The production of sulfide due to sulfate reduction is the phenomenon widely detectable in microbial ecosystems under anaerobic conditions. Such phenomenon has been reported on marine and fresh-water environments, paddy soil, rumen, sludge digestion tanks and so on. It has been attributed to the presence and the activity of the specific bacteria, i.e. the sulfate-reducing bacteria. Recent investigations on the physiology and biochemistry of the sulfate-reducing bacteria have shown that only a few compounds such as lactate, pyruvate or molecular hydrogen are utilized by these bacteria as hydrogen donor for sulfate reduction.

In previous papers, however, the author has shown that a sulfate-reducing bacterium Desulfovibrio desulfuricans can, in natural mixed populations, reduce sulfate to sulfide in the presence of a wide variety of organic compounds including carbohydrates, amino acids, proteins, organic acids, alcohols, etc., although this bacterium in single culture utilizes only a few compounds mentioned above.

From the above facts, it appears possible that there exists certain interaction between the sulfate-reducing bacteria and other heterotrophic bacteria constituting a microbial community.

The present work was initiated to ascertain such possibility. As the result, it became evident that the mixed cultures of the sulfate-reducing bacterium Desulfovibrio desulfuricans and other heterotrophic bacteria such as Escherichia coli, Paracolobactrum aerogenoides or Proteus morganii, can reduce sulfate in the presence of various organic compounds which are not utilized in the case of the pure culture of this sulfate-reducing bacterium. This paper is dealing with such an interaction.

**Materials and Methods**

**MATERIALS** Four bacterial species were used; one strain of the sulfate-reducing bacterium Desulfovibrio desulfuricans isolated by the author from the polluted river-water of the Sumida, Tokyo, Escherichia coli strain B(S) presented by other laboratory of this university, Paracolobactrum aerogenoides and Proteus morganii. The latter two species were isolated by the author from the same river-water, and identified tentatively according to the following cultural, morphological and biochemical properties.


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Methyl red test; negative. Acetymethylcarbinol; produced. Litmus milk; delayed coagulation. Urea; hydrolyzed. Nitrate; reduced to nitrite. Na-citrate; utilized as a sole source of organic carbon. Acid and gas from glucose, fructose, galactose, mannose, maltose, sucrose, mannitol and glycerol. Fermentation of lactose; consistently delayed. Starch; not fermented.

These characters are similar to those of *Aerobacter aerogenes* except delayed fermentation of lactose. Therefore, this bacterium was classified tentatively as *P. aerogenoides* according to the *Bergey's Manual*.


These properties coincide well with those of *Pr. morganii* described in the *Bergey's Manual*. In addition to the above properties, this bacterium was found to be able to ferment L-aspartate, L-glutamate, L-threonine, L-cysteine, L-serine, L-tyrosine, L-ornithine and L-methionine in the presence of a small amount of yeast extract.

**Methods** Four organic compounds, *i.e.* glucose, sodium citrate, glycerol and sodium glutamate, were tested as the substrates for mixed cultures. None of these compounds was utilized as hydrogen donor for the growth of the sulfate-reducing bacterium in pure culture.

The basal medium to which the above substrates were added, had the following composition: \( NH_4Cl, 2.0 \text{ g}; MgCl}_2\cdot6\text{H}_2\text{O, 0.7 g; CaCl}_2, 0.1 \text{ g; KH}_2\text{PO}_4, 0.5 \text{ g; Na}_2\text{SO}_4, 2.0 \text{ g; (NH}_4)_2\text{Fe(SO}_4)_2\cdot6\text{H}_2\text{O, 0.1 g; H}_2\text{O, 1000 ml (pH 7.2).}\)

The amount of culture media and the kind of culture vessels used were different from experiment to experiment, but all cultures were grown under anaerobic conditions at 35°. In the mixed culture on glucose, cotton-plugged test tubes containing 10 ml of medium were used as culture vessel. In this case, the test tubes were incubated in an atmosphere of nitrogen. In the mixed culture on citrate, evacuated Thunberg tubes containing 10 ml of medium were used. In the mixed cultures on glycerol and glutamate, evacuated 100 ml Erlenmeyer flasks with a side arm were used, and, in these cases, 50 ml of media was added to the flasks. In those experiments, where glucose and glycerol were tested, calcium carbonate was added to the basal medium, in order to avoid lowering of the pH of the medium during fermentation. In the mixed culture on glutamate, where *D. desulfuricans* and *Pr. morganii* were cultured, a small amount of Difco yeast extract was added to the basal medium, since *Pr. morganii* was found to require certain growth factor.

The amount of inoculum was the same in all experiments, *i.e.* 0.1 ml of the culture solution of *D. desulfuricans* and one drop of the suspension of other bacteria in sterilized water were added to the media.

In those experiments, where Thunberg tubes or Erlenmeyer flasks with a side arm were used, 1 ml of 2N zinc acetate solution was added to the side arm, in order to determine the amount of sulfide produced. In glucose medium, the production of sulfide was determined only qualitatively by observing the formation of the black
The precipitation of ferrous sulfide.

The amounts of sulfide and organic acids after fermentation were determined according to the methods described in a previous paper13).

Result and Discussion

The results of the experiments were shown in Table 1 and Fig. 1. Since D. desulfuricans in single culture cannot grow on these organic substances13), the data of this table show clearly that there exists a commensalism between the sulfate-reducing bacterium D. desulfuricans and other heterotrophic bacteria. Namely, D. desulfuricans can reduce sulfate to sulfide under the influence of other heterotrophic bacteria in the presence of various organic compounds, which are not utilized by the single culture of D. desulfuricans but are utilized by other heterotrophic bacteria. Although only a few organic compounds were tested in this study, a wide variety of other organic compounds seems to be utilized for sulfate reduction by these mixed cultures, since E. coli, P. aerogenoides and Pr. morganii can degrade various kinds of organic compounds. Besides E. coli, P. aerogenoides and Pr. morganii used as experimental materials in this study, many other anaerobic bacteria must, in nature,

<table>
<thead>
<tr>
<th>Kind of mixed culture</th>
<th>Substrates added (μmole)</th>
<th>Incubation time (hr.)</th>
<th>Sulfide produced (μmole)</th>
<th>Organic acid produced (μmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. desulfuricans + E. coli</td>
<td>Glucose: 110</td>
<td>72</td>
<td>+</td>
<td>Acetate: 67, Lactate: 39</td>
</tr>
<tr>
<td>D. desulfuricans + P. aerogenoides</td>
<td>Citrate: 100</td>
<td>65</td>
<td>13</td>
<td>Acetate: 136</td>
</tr>
<tr>
<td>D. desulfuricans + P. aerogenoides</td>
<td>Glycerol: 1000</td>
<td>72</td>
<td>64</td>
<td>Acetate: 254</td>
</tr>
<tr>
<td>D. desulfuricans + Pr. morganii</td>
<td>Glutamate: 200</td>
<td>120</td>
<td>13</td>
<td>Acetate: 124</td>
</tr>
</tbody>
</table>

Fig. 1. Right: Sulfate reduction by the mixed culture of Desulfovibrio desulfuricans and Paracolobactrum aerogenoides on glycerol. The culture is black, owing to the formation of ferrous sulfide. Left: The single culture of P. aerogenoides on the same medium.
have a similar relation to the sulfate-reducing bacteria. Such a commensalism mentioned above, whatever its mechanism may be, can explain reasonably the reason why natural mixed populations including sulfate-reducing bacteria\(^4,14,15\) or crude culture of the sulfate-reducing bacteria\(^12\) can reduce sulfate to sulfide in the presence of a wide variety of organic compounds.

There is no doubt that such a commensalism mentioned above is brought about by the utilization by the sulfate-reducing bacteria of certain metabolic product excreted by other heterotrophic bacteria. Judging from the nutrition and metabolism of this sulfate-reducing bacterium\(^13\), it is reasonable to assume that one or more of the following compounds, \textit{i.e.} lactate, pyruvate and molecular hydrogen (or reduced coenzyme), must be produced by other heterotrophic bacteria and utilized as hydrogen donor for sulfate reduction by the sulfate-reducing bacterium. This possibility is supported by the formation of acetate by the mixed cultures. Acetate is a final metabolic product of \textit{D. desulfuricans} in pure culture on lactate or pyruvate\(^13\).

Aside sulfate reduction itself, other two interesting facts became evident from the foregoing experiments. The first is that, when \textit{D. desulfuricans} is cultured together with other heterotrophic bacteria, it is not necessary to control redox potential of the culture media. As reported in previous paper\(^13\), the pure culture of this bacterium requires a low redox potential for its initial growth. Probably, other heterotrophic bacteria in mixed cultures bring about a low redox potential necessary for the growth of \textit{D. desulfuricans}. The second is that, although the pure culture of the sulfate-reducing bacterium used here requires certain growth factor for its growth, this bacterium in mixed cultures does not. Presumably, other heterotrophic bacteria, \textit{e.g.} \textit{E. coli}, can synthesize and excrete certain growth factor necessary for the growth of the sulfate-reducing bacterium.

Details of such a commensalism are unknown and, hence, deserve further investigation.

**Summary**

There exists a commensalism between the sulfate-reducing bacterium \textit{Desulfovibrio desulfuricans} and other heterotrophic bacteria. Namely, the mixed cultures of \textit{D. desulfuricans} and other heterotrophic bacteria such as \textit{Escherichia coli}, \textit{Paracolobacterium aerogenoides} or \textit{Proteus morganii}, can reduce sulfate to sulfide in the presence of various organic compounds such as glucose, citrate, glycerol or glutamate, which are not utilized by the pure culture of \textit{D. desulfuricans}.

As for such a commensalism, two other interesting facts became evident from the mixed culture experiments. Namely, although the pure culture of \textit{D. desulfuricans} requires both the addition of growth factor and regulation of the redox potential of the culture media, this bacterium in mixed cultures requires none of these treatments.

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References