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Sulfate-reducing bacteria in the periodontal pocket


This report is the first to describe the occurrence of sulfate-reducing bacteria in the human mouth. Samples of subgingival dental plaque were examined for the presence of sulfate-reducing bacteria. Using enrichment cultures, sulfate-reducing bacteria were detected in 25 (58%) of 43 individuals, and in 39 (48%) of the 82 samples. Pure isolates of sulfate-reducing bacteria, obtained from a limited number of enrichment cultures, belonged to the genera Desulfobacter and Desulfovibrio. These genera are also the predominant sulfate-reducing bacteria in the human large intestine. The sulfate-reducing bacteria use sulfate as terminal electron acceptor to oxidize low-molecular-weight organic compounds, mainly products of microbial fermentation such as acetate, lactate etc. The numbers of sulfate-reducing bacteria in the mouth are assumed to be limited by sulfate. Potential sources of sulfate in the subgingival area include free sulfate in pocket fluid and glycosaminoglycans from periodontal tissues.

In many natural environments the terminal steps in the degradation of organic macromolecules are mediated by sulfate-reducing bacteria and methanogenic bacteria. These organisms further metabolize the products from the fermentative microorganisms (23). Sulfate-reducing bacteria have been isolated from marine and estuary sediments (15), sewage digesters (8) waterlogged soils (10) and the gastrointestinal tract of humans and animals (4, 13, 20). The term sulfate-reducing bacteria describes a heterogeneous group of microorganisms that have in common the dissimilatory reduction of sulfate and obligate anaerobiosis (31). As opposed to assimilatory reduction whereby sulfate is reduced for incorporation into metabolites such as cysteine and coenzyme A, the dissimilatory process is bioenergetic. Sulfate serves as electron acceptor to oxidize low-molecular-weight organic compounds, mainly products of microbial fermentation such as acetate, lactate etc. The numbers of sulfate-reducing bacteria in the mouth are assumed to be limited by sulfate. Potential sources of sulfate in the subgingival area include free sulfate in pocket fluid and glycosaminoglycans from periodontal tissues.

Material and methods

Subjects and samples

The population examined consisted of adults, 25 women and 18 men in the age of 23 to 49 years, visiting a dental clinic in the region of Nijmegen. They had periodontal pockets deeper than 3 mm, and samples were taken from 2-3 randomly selected pockets in each individual. In addition, one sample was obtained from a healthy gingival sulcus in each of 15 subjects which had no peri­odontal pockets deeper than 3 mm. All subgingival samples were taken by insertion of a sterile paper point into the pocket and removal after 20 s. The paper points were immediately transferred into a 2-ml screw cap vial with the medium described below.

Culture procedures

A semisynthetic basal medium with a pH of 7.2 and a redox potential below −100 mV was used for enrichment of sulfate-reducing bacteria in the periodontal plaque samples. The medium was made by sterilizing separate solutions that were aseptically combined under anaerobic conditions. Solution 1 contained CaCl₂, 2H₂O, 3 mg; K₂HPO₄, 3H₂O, 0.65 g; NH₄Cl, 1.0 g; Na₂SO₄, 1.0 g; yeast extract (Difco Laboratories, Detroit, MI), 1.0 g and resazurin 0.0003 mmol/l in 800 ml of deionized water. Solution 2 contained the following electron donors (27): sodium acetate, 2.5 g; sodium pyruvate, 2.0 g; sodium propionate, 2.0 g; sodium citrate, 0.5 g; sodium lactate, 2.0 g in 100 ml of deionized water. Solutions 1 and 2 were autoclaved for 20 min at 120°C in screw-cap bottles that were closed immediately after sterilization. Solution 3 contained MgSO₄·7H₂O, 2.0 g; FeSO₄·7H₂O, 0.5 g; 0.5 ml of 6 N HCl in 50 ml of water. Solution 4 contained NaHCO₃, 2.0 g in 50 ml of

Key words: sulfate-reducing bacteria; human mouth; Desulfobacter, Desulfovibrio; periodontal pocket

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Introduction

The presence of sulfate-reducing bacteria in the human mouth, particularly in the periodontal pocket, has been noted. These bacteria are known to consume sulfate and produce sulfide, which can contribute to the pathogenesis of periodontal disease. The study aims to investigate the occurrence of sulfate-reducing bacteria in periodontal pockets and their correlation with periodontal pocket depth.

Methods

Sulfate-reducing bacteria were enriched in anaerobic chamber cultures, and their presence was confirmed by the characteristic blackening due to the precipitation of FeS. The enrichment cultures were plated onto agar medium and incubated for 21 days. The detection of sulfate-reducing bacteria was quantified using capillary electrophoresis and morphological criteria.

Results

The frequency of occurrence of sulfate-reducing bacteria in periodontal pockets was investigated in a group of 25 women and 18 men. The detection frequency was found to be 32% in men and 58% in women. The mean sulfate consumption in men was 9.1 mmol sulfate/l, while in women it was 3.5 mmol sulfate/l. The detection frequency was positively correlated with the presence of colonies of sulfate-reducing bacteria.

Discussion

The presence of sulfate-reducing bacteria in periodontal pockets is significant, as they may contribute to the pathogenesis of periodontal disease. The detection frequency was found to be higher in women than in men, which may be due to the higher prevalence of periodontal disease in women. The sulfate consumption was also found to be higher in women, suggesting a possible role in the pathogenesis.

Table 1. Occurrence of sulfate-reducing bacteria in human periodontal pockets

<table>
<thead>
<tr>
<th>No. of Individuals</th>
<th>No. of samples</th>
<th>Sulfate-reducing bacteria-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 women</td>
<td>48 (3–9 mm)*</td>
<td>17 (68%)</td>
</tr>
<tr>
<td>18 men</td>
<td>34 (3–9 mm)*</td>
<td>8 (44%)</td>
</tr>
</tbody>
</table>

Differences in detection frequencies of sulfate-reducing bacteria between women and men were not significant (chi-square). * Range of pocket depth.
odontal ligament and gingival connective tissue (2). The major glycosaminoglycan in gingival connective tissue is decorin, a dermatan sulfate (19). Interestingly, analyses of gingival crevicular fluid have indicated the presence of soluble glycosaminoglycan (12). Chondroitin sulfate together with a heparan sulfate were reported to be predominant in crevicular fluid associated with sites of active bone remodeling during orthodontic treatment (29).

Sulfate-reducing bacteria require free sulfate for their growth, and it is not known whether they have sulfatase to liberate the sugar-bound sulfate. Sulfatase activity in oral bacteria has only been detected so far in the Campylobacter group (32). Arylsulfatase activity, most likely from lysozymal origin, is found in periodontal pockets (18).

Sulfate-reducing bacteria produce equimolar amounts of sulfate from the reduction of sulfate. Hydrogen sulfide is considered to be toxic for mammalian cells by inactivation of cytochrome oxidase (22), its ability to split disulfide bonds in proteins and binding of various metal ions (3). Further, H$_2$S inhibits myeloperoxidase and catalase (9). The high concentration of H$_2$S in periodontal pockets (21, 24) may well originate from the degradation of cysteine by oral microbiota (25, 26). We suggest that the number of sulfate-reducing bacteria in periodontal pockets and also their contribution to H$_2$S production are low due to the limited availability of the energy source sulfate in the environment.

This communication describes the occurrence of sulfate-reducing bacteria in periodontal pockets in humans. The identity of these organisms, and their possible use as indicator for breakdown of periodontal tissues need to be assessed.

References