The Morphology of Leptotrichia Species

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SUMMARY: The morphology is described of two species of filamentous oral bacteria, both of which have in the past been accorded the same name, Leptotrichia buccalis. Although these have certain characters in common, it is concluded that they are entirely distinct. Both show signs of a residual life-cycle, suggesting a remote relationship with more complex saprophytes.

The specific name Leptotrichia buccalis was proposed by Trevisan (1879) to replace Leptothrix buccalis (Robin, 1858) on the grounds that the usage of Leptothrix, originally signifying filamentous iron bacteria (Kützing, 1848), had become unworkably confused. The early descriptions of the species were clearly recognizable, but this generic name became in its turn corrupted by application to any micro-organism capable of growing in culture in the form of unbranched filaments, so that even sporing bacilli (Gifford, 1920) have been assigned to it, irrespective of origin, whereas true Leptotrichia are exclusively oral parasites and have a very characteristic morphology, so characteristic indeed as to be clearly recognizable in the drawings of Antonj van Leeuwenhoek. The descriptions of Robin (1858), Trevisan (1879) and Goadby (1908), especially the latter, make it clear that the name correctly refers to the Gram-positive, fusiform, anaerobic organism which forms so large an element in smears of the materia alba of the teeth.

Confusion has arisen especially in two respects, by the failure to distinguish between Leptotrichia buccalis and Fusobacterium species (Hine & Berry, 1937; Omata & Disraely, 1950), and by application of the name to the second type of micro-organism described in this paper, a filamentous branched aerobe (Bulleid, 1925; Bibby & Berry, 1939; Ludwig, 1955). For this species, the name L. dentium has been proposed (Davis & Baird-Parker, 1958). The purpose of this paper is to describe its morphology and cytology, to compare it with L. buccalis and to suggest their possible relationships with one another and with other bacterial groups.

MATERIALS

Strains of Leptotrichia buccalis were isolated in McIntosh and Fildes jars containing an atmosphere of 90% (v/v) hydrogen + 10% (v/v) carbon dioxide. The medium employed contained (% w/v) proteose peptone (Difco), 1; yeast extract (Oxoid), 0.1; Lab-Lemco, 0.3; glucose, 1; soluble starch (Analar), 0.2; L-cysteine hydrochloride, 0.05; anhydrous Na₂HPO₄, 0.5; agar, 1.5; dissolved in distilled water. This medium was autoclaved at 10 lb./sq.in. for 20 min. After autoclaving the following additions were made: 5% (v/v) Seitz-filtered serum; sulphathiazole to 0.05 mg./ml.; ethyl violet to a final concentration of 1:15,000.
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The pH of the medium after autoclaving was 7.6. Plates were poured immediately before use, and inoculated with saliva or *materia alba*. Colonies appeared after 2–3 days of incubation at 37°C. Isolated colonies were replated on the same medium with the omission of the ethyl violet and sulphathiazole.

Strains of *Leptotrichia dentium* were isolated on a medium containing (% w/v): peptone (Oxoid), 1; yeast extract (Oxoid), 0.3; soluble starch (Analar), 0.2; sodium chloride, 0.5; agar, 1.5; in beef heart broth and adjusted to pH 7.6. The medium was autoclaved at 10 lb./sq.in. for 20 min. Plates of this medium were poured at c. 45° and inoculated with 1 ml. of a suspension of *materia alba* well dispersed in normal saline, and incubated for 2–3 days aerobically at 37°C. Submerged colonies of *L. dentium* were removed by cutting out a small piece of agar and used to inoculate further poured plates. Surface plating was used when a reasonably pure inoculum had been obtained.

Morphological studies were made upon material stained for cell walls by the method of Hale (1953) in the case of *Leptotrichia dentium*, and by tannic-acid and crystal violet (Robinow, 1945) for *L. buccalis*, which gave better results by this method. Colonies were photographed *in situ*.

**RESULTS**

The colonies of *Leptotrichia buccalis* shown in Pl. 1., figs. 1 and 2 are of a mature (8-day) and a growing (24 hr.) colony, respectively; the 'Medusa-head' appearance is characteristic. By comparison, the colonies of *L. dentium* more closely resembled those of actinomycetes. The submerged colonies of *L. dentium* on primary isolation had a tangled appearance (Pl. 1, fig. 3) and all growth under these microaerophilic conditions was thin and sparse (Pl. 1, fig. 4). Aerobic surface colonies grew much more profusely (Pl. 1, fig. 5), but in all cases the edge consisted of outgrowing, branched filaments.

On first isolation the constituent elements of the colonies of *Leptotrichia buccalis* were fusiform, with very occasional small branches (Pl. 1, fig. 6). At later stages of culture, filaments with side-branches were sometimes found (Pl. 1, fig. 7), but these were never common, and the branches were of the impermanent type, rapidly separated by a septum. L-Form production was very common in these cultures. In the initial stage of L-form production a section of filament showed signs of spiral twisting (Pl. 1, fig. 8), and then swelled into a large, almost globular structure, from which presumed L-forms were released (Pl. 1, fig. 9).

In *Leptotrichia dentium* branching was frequent at most stages of culture, and could, as already noted, be observed at the edges of the colonies (Pl. 1, fig. 4; Pl. 2, fig. 10). This species not only shows normal dichotomous branching of this type, but reproduces by branching in a very characteristic manner; this is illustrated in Pl. 2, fig. 11, and develops in the following manner (Fig. 1): a filament becomes segmented (a, b) and proceeds to break at the points of division, in a zig-zag manner (c). At the breaks, much narrower filaments grow outwards (d, e), and eventually the sections separate, each consisting of a short filament with a wider and a narrower portion (f). These 'whip-handles' are
diagnostic of *L. dentium* and may be seen in the majority of figures in Pl. 2. As the thinner parts of the filaments extend the diameter increases until the original diameter is restored (Pl. 2, figs. 12, 18). The wide filaments also reproduce by breaking down into spore-like bodies, in a manner strongly reminiscent of the sporogenous filaments of *Streptomyces* (Pl. 2, figs. 14, 15). These ‘spores’ germinate by a tube, and one is visible in Pl. 2, fig. 12 (arrowed). As in the case of *Leptotrichia buccalis*, *L. dentium* readily yields apparent L-forms, and in this case also the first sign is a spiral formation of the filaments (Pl. 2, fig. 16) followed by the production of swollen bodies (Pl. 2, fig. 17).

**DISCUSSION**

The morphology of *Leptotrichia buccalis* and *L. dentium* (previously described as *L. buccalis*) is shown to be distinct, although resemblances exist. *L. buccalis* branches very sparingly whereas *L. dentium* does so freely, and also retains the potentiality to form occasional chains of spores. In addition to these Streptomyces-like characters, the curious alternation between thicker and thinner filaments, the latter growing out almost like germination tubes from the former, is known to occur in Streptomyces. Very similar appearances have been described from submerged cultures of Streptomyces by Neukirch (1902) and by Pénaud, Hagemann, Velu & Peyré (1954). The *Vierhyphensporen* of Lieske (1921), also bear a close resemblance in general form. Bisset (1957) showed that the vegetative mycelium of Streptomyces was capable of producing forms reminiscent of Micromonospora, Actinomycetes and Nocardia. Upon this evidence the suggestion was made that the parasitic actinomycetes may have evolved by a degenerative process from Streptomyces, or some common ancestor. It is possible that the two species of *Leptotrichia* described...
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in this study may also represent degenerate forms of actinomycetes. How close the relationship between Leptotrichia buccalis and L. dentium may be is a matter of doubt. Almost certainly, if they are related, the former is an even more degenerate parasitic form than the latter, having become anaerobic and lost almost entirely its power of branching.

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REFERENCES


EXPLANATION OF PLATES

Plate 1

Fig. 1. Leptotrichia buccalis, 3-day colony on agar; × 16.

Fig. 2. As fig. 1 at 24 hr.; × 32.

Fig. 3. L. dentium, submerged colony on primary isolation pour plate, 3 days old; × 16.

Fig. 4. L. dentium, anaerobic surface colony, 3 days old. × 32.

Fig. 5. L. dentium, aerobic 2-day colony on agar surface; × 16.

Figs. 6–9. L. buccalis, stained tannic acid/crystal violet; × 1600.

Fig. 6. Typical cell forms and example of apical crutch formation.

Fig. 7. Filament bearing impermanent lateral branches.

Fig. 8. Filament showing spiral form in early stage of L-cycle.

Fig. 9. Presumed large-body formation in later stage of L-cycle.
Plate 2

Figs. 10–17. *L. dentium*, stained phosphomolybdic acid/methyl green. Figs. 11 and 17 from broth cultures, the rest from agar; ×1600.

Fig. 10. Filament showing variation in diameter and permanent branching.

Fig. 11. Germination of elements formed by segmentation of filament (see fig. 1).

Figs. 12, 13. Typical ‘whip-handle’ forms showing variation in diameter of filaments.

Fig. 14. Streptomycetes-like chain of spores.

Fig. 15. Group of presumed spores including one showing germination tube.

Fig. 16. Twisting of filaments in early stage of L-cycle.

Fig. 17. Presumed large body stage of L-cycle.

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Plate 1

(Facing p. 450)