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THE MORPHOLOGY OF AN ANAEROBIC ORAL FILAMENTOUS MICROORGANISM AS REVEALED BY ELECTRON MICROSCOPY

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In a recent study (*Theilade & Gilmour* 1961) an anaerobic oral filamentous microorganism was characterized which differed from earlier described types in certain morphological and biochemical characteristics. Most of the cells seen in wet mounts were short, straight or slightly curved filaments, 15 to 35μ by 1.0 to 1.2μ, with blunt ends. Additionally, rods 8 to 10μ long were frequently found in chains of 2 to 6 cells, and a few filaments 100 to 200μ long were generally present. Usually a few of the cells showed non-refractile, darkly staining, oval, central or subterminal swellings which did not take up a spore stain. Observation in the light microscope of stained smears, unstained wet mounts, and colonies *in situ* on agar plates indicated that the relative number of cells with swellings was independent of the age of the culture. In order to further investigate the general morphology of the organism and with the hope of clarifying the structure of the swellings, broth cultures of the organism were prepared for electron microscopy.

MATERIALS AND METHODS

Three representative strains of the filamentous microorganism were studied; however, they proved to be quite similar in morphology as well as in all physiological characteristics tested. Cultures were grown in a broth medium of the following composition, modified from *Wood & Gunsalus* (1942): tryptone (Difco), 10 g; yeast extract (Difco), 10 g; dextrose, 2 g; monobasic potassium phosphate, 5 g; water, 1000 ml. The pH was adjusted to 7.4 and, after autoclaving, a hemin solution was added to a final concentration of 1 μg per ml. Following inoculation, 10 ml aliquots of medium were incubated at 37°C under a pyrogallol-sodium carbonate seal (*Howell & Pine* 1956) to give anaerobic conditions and increased carbon dioxide tension. Maximum growth was obtained in 7 days. After incubation periods from 5 days to 3 weeks the cells were washed 8 to 10 times by centrifugation and resuspension in distilled water and then fixed in one per cent buffered osmic acid at pH 7.3. Some of the cells were then suspended in distilled water and microdrops placed on carbon coated specimen screens, dried, and shadowed with palladium. The rest of the fixed cells were embedded in a mixture of methyl and butyl methacrylates and sectioned on a Porter-Blum microtome with glass knives.

RESULTS

Examination of the shadowed preparations in the electron microscope confirmed the earlier finding that the filaments generally were of quite uniform thickness and varying length, some of them segmented into chains of shorter filaments and long rods (Figs. 1 and 2). Branching was not observed. At higher magnification (Figs. 3 and 4) the majority of the cells were seen to have an even outline suggesting a homogeneous or only slightly granular cytoplasm. However, in a few cells of each culture the cytoplasm was extremely granular causing a pronounced unevenness in surface contour. The ends of the organisms were blunt or rounded, never tapered, and no flagella or fimbriae were present.

Characteristic swellings (Figs. 5—7) were observed in about

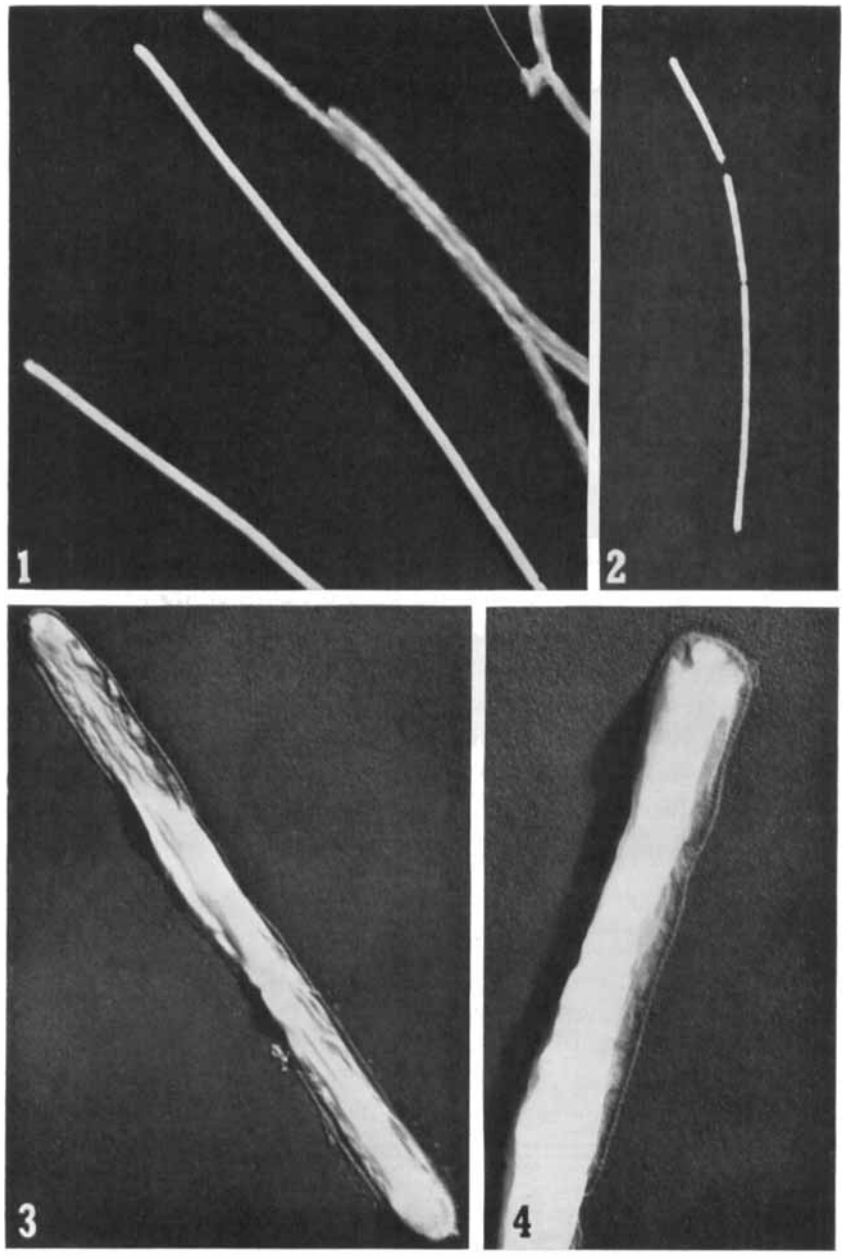

Fig. 1. Unsegmented filaments. Shadowed preparation. ×4700.

Fig. 2. Fragmentation into a chain of rods. Shadowed preparation. ×4000.

Fig. 3. Higher magnification showing a single rod with rounded ends. Shadowed preparation. ×12600.

Fig. 4. High magnification showing a typical blunt end of a filament. Shadowed preparation. ×21600.

one per cent of the cells. Most often the swelling was located near the end of the filament as shown in Fig. 5, rarely at the very end (Fig. 6). Quite frequently it was found centrally on the filament, occasionally associated with a beginning cell division (Fig. 7). Isolated ovoid or spherical bodies were never encountered. No relation was found between the age of the culture and cell length, granularity of the cytoplasm, or occurrence and location of swellings. However, this may be explained in part by the fact that large inocula were necessary to obtain even slow growth of the broth cultures studied, so that young cultures contained many old cells.

In thin sections the organisms were seen to contain irregular, dense granules scattered throughout the cytoplasm inside the cell wall. Of special interest were longitudinal sections of filaments with swellings (Figs. 8 and 9). They showed that the latter were produced by a thickening of the cytoplasm which was covered by a normal cell wall. There were no cross walls separating the swelling from the remainder of the cell. In a few instances a large dense granule was observed in the swelling (Fig. 8); such dense material did not occur more frequently in these areas than in other parts of the cells. Most often the structure in the swelling was like that of the .rest of the cytoplasm. (Fig. 9).

DISCUSSION

Although the general cell morphology and dimensions of the present microorganism are rather similar to that of *Leptotrichia buccalis* as described by *Thjøtta, Hartmann & Bøe* (1939), *Takazoe & Frostell* (1960), and in reports, including electron micrographs, by *Hamilton & Zahler* (1957) and *Kasai* (1961), there are certain significant differences between them. Both organisms can be seen as rods as well as shorter and longer filaments. However, in *L. buccalis* it is very common to find two rods joined with blunt ends towards each other and the free ends tapered, whereas in the present microorganism chains of two rods are rare, and the ends of the cells are always blunt or rounded, never tapered. Although the above mentioned publications apparently discuss the very same organism *(L. buccalis)* only *Kasai* and *Takazoe & Frostell* mention that large coccoid bodies or bulbous

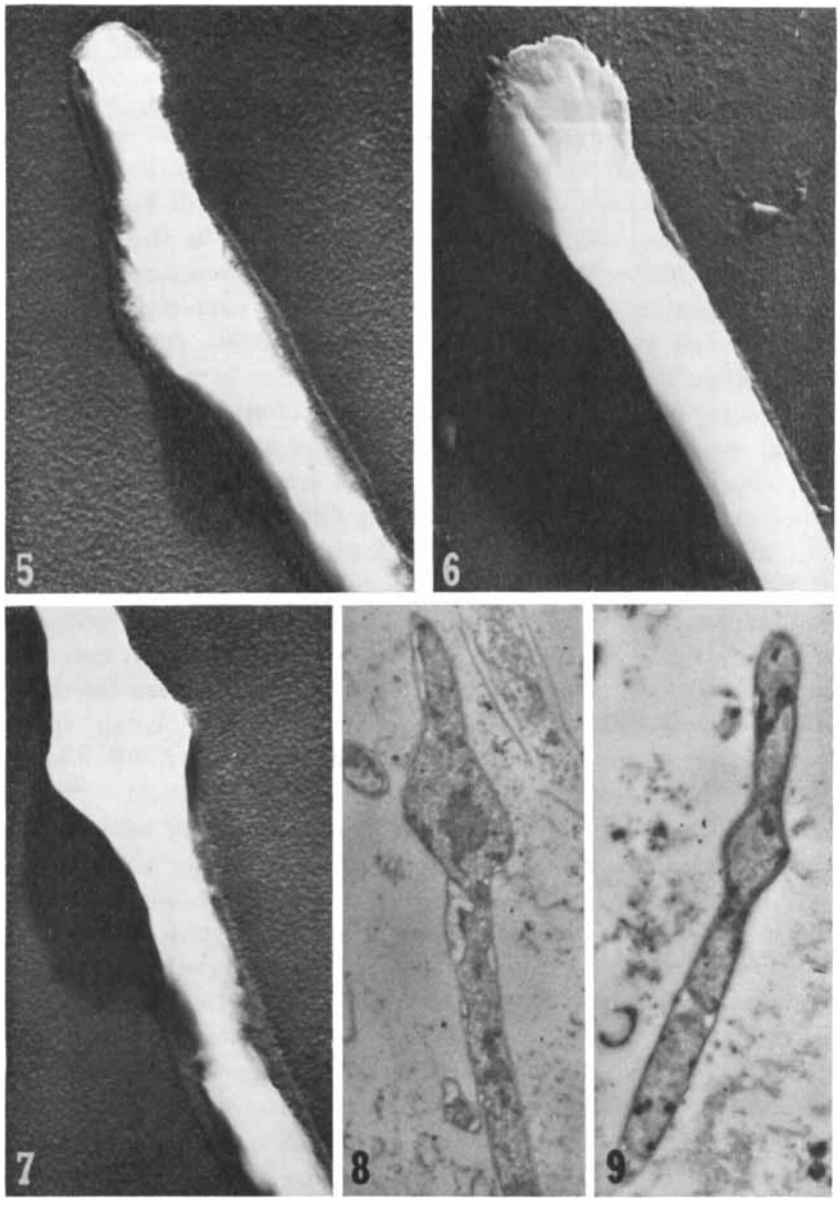

Fig. 5. Filament with subterminal swelling. ×20800.

Fig. 6. Swolling end of a filament. Shadowed preparation. ×25100.

Fig. 7. Swelling located centrally on a filament. Beginning cell division is seen below the swelling. Shadowed preparation. ×24300.

Fig. 8. Thin section of a filament with a large granule in the cytoplasm at the site of the swelling. ×12600.

Fig. 9. Thin section of a filament with a centrally located swelling showing the common uniform distribution of granules throughout the cytoplasm. ×12100.

swellings at the end, or in the middle of a filament, were occasionally encountered. According to *Kasai,* these structures were not heat resistant and did not take up spore stains. Similar swellings have been observed in some strains of *L. buccalis* by *Howell* (1962) using the light microscope. The drawings of *Wherry & Oliver* (1916) from cultures of a microorganism which may be identical to *L. buccalis,* clearly show subterminal swellings as well as free pyriform and ovoid bodies. *Bibby* (1935) isolated six groups of morphologically and biochemically distinct oral filamentous microorganisms, several of which showed occasional swellings. Certainly this feature is not characteristic of any one type. However, it is at present impossible to determine whether the swellings observed in the different organisms are of the same nature, and any function they might have also remains to be revealed.

Whereas the oral filamentous types mentioned above have the same general cell morphology, there are differences in colonial morphology, as well as striking biochemical characteristics, which clearly differentiate the organism described herein from *L. buccalis,* the only well defined type in the group. Routine tests for biochemical characteristics (*Theilade & Gilmour* 1961) showed that the present organism produces indole, whereas *L. buccalis* does not. Although both are active fermenters, with only a few differences in the carbohydrates attacked, *L. buccalis* produces acid without gas, whereas the present organism produces acid with large quantities of gas. Preliminary results of carbon balance studies now in progress have shown that *L. buccalis,* in the medium used, gave a homolactic fermentation of glucose, producing about 1.8 moles of lactic acid per mole of glucose utilized. The present organism, contrariwise, in the same medium, produced a mixture of carbon dioxide, lactic, formic, acetic, and butyric acids, ethanol, and a higher alcohol, presumably butanol. This gas-producing organism may be identical to the strains described by *Takazoe & Frostell* (1960) as *L. buccalis* Type II, although the available information is insufficient for full identification.

In a recent review of organisms termed *L. buccalis, Gilmour, Howell & Bibby* (1961) proposed that the genus *Leptotrichia* Trevisan, 1879, which was ignored in the seventh edition of

Bergey's Manual (*Breed, Murray & Smith,* 1957), be redefined by amending *Trevisan's* definition of the genus by the addition of the additional characteristics described by *Thjøtta et al.* (1939) and by *Hamilton & Zahler* (1957). *Gilmour et al.* (1961) agreed with *Hamilton & Zahler* that this genus should be included in the family *Lactobacillaceae,* tribe *Lactobacilleae.* Thus far, *L. buccalis* Trevisan is the only species in this genus. The organism described in the present investigation fits this general description of the genus *Leptotrichia,* as proposed by *Gilmour et al.,* and might be classified as a new species of this genus. However, it seems questionable whether or not an organism with such a fundamentally different type of metabolism of glucose can be included in the same genus as one which gives a homofermentative type of fermentation under identical conditions. It is hoped that continued investigations, to be reported later, on the metabolism of the organism will clarify this problem.

SUMMARY

Osmic acid-fixed cells from broth cultures of the organism were studied in the electron microscope in shadowed dried suspensions as well as in thin sections. The shadowed preparations showed that the filaments generally were of quite uniform thickness, 1.0—1.2μ, and of varying length, often segmented into chains of shorter filaments and long rods. The ends of the cells were blunt or rounded, never tapered; no flagella or fimbriae were present, and branching was not observed. The cytoplasm of the majority of cells appeared homogeneous, whereas in a few cells of each culture the cytoplasm was granular, causing a pronounced unevenness in the contour of the cell. About one per cent of the cells showed characteristic swellings, generally located near the end of the filament, sometimes centrally, and rarely at the very end of the cell. In thin sections smaller dense granules were seen scattered throughout the cytoplasm inside the cell wall. The swellings consisted of a thickening of the cytoplasm, which occasionally contained a large dense granule, although the structure in the swellings most often was similar to that of the rest of the cytoplasm. The swellings were covered by a normal cell

wall and were not separated from the remainder of the cell by any cross walls. The taxonomic position of the microorganism is discussed in relation to *Leptotrichia buccalis*.

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RÉSUMÉ

MORPHOLOGIE D'UN MICRO-ORGANISME FILAMENTEUX ANAÉROBIE DE LA CAVITÉ BUCCALE D'APRÈS L'EXAMEN AU MICROSCOPE ÉLECTRONIQUE

Des cellules fixées à l'acide osmique et provenant de cultures de ce micro-organisme sur bouillon ont été examinées au microscope électronique en suspensions ombrées et séchées d'une part, et en coupes minces d'autre part. Les préparations ombrées ont montré que les filaments étaient en général d'une épaisseur assez uniforme, 1,0—1,2μ, et de longueur variable, souvent segmentés en chaînes de filaments plus courts et de longs bâtons. Les extrémités des cellules étaient émoussées et arrondies, jamais pointues; il n'y avait ni flagelles ni franges, et on n'a pas constaté de ramification. Le cytoplasme de la plupart des cellules a paru homogène, tandis que quelques cellules dans chacune des cultures présentaient un cytoplasme granuleux déterminant des inégalités prononcées du contour de la cellule. Chez environ 1 pour cent des cellules, on a observé des renflements caractéristiques, situés en général près de l'extrémité du filament, parfois au centre, et rarement à l'extrémité même de la cellule. Sur les coupes minces, on a observé de petites granulations denses dispersées dans le cytoplasme en dedans de la paroi cellulaire. Les

renflements consistaient en un épaississement du cytoplasme, qui contenait parfois une grosse granulation dense, bien que la structure du renflement fût le plus souvent semblable à celle du reste du cytoplasme. Les renflements étaient recouverts par une paroi cellulaire normale et n'étaient séparés du reste de la cellule par aucune cloison. La situation de ce micro-organisme par rapport au *Leptotrichia buccalis* en ce qui concerne la classification fait l'objet d'une discussion.

ZUSAMMENFASSUNG

MORPHOLOGIE EINES ANAËROBEN, FADENFÖRMIGEN MIKROORGANISMUS AUS DER MUNDHÖHLE, WIE DURCH ELEKTRONENMIKROSKOPIE UNTERSUCHT

Zellen aus in Brühe gezüchteten Kulturen dieses Organismus wurden nach Fixierung mit Osmiumsäure teils als beschattet eingetrochnete Suspension, teils als dünne Schnitte präpariert und im Elektronenmikroskop untersucht.

Die beschattet getrockneten Präparate zeigten, dass die fadenförmigen Organismen im allgemeinen von ganz einheitlicher Dicke – 1,0 bis 1,2 μ – aber von schwankender Länge waren und oft Ketten aus kürzeren Fäden und lange Stäbe bildeten.

Die Enden der Zellen waren stumpf oder abgerundet, niemals spitz zulaufend; es wurden keine Geisseln oder Fimmelhaare beobachtet, wie auch keine Verzweigungen festgestellt wurden. Beim grössten Teil der Zellen schien das Zytoplasma homogen zu sein, aber in jeder Kultur wurde bei einigen wenigen Zellen ein körniges Zytoplasma vorgefunden, das die Kontur der Zelle stark uneben machte. Etwa 1 % der Zellen wies charakteristische Erweiterungen auf, gewöhnlich in der Nähe des Endes, ab und zu zentral gelegen, und in Einzelfällen am Ende der Zelle selbst.

Bei den aus dünnen Schnitten bestehenden Präparaten wurden kleinere, massive Körper beobachtet, die innerhalb der Zellenwand über das ganze Zytoplasma verstreut lagen. Die Erweiterungen bestanden aus einer Verdickung des Zytoplasmas, die in einigen Fällen ein grosses, massives Korn enthielt, obgleich

das Gefüge der Erweiterung meist dem des sonstigen Zytoplasmas entspricht. Die Erweiterung war von einer normalen Zellenwand gedeckt und durch keine Querwand von der restlichen Zelle getrennt.

Die taxonomische Einordnung des Mikroorganismus im Verhältnis zu *Leptotrichia buccalis* wird erörtert.

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