ISOLATION OF AN ORAL FILAMENTOUS MICROORGANISM 1, 2

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In the past, a wide variety of unrelated oral filamentous and rod shaped bacteria were placed either in the genera Leptothrix or Leptothricia. The confusion resulted from meager studies of many of these organisms, inadequate generic descriptions, and use of the ill-defined word "filamentous." Bergey’s Manual of Determinative Bacteriology in 1948 stated, “the genus Leptothricia Trevisan, 1879 is no longer recognized as a valid genus,” and in 1957 completely neglected it. The genus Leptothrix has been redefined and would now (Breed et al., 1957) exclude the oral forms previously placed in it.

The confusion concerning classification of oral forms is well illustrated by the fact that within the past twenty-five years two entirely different organisms have been termed Leptothrix buccalis. One of these, described by Thijseta et al. (1939), B3e and Thijsetta (1944), Hamilton and Zahler (1957), and others, is a rod, generally 10 μ long, and normally two rods chain to form short filaments. In fluid media, filaments of up to 200 μ in length can be found. The organism is anaerobic or microaerophilic, gram-positive only in very young cultures, and does not branch. The second type of organism, described by Bibby (1935), Bartels (1943), and Morris (1954), is a filament with a bacillus-like body attached to one end. According to Bibby (1935), the unsegmented filaments vary from 0.8 to 1.5 μ in diameter and may be more than 100 μ long. This organism is anaerobic or facultative, gram-positive, and branches very rarely in anaerobic cultures. As described by Bibby, this organism resembles the generic description for Leptothricia (Bergey et al., 1934), whereas the organism described by Thijsetta resembles the type species chosen for this genus.

As a possible contribution towards a better classification of parasitic bacteria previously placed in the genera Leptothrix and Leptothricia, a systematic investigation of organisms resembling those described by Bibby (1935) has been undertaken. Difficulties in their isolation and particularly their subculture have been reported (Morris, 1954). It is therefore deemed worthwhile to describe the isolation, culture requirements, and colonial and cellular morphologies of this type of organism.

MATERIALS AND METHODS

Pure cultures of sixty strains were isolated from carious lesions, calculus, matura alba, and plaque material of twenty subjects. An inoculum of the source material was prepared by grinding in 0.5 ml of 0.85 per cent sterile saline and diluting 1:10, 1:100, and 1:1000. Two methods were found satisfactory for primary isolation: (a) One-tenth ml aliquots of the diluted and undiluted suspensions were dispensed into sterile petri dishes, and pour plates prepared using brain-heart-infusion (Difco) agar supplemented with 0.2 per cent yeast extract. The plates were incubated aerobically 3 to 4 days at 37 C. (b) The undiluted material was streaked onto plates of brain-heart-infusion (Difco) agar supplemented with 0.2 per cent yeast extract and 7 per cent citrated sheep blood (hereafter referred to as blood agar). The plates were incubated 3 to 4 days under strict anaerobic conditions employing a McIntosh and Fildes jar and an atmosphere of 95 per cent hydrogen and 5 per cent carbon dioxide. To check on anaerobiosis, test plates inoculated with either Bacillus subtilis or Nocardia asteroides, strain 328 (supplied by Dr. A. Howell, National Institute of Dental Research) were incubated in the jars. Typical colonies from the pour plates and the anaerobic blood agar streak plates were purified by serial restreaking. Subculture was performed on blood agar plates incubated anaerobically or plates of brain-heart-infusion agar supplemented with yeast extract and 1 per cent glucose and

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Figure 1. Pour plate colonies in brain-heart-infusion agar + yeast extract incubated aerobically for 48 hr, showing filamentous edge.

Figure 2. Surface colonies on blood agar incubated anaerobically 72 hr showing rhizoid colonial morphology. (Culture from Dr. B. G. Bibby.)
Figures 3 and 4. Surface colonies on brain-heart-infusion agar + yeast extract incubated aerobically for 72 hr.

incubated either aerobically or anaerobically. Although some cultures appeared pure after serial restreaking, they were in fact often contaminated with actinomycetes or diphtheroid organisms. Therefore, a dilution procedure was employed to ensure purity of stocks. For this purpose rubber stoppered brain-heart-infusion with yeast extract plus 1 per cent glucose broth cultures were incubated with vigorous agitation on a reciprocating shaker to obtain
diffuse growth. The cells were counted in a hemocytometer, and a broth dilution of two cells per ml prepared. One-ml aliquots of the diluted suspension were distributed into sterile Kahn tubes and incubated aerobically at 37 C for 48 hr. Tubes showing least turbidity were chosen for subculture.

RESULTS

Colonial morphology. (1) Pour plates:—Upon initial isolation, pour plate colonies have the appearance of a tight ball of hair (figure 1), and upon continued incubation resemble a white fluffy ball of cotton wool as noted by Bibby (1935). The opacity of the colony is less in the depths of the agar, and greater on the surface.

(2) Anaerobic streak plates:—Colonial morphology on anaerobically incubated plates is influenced by the medium employed. All strains yield translucent flat rhizoid colonies with a slightly raised central area on blood agar (figure 2), whereas on brain-heart-infusion (with yeast extract) agar supplemented with 1 per cent glucose, both flat rhizoid colonies and colonies of the type illustrated in figure 3 are found. The latter type is also found on aerobic plates.

(3) Aerobic streak plates:—Colonies of aerobic cultures on brain-heart-infusion (with yeast extract) plates exhibit marked heterogeneity not only between strains but even on plates streaked with pure cultures. The types of colonies generally encountered are translucent, flat, filamentous, 2 mm or less in diameter, resembling the pour plate colonies in figure 1, opaque white or gray white, 0.5 to 2 mm in diameter (figures 3 and 4), and opaque white or gray white, 0.5 to 1 mm in diameter, differing from figure 4 in having a raised rolled border between the opaque center and filamentous edge. In addition, one strain gave somewhat conical, opaque, cream colored, butyrous, radiate, lobate edged colonies on aerobic brain-heart-infusion (with yeast extract) streak plates.

Cell morphology. The predominating cell type is a nonseptate filament with one end attached to a bacillus-like body. However, in any one colony, cells of three types can be found: (a) one or more filaments of varying lengths attached to a bacillus-like body, (b) the bacillus-like body alone, and (c) filaments (figure 5). Cell wall staining (Chance, 1953) has shown that the bacillus-like body is generally separated from its attached filaments by a septum. Very occasionally zigzagged rows of bacillus-like bodies are found, possibly representing a reproductive phase of the filaments (figure 6).

Cell size seems to be a strain trait which is also influenced by cultural conditions. The filaments are 1 to 2.3 μ wide and 20 μ to more
Figure 6. Unstained cells from a continuously observed microcolony illustrating fragmentation of the filament to form a zigzagged row of bacillus-like bodies.

Figure 7. Unstained cells in a continuously observed microcolony illustrating the forking type of branching.
Figure 8. Cells stained by Laybourn’s method showing metachromatic granules.

than 100 μ long; the bacillus-like bodies are 1.3 to 2.5 μ wide and 2.5 to 10 μ long.

Dichotomous branching has been observed. As shown in figure 7 there are no septa between the branches and the mother filament. However, continuous observation of growing cells has often shown that eventually septa are formed at these points. Branching occurs regularly in aerobically incubated cultures, whereas it occurs extremely rarely in colonies on anaerobic plates.

The staining reactions are as previously described (Bibby, 1935). Young cells are gram-positive, and in older cultures, the cells have the appearance of a pink sheath containing small purple coccolid elements. Metachromatic granules are observed with the Laybourn (1924) metachromatic stain (figure 8).

Growth conditions. (1) Media:—Good growth is obtained on brain-heart-infusion agar (Difco) supplemented with 0.2 per cent yeast extract. One per cent glucose enhances the growth rate and is employed in all growth conditions subsequent to the primary isolation plate. Satisfactory growth also occurs on nutrient broth or agar supplemented with 0.2 per cent yeast extract and 1 per cent glucose.

(2) Gaseous requirements:—Upon initial isolation, optimal growth takes place with a slightly reduced oxygen tension. Following continued subculture, good growth occurs under both aerobic and strict anaerobic conditions. However, changes in colony morphology and cell size occur more frequently under aerobic than anaerobic circumstances. Carbon dioxide is required for growth on brain-heart-infusion or blood agar plates incubated anaerobically.

(3) Maintenance of cultures:—Cultures have been maintained for one year on slants of brain-heart-infusion (with yeast extract) and transferred monthly. Stock cultures have also been successfully lyophilized.

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SUMMARY

Sixty strains of a mouth organism resembling types previously termed Leptotrichia or Leptothrix and often reported difficult to subculture, were isolated in pure culture from materia alba, plaques, calculus, and carious lesions. The organism is a filament with a bacillus-like body attached at one end. Good growth of all strains occurred on brain-heart-infusion agar supplemented with 0.2 per cent yeast extract, and cultures could be maintained on this medium. All strains grew under both aerobic and strict anaerobic conditions. Dichotomous branching
occurred regularly in aerobically incubated cultures, and extremely rarely in anaerobically incubated cultures. The taxonomic positioning of this organism remains for future work.

REFERENCES

Bartels, H. A. 1943 A filamentous microorganism isolated from stained teeth. J. Dental Research, 22, 97–102.


