Description of Strains of *Peptostreptococcus anaerobius* Isolated from Subcutaneous Abscesses in Cats

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Strains of *Peptostreptococcus*, *Streptococcus* and of a Gram-positive coccus, which was initially isolated as an anaerobe but grew subsequently as a facultative organism, were isolated from subcutaneous abscesses in cats. The cat strains of *Peptostreptococcus* gave metabolic fermentation products in combinations described for *P. anaerobius*. The *Streptococcus* strains conformed to the group *S. intermedius*. The facultative organism described had the metabolic products of *P. anaerobius* but the distinctly different biochemical characteristics of *S. intermedius* and fits neither of the genera strictly.

INTRODUCTION

Controversy surrounds the classification of some of the Gram-positive anaerobic cocci (Rogosa, 1974; Sutter *et al.*, 1975). Rogosa (1974) suggests that the organisms which were formerly classified as *Peptostreptococcus intermedius* but which are not strict anaerobes, i.e. they will grow on the surface of agar plates after several subcultures, should be removed from the genus *Peptostreptococcus* and placed in the genus *Streptococcus*. This view is held by others who now classify this organism as *Streptococcus intermedius* (Holdeman *et al.*, 1977; Sutter *et al.*, 1975) as it is primarily saccharoclastic with lactic acid as the sole major product of fermentation. We have isolated and characterized another facultative Gram-positive coccus which does not appear to fall strictly into any of the genera so far described.

METHODS

Ten strains of *Peptostreptococcus*, four strains of *Streptococcus* and three strains of a Gram-positive coccus, which was initially isolated as an anaerobe on supplemented brain heart infusion agar (Holdeman & Moore, 1975) but grew subsequently as a facultative organism, were isolated from subcutaneous abscesses in cats. The reference strain ATCC 27337 of *Peptostreptococcus anaerobius* was included for comparison. Pure cultures of each strain were grown anaerobically in peptone/yeast extract/glucose (PYG) medium (Holdeman & Moore, 1975) and in cooked meat plus BVF-OS broth (Turner *et al.*, 1935) supplemented with 0.4% (w/v) glucose, 0.1% (w/v) cellobiose, 0.1% (w/v) maltose and 0.1% (w/v) starch (CMC medium). Facultative organisms were also grown aerobically in CMC and PYG (not pre-reduced) media and their metabolic products were analysed. Selected strains of each group were tested for their fermentation products from peptone/yeast extract/threonine (PY-T) and peptone/yeast extract/pyruvate (PY-P) media (Holdeman *et al.*, 1977). All metabolic products were extracted according to the procedures of Holdeman & Moore (1975) and analysed by gas-liquid chromatography (g.l.c.) using a Hewlett-Packard gas chromatograph (model 5830A) fitted with a glass column (160 cm × 2 mm i.d.) packed with either 10% (w/w) AT1200 (Alltech Associates, Illinois, U.S.A.) plus 1% (w/w) H₃PO₄ on Chromosorb (Johns-Manville Inter Corp., Colorado, U.S.A.) W-AW, 80/100 mesh, which was used for routine analyses, or 5% (w/w) FFAP (Deltron, Summer Hill, N.S.W., Australia) on Chromosorb W-HP, 80/100 mesh. The carrier gas (nitrogen) flow rate was 30 ml min⁻¹, the oven temperature was 115 °C, and the flame ionization detector was at 225 °C. The machine was computer programmed to quantify the products of fermentation against standards of 1 mequiv./100 ml values.

The biochemical tests performed were those described for the anaerobic cocci by Holdeman *et al.* (1977). Peptone/yeast extract/sugars were pre-reduced (Holdeman *et al.*, 1977) while other biochemical tests were incubated anaerobically in the BBL GasPak Anaerobic System in which the catalysts had been rejuvenated by heating before each use (Holdeman *et al.*, 1977).

RESULTS AND DISCUSSION

The strains of strictly anaerobic Gram-positive cocci isolated from cats, as well as the reference strain of *P. anaerobius*, were similar in cultural characteristics. They were inactive biochemically, i.e. they did not ferment a wide range of sugars, did not hydrolyse aesculin and did not react in the range of other tests described by Holdeman *et al.* (1977). In both PYG and CMC media, incubated anaerobically, *P. anaerobius* ATCC 27337 produced acetic and isobutyric acids (≥ 1 mequiv./100 ml) and butyric acid (< 1 mequiv./100 ml). The cat strains of *P. anaerobius* produced acetic, isovaleric and isocaproic acids (≥ 1 mequiv./100 ml) and propionic, isobutyric, butyric and valeric acids (< 1 mequiv./100 ml). From pyruvate they produced acetic, isobutyric, isovaleric and isocaproic acids (< 1 mequiv./100 ml), and threonine was converted to propionic acid. Strains of *S. intermedius* produced acetic acid (< 1 mequiv./100 ml) and lactic acid (≥ 1 mequiv./100 ml) when grown both aerobically and anaerobically, produced acetic and lactic acids from pyruvate but did not convert threonine to propionic acid. They had the biochemical and morphological characteristics described for *S. intermedius* (Holdeman *et al.*, 1977), including hydrolysis of aesculin and acid formation from a wide range of sugars.

The third group of Gram-positive cocci were isolated as anaerobes but, after several subcultures, grew well aerobically. These strains had morphological and biochemical characteristics similar to *S. intermedius*, i.e. they produced acid from glucose, lactose, maltose, sucrose and cellobiose and hydrolysed aesculin. However, their metabolic fermentation products were similar to those of the cat strains of *P. anaerobius*. These products were formed in PYG, CMC and PY-P media incubated both aerobically and anaerobically, although in all strains the amounts of each product were greatest in CMC medium. Threonine was converted to propionic acid.

A feature of *P. anaerobius* and the uncharacterized organism, but not of streptococci, was the production of very large amounts of isocaproic acid. This acid was detected as its methylated derivative by g.l.c. on AT1200 (but not FFAP) columns, and its identity was confirmed by g.l.c.-mass spectrometry. It was produced in cultures of cat strains and the reference strain of *P. anaerobius* grown anaerobically in PYG and CMC media and by the facultative organism under both anaerobic and aerobic conditions in CMC and PYG media.

The metabolic products of the cat strains of *P. anaerobius* were diverse but were present in combinations described for the genus (Rogosa, 1974). The strains which remained anaerobic despite continued subculture are true members of the genus *Peptostreptococcus*. *Streptococcus*, on the other hand, is a facultative organism and its major fermentation product is lactic acid (Deibel & Seeley, 1974). It seems correct, therefore, to group *S. intermedius* according to its lactic acid production despite its reluctance to grow aerobically until multiple subculturing has taken place. The facultative organisms described here have the metabolic products of *P. anaerobius* but the distinctly different biochemical characteristics of *S. intermedius*. It is also clear that these facultative organisms fit neither of these genera strictly. If the emphasis is to be placed on metabolic fermentation products rather than growth requirements and biochemical tests, they should be grouped with the peptostreptococci and may be 'aerotolerant strains' of *P. anaerobius* with different biochemical reactions. As with *P. anaerobius*, isocaproic acid is a major metabolite. If, however, the genus contains only strict anaerobes they must be placed elsewhere.

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REFERENCES

- DEIBEL, R. H. & SEELEY, H. W. (1974). Genus I. *Streptococcus*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, p. 490. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- HOLDEMAN, L. V. & MOORE, W. E. C. (1975). *Anaerobe Laboratory Manual*, 3rd edn. Blacksburg, Va: Virginia Polytechnic Institute and State University.
- HOLDEMAN, L. V., CATO, E. P. & MOORE, W. E. C. (1977). *Anaerobe Laboratory Manual*, 4th edn. Blacksburg, Va: Virginia Polytechnic Institute and State University.
- ROGOSA, M. (1974). Genus II. *Peptostreptococcus*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- SUTTER, V. L., VARGO, V. L. & FINEGOLD, S. M. (1975). *Wadsworth Anaerobic Bacteriology Manual*, 2nd edn. Los Angeles: Department of Continuing Education in Health Sciences, University Extension and the School of Medicine, University of California.
- TURNER, A. W., CAMPBELL, A. D. & DICK, A. T. (1935). Recent work on pleuro-pneumonia contagiosa bovis in North Queensland. *Australian Veterinary Journal* **11**, 63-70.