NOTES

Eubacterium yurii subsp. schtitka subsp. nov.: Test Tube Brush Bacteria from Subgingival Dental Plaque

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Representative strains of the test tube brush bacterium Eubacterium yurii, previously recovered from subgingival dental plaque of chronic adult periodontitis patients, were examined by determining their serological reactivities, protein electrophoretic patterns, deoxyribonucleic acid homologies, and bone resorptive activities in vitro. Our results indicate the existence of a third subspecies, E. yurii subsp. schtitka subsp. nov., in addition to the two previously described subspecies, E. yurii subsp. yurii and E. yurii subsp. margaretiae. The third subspecies is phosphatase negative and asaccharolytic and does not stimulate bone resorption in vitro. The type strain of E. yurii subsp. schtitka is strain ATCC 43716.

Several investigators have reported observing “brush-like” bacterial aggregates in subgingival dental plaque from periodontally involved sites. Listgarten (2) described at least two different types of “brushes,” coarse and fine, during his microscopic study of subgingival plaque. The brushes were composed of gram-negative fusiform or filamentous rods and typically contained a morphologically mixed population of cells. Keyes and Rams (1) found “rosette-like” bacterial structures in the subgingival plaque in association with destructive periodontitis. Recently, we reported (4) the isolation, from subgingival dental plaque of chronic adult periodontitis patients, of 18 strains of gram-positive, anaerobic rods that formed three-dimensional, monospecific brushlike structures. These organisms were asaccharolytic or weakly saccharolytic and biochemically rather inactive. The major volatile fatty acid produced in peptone-yeast extract-glucose broth was butyrate; lesser amounts of propionate and acetate were also produced. Ribonuclease, H$_2$S, and indole production were positive for all strains. Cells for the experiments described below, cultures were harvested by centrifugation at 5°C, and washed in phosphate-buffered saline (pH 7.2) as previously described (4). Whole-cell lysates were prepared from washed cells (10%, vol/vol) by two sequential passes through a French pressure cell at 20,000 lb/in$^2$. Protein concentrations were determined by the procedures of Lowry et al. (3).

Reaction by double diffusion in agar (6) of rabbit antisera to strains of each of the three groups with whole-cell lysates of each group produced three distinct patterns of reactivity (Table 1). The anti-E. yurii sera (groups I, II, and III) were then reacted with whole cells of the various strains by a modification of the enzyme-linked immunosorbent assay technique (8). In both serological tests E. yurii subsp. yurii (group I) and E. yurii subsp. margaretiae (group II) antisera reacted only with their homologous strains. The third group antisera reacted with both homologous and group I strains, suggesting that E. yurii subsp. schtitka shares an antigenic determinant that is also present on E. yurii subsp. yurii (group I) organisms; however, it also has an unshared antigenic determinant of its own.

Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7) of whole-cell lysates of the three groups showed many common protein bands. However, as Fig. 1 shows, several consistent differences also emerged. Each group displayed a unique pattern in the 93- to 183-kilodalton (kDa) range; groups I and II produced a more prominent 25-kDa band and a less pronounced 24-kDa band than did group III. Group III displayed a unique 59-kDa band and also a slightly different protein pattern in the 28- to 38-kDa range.

**Table 1. Serological reactivity among E. yurii strains as determined by double diffusion in agar**

<table>
<thead>
<tr>
<th>E. yurii group</th>
<th>Reaction with antiserum to:</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (E. yurii subsp. yurii CSF and SM14$^{1}$)</td>
<td>+*</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>II (E. yurii subsp. margaretiae SM65$^{2}$)</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>III (E. yurii subsp. schtitka SMN$^{3}$)</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* +, Positive; –, negative.

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TABLE 2. Levels of DNA-DNA homology among the three groups of *E. yurii*

| Group | I (E. yurii subsp. yurii CSF and SM14<sup>T</sup>) | Group II | Group III
|-------|---------------------------------|----------|----------
| % Homology to: | 100.2 | 56.6 | 58.3 |
| II (E. yurii subsp. margaretiae SM6<sup>T</sup>) | 98.0 | 63.0 |
| III (E. yurii subsp. schitika SM<sup>T</sup>) | 99.3 |

Free solution DNA-DNA renaturation studies (5) among representative strains from each group confirmed the presence of the third group (Table 2). There was a high degree of intragroup homology; however, only 60% homology existed between groups.

In a 45Ca-labeled fetal rat bone bioassay (B. S. Margaret, G. N. Krywolap, and J. R. Heath III, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, B200, p. 57) differences among the groups in the bone resorptive activity of methanol extracts of *E. yurii* whole cells further supported the evidence for the existence of the third subspecies. *E. yurii* subsp. *yurii* (group I) exhibited a moderate level of activity, whereas *E. yurii* subsp. *margaretiae* (group II) stimulated statistically significant bone resorption at concentrations as low as 1.25 μg/ml; group III organisms failed to stimulate bone resorption.

An examination of the clinical data describing the periodontal pockets from which these organisms were initially isolated (Margaret and Krywolap, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, R-3, p. 211) showed that, in contrast to the other groups, group III organisms were recovered from relatively shallow pockets (approx. 4 mm) with a lesser degree of bone loss (approx. 50%) in which high numbers of black-pigmented *Bacteroides* species were conspicuously absent (colony count, <10<sup>4</sup> colonies per plaque sample).

In conclusion, patterns of serological reactivity, protein profiles, the results of DNA reassociation studies, stimulation of in vitro bone resorptive activity, and characteristics of the recovery site suggest the existence of a third subspecies of *E. yurii*. We propose to name this taxon *E. yurii* subsp. *schitika* (schit'ka. Urkainian schitika brush). Into this subspecies we suggest placing those strains of *E. yurii* that are phosphatase negative and asaccharolytic and do not stimulate bone resorption in vitro. The type strain of *E. yurii* subsp. *schitika* is strain ATCC 43716.

LITERATURE CITED