STREPTOTHRIX INTERPROXIMALIS. N. SP. AN OBLIGATE MICROAEROPHILE FROM THE HUMAN MOUTH

ERIC A. FENNEL

From the Laboratory of Bacteriology and Hygiene, University of Cincinnati, Cincinnati

An organism of exceedingly interesting morphology and cultural characteristics has been isolated from the mouth. The mouths from which this organism was isolated would be classed as "habitually unclean mouths with incipient caries and a liberal deposit of salivary calculus."

After having worked with mouth organisms on agar and glucose agar under aerobic and anaerobic conditions, and finding them unfavorable to the growth of the thread forms so common in the oral flora, I substituted Martin's agar[1] and pleuritic fluid, and used the system of partial oxygen tension of Wherry and Oliver[2] during incubation.

Isolation.—Materia alba from the gingival and interproximal portions of the central lower incisors was emulsified in sterile water, by very diligent shaking. Proportionate amounts of this emulsion were inoculated into tubes of equal parts of melted Martin's agar and pleuritic fluid, and the tubes carefully manipulated to make roll tubes. These tubes were then connected by airtight rubber tubing, to cultures of freshly inoculated B. subtilis, and grown at 35 C. (average mouth temperature) for 5 days when they were opened for examination.

A small, round, white, elevated colony was fished and transplanted to slants of the same medium, and grown aerobically, anaerobically and at partial tension. Very slowly, during 5 days, the partial tension culture grew, to form white, slightly raised, rather dry but tenacious colonies. The aerobic and anaerobic tubes gave absolutely no growth.

Subcultures were made on the same medium, and grown under the three varying oxygen pressures, with the uniform result that the partial tension tube gave a growth, while the aerobic and anaerobic tubes were consistently without growth. The colonies were practically invisible until the 4th day, and attained their maximum growth by the 5th or 6th day.

Six consecutive subcultures were made, at the end of 5 days each, from the partial tension tubes, when finally the aerobic tube showed a faint growth. This tube, however, was tightly capped with a rubber hood to prevent drying of mediums, and in this manner a somewhat reduced oxygen pressure may have been produced. This aerobic strain was carried through 2 subcultures, when it died out.

Received for publication Jan. 25, 1918.

[1] Martin, W. B. M.: Jour. Path. and Bacteriol., 1911, 15, p. 76.

[2] Jour. Infect. Dis., 1917, 20, p. 28.

Subcultures from Martin's medium were carried over to glucose agar with pleuritic fluid, and later to glucose agar (all at partial tension), and the latter medium was used for all stock cultures.

Morphology.—A highly pleomorphic organism, growing in plaquelike colonies of intertwined and matted threads, it varies in its morphology rather consistently with the age of the culture.

1. A 5-day culture shows rather heavy, long, intertwined threads. These threads are about 2-4 microns in width, and vary much in length, probably being broken up in smear preparations, since the colonies are very tenacious. The threads vary from 5-80 microns in length. They are not of a constant diameter, reminding one of a grasshopper's leg. Straight and curved, unbranched fibers are the rule, but occasional branched forms are noted. Many of these fibers are of the same diameter throughout, but an almost equal number present either distal enlargements or segmental indentations, or both. Many of the shorter threads are apparently only fragments of the longer threads, broken in making smears. An occasional knoblike end is noted.

The branching forms, not at all unusual, might be divided into three types: (*a*) Threads ending in two equal branches; (*b*) threads showing one or many lateral knobs, and (*c*) threads showing lateral branching of equal size, but with a very marked constriction at the point of origin (Figs. 1 and 2).

The strain which grew aerobically a short time presented these forms and differed only in the greater number of branching forms.

The ends of the threads were either straight and broken off, slightly rounded, or bulbous, and a few were knobshaped. This picture was quite constant for all 3-5 day cultures on all the mediums.

2. A culture, grown 2 weeks at partial tension, presented an organism of an entirely different form. The involution forms were varied, and fantastic in the extreme. Knobs, commas, heavy crutchlike forms, horns, scrolls, and plain and spotted rods were present. The crutchlike forms were suggestive of the dichotomous branched forms of the previous type; the spotted rods had been suggested by a few forerunners in the 3-5 day culture. Nothing was suggestive of spore formation, except these rounded or ovoid masses in a shell that had been a thread. These masses, however, stained with the greatest ease and subcultures from this type gave healthy growth (Fig. 3).

3. Cultures allowed to remain in the incubator 2 months, at partial tension, showed no increase in growth after the 5th day. Examination showed rod forms, 2 microns by 10-20 microns, some straight, some curved, but none branched, with a poorly staining wall, containing highly staining granules, almost round, usually situated at the ends of the rod forms, and from 2-8 intermediate. I was not successful in subculturing this type (Fig. 4).

Staining.—For routine work, Loeffler's methylene blue, aqueous safranine and gentian violet do well.

By far the greater number of fibers retain the stain by Gram's method, the swollen ends and the swollen portions just adjacent to the indented portions, being stained intensely violet. Some of the threads, throughout their length, take the counterstain. A very considerable number of the threads retain the violet at one end, and progressively take more of the counterstain, as the other end is approached. A few of the fibers, most of them rather short, stained irregularly, with a resultant ground glass appearance.

Type 2 stained very irregularly, and while some forms retained the stain by Gram's method, others took the counterstain. Some forms showed evidences

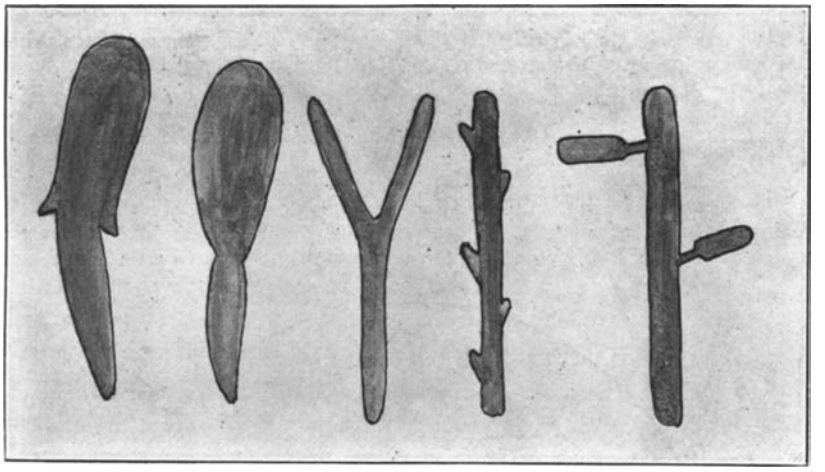

Fig. 1.—Typical forms in a five day culture.

Fig. 2.—Five days' culture.

of both stains, in varying densities. Some forms took practically no stain. The granular type was noticeable for the deep violet the contained granules retained.

Type 3 consisted of a shell which took no stain or a faint counterstain, while the round or ovoid granules retained the violet by Gram's stain. These granules sometimes seemed to be in the form of platelets or plaques, peripherally located in the shell of the fiber.

None of these forms are acid fast, when stained with carbolfuchsin, nor do any give the granulose reaction.

The organism is not motile. It grows not at all anaerobically, with the greatest difficulty aerobically, and rather slowly under a condition of partial oxygen pressure. It is not a chromogenic organism; grows best at 35 C. and

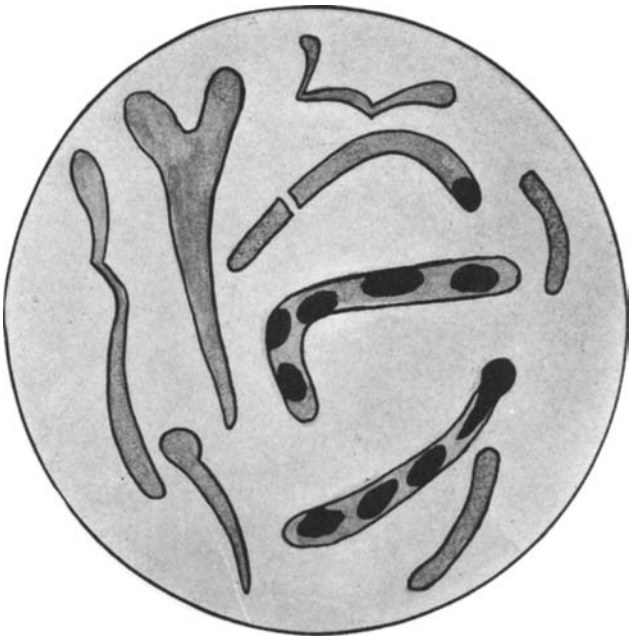

Fig. 3.—Two weeks' culture.

not at all at room temperature. It does not form endospores; does not give rise to any odors on any medium, and is best isolated on a serum agar, though I have once isolated it on dextrose agar. Great difficulty in making subcultures was experienced, due to the tenacity of the colonies. It was later found better to remove the colony entire, emulsify in normal salt solution, and make subcultures from the emulsion. Dextrose agar gives a good and typical growth; maltose agar is almost equally good.

On dextrose agar slant in 2 days a very faint change in the surface of the medium may be noted. In 3 days many pinpoint, white, translucent, hemispherical colonies (like half-pearls) with smooth edge, appear. These colonies grow to pinhead size by the 4th or 5th day, but do not become confluent. After

6 days each colony presents a nipplelike elevation in the center, which has a rounded apex. The pearly white appearance of the colonies continues, the surface being smooth and glistening, in contradistinction to dry and chalky. The colony grows slightly in size up to this time, the largest colony attaining a diameter of only 1.5-2 mm., but growth discontinues from this time on. By transillumination the colonies are dark brown, with a lighter halo.

Dextrose agar stab, under aerobic conditions, gives no deep growth, nor any surface growth, but a faint growth in the upper 5 mm. of the stab.

Plain agar slant gives the same as the dextrose agar, except that the growth is not so profuse, and that the individual colonies are very minute.

Maltose agar yields results identical with those on dextrose agar.

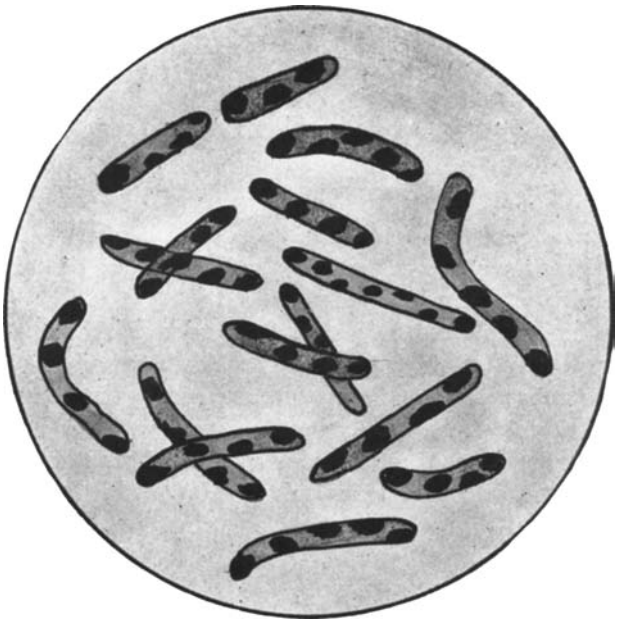

Fig. 4.—Two months' culture.

On Loeffler's blood serum, the usual growth takes place, but without liquefaction.

On blood agar, typical growth results, without hemolysis.

Neither on gelatin nor potato does growth take place.

In neither sugar-free broth, glycerol broth, starch broth nor milk, after repeated efforts, could growth be obtained.

In dextrose litmus broth, there is no cloudiness, no scum, no precipitate, nor any visible evidence of growth at any time, though the organism was later recovered from the medium. A slight acidity was noticed after 48 hours, which increased until, after 4 days, the acidity was marked.

On maltose litmus broth a slight acidity in 2 days; marked acidity in 4 days.

In lactose litmus broth, no acidity, even after 10 days.

In galactose litmus broth, slight acidity after 2 days, with slight increase after 4 days.

In saccharose litmus broth acid production after 2 days was definite and after 4 days, marked.

The organism, during 5 days' incubation, ferments the sugars with a proportionate ease and quantity of acid expressed in the following order: dextrose, saccharose, maltose, galactose, but lactose not at all.

This organism differs from Goadby's Streptothrix buccalis in many important details. The inability of this organism to grow either under aerobic or anaerobic conditions, the presence of dichotomous branching, an absence of the "white powdery gonidia," on the colonies, its inability to grow at room temperature and liquefy gelatin, and its lack of growth on potato and in milk, serve to differentiate it from Streptothrix buccalis, the only organism it resembles. I have, therefore, tentatively, for the purpose of description and study, named it Streptothrix interproximalis.

CONCLUSIONS

Streptothrix interproximalis has its natural habitat in the unclean mouth, and may play some part in the etiology of dental caries or chronic peridental inflammation.

It is a highly pleomorphic organism, with fairly constant types representing 5 days', 3 weeks' and 2 months' cultures.

It grows only under conditions of reduced oxygen pressure.

It is worthy of study, chiefly because it indicates by its strict cultural requirements, that a method for cultivating many of the heretofore uncultivated organisms, particularly of the oral flora, may have been found.