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 Desulfohabter 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 digestors (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 donor in the form of low-molecular-weight fatty acids are present (6), while sulfate might be limiting in the periodontal pocket.

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 individuals samples

with a 50-cm glass column with an inter-
face active agent cetyltrimethyl am­
dium bromide (CTAB, 0.1 mol/1).

Sulfate reduction in the primary en­
richment cultures was indicated by
strong blackening due to precipitation of FeS. Tenfold serial dilutions of
the enrichment culture were plated onto
agar medium and incubated for 21
days. Blackening of the plates indicated
the presence of colonies of sulfate-redu­
cing bacteria. All incubation was
done in the anaerobic chamber.

Identification of sulfate-reducing bacteria

Isolated colonies of sulfate-reducing bacteria were streaked twice onto agar
plates, and final colonies were grown in
liquid media containing various elec­
tron donors. Growth in the liquid me­
dium was indicated by measurement of
sulfate, production of FeS and micro­
scopy. Preliminary identification of the
isolated strains was done using morphological criteria and substrate
utilization tests (16).

Chemical analyses

Sulfate and volatile fatty acids were measured using capillary elec­
rophoresis. Samples consisted of cell-free
culture supernatant fluids obtained by
centrifugation and filtration (pore size
0.2 μm, Schleicher and Schuell, Dassel,
Germany; type FP 030/3). Analyses were
done in a P/ACE System 2210 (Beckman
Instruments, Berkeley, CA) provided with a 50-cm glass column with an inter­
nal diameter of 50 μm and an ultraviolet
detector. Separations were carried out at
20 kV in a histidine-phthalic acid buffer
(pH 5.6; 20 mmol/l) containing the sur­
face active agent cetyltrimethyl am­
nonium bromide (CTAB, 0.1 mol/l).
The time of analysis was approximately
6 min.

Results

The frequency of occurrence of sulfate­
reducing bacteria in periodontal
pockets in human adults is given in

Table 1. Sulfate-reducing bacteria tended to occur more frequently in
women than in men, but the difference
was not statistically significant using the
chi-square test. No relationship be­
tween the occurrence of sulfate-reduc­
ing 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 individ­
uals 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 en­
richment cultures were not always suc­
cessful. Preliminary characterization of
the first 10 isolates obtained from differ­
ent individuals revealed the following.
Eight strains were gram-negative non­
motile coccobacilli. These organisms re­
duced sulfate to sulfide, as indicated by
FeS precipitation. They consumed acet­
ate, some pyruvate and no propionate and
might resemble Desulfovibacter spe­
cies (30, 31). Acetate consumption and
sulfate reduction were highly correlated
(r=0.92) for these strains. Two other is­
olates were gram-negative motile curved
rods 4–7 μm in length. Lactate, pyruvate,
but no acetate consumption suggested
that they belonged to the genus Desul­
fovibrio (28).

Discussion

This report is the first to describe the
presence of sulfate-reducing bacteria in
the human mouth, particularly the peri­
donatal pocket. The detection of sul­
fate-reducing bacteria in 32% (men) to
58% (women) of the samples indicated
that these organisms are a common in­
habitant 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 con­
ditions required for the growth of these
bacteria, including an anaerobic en­
vironment 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 individ­
uals might be due to the relatively high
redox potential (17). The genera Desul­
fovibacter and Desulfovibrio 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 deter­
mined to get insight into the ecological
role of sulfate reduction in the pocket.
In several ecosystems, including the hu­
man gut (13, 14), the numbers of sul­
fate-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 (Bio­
chemisches Taschenbuch, Springer Ver­
lag, Berlin, 1964), and sulfate from
serum might be available in the pocket
fluid. It is tempting to assume that the
sulfate-containing proteoglycans repre­
sent 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 heteropo­
l Yasacharide chains called glycosaminogly­
cans are linked. Sulfate is bound to
glucosamine and galactosamine resi­
dues in glycosaminoglycans. Proteo­
glycan species are characterizedly dis­
tributed among the periodontal tissues.
Heparan sulfate is the predominant gly­
cosaminoglycan in gingival epithelium
(1) and a minor constituent in peri­

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

<table>
<thead>
<tr>
<th>No. of</th>
<th>No. of</th>
<th>Sulfate-reducing bacteria-positive</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>individuals</td>
</tr>
<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, 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