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Unusual Cell Wall Ultrastructure of *Leptotrichia buccalis*

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Leptotrichia buccalis was examined by transmission electron microscopy. Its cell wall structure is generally compatible with that of gram-negative bacteria. However, the scale-like membranous folds associated with the external surface of the outer cell membrane appear to be sufficiently unusual to serve as a useful morphological criterion in the identification of *L. buccalis* cells.

In 1939, Thjøtta et al. (13) published a comprehensive description of *Leptotrichia buccalis*. Gilmour et al. (2) and later Kasai (9) established criteria to differentiate between *L. buccalis* and *Bacterionema matruchotti*, an organism first described as *Leptothrix buccalis* by Kligler (10). Suggestions have been made that *L. buccalis* be classified with the gram-positive lactobacilli (2, 3, 8), whereas others favored a classification with the gram-negative fusobacteria (1, 5). Although *L. buccalis* tends to be gram positive in young cultures and gram negative in older cultures (3), it is likely that is should be classified as a gram-negative microorganism (4, 5, 6).

Recent ultrastructural studies in our laboratory indicated that several *L. buccalis* strains demonstrate a rather unique cell wall structure which appears to be common to all the strains we have examined thus far and is different from that of other genera of microorganisms including *Bacterionema*, *Fusobacterium* and *Lactobacillus*. Since these morphological characteristics may be of the value as an additional criterion for the identification and classification of *L. buccalis*, the salient features will be described.

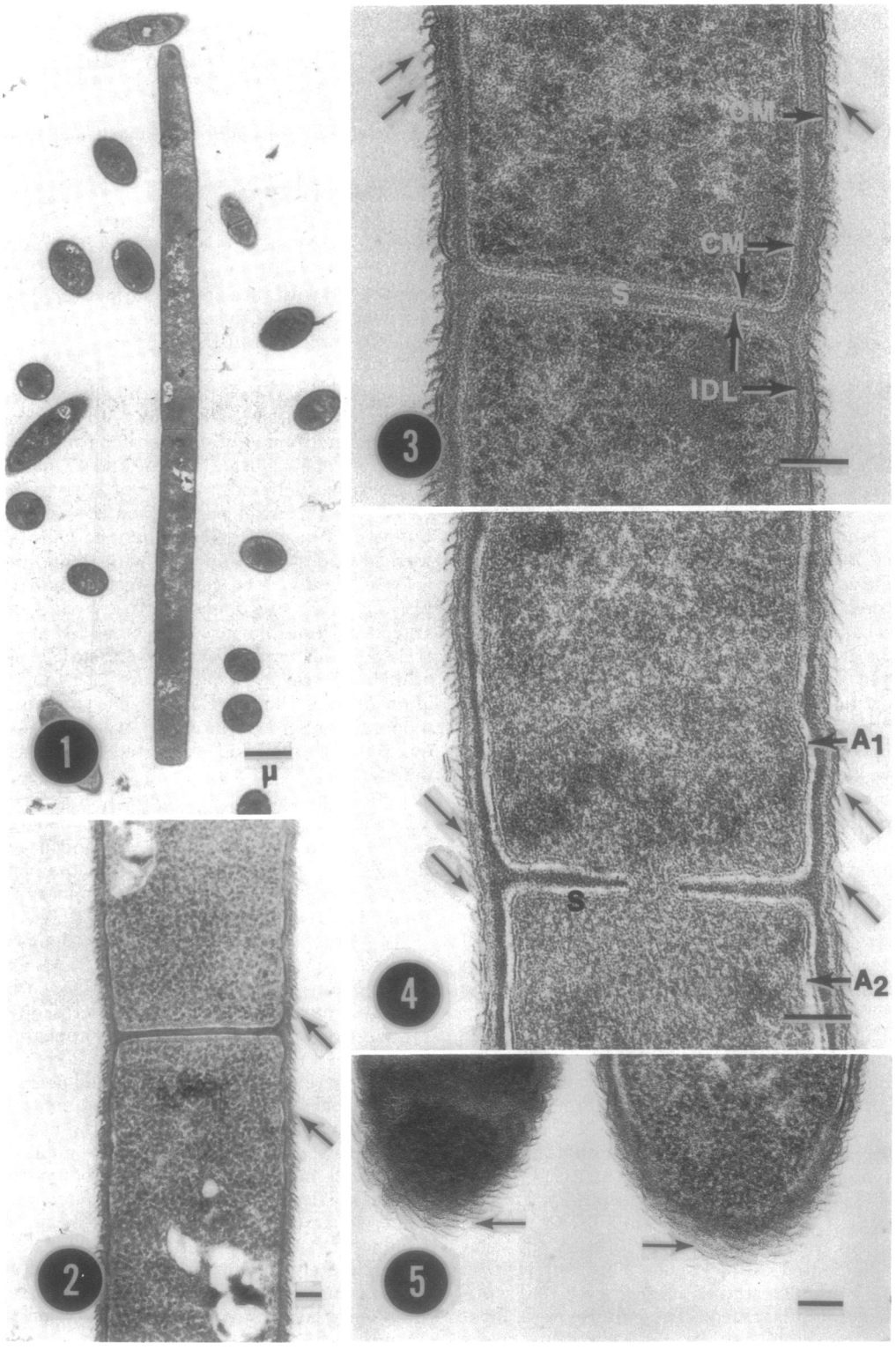
The following strains of *L. buccalis* were examined: ATCC strain 14201 (courtesy of B. Hammond, University of Pennsylvania), ATCC strain 19616 (courtesy of M. N. Gilmour, Eastman Dental Center, Rochester, N.Y.) and recently characterized isolates from patients with periodontal disease, namely CS1/AC, CS3/AC and CS4/AC (courtesy of S. Socransky, Forsyth Dental Center, Boston, Mass.). All strains studied were grown on Trypticase soy agar supplemented with 5% sheep blood cells (Difco). Cells were also studied after growth in Trypticase soy broth (Difco). The cultures were incubated anaerobically in gas generating packs (BBL) for 2 days at 37 C.

After 2 days, cells grown on blood agar plates were removed as entire colonies with a thin agar support and immediately fixed for 1 h at 4 C in a mixture of 5% glutaraldehyde and 4% paraformaldehyde, buffered to pH 7.3 with sodium cacodylate (7). After postfixation in s-collidine-buffered 2% osmium tetroxide, the cells were washed and stained en bloc with 0.5% uranyl acetate dissolved in veronal-acetate buffer at pH 5.2. The specimens were dehydrated in graded ethanol solutions and embedded in epon (11). Sections cut at 0.1 μm were stained with uranyl acetate and lead citrate (12), and examined in a Philips EM-300 electron microscope. Cells grown in broth were harvested by centrifugation and processed in the same manner.

Individual cells of *L. buccalis* appeared as relatively straight rods, 4 to 15 μm in length, with a mean diameter of 0.6 μm and a range from 0.4 to 1.0 μm. In measuring diameters, cross-sections which appeared to be close to the tapered end were avoided.

Cells generally occurred in pairs with one end tapered and the other square (Fig. 1). Although longer chains containing several cells could be observed in wet mounts by light microscopy, in thin sections most microorganisms appeared to be sectioned transversely.

The general arrangement of the cell periphery was compatible with that reported for gram-negative microorganisms. The cytoplasm was limited by a typical cytoplasmic membrane (inner cell membrane) consisting of two electron-dense laminae each approximately 3 nm thick, separated from one another by an electronlucent space of similar width. A space approximately 10 nm wide separated the cytoplasmic membrane from an intermediate electron-dense layer ranging in width between 20 to 35 nm. Both the cytoplasmic membrane and the intermediate dense layer contributed to


1
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the formation of transverse septa (Fig. 2-4). External to the intermediate dense layer and separated from it by a variable distance of 0 to 60 nm an undulating outer cell membrane was evident with a typical trilaminar structure consisting of two electron-dense laminae each approximately 3 nm thick, separated by an electronlucent space of similar width.

Characteristically, all strains exhibited an unusual structural modification of the outer cell membrane. In longitudinal sections of the cells and to a lesser extent in cross-sections, structures resembling hair-like protrusions seeemed to sprout from the external lamina of the outer cell membrane. In some sections they appeared to originate between the electron-dense laminae of the outer cell membrane. Inspection of tangentially sectioned cells (Fig. 5) indicated that these protrusions were in reality membranous folds, arranged in a pattern somewhat reminiscent of fish scales. Although these "scales" tended to generally point away from the tapered and toward the square end of cellular pairs (Fig. 1 and 2) this was not a constant observation. In Fig. 4, the direction of the "scales" is actually reversed on opposite sides of a forming septum. Attempts to resolve these scale-like membranous folds by scanning electron microscopy of freeze-dried and platinum-shadowed cells were unsuccessful. Our results are compatible with those of Hofstad and Selvig (6) who reported a typical gram-negative cell wall for *L. buccalis* strain L11. However, these investigators were not able to preserve the unusual and rather characteristic scale-like folds of the outer cell membrane. Although Hofstad was kind enough to send us a sample of strain L11, we experienced difficulty in its cultivation and were not able to include it in this report.

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LITERATURE CITED

1. Bøe, J., and T. Thjøtta. 1944. The position of *Fusobacterium* and *Leptotrichia* in the bacteriological system. Acta Pathol. Microbiol. Scand. **21**:441-450.
2. Gilmour, M. N., A. H. Howell, Jr., and B. G. Bibby. 1961. The classification of organisms termed *Leptotrichia* (*Leptothrix*) *buccalis*. I. Review of the literature and proposed separation into *Leptotrichia buccalis* Trevisan, 1879, and *Bacterionema* Gen. Nov., *B. matruchotii* (Mendel, 1919) Comb. Nov. Bacteriol. Rev. **25**:131-141.
3. Hamilton, R. D., and S. A. Zahler. 1957. A study of *Leptotrichia buccalis*. J. Bacteriol. **73**:386-393.
4. Hofstad, T. 1967. An anaerobic oral filamentous organism possibly related to *Leptotrichia buccalis*. I. Morphology, some physiological and serological properties. Acta Pathol. Microbiol. Scand. **69**:543-548.
5. Hofstad, T. 1970. *Leptotrichia buccalis*. A gram-negative bacterium. Int. J. Syst. Bacteriol. **20**:175-177.
6. Hofstad, T. and K. A. Selvig. 1969. Ultrastructure of *Leptotrichia buccalis*. J. Gen. Microbiol. **56**:23-26.
7. Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. **27**:137A-138A.
8. Kasai, G. J. 1961. A study of *Leptotrichia buccalis*. I. Morphology and preliminary observations. J. Dent. Res. **40**:800-811.
9. Kasai, G. J. 1965. A study of *Leptotrichia buccalis*. II. Biochemical and physiological observations. J. Dent. Res. **44**:1015-1022.
10. Kligler, I. J. 1915. A biochemical study and differentiation of oral bacteria with special reference to dental caries. J. Allied Dent. Soc. **10**:282-330.
11. Luft, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9**:409-414.
12. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J. Cell Biol. **17**:208-213.
13. Thjøtta, T., Hartmann, O. and Bøe, J. 1939. A study of the *Leptotrichia* Trevisan. History, morphology, biological and serological characteristics, no. 5. Avhandl. Norske Videnskaps-Akad., Oslo.

FIG. 1-5. *Unless noted otherwise, the bar represents 0.1 μm.*

FIG. 1. *L. buccalis ATCC strain 14201 grown in broth. Note the square and tapered ends.*

FIG. 2. *Higher magnification of Fig. 1. Note angulation of surface folds (arrows) in relation to the tapered and square ends.*

FIG. 3. *Longitudinal section through septal region of L. buccalis ATCC strain 19616. Note typical gram-negative cell wall structure and associated hair-like membranous folds (unlabeled arrows) of the outer cell membrane (OM). Septum (S) consists of cytoplasmic membranes (CM) and a layer continuous with the intermediate dense layer (IDL).*

FIG. 4. *Section through partially formed septum (S) of L. buccalis ATCC strain 19616. Note that the membranous folds associated with the outer cell membrane have different orientations (arrows) on either side of the cell. Note separation artifact between intermediate dense layer and cytoplasmic membrane (A1) and between the cytoplasm and adjacent membrane (A2).*

FIG. 5. *Tangentially sectioned cells of L. buccalis reveal the fish scale-like appearance of the membranous surface folds (arrows).*