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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 *Desulfovirbrio*. 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 5 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...
Sulfate-reducing bacteria-positive samples

Monium bromide (CTAB, 0.1 mol/l). The frequency of occurrence of sulfate-reducing bacteria and pocket depth was found. Sulfate-reducing bacteria were detected in 55% of pockets with a depth up to 5 mm and in 43% of pockets deeper than 5 mm. Sulfate-reducing bacteria were only occasionally found in the healthy gingival sulci of individuals without periodontal pockets deeper than 3 mm (1 positive sample out of 28 samples from 16 individuals).

The mean sulfate consumption in the plaque-enrichment cultures positive for sulfate-reducing bacteria was 9.1 (SD 3.5) mmol sulfate/l.

Attempts to obtain pure cultures from the sulfate-reducing bacteria positive enrichment cultures were not always successful. Preliminary characterization of the first 10 isolates obtained from different individuals revealed the following. Eight strains were gram-negative non-motile coccobacilli. These organisms reduced sulfate to sulfide, as indicated by FeS precipitation. They consumed acetate, some pyruvate and no propionate and might resemble Desulfobacter species (30, 31). Acetate consumption and sulfate reduction were highly correlated (r=0.92) for these strains. Two other isolates were gram-negative motile curved rods 4–7 μm in length. Lactate, pyruvate, but no acetate consumption suggested that they belonged to the genus Desulfovibrio (28).

Discussion

This report is the first to describe the presence of sulfate-reducing bacteria in the human mouth, particularly the periodontal pocket. The detection of sulfate-reducing bacteria in 32% (men) to 34% (women) of the samples indicated that these organisms are a common inhabitant of sites showing periodontal destruction. Sulfate-reducing bacteria were only occasionally found in healthy sites in periodontitis-free individuals. The search for sulfate-reducing bacteria was undertaken because several conditions required for the growth of these bacteria, including an anaerobic environment with a low redox potential (17), the presence of low-molecular-weight fermentation products (6) and a neutral to slightly alkaline pH (11), are met in the periodontal pocket. The low detection frequency of sulfate-reducing bacteria in the sulci in healthy individuals might be due to the relatively high redox potential (17). The genera Desulfovibrio and Desulfovaria found in periodontal pockets have also been found to occur in the intestinal tract of humans (13).

The numerical significance of sulfate-reducing bacteria in relation to the total microbial counts needs to be determined to get insight into the ecological role of sulfate reduction in the pocket. In several ecosystems, including the human gut (13, 14), the numbers of sulfate-reducing bacteria seem to be limited by the energy source sulfate. Sources of sulfate in the periodontal pocket include the pocket fluid, mainly a transudate of periodontal tissues, the constituents of which are derived from serum, inflammatory cells and host tissue. The mean concentration of free sulfate in serum is 0.3 mmol/l (Biochemisches Taschenbuch, Springer Verlag, Berlin, 1964), and sulfate from serum might be available in the pocket fluid. It is tempting to assume that the sulfate-containing proteoglycans represent a source of sulfate. Proteoglycans constitute a major component in the extracellular matrix of connective tissues. They consist of a central protein core to which highly anionic heteropolysaccharide chains called glycosaminoglycans are linked. Sulfate is bound to glucosamine and galactosamine residues in glycosaminoglycans. Proteoglycan species are characteristically distributed among the periodontal tissues. Heparan sulfate is the predominant glycosaminoglycan in gingival epithelium (1) and a minor constituent in peri-

<table>
<thead>
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<th>Table 1. Occurrence of sulfate-reducing bacteria in human periodontal pockets</th>
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<td>No. of Individuals</td>
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<tr>
<td>25 women</td>
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<td>18 men</td>
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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 lysosomal 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, H2S inhibits myeloperoxidase and catalase (9). The high concentration of H2S 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 H2S 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


