Isolation and Characterization of a Sulfur-Oxidizing Chemolithotroph Growing on Crude Oil under Anaerobic Conditions

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Molecular approaches have shown that a group of bacteria (called cluster 1 bacteria) affiliated with the ε subclass of the class Proteobacteria constituted major populations in underground crude-oil storage cavities. In order to unveil their physiology and ecological niche, this study isolated bacterial strains (exemplified by strain YK-1) affiliated with the cluster 1 bacteria from an oil storage cavity at Kuji in Iwate, Japan. 16S rRNA gene sequence analysis indicated that its closest relative was Thiomicrospira denitrificans (90% identity). Growth experiments under anaerobic conditions showed that strain YK-1 was a sulfur-oxidizing obligate chemolithotroph utilizing sulfate, elemental sulfur, thiosulfate, and hydrogen as electron donors and nitrate as an electron acceptor. Oxygen also supported its growth only under microaerobic conditions. Strain YK-1 could not grow on nitrite, and nitrite was the final product of nitrate reduction. Neither sugars, organic acids (including acetate), nor hydrocarbons could serve as carbon and energy sources. A typical stoichiometry of its energy metabolism followed an equation: S^{2-} + 4NO_3^- \rightarrow SO_4^{2-} + 4NO_2^- (\Delta G^0 = -534 \text{ kJ mol}^{-1}). In a difference from other anaerobic sulfur-oxidizing bacteria, this bacterium was sensitive to NaCl; growth in medium containing more than 1% NaCl was negligible. When YK-1 was grown anaerobically in a sulfur-depleted inorganic medium overlaid with crude oil, sulfate was produced, corresponding to its growth. On the contrary, YK-1 could not utilize crude oil as a carbon source. These results suggest that the cluster 1 bacteria yielded energy for growth in oil storage cavities by oxidizing petroleum sulfur compounds. Based on its physiology, ecological interactions with other members of the groundwater community are discussed.

Underground cavities have been used for long-term storage of crude oil in several countries, including those situated at Kuji in Iwate, Japan. Since these cavities have been constructed in groundwater-rich rocky strata, groundwater migrates into and accumulates at the bottom of these cavities (this groundwater is called cavity groundwater [26]). This flow of groundwater facilitates establishing a continuous culture of microorganisms in cavity groundwater; the cell count was constantly over 10^6 ml^{-1}, which was 100 times greater than the counts in control groundwater obtained around these cavities (26). This habitat of microorganisms can be characterized by immediate contact with a large quantity of crude oil and an excess of electron donors (i.e., hydrocarbons) but a shortage of electron acceptors. These characteristics may be similar to those of microbial habitats associated with subterranean oil reservoirs that have recently attracted strong microbiological attention (6, 8, 11, 24). Since cavity groundwater can easily be obtained at any time without contamination by surface water (26, 27), these cavities are considered to represent good models for studying the microbial ecology of subterranean oil fields.

Our previous study applied molecular phylogenetic approaches to analyzing bacterial populations that occurred in the Kuji cavity groundwater (26). It was found that a group of bacteria (called cluster 1 bacteria) affiliated with the Thiovul- lum subgroup (10) in the ε subclass of the class Proteobacteria constituted major populations, sharing 10 to 30% of the total microbial populations. We have also detected cluster 1 bacteria in oil storage cavities at Kushikino in Kagoshima, Japan (unpublished data). The Thiovulum subgroup includes three cultivated sulfur-oxidizing bacteria (SOB) (3, 7, 19) and many environmental clones obtained from hydrothermal vents (5, 12), marine sediments (2), and groundwater (15–17). In this subgroup, the cluster 1 bacteria formed a peculiar assemblage, called the groundwater bacteria assemblage, together with three environmental clones obtained from groundwater at geographically distant sites (15–17, 26). It has thus been suggested that bacteria belonging to this assemblage are widely distributed in the subterranean environment, although their physiology and ecological niche have been unknown.

The present study isolated bacterial strains (exemplified by strain YK-1) affiliated with the cluster 1 bacteria from an oil storage cavity at Kuji. In order to unveil their ecological niche in oil storage cavities, experiments were conducted to investigate physiological features of strain YK-1. In these experiments, especially provocative was the possibility that YK-1 could utilize crude oil for its growth under anaerobic conditions. Based on its physiological features, we discussed ecological interactions of the cluster 1 bacteria with other members of the cavity groundwater community.

MATERIALS AND METHODS

Groundwater sample. The cavity groundwater used for isolation of bacteria was obtained in March 1999 from the TK101 underground crude oil storage cavity situated at Kuji in Iwate, Japan. Characteristics of this groundwater have been reported previously (26).

Culture media, growth conditions, and maintenance. Culture media used in this study included DSM113 (for nitrate-reducing thiosulfate-oxidizing bacteria) (the DSM catalog provided by German Collection of Microorganisms and Cell Cultures [http://www.dsmz.de/]), Luria-Bertani (for heterotrophs [20]), dCGY (for heterotrophs [25]) and MB (an inorganic medium [25]). The MBM medium
was also used in this study, which contained (liter−1) 0.2 g of KH2PO4, 0.2 g of
NH4Cl, 0.4 g of MgCl2·6H2O, 0.2 g of KCl, 0.1 g of CaCl2·2H2O, 0.2 g of
NaNO3, 2 mg of resazurin, and 2 ml of SL-4 trace metal solution (the DSM
catalog). Solid media contained 1.5% Bactoagar (Difco). These media were
sterilized under pressure, poured, and cooled under nitrogen atmosphere. For
incubation, freshly prepared Na2S·9H2O (2 mM) was used as a reducing agent
unless otherwise specified. The pH was adjusted to 7.0. Routine cultivation was
conducted at 25°C using a bottle capped with a Teflon-coated butyl rubber
septum and sealed with an aluminum crimp seal. The vapor phase in the bottle
was filled with N2-CO2 (80%:20%) or N2-CO2-H2 (80%:10%:10%), and when
necessary, pure N2 was used. Cells in liquid cultures were counted by using
epitaxial growth technique of ClustalW version 1.7 (23) was used to align the se-
quences. A phylogenetic tree was con-
structed by visual inspection; secondary struc-
tures were considered for the re-
duced MBM medium supplemented
with either of the following electron donors (2 mM): sulfate, thiosulfate,
acetate, pyruvate, succinate, fumarate, lactate, glucose, formate, malate, gluta-
mate, benzoate, phenol, octane, toluene, benzene, or elemental sulfur (1% wt
vol−1). Elemental sulfur was sterilized as described elsewhere (29).
Microaerobic growth was tested in MBM medium without nitrate at an O2
partial pressure in the headspace of 1% (vol vol−1). The headspace gas also
contained CO2 (20%) and N2 (the rest). Electron donors tested included sulfi-
dide (2 mM) that, in co-culture, H2 (10% of N2 in the headspace was replaced)
and elemental sulfur (1% wt vol−1). Titanium(III) citrate (1.3 mM) was used as a
reducing agent (9), when the medium did not contain sulfide.
Anaerobic growth was examined in modified MBM medium containing either
sulfide (2 mM), thiosulfate (2 mM), H2 (10% in headspace), or elemental sulfur
(1%) as an electron donor and either nitrate (0.6 mM) or nitrite (0.7 mM) as an
electron acceptor. Titanium(III) nitrate (1.3 mM) was used as a reducing agent.
The headspace gas consisted of N2-CO2 (80%-20%). In a growth test with H2 as
an electron donor, the headspace gas consisted of N2-CO2-H2 (80%-10%:10%).
In a growth test with an organic compound (methanol, formate, acetate, pyru-
vote, succinate, fumarate, lactate, glucose, malate, glutamate, phenol, benzoate
or octane [2 mM]) as an electron donor, MBM medium was used in which nitrate
served as an electron acceptor.

The standard free energy generated by a metabolic reaction was calculated
from the standard free energies of reactants and products (22). The amount of
sulfate oxidized was estimated from the amount of sulfate produced, since pos-
sible intermediate metabolites (elemental sulfur, thiosulfate, and sulfite) were
not detected or detected transiently in the culture.

Growth on crude oil. Crude oil used was Arabian light. The crude oil
crude oil was sterilized and deaerated by the method of Rabus and Widdel (18). Eight mil-
liters of MBM medium supplemented with ascorbate (2 mM) was infused into a
bottle (20 ml in capacity), overlaid with 2 ml of the crude oil, and sealed with
a Teflon-coated butyl rubber septum and an aluminum crimp cap under the ni-
trogen atmosphere. In some cases, sulfide (2 mM) and bicarbonate (2 mM) were
added as an electron donor and a carbon source, respectively. The headspace
was filled with pure N2. The bottle was inoculated with approximately 0.1 ml of
strain YK-1 culture fully grown in the MBM medium by using a syringe needle. The culture
was incubated at 20°C without shaking.

Nucleotide sequence accession numbers. The 16S rDNA gene sequences of
strains YK-1, YK-2, YK-3, and YK-4 have been deposited in the GSDB, DDBJ,
EMBL, and NCBI nucleotide sequence databases under accession no.
AB080351, AB080843, AB080644, and AB080845.

RESULTS AND DISCUSSION

Isolation. Colonies formed on agar plates of several different


types of media (see Materials and Methods) under aerobic,

microaerobic, and anaerobic conditions were subjected to the
genetic screening by means of DGGE of PCR-amplified 16S

rDNA fragments. A DGGE band derived from a colony

was compared to a band representing cluster 1 bacteria in the

DGGE profile for the Kuji cavity groundwater (see reference
26). Bands obtained from several very tiny colonies formed

on the DSM113 plates migrated to the same position as the band

of the cluster 1 bacteria; these colonies were picked and puri-

fied by restreaking them on the DSM113 plates. The bacterial

strains thus obtained were designated YK-1, YK-2, YK-3,

and YK-4. Their 16S rDNA sequences were similar and were af-

liated with the cluster 1 bacteria (Fig. 1). In addition, prelim-

inary tests showed that their physiological characteristics were

identical (data not shown). We therefore describe in the fol-

lowing section characteristics of strain YK-1 as the represen-

tative. Strain YK-1 was deposited in the Japan Collection

of Microorganisms under strain number JCM 11577.

The database search with the 16S rDNA sequences showed

Thiomicrospira denitrificans to be the closest relative of strain

YK-1, as previously reported for the cluster 1 bacteria (26),

Growth of strains YK-1, YK-2, and YK-3 on the DSM113 medium was

examined (Table 1). The strains used here were previously reported

to be the closest relative of strain YK-1, as previously reported for the cluster 1 bacteria (26),
although the identity was approximately 90%. Recently, another *Thiomicrospira* bacterium, strain CVO, was isolated from the production water of a Canadian oil well (3). The identity between the 16S rDNA sequence of strain YK-1 and that of strain CVO was also 90%.

DSM113 medium has been used to cultivate *T. denitrificans* (7). Thiosulfate was supplemented as an electron donor in this medium, while sulfide added as a reducing agent could also serve as an electron donor. Growth of strain YK-1 in the liquid DSM113 medium was very slow, however, with the doubling time being approximately 15 days. We assumed that this slow growth was attributable to a high ionic strength of medium DSM113, because this medium had been designed for *T. denitrificans*, a marine bacterium. Actually, the ionic strength of the cavity groundwater was very low (its electric conductivity was 250 to 300 μS). When YK-1 was grown in the MBM medium (a low-ionic-strength medium developed in this study) supplemented with bicarbonate (2 mM) under N2 atmosphere, the doubling time of YK-1 was shortened to approximately 1 day (Fig. 2). MBM-based media were hence used in the subsequent experiments.

Physiological and taxonomic characteristics of strain YK-1.

Electron microscopy showed that the strain YK-1 cell was a curved rod with a length of 1 to 2 μm and a diameter of approximately 0.4 μm. The transmission scanning electron microscopy picture showed a single flagellum attaching at one pole.

Physiological and taxonomic characteristics of strain YK-1 are summarized in Table 1, in which characteristics of *T. denitrificans* (7) and strain CVO (3) cited from the literature are also presented for the comparison. The data in this table illustrate that YK-1 is a sulfur-oxidizing obligate chemolithotroph growing under microaerobic and anaerobic conditions. We found several distinct features of YK-1, which could clearly separate YK-1 from strains of *Thiomicrospira*; these features include motility, NaCl sensitivity, inability to utilize acetate as a carbon source, ability to utilize hydrogen gas as an electron donor, the product of nitrate reduction, the end product from sulfide oxidation, and inability to utilize nitrite as an electron acceptor. Another isolated member of the *Thiovulum* subgroup, *Thiovulum* sp., is also a SOB; however, its features described in the literature (19) are quite different from those of strain YK-1. *Thiovulum* grows only under microaerobic conditions by reducing molecular oxygen, and cells are round or ovoid (5 to 25 μm in diameter). The higher sensitivity to NaCl of YK-1 than of the other anaerobic SOB is understandable, because YK-1 is the only strain isolated from fresh groundwater. From these results, YK-1 is considered to represent a novel genus in the ε subclass of the class *Proteobacteria*, although further studies are needed to confirm this idea.

A typical growth pattern of strain YK-1 in MBM medium at 25°C under the N2 atmosphere is shown in Fig. 2, where sulfide was an electron donor and nitrate was an electron acceptor. This organism did not show a typical exponential growth pattern, probably due to its two-step oxidation of sulfide; i.e., it initially transformed sulfide to elemental sulfur and subsequently started to produce sulfate. A stoichiometric analysis of the ions consumed and produced during this growth suggests that the energy metabolism of YK-1 follows an equation: $S^2^- + 4NO_3^- \rightarrow SO_2^{2-} + 4NO_2O_2$ ($AG = -534$ kJ mol$^{-1}$). As shown in Fig. 2, growth of YK-1 terminated below a cell count of $10^7$ ml$^{-1}$. This low cell count is considered to be ascribable in part to the accumulation of nitrite. Its growth on sulfide and nitrate in MBM medium was inhibited, when nitrite was added at concentrations above 0.7 mM (data not shown). Nitrite was always the terminal product of nitrate reduction, even when the $HS^-/NO_3^-$ ratio was changed from 0.2 to 5.

In addition to sulfide, thiosulfate, elemental sulfur, and hydrogen also supported growth of YK-1 as electron donors. The strain was also capable of utilizing molecular oxygen as an electron acceptor under microaerobic conditions.
variety of energy metabolism could allow this type of organisms (e.g., those affiliated with the groundwater bacteria assemblage [26]) to occur widely in subterranean environments where the organic nutrient is limited.

Ecological niche. We next investigated growth of YK-1 in the presence of crude oil (Fig. 3). Two major effects of crude oil on microbial growth are conceivable; first, crude oil may serve as a nutrient (i.e., carbon and energy sources), and second, crude oil may select for microorganisms that exhibit resistance to organic solvents. This experiment therefore overlaid a culture medium with an excess amount of crude oil, which may have simulated the habitat in the oil storage cavity. Figure 3 shows that YK-1 did not grow without bicarbonate even when crude oil was present, indicating that YK-1 was incapable of utilizing crude oil as a carbon source. In addition, it could not grow in bicarbonate-depleted media supplemented with pure hydrocarbons including octane, benzene, and toluene (Table 1). In contrast, when a sulfur-free inorganic medium was overlaid with crude oil, growth of YK-1 was observed, corresponding to the production of sulfate (Fig. 3). These data indicate that YK-1 grew by oxidizing sulfur compounds in crude oil as energy sources. They also indicate that YK-1 exhibited resistance to an excess amount of crude oil. It is noteworthy that this organism utilizes crude oil as an energy source (electron donor) but not as a carbon source. Crude oil contains a variety of sulfur compounds, including inorganic and organic ones, and most of the sulfur has been considered to be bound to organic compounds (14); this is especially relevant to crude oil that has been stored for a long time after it was produced. We therefore assume that YK-1 may have

### Table 1. Summary of characteristics of strain YK-1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value&lt;sup&gt;a&lt;/sup&gt; for strain:</th>
<th>YK-1</th>
<th>CVO&lt;sup&gt;b&lt;/sup&gt;</th>
<th>T. denitrificans&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Curved rod</td>
<td>Curved rod</td>
<td>Vibroid or spiral</td>
<td></td>
</tr>
<tr>
<td>Width (μm)</td>
<td>0.4</td>
<td>0.4</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Length (μm)</td>
<td>1 to 2</td>
<td>0.6 to 0.8</td>
<td>Variable</td>
<td></td>
</tr>
<tr>
<td>Spore</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Gram stain</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Oxidase test</td>
<td>+</td>
<td>+</td>
<td>ND&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Catalase test</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Growth temperature (°C)</td>
<td>10 to 35 (25°)</td>
<td>5 to 35</td>
<td>22°</td>
<td></td>
</tr>
<tr>
<td>Growth pH</td>
<td>6.0 to 8.0 (7.0°)</td>
<td>5.5 to 8.5</td>
<td>7.0°</td>
<td></td>
</tr>
<tr>
<td>NaCl range (%)</td>
<td>0 to 1.0</td>
<td>0 to 7.0</td>
<td>3°</td>
<td></td>
</tr>
<tr>
<td>Carbon source</td>
<td>CO₂ or bicarbonate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acetate</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Glucose, octane, toluene and benzene</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Fermentative growth</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Aerobic growth</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Microaerobic growth (1% O₂)</td>
<td>HS&lt;sup&gt;−&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S₂O₃&lt;sup&gt;2−&lt;/sup&gt;</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>S₀&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>H₂</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Anaerobic growth on NO₃⁻ by oxidation of:</td>
<td>HS&lt;sup&gt;−&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S₂O₃&lt;sup&gt;2−&lt;/sup&gt;</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>S₀&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>H₂</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Organic compounds</td>
<td>–&lt;sup&gt;f&lt;/sup&gt;</td>
<td>–&lt;sup&gt;f&lt;/sup&gt;</td>
<td>–&lt;sup&gt;j&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Denitrification product from NO₃⁻ by oxidation of HS&lt;sup&gt;−&lt;/sup&gt;</td>
<td>NO₂&lt;sup&gt;−&lt;/sup&gt;</td>
<td>NO₂&lt;sup&gt;−&lt;/sup&gt;, NO, N₂</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>End product from HS&lt;sup&gt;−&lt;/sup&gt; oxidation</td>
<td>SO₂&lt;sup&gt;2−&lt;/sup&gt;</td>
<td>SO₂&lt;sup&gt;2−&lt;/sup&gt;, S²O³&lt;sup&gt;−&lt;/sup&gt;</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Anaerobic growth on NO₂&lt;sup&gt;−&lt;/sup&gt; by oxidation of:</td>
<td>HS&lt;sup&gt;−&lt;/sup&gt;</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S₂O₃&lt;sup&gt;2−&lt;/sup&gt;</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td></td>
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<tr>
<td>S₀&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td></td>
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<tr>
<td>H₂</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>+, positive; –, negative.
<sup>b</sup>Data are cited from reference 3.
<sup>c</sup>Data are cited from reference 7.
<sup>d</sup>Nonmotile or weakly motile.
<sup>e</sup>ND, not determined.
<sup>f</sup>A value for the optimum growth.
<sup>g</sup>Substrates tested were described in the Materials and Methods section.
<sup>h</sup>Substrates tested included pyruvate, succinate, lactate, fumarate, malate, and aspartate.
<sup>i</sup>Substrates tested included S₂O₃<sup>2−</sup>, S⁰, acetate, succinate, lactate, fumarate, malate, formate, pyruvate, glucose, and glycerol.
<sup>j</sup>Substrate tested included thiosulfate, lactate, and glucose.
<sup>k</sup>Substrates tested included formate, acetate, propionate, pyruvate, succinate, fumarate, lactate, and glucose.
<sup>l</sup>A substrate tested was glucose.
<sup>m</sup>The end product changes according to the initial HS<sup>−</sup> concentration.
utilized some organosulfur compounds to produce sulfate. We are currently carrying out further experiments to identify types of sulfur compounds, including various organosulfur compounds, which are available for strain YK-1.

Recently we reevaluated the bacterial biodiversity in the oil storage cavity at Kuji by cloning and sequencing of 16S rRNA gene fragments that were amplified by PCR using universal primers (28). In order to examine if major rRNA sequence types obtained actually represented abundant populations in the groundwater, we applied quantitative competitive PCR with specific primers (28). In these experiments, bacterial populations detected in abundance were related to sulfate-reducing bacteria (SRB) (affiliated with Desulfovibrio and Desulfotomaculum), denitrifying bacteria (Azoarcus/Zoogloae group), acetogens (Acetobacterium), and the cluster 1 bacteria. Together with the results obtained in the present study, it can be assumed that the cluster 1 bacteria may compete for nitrate with the Azoarcus/Zoogloae population (31). At the same time, the Azoarcus/Zoogloae population may scavenge nitrite produced by the cluster 1 bacteria (31), which may be advantageous to the cluster 1 bacteria. Currently, we are trying to isolate bacteria which represent the Azoarcus/Zoogloae-group bacteria.

We also consider the possibility that sulfate produced from crude oil by the cluster 1 bacteria (YK-1) could promote growth of SRB in the oil storage cavity. This notion is based on our finding that although the molecular analyses detected a significant level of SRB, sulfate concentrations in inflow groundwater and in groundwater that had accumulated at the bottom of the cavities were at the same level (4 to 8 mg liter$^{-1}$ [26]). The SRB affiliated with the same genera as those detected from the oil storage cavity had also been detected and isolated from subterranean oil fields (11, 24), and some representative organisms of these genera are known to be capable of anaerobic hydrocarbon degradation (30). The cluster 1 bacteria could utilize sulfide produced by such SRB, suggesting the presence of a sulfur cycle in the oil storage cavity. A similar sulfur cycle has previously been suggested for a Canadian oil reservoir (24), and anaerobic SOB, strains CVO and FWKO B, were isolated from it (3). In the case of the Canadian reservoir, however, the injection with sulfate-containing surface water has been considered essential for maintaining the sulfur cycle (24). In contrast, the results of the present study suggest that the sulfur cycle in the oil storage cavity can be supported by petroleum sulfur compounds from which sulfate is produced by anaerobic SOB. According to this idea, we hypothesize that the sulfur cycle involving SOB and SRB operates more ubiquitously in subterranean oil fields than hitherto believed.

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