes them from the Bacteroides and the intestinal fusiform *F. biacutus*.

The filamentous nature of the fusobacteria suggests their inclusion in the Actinomycetales (Prévot, 1938; Bisset, 1952). Their Gram-negative state is apparently secondary as indicated by their sensitivity to antibiotics such as penicillin (Lavergne, Burdin, Schmitt & le Moynes, 1956; Baird-Parker, 1959). Further evidence for inclusion with the Gram-positive genera is the absence of detectable sulphur-containing amino acids and the presence of a high proportion of sugar in the cell wall (Cummins, 1956). Size of organism, septation and the lack of acid-fastness indicates a relationship to the Actinomycetaceae. It is possible to regard the oral fusobacteria as degenerate actinomycetes which, in undergoing adaptation to parasitism, have lost their Gram-positive state, catalase and some synthetic ability. It was suggested by Bisset (1959) that this process is commonly found in parasitic bacteria of Gram-positive origin.

I wish to thank Dr K. A. Bisset for his advice and encouragement throughout this work.

REFERENCES

- AYERS, H. S. & RUPP, P. (1922). Differentiation of haemolytic streptococci from human and bovine sources by the hydrolysis of sodium hippurate. *J. infect. Dis.* **30**, 388.
- BAIRD-PARKER, A. C. (1957). Isolation of *Leptotrichia buccalis* and *Fusobacterium* species from oral material. *Nature, Lond.* **180**, 1056.
- BAIRD-PARKER, A. C. (1959). *The classification of oral Leptotrichia and Fusobacterium species*. Ph.D. Thesis; University of Birmingham.
- BAIRD-PARKER, A. C. & DAVIS, G. H. G. (1958). The morphology of *Leptotrichia* species. *J. gen. Microbiol.* **19**, 446.
- BARNETT, J. A., INGRAM, M. & SWAIN, T. (1956). The use of β -glucosides in classifying yeasts. *J. gen. Microbiol.* **15**, 529.
- BARRETT, M. M. (1936). The intensification of the Voges-Proskauer reaction by the addition of α -naphthol. *J. Path. Bact.* **42**, 441.
- BEERENS, H., GUILLAUME, J. & PETIT, H. (1959). Étude de la fermentation propionique de la L-thréonine par 45 souches de bactéries anaérobies non sporulées à Gram-négatif. *Ann. Inst. Pasteur*, **96**, 211.
- BERGER, U. (1956). Untersuchungen an Fusobakterien. I. Mitteilung. Systematik, Züchtung und Morphologie. *Zbl. Bakt. (1. Abt.)*, **166**, 484.
- BISSET, K. A. (1952). *Bacteria*. Edinburgh and London: E. and S. Livingstone.
- BISSET, K. A. (1959). Characters associated with parasitism in Gram-positive bacteria. *Nature, Lond.* **184**, B.A. 29.
- BØE, J. (1941). *Fusobacterium*. Studies on its Bacteriology, Serology and Pathogenicity. *Norske. Videnskaps-Akad. I. Oslo I. Mat-Naturv. Klasse*, no. 9, p. 1.
- BUCHANAN, R. E., COWAN, S. T. & WIKÉN, T. (1953). Status of generic names of bacteria proposed by Trevisan. *Int. Bull. bact. Nomencl.* **3**, 96, 103.
- CUMMINS, C. S. (1956). The chemical composition of the cell-wall. *Int. Rev. Cytol.* **5**, 25.
- CUMMINS, C. S. & HARRIS, H. (1958). Studies on the cell-wall composition and taxonomy of Actinomycetales and related groups. *J. gen. Microbiol.* **18**, 173.
- DAVIS, G. H. G. & BAIRD-PARKER, A. C. (1959a). Cell-wall composition of *Leptotrichia* spp. *Nature, Lond.* **183**, 1206.
- DAVIS, G. H. G. & BAIRD-PARKER, A. C. (1959b). *Leptotrichia buccalis*. *Brit. dent. J.* **106**, 70.
- DAVIS, J. G. & ROGERS, H. T. (1939). The effect of sterilization upon sugars. *Zbl. Bakt. (2. Abt.)*, **101**, 102.
- ECKFORD, M. O. (1927). Measurement of diastatic action. *Amer. J. Hyg.* **7**, 201.
- FRAZIER, W. C. (1926). A method for detection of changes in gelatin due to bacteria. *J. infect. Dis.* **39**, 302.
- GIBSON, T. & ABD-EL-MALEK, Y. (1946). The formation of carbon dioxide by lactic acid bacteria and *Bacillus licheniformis* and a cultural method of detecting the process. *J. Dairy Res.* **14**, 35.
- HASTINGS, E. G. (1904). The action of various classes of bacteria on casein as shown by milk-agar plates. *Zbl. Bakt. (2. Abt.)*, **12**, 590.
- HAYWARD, A. C. (1957). Detection of gas production from glucose by heterofermentative lactic acid bacteria. *J. gen. Microbiol.* **16**, 9.
- HINE, M. K. & BERRY, G. P. (1937). Morphological and cultural studies of the genus *Fusiformis*. *J. Bact.* **34**, 517.
- HOARE, D. S. & WORK, E. (1957). The stereoisomers of $\alpha\epsilon$ -diaminopimelic acid. II. Their distribution in the bacterial order Actinomycetales and in certain Enbacteriales. *Biochem. J.* **65**, 441.

- HOFFMAN, H. (1957). In *Bergey's Manual of Determinative Bacteriology*, ed. R. S. Breed, E. G. D. Murray & N. R. Smith, 7th edition, p. 436. London: Baillière, Tindall and Cox.
- ISENBERG, H. D. & SUNDHEIM, L. H. (1958). Indole reactions in bacteria. *J. Bact.* **75**, 682.
- JACKINS, H. C. & BARKER, H. A. (1951). Fermentative processes of the fusiform bacteria. *J. Bact.* **61**, 101.
- JEYNES, M. H. (1955). Taxonomic position of the genus *Selenomonas* (von Prowazek). *Nature, Lond.* **176**, 1077.
- JEYNES, M. H. (1956). Analysis of the genus *Selenomonas* with respect to its transfer to the protozoa. *Int. Bull. bact. Nomencl.* **6**, 53.
- JONSEN, J. & THJÖTTA, TH. (1948). Studies on *Bacteroides*. II. *B. funduliformis* and its relation to *Necrobacterium necrophorus* (*Actinomyces necrophorus*). *Acta path. microbiol. scand.* **25**, 688.
- KNORR, M. (1923). Über die Fusospirillare Symbiose, die Gattung *Fusobacterium* (Lehmann) und *Spirillum sputigenum*. II. Mitteilung. Die Gattung Fusobacterien. *Zbl. Bakt.* (1. Abt.), **89**, 4.
- LAHELLE, O. (1947). *Necrobacterium*. A study of its bacteriology, serology and pathogenicity, and its relationship to *Fusobacterium*. *Acta path. microbiol. scand.* Suppl. **67**, 1.
- LAVERGNE, E. DE, BURDIN, J. C., SCHMITT, J. & LE MOYNE, M. T. (1956). Étude du comportement de 17 espèces anaérobies strictes vis-à-vis de 11 antibiotiques. *Ann. Inst. Pasteur*, **91**, 631.
- LEWIS, K. H. & RETTGER, L. F. (1940). Non-sporulating anaerobic bacteria of the intestinal tract. I. Occurrence and taxonomic relationships. *J. Bact.* **40**, 287.
- MACDONALD, J. B. (1953). *The Motile Non-Sporulating Anaerobic Rods of the Oral Cavity*. Toronto: University of Toronto Press.
- MACKIE, T. J. & MCCARTNEY, J. E. (1953). *Handbook of Practical Bacteriology*. 9th edition. London: E. and S. Livingstone Ltd.
- MCCLUNG, L. S. & TOABE, R. (1947). The egg yolk plate reaction for the presumptive diagnosis of *Cl. sporogenes* and certain species of the gangrene and botulinum group. *J. Bact.* **53**, 139.
- MULLANEY, P. J. (1956). A simple fermentation medium for *Neisseria gonorrhoeae*. *J. Path. Bact.* **71**, 516.
- OLITZKI, A. L. (1954). Hydrogen sulphide production by non-multiplying organisms and its inhibition by antibiotics. *J. gen. Microbiol.* **11**, 160.
- OMATA, R. & DISRAELY, M. N. (1956). A selective medium for oral fusobacteria. *J. Bact.* **72**, 677.
- PRÉVOT, A. R. (1938). Études de systématique bactérienne. III. Invalidité du genre *Bacteroides*. Castellani et Chalmers, démembrement et réclassification. *Ann. Inst. Pasteur*, **60**, 285.
- PRÉVOT, A. R. (1940). Recherches sur la flore anaérobie de l'intestin humain. *C.R. Soc. Biol., Paris*, **133**, 246.
- PRÉVOT, A. R. & TAFFANEL, J. (1942). Recherches biochimiques sur *F. fusiformis*. *Ann. Inst. Pasteur*, **68**, 420.
- PRÉVOT, A. R., TARDIEUX, P., JOUBERT, L. & DE CADORE, F. (1956). Recherches sur *Fusiformis nucleatus* (Knorr) et son pouvoir pathogène pour l'homme et les animaux. *Ann. Inst. Pasteur*, **91**, 787.
- ROBINOW, F. C. (1945). In Dubos's *The Bacterial Cell*. Cambridge, Mass.: Harvard University Press.
- SCHWABACHER, H., LUCAS, D. R. & RIMINGTON, C. (1947). *Bacterium melaninogenicum*—a misnomer. *J. gen. Microbiol.* **1**, 109.
- SÉGUIN, P. (1928). Culture du *Fusobacterium plauti*, forme mobile du bacilli fusiforme. *C.R. Soc. Biol., Paris*, **99**, 439.

- SLANETZ, L. W. & RETTGER, L. F. (1933). A systematic study of the fusiform bacteria. *J. Bact.* **26**, 599.
- SOCIETY OF AMERICAN BACTERIOLOGISTS (1957). *Manual of Microbiological Methods*. New York: McGraw-Hill.
- SPAULDING, E. H. & RETTGER, L. F. (1937). The *Fusobacterium* genus. I. Biochemical and serological classification. *J. Bact.* **34**, 535.
- TITSLER, R. P. & SANDHOLZER, L. A. (1936). The use of semi-solid agar for the detection of bacterial motility. *J. Bact.* **31**, 575.
- Topley and Wilson's Principles of Bacteriology and Immunity* (1929). London: Arnold.
- Topley and Wilson's Principles of Bacteriology and Immunity* (1946, 1955). 3rd and 4th ed. Ed. G. S. Wilson & A. A. Miles. London: Arnold.
- TREVISAN, V. (1879). Prime Linee d'introduzione allo Studio dei Batterj Italiani. *Reale Istituto Lombardo di Scienze e Lettere*, Ser. II, **12**, 133.
- VARNEY, P. L. (1927). Serological classification of fusiform bacilli. *J. Bact.* **13**, 275.
- WEINBERG, M., NATIVELLE, R. & PRÉVOT, A. R. (1937). *Les Microbes Anaérobies*. Paris: Masson et Cie.
- WEISS, J. E. & RETTGER, L. F. (1937). The Gram-negative bacteroides of the intestine. *J. Bact.* **33**, 423.

EXPLANATION OF PLATE

- Fig. 1. *Fusobacterium polymorphum*, typical 3-day colony on *Fusobacterium* medium, $\times 50$. Also characteristic of some *F. nucleatum* strains, and all group III strains.
- Fig. 2. *Fusobacterium nucleatum*, typical 3-day colony on *Fusobacterium* medium, $\times 50$.
- Fig. 3. *Fusobacterium nucleatum*, type III (Table 2). 1-day colonies on *Fusobacterium* medium, $\times 60$.
- Fig. 4. *Fusobacterium nucleatum*, cell-wall stain of 18 hr. culture, $\times 2500$.
- Fig. 5. *Fusobacterium polymorphum*, cells from 3-day colony stained with strong carbolfuchsin to demonstrate basophilic, intracellular granules, $\times 2500$.
- Fig. 6. *Fusobacterium polymorphum*, cell-wall stain of 24 hr. culture, $\times 2500$.

(Received 16 September 1959)