Strict anaerobic organisms resembling *Wolinella* isolated from human gingival crevice

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The existence of gram negative asaccharolytic rod-shaped bacteria in the human oral cavity has been reported by a number of investigators. However, the data on motile anaerobic bacteria are considered to be less numerous. Van Palenstein Helderman\(^1\) isolated hydrogen dependent organisms resembling *Vibrio succinogenes* which had been previously reported by Wolin et al.\(^2\) Recently, Tanner et al. isolated anaerobic gram negative asaccharolytic bacteria with a polar flagellum from the human dental plaque and proposed a new genus *Wolinella*\(^3\). We also isolated strict anaerobic gram negative, motile, asaccharolytic rods from the human gingival crevice area. They were similar, in some biochemical characters, to the organisms reported by Van Palenstein Helderman and Rosman and *Wolinella*, but were distinct from them, especially in their morphological characters. In this communication, the morphology and some biochemical characteristics of the newly isolated organisms are reported and discussed.

Fourteen strains of bacteria were isolated from the clinical healthy gingival crevice of man in a modified CBRCA medium described by Van Palenstijn and Winkler\(^4\) in an anaerobic glove box (Anaerobox, Hirasawa, Tokyo) under a gaseous atmosphere of \(N_2: H_2: CO_2 = 80:10:10\). They were purified in a RCA medium (Oxoid), and maintained in the RCA medium or in a BHI agar medium (Difco).

The gaseous requirements of the isolated organisms are shown in Table 1, in which the characters were compared with those of the strains of *Campylobacter, Wolinella* and the organisms described by Van Palensterin Helderman and Rosman. As shown in Table 1, the isolates did not grow in the atmosphere in the presence of oxygen, and were considered to be strictly anaerobic and to require \(CO_2\) in the atmosphere.

The biochemical characteristics of the isolates are shown in Table 2. PY agar medium\(^6\) was used for the basal medium and all cultures were incubated in an anaerobic glove.
Table 1  Gaseous requirements of 14 strains isolated and the reference organisms

<table>
<thead>
<tr>
<th></th>
<th>BHI-blood agar in</th>
<th>Semsolid medium in</th>
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<tbody>
<tr>
<td></td>
<td>Isolates (14 strains)</td>
<td>C. sputorum subsp. sputorum</td>
</tr>
<tr>
<td>Air</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Candle Jar</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1/4 Air, 3/4 CO₂</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>100% N₂</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100% CO₂</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>95% N₂, 5% CO₂</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steel Wool</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anaerobic*</td>
<td>+</td>
<td>±</td>
</tr>
</tbody>
</table>

*: 10% CO₂, 8% H₂, 82% N₂  
**: C. sputorum subsp. sputorum #1  
***: C. fetus ATCC 27374, 29428  
****: Description of Ref. 1  
******: Description of Ref. 3

box in a gaseous atmosphere of N₂·H₂·CO₂ = 80:10:10. To determine H₂S production, BHI agar slant supplemented with 0.05% cysteine was used and H₂S was detected with lead acetate paper. The isolates decomposed H₂O₂ but not produced the oxidase. All strains produced H₂S and reduced the nitrate and nitrite. They were tolerant to 1% bile, 1% glycerine and 1% NaCl but not to 2% or 3.5% NaCl. No carbohydrates were fermented by any of the strains. G+C contents of DNA determined by buoyant density was 46 mol%.

These results indicated that the fourteen strains isolated in this experiment can be considered to be distinct from Wolinella and the organisms reported by Van Palenstein Helderman and Rosman, although our isolates are closely related to them, especially in their biochemical characteristics. The studies on characters of hydrogen dependency and energy metabolism are now in progress and a taxonomical proposition will be reported elsewhere.

References


Fig. 1 Electronmicrograph of the isolated organisms. (1)–(3): Preparations negatively stained with PTA. A tuft of polar flagella (2 to 4) is present at one end of the cell. Cells are slightly curved with tapered ends. (4): Shadow-casted preparation.