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1	Vertical distribution of major sulfate-reducing bacteria in a eutrophic
2	shallow meromictic lake
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18	Running title: SRB in a shallow meromictic lake

19

Abstract

20 The vertical distribution of sulfate-reducing bacteria was investigated in a shallow, eutrophic, 21 meromictic lake, Lake Harutori, which is located in a residential area of Kushiro, Japan. A 22 steep chemocline, which is characterized by gradients of oxygen, sulfide, and salinity, was 23 found at a depth of 3.5–4.0 m. The sulfide concentration at the bottom of the lake was high 24 (up to a concentration of 10.7 mM). Clone libraries were constructed using the *aprA* gene, 25 which encodes adenosine-5'-phosphosulfate reductase subunit A to monitor sulfate-reducing 26 bacteria. In the *aprA* clone libraries, the most abundant sequences were those from the 27 Desulfosarcina-Desulfococcus (DSS) group. A primer set for a DSS group-specific 16S rRNA 28 gene was used to construct another clone library, analysis of which revealed that the 29 uncultured group of sulfate-reducing bacteria, SEEP SRB-1, accounted for nearly half of the 30 obtained sequences. Quantification of the major bacterial groups by catalyzed reporter 31 deposition-fluorescence in situ hybridization demonstrated that the DSS group accounted for 32 3.2–4.8% of the total bacterial community below the chemocline. Our results suggest that 33 below the chemocline of Lake Harutori, DSS group is one of the major groups of sulfate-34 reducing bacteria and that these presumably metabolically versatile bacteria might play an important role in sulfur cycling in the lake. 35

36

37 Keywords:

Meromictic lake, sulfate-reducing bacteria, aprA, sulfide

38

Introduction

39 The global expansion of anoxic aquatic environments has a massive influence on 40 biological activities. For instance, in the California Current marine ecosystem, serious damage 41 to the fish and benthic invertebrate communities is reported to have occurred due to anoxic 42 water, and there is concern about possible further damage to the highly productive coastal 43 environment [7]. The formation of anoxic water masses is thought to be connected to human-44 induced eutrophication and global warming [1], and it is speculated that the occurrence of 45 hypoxia will increase in the coming decades [21]. Sulfide accumulation in anoxic water 46 columns is an additional concern. In anoxic environments, sulfides are normally produced via 47 microbial sulfate reduction. Especially in the marine environment, the sufficient supply of 48 sulfate from seawater and of organic compounds support the growth of sulfate-reducing 49 bacteria. Most of the sulfide is usually converted into colloidal sulfur or sulfate by bacteria, 50 via either anaerobic or aerobic oxidation before it reaches the surface layer of the water 51 column [21]. Therefore, the analysis of microbial sulfur cycling in oxic-anoxic interfaces is 52 attracting increasing attention.

53 Meromictic lakes can provide a suitable environment to investigate the potential importance of sulfate-reducing bacteria (SRB) in anoxic water columns. In meromictic lakes, 54 55 the surface layer of the lake, called the mixolimnion circulates but does not intermix with the 56 deeper layer, called monimolimnion. Therefore, such lakes stay stratified throughout the year. 57 At the interface between the mixolimnion and the monimolimnion, a steep chemical gradient 58 called a chemocline is formed. A clear shift of the microbial community structures below and 59 above the chemocline has been shown in many meromictic environments [17, 38]. Lake 60 Harutori is located in a residential area of Kushiro, Hokkaido, Japan. This meromictic lake is 61 consisting of water layers based on salinity. The bottom layer of Lake Harutori is known to

contain a very high amount of sulfide. The lake was reported to contain up to 670 mg L^{-1} 62 sulfide at one point [44], so that the toxic effects on indigenous animals and plants were a 63 64 matter of concern. In addition, most of the wastewater from the adjoining residential area was 65 directly discharged into the lake; therefore, the accumulation of organic compounds was an environmental problem. An analysis of photosynthetic pigments revealed anaerobic 66 67 anoxygenic phototrophic bacteria (green sulfur bacteria) in the Lake Harutori chemocline [35]. 68 Aside from that, not much is known about the microbial community in the lake. 69 In this study, we aimed to identify and quantify the main sulfate-reducing bacteria 70 beneath the Lake Harutori chemocline. To find the key players in sulfur cycling in Lake 71 Harutori, we investigated the phylogenetic diversity of sulfate-reducing and sulfur-oxidizing 72 bacteria by sequence analysis of the gene encoding adenosine 5'-phosphosulfate reductase 73 (*aprA*). Furthermore, the vertical distribution of the predominant group in 74 Deltaproteobacteria was revealed by using catalyzed reporter deposition-fluorescence in situ 75 hybridization (CARD-FISH). The study shows that *Desulfosarcina-Desulfococcus* (DSS) 76 group is one of the major groups of sulfate-reducing bacteria in the monimolimnion of Lake 77 Harutori.

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Materials and methods

80 Study sites and sampling

Lake Harutori is a shallow meromictic lake located in Kushiro, Hokkaido, Japan (Fig. 1). The lake (surface area 0.36 km²) is a coastal lagoon in which the low salinity water is overlaying water of marine origin, resulting in a permanent stratification of the water body [2]. The lake is completely covered with ice in winter, usually from December to the end of March [2]. The center of the lake (N42°58'20.6", E144°24'6.6"), which has a maximum depth of 5.75 m, was 86 selected as the sampling point. Sampling took place in May 2012 after the ice had melted. 87 Water samples were collected using a horizontal Van Dorn water sampler (3L, Rigosha, 88 Tokyo, Japan) along the depths. Vertical profiles of temperature, specific conductivity and pH 89 were measured in situ, with a multiparameter sensor (YSI 600XLM; YSI Inc., Yellow Springs, 90 OH, USA). Photosynthetically active radiation (400–700 nm) was measured using a quantum 91 meter (Model QMSS-S; Apogee instruments Inc., Logan, UT, USA). The dissolved oxygen 92 profile was measured in situ with an optical dissolved oxygen meter (ProODO; YSI Inc., 93 Yellow Springs, OH, USA).

94

95 *Chemical measurements*

96 Sulfide concentrations were measured colorimetrically with the methylene blue formation 97 method [3]. To measure dissolved organic carbon (DOC), sulfate, and chloride, water samples 98 were filtered through cellulose acetate syringe filter units with a 0.2 μm pore size (DISMIC-99 13CP; Toyo Roshi Kaisha, Tokyo). Concentrations of DOC were measured with a total 100 organic carbon analyzer (TOC-V; Shimadzu, Kyoto, Japan). Sulfate and chloride 101 concentrations were determined using an ion chromatograph (ICS-1500, column: 102 IonPacAS12A, Dionex, Sunnyvale, CA, USA) with appropriate dilutions.

103

104 DNA extraction

105 The water samples (approximately 200 mL for each sample) were filtered through Sterivex-

106 GV 0.22 µm pore-sized filter cartridges (Millipore, Billerica, MA, USA) immediately after

- 107 the collection. The filter cartridges were frozen on dry ice and brought back into the
- 108 laboratory without thawing, and stored at -20°C until DNA extraction. The filters were
- 109 transferred into a 2 mL screw-capped tube with 0.5 g of sterile glass beads, 0.6 mL TE buffer

110 (10 mM Tris-HCl, 1 mM EDTA, pH 8), 30 µL 20% SDS and 0.6 mL phenol-chloroform-

111 isoamyl alcohol (25:24:1; v/v/v). The tube was vigorously shaken with a beads-beater

112 (FastPrep24; MP Biomedicals, Santa Ana, CA, USA) twice at 4.0 m s⁻¹ for 30 sec. DNA in

113 the water phase was purified using the CTAB method followed by isopropanol precipitation

114 [43]. Extracted DNA was stored at -20°C until use.

115

116 *aprA clone libraries and sequence analysis*

117 For amplification of *aprA* genes, the primers AprA-1-FW and AprA-5-RV [25] were used. 118 PCR conditions were as follows: an initial denaturation step at 94°C for 2 min, followed by 34 119 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 120 72°C for 45 sec, with a final extension step at 72°C for 10 min. PCR products were inserted 121 into the pCR2.1-TOPO vector (TOPO TA cloning kit; Invitrogen, Carlsbad, CA, USA) and 122 cloned into competent Escherichia coli TOP10 cells according to the manufacturer's 123 instructions. Clones that had inserts of the predicted size were screened by the direct PCR 124 amplification with vector primers (M13F and M13R), and the PCR products were purified 125 with isopropanol precipitation and used for sequencing reaction. The sequencing reactions 126 were done with the ABI BigDye chemistry and analyzed with an ABI 3130 Genetic Analyzer 127 (Applied Biosystems, Tokyo, Japan). The aprA sequences were translated and the deduced 128 amino acid sequences were aligned using ClustalW implemented in MEGA5.05 software [36]. 129 A pairwise distance matrix was calculated based on the Poisson model. Based on the distance 130 matrix, sequences were classified into operational taxonomic units (OTUs) using mothur 131 software [32], with 97% sequence identity as a threshold. The AprA tree was constructed 132 from deduced amino acid sequences using the neighbor-joining method with bootstrap 133 analysis with 1,000 replications.

134

135 DSS-specific 16S rRNA gene clone library

136 The primers DCC305 and DCC1165 [4] were used to amplify DSS-specific 16S rRNA genes. 137 The PCR conditions were as follows: an initial denaturation step at 95°C for 5 min, followed 138 by 30 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 1 min, and elongation at 139 72°C for 1 min, with a final extension step at 72°C for 7 min. The PCR products were cloned 140 and sequenced as described above. The sequences were aligned using ClustalW implemented 141 in MEGA5.05 software and classified using mothur software into OTUs based on 98% 142 sequence identity. Final phylogenetic analyses of the 16S rRNA genes were performed with 143 the ARB software package [23] using the ARB reference database SILVA SSU release 108. 144 All sequences were automatically aligned with the SINA web aligner (http://www.arb-145 silva.de) [28] and the alignments were subsequently optimized manually. The phylogenetic 146 tree was constructed by neighbor-joining and maximum-likelihood analysis with different sets 147 of filters. For construction of the reference tree, only nearly full-length sequences (>1300 bp) 148 were considered. Partial sequences obtained in this study were inserted into the tree by 149 parsimony criteria without allowing changes in the overall tree topology. 150 151 *Nucleotide sequence accession numbers*

152 Sequence data reported in this study are available in the DDBJ, GenBank and EMBL

- 153 databases under the following accession numbers: 16S rRNA genes (AB894629-AB894655),
- 154 and *aprA* genes (AB894656-AB894821).

155

156 Probe design

157 An oligonucleotide probe SEEP1d-468 was designed using the probe design tool in the ARB 158 software package [23]. The probe specifically targets the 16S rRNA sequences of SEEP SRB-159 1d group obtained in Lake Harutori and several environmental clones (Fig. 4). The probe was 160 tested for coverage (target group hits) and specificity (outgroup hits) in silico with the ARB 161 probe match tool [23]. Probe specificity was checked on 618,442 prokaryotic sequences of the 162 SILVA SSU Ref dataset Release 108 [29]. Specific CARD-FISH conditions were determined 163 by hybridizing the probe to the lake water sample at formamide concentrations of 40%, 50%, 60%, and 70%, as explained in the following section. A deltaproteobacterial strain PL12 [8] 164 165 was used as a control having a single mismatch.

166

167 Catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH)

168 Water samples were fixed with paraformaldehyde solution (final concentration 0.9% 169 [v/v]) at 4°C. Afterwards, 200 µL aliquots were filtered onto polycarbonate membrane filters 170 (Cyclopore Track Etched Membrane; pore size, 0.22 µm; 25-mm diameter; Whatman, NJ, 171 USA) within 40 hours and stored at -20°C until further processing. Inactivation of endogenous 172 peroxidases was done by incubating the filters in 0.01 M HCl with 0.3% H₂O₂ for 10 min at room temperature. Cells were permeabilized by incubating the filters in 15 μ g mL⁻¹ proteinase 173 K (dissolved in 0.1 M Tris-HCl, 0.05 M EDTA [pH 8.0]) for 3 min at 37°C [37]. CARD-174 175 FISH and subsequent staining with 4',6-diamidino-2-phenylindole (DAPI) followed a 176 previously published protocol [27] with the following modification: hybridization was 177 performed for 2 h, and tyramide signal amplification was performed for 15 min, both at 46°C. 178 The washing temperature was 48°C. For the probes SEEP1d-1420 and SEEP1d-468, 179 hybridization and washing temperatures were 35°C, and tyramide signal amplification was 180 performed for 30 min at room temperature. Oligonucleotide probes labeled with horseradish

181 peroxidase were purchased from Biomers (Ulm, Germany). Probe sequences, probe 182 concentrations and formamide concentrations required for specific hybridization are given in 183 Table S1. Hybridized samples were examined with an epifluorescence microscope 184 (Axioplan2; Zeiss, Germany). The given CARD-FISH counts are the means calculated from 185 40–70 randomly chosen microscopic fields, corresponding to 2,000 DAPI-stained cells.

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Results

188 Physico-chemical properties of Lake Harutori

189 In Lake Harutori, a steep chemocline was found between 3.5 and 4.0 m below the surface. 190 Physicochemical parameters are summarized in Figure 2. The thermocline almost coincided 191 with the chemocline. The temperature of the lake bottom was 9°C. Photosynthetically active 192 radiation decreased with depth in the mixolimnion and, in the chemocline, declined to 0% of 193 the surface intensity. Oxygen and sulfide formed opposite concentration gradients. The sulfide 194 concentration increased below the chemocline, reaching a concentration of 10.7 mM at the 195 bottom of the lake. The chloride concentration in the middle of the monimolimnion was about 196 half of the concentration of that in seawater, meaning that the water was brackish. The sulfate 197 concentration was much lower than that of seawater and the maximal concentration was 1.4-198 2.1 mM in the chemocline. The concentration of DOC increased below the chemocline.

199

200 *AprA diversity in around the chemocline*

201 Clone libraries for *aprA* were generated from water taken from three different depths (3.0, 3.5,

- and 4.0 m) and contained 62, 42, and 62 clones, respectively. Based on a 97% identity cut-off
- 203 of the deduced amino acid sequences, 51 distinct OTUs were identified (Table 1).
- 204 Representative sequences of each OTU were selected to be shown in the phylogenetic tree in

205 Figure 3. The phylogenetic analysis of the deduced AprA amino acid sequences revealed a 206 clear difference in the dominant sequences in each library. In the 3.0 m library, from water 207 taken from above the chemocline, OTU A2 was the most dominant OTU (Table 1). The 208 closest cultured species to OTU A2 was Desulfatitalea tepidiphila (94-97% sequence 209 identity) [9]. In the upper part of the chemocline at 3.5 m, OTU A1, A3, and A7 were the 210 dominant OTUs (Table 1). Sequences from OTU A3 were closely related to *Desulfocapsa* 211 *thiozymogenes* (98–100%), which is known to disproportionate inorganic sulfur compounds 212 [10]. Sequences of OTU A7 were related to Desulfobacterium vacuolatum (96–97%), which 213 can oxidize a wide variety of organic acids completely [20]. OTU A1 was also the most 214 abundant OTU in the lower part of the chemocline (4.0 m). The closest, but still distantly 215 related, cultured organism was *Desulfofaba gelida* (88–92%), which is a psychrophilic 216 incomplete oxidizer [16]. All the above-mentioned species and OTUs belong to the family 217 Desulfobacteraceae. A few sequences related to spore-forming sulfate reducers in the genus 218 Desulfotomaculum were found, however, they were all distantly related to cultured species 219 (Table 1). Sequences related to those of known sulfur oxidizers were found at a depth of 3.0-220 3.5 m, which is above and in the upper part of the chemocline. Of these sulfur-oxidizing 221 bacteria, OTU A11 was related to "Candidatus Pelagibacter ubique" (85% identity; 222 EAS84493; Fig. S3), which is one of the most abundant and common species in the pelagic 223 zone of the ocean [30]. Some OTUs were related to Sulfuricella denitrificans [18] (Fig. S3), 224 and OTU A5 was related to Thiobacillus denitrificans [12] (Fig. S3). OTUs A13 and A20 225 formed a distinct clade with other environmental clones; however, their function is uncertain 226 (Fig. 3a).

227

228 Abundance of SEEP SRB-1d in DSS-specific 16S rRNA gene clone library

229 The most abundant sequences in the *aprA* clone libraries were those related to the DSS group 230 (Fig. 3b; OTU A1). Therefore, a 16S rRNA gene clone library using a DSS-specific primer set 231 was constructed from a water sample taken at 4.5 m. This clone library contained a total of 27 232 sequences of ca. 820 bp in size. The sequences are grouped into 10 different OTUs based on 233 98% identity (Fig. 4). All but one of the sequences (OTU7) are in the DSS group. Almost half 234 of the sequences (13 out of 27 sequences) were grouped into OTU1, which is distantly related 235 to Desulfofaba fastidiosa (94.2-94.7% identity). An environmental clone obtained in the 236 Guaymas Basin has high sequence identity (98.5–99.6%) with the sequences in OTU1. OTU1 237 sequences were grouped into the SEEP SRB-1d cluster in the phylogenetic tree shown in 238 Figure 4, and had no mismatch with the probe DSS658 (Fig. S1) used for the CARD-FISH 239 experiments.

240

241 Design and evaluation of new probe SEEP1d-468

242 An oligonucleotide probe SEEP1d-468 was designed to specifically detect OTU1 in SEEP 243 SRB-1d group, which comprised nearly half of the sequences obtained from the above 244 mentioned DSS group-specific 16S rRNA gene clone library (Fig. 4). The new probe 245 SEEP1d-468 has coverage of nearly half of the SEEP SRB-1d group and two outgroup hits in OTU4 (Fig. 4). When the probe tested with 20% formamide concentration with hybridization 246 247 temperature at 46°C, only spotty signals were observed. Bright signals were observed at 40% 248 formamide concentration with the hybridization and washing temperature at 35°C. No signal 249 was observed from the reference strain PL12, which has a single mismatch to probe SEEP1d-250 468, under the same hybridization condition. A previously published probe SEEP1d-1420 251 [33] showed bright signals in the lake water sample with the same hybridization condition as 252 the probe SEEP1d-468. Since the sequence information about the probe-binding site of

- 253 SEEP1d-1420 was not available from the DSS specific clone library, the newly designed
- 254 probe SEEP1d-468 was mainly used for the further analyses.
- 255

256 Abundance of DSS bacteria in the monimolimnion

Total cell numbers (DAPI) ranged from 1.3×10^7 to 1.5×10^7 cells mL⁻¹ in the mixolimnion, 257 258 and decreased somewhat in a depth of 3.0 to 3.5 m (Fig. 5). Total cell numbers increased below 3.5 m, reaching up to 3.5×10^7 cells mL⁻¹ in the bottom part of the water column (4.8 259 m depth). Total bacterial cell numbers (probe EUB I-III) also increased below the chemocline, 260 reaching up to 1.9×10^7 cells mL⁻¹ at 4.8 m. Deltaproteobacteria comprised 7.2–22.1% of 261 total bacteria below 3.0 m (Fig. 5). Among deltaproteobacteria, cells from the DSS group 262 263 were predominant (probe DSS658). At 4.5 m, in the middle of the monimolimnion, the ratio of DSS was 67% of the deltaproteobacteria (Fig. 6). The cell numbers of SEEP SRB-1d group 264 (probe SEEP1d-468) ranged from $2.3 \times 10^5 - 6.3 \times 10^5$ cells mL⁻¹ below the chemocline, and 265 266 comprised 43.5–79.0% of DSS group (Fig. 6). Another probe SEEP1d-1420 also showed the similar cell numbers, which ranged from 2.6 \times 10⁵ – 5.7 \times 10⁵ cells mL⁻¹ below the 267 chemocline. The typical morphology of the hybridized cells with probes DSS658 and 268 269 SEEP1d-468 were coccoid (2–3 µm), as shown in Figure S1. Cells were dispersed or formed 270 small aggregates.

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Discussion

273 Unique characteristics of Lake Harutori

The monimolimnion of Lake Harutori contained a very high concentration of sulfide—up to 10.7 mM. Below the chemocline, the seawater seems to be diluted because the chloride concentration is nearly half of the seawater. If the water were diluted homogeneously, the sulfate concentration should remain at half of that in seawater, around 14 mM. However, only
0.4–2 mM of sulfate was detected *in situ* (Fig. 2). The sum of the sulfate and sulfide
concentrations comes close to 13 mM, which possibly means that most of the sulfate in the
monimolimnion had been converted into sulfide. Active sulfate reduction by bacteria would
explain the presence of such a high sulfide concentration in the monimolimnion.

282

283 Diversity of sulfate-reducing and sulfur-oxidizing bacteria in Lake Harutori

284 Analysis of the *aprA* clone libraries revealed a high diversity of sulfate-reducing and sulfur-285 oxidizing bacteria. The dominant groups of those bacteria differ around the three different 286 depths of the chemocline. It appears that the availability of electron acceptors and salinity 287 affected the composition of the bacterial community in the lake. Sulfur-oxidizing bacteria 288 were found only where oxygen was available. Among those, some sequences obtained from 289 3.0 m were related to Sulfuricella denitrificans [18] and Thiobacillus denitrificans [12], which 290 have been isolated from freshwater. Both of them are facultatively anaerobic 291 chemolithoautotrophs. It has been shown that not all of sulfur oxidizers have *aprA* gene [24]; 292 therefore we cannot exclude other sulfur oxidizers, such as Epsilonproteobacteria, are also 293 present. 294 In the deeper layer of the chemocline, the sulfate-reducing bacterial community is 295 dominated by a single OTU, OTU A1 (Table 1). OTU A1 is in the family Desulfobacteraceae

and is distantly related to species in the DSS group.

297

298 Abundance of the DSS group in sulfate-reducing bacteria

299 In the high diversity of sulfate-reducing and sulfur-oxidizing bacteria observed in the *aprA*

300 clone library, the most dominant sulfate-reducing bacterial OTU was distantly related to

species in the DSS group (OTU A1, Table 1). Nearly half of the sequences obtained from the
 DSS-specific 16S rRNA gene clone library were related to an uncultured group of

303 Deltaproteobacteria (OTU1, Fig. 4), previously called as SEEP SRB-1d [33]. Both OTUs 1

and A1 are members of the *Desulfobacteraceae*, and the relative abundance of the sequences
was very high in the libraries from the anoxic monimolimnion. The OTUs might correspond
to each other, but require additional evidence to demonstrate genomic linkage.

307 Members of the DSS group are known as nutritionally versatile SRB [42]. Most of them 308 oxidize organic compounds completely to CO₂, and several species can grow autotrophically 309 with H₂ and sulfate. The DSS group of bacteria is abundant in the upper layer of sediments in 310 many aquatic environments, such as permanently cold marine sediment [31], sediments above 311 gas hydrate [15], temperate tidal flat sediment [26], and a meromictic Lake Cadagno sediment 312 [34]. However, in anoxic water columns, only a few reports about the abundance of the DSS 313 group are available. For example in a permanently anoxic water column in the Black Sea, 314 SRB detected with the SRB385 probe are abundant below 100 m, where sulfide appears, and 315 comprise up to 2–8% of total DAPI counts [22]. The abundance of DSS almost coincides with 316 the cell counts obtained with the SRB385 probe [22, 40]; therefore, DSS is the most dominant 317 group of SRB in the anoxic layer of the Black Sea water column. Some sequences from the 318 DSS group are found in an anoxic brine pool of the Gulf of Mexico [11], below the 319 chemocline of the stratified lagoon in Clipperton atoll [6], and below the chemocline of the 320 meromictic Lake Suigetsu [19]. In contrast, very few sequences from the DSS group have 321 been detected in seasonal anoxic water masses such as those in Saanich Inlet [41], and the 322 Arabian Sea [5], which indicates that the DSS group might prefer permanently anoxic 323 environments in which reduced sulfur compounds can accumulate.

324 Many SEEP SRB-1d cells were observed in the anoxic layer of the water column (Fig. 6). 325 It has been suggested that SEEP SRB-1 subgroups interact with ANME-2 [15, 33] or use 326 hydrocarbons including short-chain alkanes [13,14]. However, in Lake Harutori, we could not 327 detect ANME-2 cells by CARD-FISH with the specific probe ANME-2 538 [39] (data not 328 shown). In addition, the cells detected with the probe SEEP1d-468 and DSS658 were often 329 dispersed as single cells or formed chain-like small aggregates by themselves. Hence, the 330 specific association of the SEEP SRB-1d with ANME-2 cells is unlikely in this lake. There is 331 a possibility that SEEP SRB-1 have a preference for the availability of hydrocarbons. We 332 detected methane (approximately 1.5 mM) in the monimolimnion of Lake Harutori (data not 333 shown), but no data is available for other hydrocarbons. Further study will be required to 334 confirm this hypothesis.

The DSS group in the chemocline of Lake Harutori can probably use various organic compounds including persistent substances and might prefer a permanently anoxic environment, and are therefore able to be one of the major SRB in the bottom of Lake Harutori. Further cultivation and incubation studies will be required to estimate the contribution of these SRB to sulfide production and carbon remineralization in anoxic water columns, which may expand in the near future.

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Acknowledgments

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497

Figure captions

- 498 Fig. 1 Map of Lake Harutori in Kushiro, Hokkaido, Japan.
- Fig. 2 Depth profiles of temperature, pH, specific conductivity, concentrations of chloride,
 dissolved oxygen, sulfate, sulfide, and dissolved organic carbon in the water
 column of Lake Harutori in May 2012.
- 502 Fig. 3 Phylogenetic tree based on deduced AprA amino acid sequences retrieved from 503 Lake Harutori water column. The tree was constructed based on a distance matrix 504 (119 amino acid positions) by using the neighbor-joining method. Bootstrap value 505 estimation was based on 1000 replicates (only values above 50% are shown). 506 Numbers of sequences obtained in each clone library were indicated in parentheses; 507 3.0 m, 3.5 m, and 4.0 m in that order. a) Overview tree. b) Magnified tree of 508 Desulfobacteraceae and Desulfobulbaceae. The bar represents 5% estimated 509 sequence divergence.
- Fig. 4 Phylogenetic tree showing the affiliation of Lake Harutori 16S rRNA sequences to
 selected reference sequences of *Desulfobacteraceae* of *Deltaproteobacteria*.
 Representative sequences from each OTU are in boldface type. The name of the
 representative clone and the number of the grouped sequences are in parentheses.
 Probe specificity is indicated by the vertical lines. The bar represents 10%
 estimated sequence divergence.
- 516 Fig. 5 Abundance of microbial cells in Lake Harutori water column determined by
 517 CARD-FISH. DAPI (closed circle), EUBI-III (closed triangle), Delta495a (closed
 518 square), and DSS658 (open circle).

Fig. 6 Abundance of *Deltaproteobacteria* (probe Delta495a), DSS group (probe DSS658)
and SEEP SRB-1d cells (probes SEEP1d-1420 and SEEP1d-468) in the anoxic
layer of Lake Harutori, determined by CARD-FISH.



Fig. 1 Kubo et al.





Fig. 3a Kubo et al.





10%

Fig. 4 Kubo et al.



Fig. 5 Kubo et al.



Fig. 6 Kubo et al.

Table 1. Phylogenetic affiliation of classified OTUs of aprA clone sequences and the number of clones in each clone libraries.

Putation Physiquenci attuintion 0100 Colesed (June 4 start) Berland (M) 30 m 30 m 40 m No 0.100.4 Conscionting (M) Reading (M) 86.407 10 8.6 0 No Conscionting (M) Conscionting (M) Conscionting (M) 0.00 8.6 0 0 8.6 0 0 8.6 0 0 8.6 0			ΟΤυ	Closest cultured strain ^a	Identity (%)	Number of clones		
Suifate malution 07UA1 Derus/database plot/97025 (JAM/1556)) 69.67 0 8 41 07UA2 Derus/database plot/9105 (S2012) (JAM/1556)) 69.67 0 0 8 07UA2 Derus/database plot/9105 (S2012) (JAM/1556)) 69.67 0 7 0 07UA7 Derus/database plot/9105 (S2012) (JAM/1556)) 69.69 0 1 4 0 0 8 07UA7 Derus/database plot/9105 (JAL/7305) 69.69 1 4 0 0 3 07UA8 Derus/database plot/9104 (JAL/7305) 69.0 1 1 0 0 3 07UA25 Derus/database plot/9104 (JAL/7305) 69.0 1 0 0 1 0 07UA25 Derus/database plot/9104 (JAL/7305) 69.0 1 0 0 1 0 07UA25 Derus/database plot/9104 (JAL/7305) 69.0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 <td>Putative function</td> <td>Phylogenetic affiliation</td> <td>3.0 m</td> <td>3.5 m</td> <td>4.0 m</td>	Putative function	Phylogenetic affiliation				3.0 m	3.5 m	4.0 m
Suffair module OTU A2 Parameters (C) (A) (A) (A) (A) (A) (A) (A) (A) (A) (A			OTU A1	Desulfofaba gelida PSv29 (AAL57385)	88-92	0	8	41
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Suffate meducino Orbot Description Orbot Description Orbot Description Description <thdescription< th=""> Description Descrip</thdescription<>				Desulfofaba gelida PSv29 (AAL57385)	04.06	1	4	0
Suffate reduction 010 A14 02800000000000000000000000000000000000				Desulfabastarium indelieum InO4 (AAL 57200)	94-90	0	4	0
Suffar Induction 0101/10 02800000000000000000000000000000000000			OTUA14	Desuffolia eterium indolicum InO4 (AAL57390)	90	0	0	3
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Desultation of unable is exploited as the point S280 F (AAM 1567) 97 0 1 0 Suffate reduction 011/422 Desultationations 04 (ALS 7390) 94 00 1 0 Suffate reduction 011/432 Desultationations 04 (ALS 7390) 94 00 01 1 011/432 Desultationations 050(1 (GAM 15568) 92 00 01 1 011/433 Desultationate tepidphils 3520(1 (GAM 15568) 92 00 01 01 011/443 Desultationate tepidphils 5520(1 (GAM 15568) 92 01 01 01 011/443 Desultationate tepidphils 5520(1 (GAM 1558) 93 1 0 0 011/443 Desultationate tepidphils 520(1 (GAM 1558) 94 1 0 0 011/450 Desultationate tepidphils 520(1 (GAM 1558) 91 1 0 0 011/450 Desultationase tepidphils 520(1 (GAM 1558) 91 1 0 0 011/4			OTU A26	Desulfofaba gelida PSv29 (AAL57385)	92	0	1	0
Sulfate reduction OTU A28 Desufabeterium indolum inol (ALS7390) 94 0 1 0 Sulfate reduction OTU A32 Desufabeterium indolum inol (ALS7390) 93 0 0 1 OTU A33 Desufabitable spidphilis S280.1 (BAM15568) 92 0 0 1 OTU A30 Desufabitable spidphilis S280.1 (BAM15568) 92 0 0 1 OTU A83 Desufabitable spidphilis S280.1 (BAM15568) 92 0 0 1 OTU A80 Desufabitable spidphilis S280.1 (BAM15568) 92 1 0 0 OTU A80 Desufabitable spidphilis S280.1 (BAM15568) 92 1 0 0 OTU A80 Desufabitable spidphilis S280.2 (BAM15569) 94 1 0 0 OTU A10 Desufabitable spidphilis S280.2 (BAM15569) 94 1 0 0 Desufabitable spidphilis S280.2 (BAM15569) 94 1 0 0 0 Desufabitable spidphilis S280.2 (BAB5249) 96 2 0 0 0 0 <td></td> <td>Desulfobacteraceae</td> <td>OTU A27</td> <td>Desulfatitalea tepidiphila S28bF (BAM15567)</td> <td>97</td> <td>0</td> <td>1</td> <td>0</td>		Desulfobacteraceae	OTU A27	Desulfatitalea tepidiphila S28bF (BAM15567)	97	0	1	0
Sultate reduction 0-11 0-11 0-11 Sultate reduction 0-11 0-11 0-11 Sultate reduction 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11 0-11			OTU A28	Desulfobacterium indolicum InO4 (AAL57390)	94	0	1	0
Suitate reduction OTU A30 Desuffabilities tepiciphile S280.1 (BAM15666) 03 00 01 Suitate reduction OTU A30 Desuffabilities tepiciphile S280.1 (BAM15666) 02 00 00 1 OTU A30 Desuffabilities tepiciphile S280.1 (BAM15666) 02 00 00 1 OTU A30 Desuffabilities tepiciphile S280.1 (BAM15666) 92 00 00 0 OTU A40 Desuffabilities tepiciphile S280.1 (BAM15666) 92 0 0 0 OTU A40 Desuffabilities tepiciphile S280.2 (BAM15669) 91 1 0 0 OTU A50 Desuffabilities tepiciphile S280.2 (BAM15690) 91 1 0 0 OTU A50 Desuffabilities tepiciphile S280.2 (BAM15690) 96 2 0 0 Syntrophacese OTU A5 Desuffabilities tepiciphile S280.2 (BAM15690) 96 2 0 0 Syntrophacese OTU A50 Desuffabilities tepiciphile S280.2 (BAM15690) 96 2 0 0 Syntrophacese OTU A50 Desuffa			OTU A32	Desulfobacterium indolicum InO4 (AAL57390)	94	0	0	1
Sulfate reduction OTU A35 Desulfatibales tepci/phile S280C1 (BAM15568) 92 00 00 1 Sulfate reduction OTU A35 Desulfatibales tepci/phile S280C1 (BAM15568) 92 00 00 1 Sulfate reduction OTU A35 Desulfatibales tepci/phile S280C1 (BAM15568) 92 00 0 1 OTU A35 Desulfatibales tepci/phile S280C1 (BAM15568) 93 11 00 00 OTU A36 Desulfatibales tepci/phile S280C1 (BAM15568) 92 11 0 00 OTU A35 Desulfatibales tepci/phile S280C1 (BAM15569) 91 1 0 0 OTU A35 Desulfatibales tepci/phile S280C1 (BAM15569) 91 1 0 0 Desulfatibales tepci/phile S280C1 (BAM15569) 96 0 0 1 0 Sulfationpalus valiances OTU A15 Desulfatibales tepci/phile S2802 (BAM157459) 96 3 0 0 Sulfationpalue Valiances OTU A16 Desulfatibales tepci/phile S2801 (IALS74519) 86 1 0 0			OTU A33	Desulfobacterium indolicum InO4 (AAL57390)	93	0	0	1
Sultate reduction OTU A50 Deculfatibile repoliphile S280(1 (RAM15568) 92 00 00 1 OTU A50 Deculfatibile repoliphile S280(1 (RAM15568) 92 00 00 1 OTU A50 Deculfatibile gelide PS/20 (AL5738) 94 11 00 00 OTU A40 Deculfatibile gelide PS/20 (AL5738) 94 11 00 00 OTU A50 Deculfatibile repoliphile S280(2 (RAM15568) 91 1 1 00 00 OTU A50 Deculfatibile repoliphile S280(2 (RAM15568) 91 1 1 00 00 01 OTU A50 Deculfatibile repoliphile S280(2 (RAM15568) 91 0 0 0 01 01 Deculfatibile repoliphile S280(2 (RAM15748)) 98 00 0 </td <td></td> <td></td> <td>OTU A34</td> <td>Desulfatitalea tepidiphila S28OL1 (BAM15568)</td> <td>92</td> <td>0</td> <td>0</td> <td>1</td>			OTU A34	Desulfatitalea tepidiphila S28OL1 (BAM15568)	92	0	0	1
Sum Notes OTU A50 Desulfatione periodiphile S2801. (RAN15668) 92 0 0 1 OTU A47 Desulfatione periodiphile S2801. (RAN15668) 93 11 0 0 OTU A48 Desulfatione mangrum Mantpellier (AL57389) 94 1 0 0 OTU A50 Desulfationer magrum Mantpellier (AL57389) 92 1 0 0 OTU A50 Desulfationer magrum Mantpellier (AL57389) 94 1 0 0 Desulfationer magrum Mantpellier (AL57389) 94 1 0 0 Desulfationer magrum Mantpellier (AL57389) 94 0 0 1 Desulfationer magrum Mantpellier (AL57389) 94 0 0 1 Desulfationer magrum Mantpellier (AL5748) 96 2 0 0 1 Desulfationer magrum Mantpellier (AL5749) 86 3 0 0 0 Suntrophacese OTU A10 Desulfationaculum karetsovii 17 (AL57419) 83 1 0 0 OTU A17 Desulfationaculum karetsovii 17 (AL57419) 83 1 0 0 <td>Sulfate reduction</td> <td></td> <td>OTU A35</td> <td>Desulfatitalea tepidiphila S28OL1 (BAM15568)</td> <td>92</td> <td>0</td> <td>0</td> <td>1</td>	Sulfate reduction		OTU A35	Desulfatitalea tepidiphila S28OL1 (BAM15568)	92	0	0	1
Suffire order OTUAR Desulfabas gelida PSv29 (AAL57365) 93 1 0 0 OTUAR Desulfabas gelida PSv29 (AAL5735) 94 1 0 0 OTUAR Desulfabas gelida PSv29 (AAL5735) 94 1 0 0 OTUAS Desulfabas gelida PSv29 (AAL5735) 94 1 0 0 OTUAS Desulfabas gelida PSv29 (AAL5735) 94 1 0 0 OTUAS Desulfabas gelida PSv29 (AAL5735) 94 1 0 0 OTUAS Desulfabas gelida PSv29 (AAL5735) 94 0 8 1 OTUAS Desulfabas gelida PSv29 (AAL5735) 94 0 8 1 Sufforphaceee OTUAS Desulfabas gelida PSv21 (AL57435) 86 3 0 0 OTUAS Desulfabas gelida PSv21 (AL57435) 86 3 0 0 0 OTUAS Desulfabas gelida PSv21 (TAL5745) 86 3 0 0 0 OTUAS Desulfabaterium animin Anit (OTU A36	Desulfatitalea tepidiphila S28OL1 (BAM15568)	92	0	0	1
Sufficiency OTU A48 Desulfonema magnum Montpellier (ALS7389) 94 1 0 0 OTU A49 Desulfonema magnum Montpellier (ALS7389) 92 1 00 0 OTU A50 Desulfonema magnum Montpellier (ALS7389) 92 1 0 0 OTU A51 Desulfonepain supmogenes Braz (ALS7437) 94 1 0 0 Desulfonbulbaceae OTU A3 Desulfonepain supmogenes Braz (ALS7437) 96 0 0 1 Syntrophaceae OTU A1 Desulfonbacits math (ALS7425) 96 83 0 0 OTU A1 Desulfonbacits mathini Ant (ALS7419) 86 86 0 0 0 OTU A1 Desulfonaccium mathini Ant (ALS7419) 83 1 0 0 0 0 OTU A2 Desulfonaccium matini Ant (ALS7419) 83 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 </td <td></td> <td></td> <td>OTU A47</td> <td>Desulfofaba gelida PSv29 (AAL57385)</td> <td>93</td> <td>1</td> <td>0</td> <td>0</td>			OTU A47	Desulfofaba gelida PSv29 (AAL57385)	93	1	0	0
Sufficiency OTU A49 Desulfonema magnum Monipellier (AAL57389) 92 1 0 0 OTU A45 Desulfobacterium autorophicum HRW2 (AAL57339) 94 1 0 0 Desulfobal baceaee OTU A31 Desulfobacterium autorophicum HRW2 (AAL5733) 98-100 0 88 11 OTU A31 Desulfobacterium autorophicum SE2 (AAL57433) 98-100 0 88 11 Syntrophaceae OTU A31 Desulfobaccae acetoxidans ASRB2 (AR92549) 96 2 0 0 Syntrophaceae OTU A16 Desulfobaccium autimi A11 (AAL57419) 86 3 0 0 OTU A12 Desulfobaccium autimi A11 (AAL57419) 86 3 1 0 0 OTU A13 Desulfobaccium autimi A11 (AAL57419) 83 1 0 0 0 0 OTU A12 Desulfobaccium autimi A11 (AAL57419) 83 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0			OTU A48	Desulfonema magnum Montpellier (AAL57389)	94	1	0	0
Suffix output OTU A50 OTU A51 OTU A51 Desulfobacterium autotrophicum HRM2 (AAL57375) 94 1 0 0 Desulfobulbaceee OTU A51 Desulfobages thiozymogenes Bra2 (AAL57433) 98-100 0 8 1 Desulfobulbaceeee OTU A31 Desulfobages thiozymogenes Bra2 (AL57433) 98-100 0 8 1 Syntrophaceee OTU A10 Desulfobace:a acetoxidans ASR62 (ABR02549) 96 2 0 0 OTU A10 Desulfobace:a acetoxidans ASR62 (ABR02549) 96 2 0 0 0 OTU A1 Desulfobace:a acetoxidans ASR62 (ABR02549) 96 2 0			OTU A49	Desulfonema magnum Montpellier (AAL57389)	92	1	0	0
Suffer output OTU A51 Desulfatiales tepidiphile S280L2 (BAM15569) 91 1 0 0 Desulfobulbacee OTU A31 Desulfobulpagenes Bra2 (ALS7433) 96-100 0 8 1 Syntrophaceae OTU A31 Desulfobulbacea acetoxidans ASRB2 (AR825433) 96 2 0 0 Syntrophaceae OTU A41 Desulfobacterium anilini An1 (AL57425) 86-88 7 2 0 OTU A41 Desulfobacterium anilini An1 (AL57425) 86-83 1 0 0 OTU A41 Desulfobacterium anilini An1 (AL57425) 86 3 1 0 0 OTU A41 Desulfobacterium anilini An1 (AL57425) 83 1 0 0 OTU A12 Desulfobaracium kuzretsovi 17 (AL57419) 79 1 0 0 OTU A31 Desulfobaracium kuzretsovi 17 (AL57419) 79 1 0 0 OTU A31 Desulfobaracium kuzretsovi 17 (AL57419) 79 1 0 0 OTU A35 Desulfobaracium kuzretsovi 17 (AL57419) 80			OTU A50	Desulfobacterium autotrophicum HRM2 (AAL57375)	94	1	0	0
Desulfobulaceae OTU A3 OTU A31 Desulfocapsa thiozymogenes Bra2 (AL57433) 98-100 0 8 1 Syntrophaceae OTU A31 Desulfobropalics vacuoletus lik 10 (ABR92543) 99 0 0 1 Syntrophaceae OTU A16 Desulfobacea aectoxidans ASRB2 (ABR92549) 96 2 0 0 Gram positive SRB and related OTU A17 Desulfobaceau mextosvii 17 (AL57419) 86 3 0 0 OTU A17 Desulfobaceau mextosvii 17 (AL57419) 83 1 0 0 0 OTU A17 Desulfobaceau mextosvii 17 (AL57419) 83 1 0 0 0 OTU A17 Desulfotomaculum kuznetsovii 17 (AL57419) 83 1 0			OTU A51	Desulfatitalea tepidiphila S28OL2 (BAM15569)	91	1	0	0
Desilfboliaccee OTUA31 Desultationality success 00 0 0 0 1 Syntrophaceae OTUA31 Desultabace accoundars ASRB2 (ABR92549) 96 2 0 0 Syntrophaceae OTUA41 Desultabace accoundars ASRB2 (ABR92549) 96 2 0 0 Gram positive SRB and related Deltaproteobacteria OTUA42 Desultatomaculum kuznetsovii 17 (AL57419) 86 3 0 0 OTUA42 Desultatomaculum kuznetsovii 17 (AL57419) 83 1 0 0 OTUA42 Desultatomaculum kuznetsovii 17 (AL57419) 79 1 0 0 OTUA43 Desultatomaculum tuznetsovii 17 (AL57419) 79 1 0 0 OTUA32 Desultatomaculum kuznetsovii 17 (AL57419) 80 0 1 0 OTUA34 Desultatomaculum kuznetsovii 17 (AL57419) 80 0 1 0 OTUA34 Desultatomaculum kuznetsovii 17 (AL57419) 80 0 1 0 OTUA35 Desultatomaculum kuznetsovii 17 (AL57419)			OTU A3	Desulfocapsa thiozymogenes Bra2 (AAI 57433)	98-100	0	8	1
Synthophaceae OTUA16 Desultabacea acetoxidans ASR2 (ARR92549) 96 2 0 0 1 Gram positive SRB and related Deltaproteobacteria OTUA16 Desultabcacea acetoxidans ASR2 (AR92549) 96 2 0 0 Gram positive SRB and related Deltaproteobacteria OTUA12 Desultatomaculum kuznetsovii 17 (AAL57419) 86 3 0 0 OTUA32 Desultatomaculum kuznetsovii 17 (AAL57419) 83 1 0 0 OTUA32 Desultatomaculum kuznetsovii 17 (AAL57419) 83 1 0 0 OTUA32 Desultatomaculum kuznetsovii 17 (AAL57419) 80 0 1 0 OTUA32 Desultatomaculum kuznetsovii 17 (AAL57419) 80 0 1 0 OTUA32 Desultatomaculum kuznetsovii 17 (AAL57419) 80 0 1 0 OTUA32 Desultatomaculum kuznetsovii 17 (AAL57419) 80 0 1 0 OTUA32 Desultatomaculum kuznetsovii 17 (AAL57419) 80 0 1 0 OTUA33 Desultatomaculum ku		Desulfobulbaceae	OTU 431	Desulforhonalus vacuolatus, Itk 10 (ABR92535)	99	0	0	1
Sufficiency OTU A4 Desultobacterium aniini Anit (AAL57425) B6 B7 Q O Gram positive SRB and related Deltaproteobacteria OTU A1 Desultobacterium aniini Anit (AAL57425) B6 3 0 0 OTU A1 Deltaproteobacteria Desultobacterium aniini Anit (AAL57425) B6 3 0 0 OTU A1 Deltaproteobacteria Desultobacterium aniini Anit (AAL57425) B3 1 0 0 OTU A2 Desultobacterium aniini Anit (AAL57439) B3 1 0 0 OTU A2 Desultobacterium aniini Anit (AAL57439) R3 0 1 0 OTU A3 Desultobacterium aniini Anit (AAL57425) B8 0 1 0 OTU A3 Desultobacterium aniini Anit (AAL57439) 80 0 1 0 OTU A3 Desultobacterium aniini Anit (AAL57439) 80 0 1 0 OTU A3 Desultobacterium aniini Anit (AAL57439) 79 4 0 0 OTU A3 Desultobacterium aniini Anit (AAU574519) 80 0 1 0		Syntrophaceae	OTU A16	Desulfohacca acetoxidans ASRB2 (ABR02540)	96	2	0	0
Suffur oxidation Cito Val Desufformaculum Xum PAIN (Pectando) 00000 1 2 0 Gram positive SRB and related Deltaproteobacteria OTU A12 Desufformaculum kuznetsovii 17 (AAL57419) 83 1 0 0 OTU A12 Desufformaculum kuznetsovii 17 (AAL57419) 83 1 0 0 OTU A12 Desufformaculum kuznetsovii 17 (AAL57419) 79 1 0 0 Detaproteobacteria OTU A13 Desufformaculum kuznetsovii 17 (AAL57419) 79 1 0 0 OTU A23 Desufformaculum kuznetsovii 17 (AAL57419) 79 1 0 0 1 OTU A32 Desufformaculum kuznetsovii 17 (AAL57419) 80 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 0 0 1		Gram positive SRB and related Deltaproteobacteria		Desulfobacterium anilini Ani1 (AAI 57425)	96.99	7	2	0
Sulfur oxidation COTU A12 Destinocontine dualina Ruzinescini (17 (AAL57419)) 0.0 0 0 0 0 OTU A17 Destinoconclum Ruzinescini (17 (AAL57419)) 83 1 0 0 0 OTU A19 Destinoconclum Ruzinescini (17 (AAL57419)) 83 1 0 0 OTU A19 Destinoconclum Ruzinescini (17 (AAL57419)) 79 1 0 0 OTU A29 Destinoconclum Ruzinescini (17 (AAL57419)) 79 1 0 0 OTU A37 Destinoconclum Ruzinescini (17 (AAL57419)) 88 0 0 1 OTU A38 Destinoconclum Ruzinescini (17 (AAL57419) 80 0 0 1 OTU A38 Destinoconclum Ruzinescini (17 (AAL57419) 80 0 0 1 OTU A31 Destinoconclum Ruzinescini (17 (AAL57419) 80 0 0 1 OTU A31 Destinoconclum Ruzinescini (17 (AAL57419) 80 0 0 1 OTU A11 Thiocapsa rosee 6611 (ABV80104) 92 1 0 0 </td <td></td> <td></td> <td>Desulfotomaculum kuznetsovii. 17 (AAI 57410)</td> <td>86</td> <td>3</td> <td>2</td> <td>0</td>				Desulfotomaculum kuznetsovii. 17 (AAI 57410)	86	3	2	0
Gram positive SRB and related Deltaproteobacteria OTU A18 Desulfotomaculum kuznetsovii 17 (AAL57419) 63 1 0 0 OTU A19 Desulfotomaculum kuznetsovii 17 (AAL57419) 79 1 0 0 OTU A20 Desulfotomaculum kuznetsovii 17 (AAL57419) 79 1 0 0 OTU A30 Desulfotomaculum kuznetsovii 17 (AAL57419) 80 0 1 0 OTU A31 Desulfotomaculum kuznetsovii 17 (AAL57419) 80 0 0 1 OTU A32 Desulfotomaculum kuznetsovii 17 (AAL57419) 80 0 0 1 OTU A32 Desulfotomaculum kuznetsovii 17 (AAL57419) 80 0 0 1 OTU A32 Desulfotomaculum kuznetsovii 17 (AAL57419) 80 0 0 1 OTU A32 Dispar osse 6611 (ABV80104) 92 1 0 0 OTU A32 Lamprocystis purpurea ThSch 12 (ABV80005) 89 0 1 0 Sulfur oxidation OTU A43 Sulfuricella denitrificans SkB26 (BAl66427) 97 1 0 </td <td></td> <td>OTUAIZ</td> <td>Desulfotomaculum kuznetoovii 17 (AAL57419)</td> <td>00</td> <td>3</td> <td>0</td> <td>0</td>			OTUAIZ	Desulfotomaculum kuznetoovii 17 (AAL57419)	00	3	0	0
Suffur oxidation OTU A18 Desuitotionaculum legunientium BSU (AAL57419) 63 1 0 0 OTU A19 Desuitotionaculum termoacetoxidans CAMZ (ABR92568) 78 0 1 0 0 OTU A29 Desuitotionaculum termoacetoxidans CAMZ (ABR92568) 78 0 1 0 0 OTU A37 Desuitotomaculum termoacetoxidans CAMZ (ABR92568) 78 0 1 0 0 OTU A38 Desuitotomaculum tuznetsovii 17 (AAL57419) 80 0 0 1 OTU A31 Desuitotomaculum tuznetsovii 17 (AAL57419) 80 0 0 1 OTU A31 Desuitotomaculum tuznetsovii 17 (AAL57419) 80 0 0 1 OTU A32 Desuitotomaculum tuznetsovii 17 (AAL57419) 80 0 0 1 0 0 OTU A32 Desuitotomaculum tuznetsovii 17 (AAL57419) 80 0 1 0 0 0 OTU A32 Desuitotomaculum tuznetsovii 17 (AAL57419) 90 1 0 0 0 0 0			OTUAT	Desulfatemanulum reatherminum BCD (AAL 57292)	00 00	1	0	0
Sulfur oxidation Lineage 2 ^b OTU A19 OTU A29 Desulfotomaculum kuznetsovii 17 (AAL57419) 79 1 0 0 Sulfur oxidation OTU A29 Desulfotomaculum thermoace(oxidans CAMZ (ABR92588) 78 0 1 0 0 OTU A37 Desulfotomaculum thermoace(oxidans CAMZ (ABR92588) 78 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 1 0 0 0 0 1 0			OTUAIS	Desulitionaculum geothermicum BSD (AAL57382)	03 70	1	0	0
Sulfur oxidation C1 U A29 Desulfobrace/lum manifini Ani (AAL57425) 88 0 1 0 OTU A37 Desulfobrace/lum manifini Ani (AAL57425) 88 0 0 1 OTU A38 Desulfobrace/lum kuznetsovii 17 (AAL57419) 80 0 0 1 V OTU A31 Thicocapsa rosea 6611 (ABV80104) 92 1 0 0 OTU A21 Thicocapsa rosea 6611 (ABV80104) 90 1 0 0 OTU A22 Thicocapsa rosea 6611 (ABV80104) 90 1 0 0 OTU A23 Lamprocystis pupurea Thickapsa rosea 6611 (ABV80104) 90 1 0 OTU A32 Lamprocystis pupurea Thickapsa rosea 6611 (ABV80005) 89 0 1 0 OTU A3 Lamprocystis pupurea Thickapsa rosea 6611 (ABV80005) 89 0 1 0 Sulfur oxidation OTU A3 Sulfuricella denitrificans skB26 (BAI66427) 97-98 4 0 0 Lineage 2 ^b OTU A43 Sulfuricella denitrificans skB26 (BAI664			010 419	Desulfotomaculum kuznetsovii 17 (AAL57419)	79	1	0	0
Sulfur oxidation OTU A37 OTU A38 Desulfobacterium nilin Anit (AAL57425) 88 0 0 1 Normal And Albert OTU A38 Desulfotomaculum kuznetsovii 17 (AAL57419) 80 0 0 1 Lineage 1° OTU A11 Thiocapsa rosea 6611 (ABV80104) 79 4 0 0 OTU A21 Thiocapsa rosea 6611 (ABV80104) 90 1 0 0 OTU A22 Thiocapsa rosea 6611 (ABV80104) 90 1 0 0 OTU A23 Lamprocystis purpurea ThSch 12 (ABV80005) 89 0 1 0 OTU A33 Sulfuricella denitrificans GP3 (ABV80033) 98-100 4 4 0 OTU A34 Sulfuricella denitrificans skB26 (BAI66427) 97-98 4 0 0 Sulfur oxidation OTU A40 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 OTU A41 Sulfuricella denitrificans skB26 (BAI66427) 92 1 0 0 OTU A42 Sulfuricella denitrificans skB26 (BAI66427) 83 <			OTU A29	Desulfotomaculum thermoacetoxidans CAMZ (ABR92588)	78	0	1	0
OTU A38 Desulfotomaculum kuznetsovii 17 (AAL57419) 80 0 0 1 Lineage 1 ^b OTU A11 Thiocapsa rosea 6611 (ABV80104) 79 4 0 0 OTU A21 Thiocapsa rosea 6611 (ABV80104) 92 1 0 0 OTU A22 Thiocapsa rosea 6611 (ABV80104) 90 1 0 0 OTU A22 Thiocapsa rosea 6611 (ABV80104) 90 1 0 0 OTU A22 Thiocapsa rosea 6611 (ABV80104) 90 1 0 0 OTU A22 Thiocapsa rosea 6611 (ABV80005) 89 0 1 0 OTU A23 Lamprocystis purpurea ThSch 12 (ABV80003) 98-100 4 4 0 OTU A3 Sulfuricella denitrificans SkB26 (BAI66427) 97-98 4 0 0 Sulfur oxidation Lineage 2 ^b OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 Uncertain Lineage 2 ^b OTU A44 Sulfuricella denitrificans skB26 (BAI66427) 83 1			OTU A37	Desulfobacterium anilini Ani1 (AAL57425)	88	0	0	1
Sulfur oxidation CTU A11 Thicocapsa rosea 6611 (ABV80104) 79 4 00 0 Sulfur oxidation Lineage 1 ^b OTU A22 Thicocapsa rosea 6611 (ABV80104) 90 1 00 0 Sulfur oxidation OTU A2 Thicocapsa rosea 6611 (ABV80104) 90 1 0 0 Sulfur oxidation OTU A2 Thicocapsa rosea 6611 (ABV80005) 89 0 1 0 Sulfur oxidation Thicocapsa rosea 6611 (ABV80005) 89 0 1 0 Sulfur oxidation Thicocapsa rosea 6611 (ABV80003) 98-100 4 4 0 OTU A5 Thiobacillus denitrificans skB26 (BAI66427) 97 1 0 0 OTU A41 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 OTU A42 Sulfuricella denitrificans skB26 (BAI66427) 91 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 83 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427)			OTU A38	Desulfotomaculum kuznetsovii 17 (AAL57419)	80	0	0	1
Lineage 1 ^b OTU A21 Thiocapsa rosea 6611 (ABV80104) 92 1 0 0 OTU A22 Thiocapsa rosea 6611 (ABV80104) 90 1 0 0 OTU A23 Lamprocystis purpurea ThSch 12 (ABV80005) 89 0 1 0 Sulfur oxidation VTU A23 Lamprocystis purpurea ThSch 12 (ABV80005) 89 0 1 0 Sulfur oxidation VTU A23 Thiobacillus denitrificans GP3 (ABV80033) 98-100 4 4 0 OTU A10 Sulfuricella denitrificans SkB26 (BAI66427) 97-98 4 0 0 OTU A39 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 OTU A41 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 OTU A42 Sulfuricella denitrificans skB26 (BAI66427) 91 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 83 1 0 0 OTU A44 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 </td <td rowspan="6"></td> <td></td> <td>OTU A11</td> <td>Thiocapsa rosea 6611 (ABV80104)</td> <td>79</td> <td>4</td> <td>0</td> <td>0</td>			OTU A11	Thiocapsa rosea 6611 (ABV80104)	79	4	0	0
Sulfur oxidation OTU A22 Thiccapsa rosea 6611 (ABV80104) 90 1 00 0 Sulfur oxidation OTU A23 Lamprocystis purpurea ThSch 12 (ABV80005) 89 0 1 0 Sulfur oxidation OTU A23 Lamprocystis purpurea ThSch 12 (ABV80033) 98-100 4 4 0 OTU A10 Sulfuricella denitrificans GP3 (ABV80033) 98-100 4 4 0 0 OTU A23 Sufuricella denitrificans GP3 (ABV80033) 98-100 4 4 0 0 OTU A10 Sufuricella denitrificans skB26 (BAl66427) 97-98 4 0 0 0 OTU A40 Sufuricella denitrificans skB26 (BAl66427) 97 1 0 0 0 OTU A42 Sufuricella denitrificans skB26 (BAl66427) 91 1 0 0 0 OTU A43 Sufuricella denitrificans skB26 (BAl66427) 83 1 0 0 0 OTU A44 Sufuricella denitrificans skB26 (BAl66427) 87 1 0 0 0		Lineage 1 ^b	OTU A21	Thiocapsa rosea 6611 (ABV80104)	92	1	0	0
Number of the second		°	OTU A22	Thiocapsa rosea 6611 (ABV80104)	90	1	0	0
Sulfur oxidation OTU A5 Thiobacillus denitrificans GP3 (ABV80033) 98-100 4 4 0 Sulfur oxidation OTU A10 Sulfuricella denitrificans skB26 (BAI66427) 97-98 4 0 0 OTU A39 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 OTU A40 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 OTU A41 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 OTU A42 Sulfuricella denitrificans skB26 (BAI66427) 91 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 92 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 83 1 0 0 OTU A44 Sulfuricella denitrificans skB26 (BAI66427) 85 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0			OTU A23	Lamprocystis purpurea ThSch 12 (ABV80005)	89	0	1	0
Sulfur oxidation OTU A10 Sulfuricella denitrificans skB26 (BAI66427) 97-98 4 0 0 Sulfur oxidation OTU A39 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 DTU A40 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 OTU A41 Sulfuricella denitrificans skB26 (BAI66427) 91 1 0 0 OTU A42 Sulfuricella denitrificans skB26 (BAI66427) 92 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 92 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 83 1 0 0 OTU A44 Sulfuricella denitrificans skB26 (BAI66427) 85 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 Uncertain Unclassified ^e OTU A13 N.A. N.A. 3 </td <td></td> <td>OTU A5</td> <td>Thiobacillus denitrificans GP3 (ABV80033)</td> <td>98-100</td> <td>4</td> <td>4</td> <td>0</td>			OTU A5	Thiobacillus denitrificans GP3 (ABV80033)	98-100	4	4	0
Sulfur oxidation OTU A39 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 DTU A40 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 DTU A40 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 DTU A41 Sulfuricella denitrificans skB26 (BAI66427) 91 1 0 0 OTU A42 Sulfuricella denitrificans skB26 (BAI66427) 92 1 0 0 OTU A42 Sulfuricella denitrificans skB26 (BAI66427) 83 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 85 1 0 0 OTU A44 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 Uncertain Unclassified ^e OTU A13 N.A. NA. 3 0 0 </td <td></td> <td>OTU A10</td> <td>Sulfuricella denitrificans skB26 (BAI66427)</td> <td>97-98</td> <td>4</td> <td>0</td> <td>0</td>			OTU A10	Sulfuricella denitrificans skB26 (BAI66427)	97-98	4	0	0
Uncertain OTU A40 Sulfuricella denitrificans skB26 (BAI66427) 97 1 0 0 Uncertain Lineage 2 ^b OTU A40 Sulfuricella denitrificans skB26 (BAI66427) 91 1 0 0 Uncertain Unclassified ^c OTU A41 Sulfuricella denitrificans skB26 (BAI66427) 92 1 0 0 Uncertain OTU A42 Sulfuricella denitrificans skB26 (BAI66427) 83 1 0 0 Uncertain OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 85 1 0 0 Uncertain Unclassified ^c OTU A13 N.A. 1 0 0 Uncertain Unclassified ^c OTU A30 N.A. 1 0 0	Sulfur oxidation		OTU A39	Sulfuricella denitrificans skB26 (BAI66427)	97	1	0	0
Lineage 2 ^b OTU A41 Sulfuricella denitrificans skB26 (BAI66427) 91 1 0 0 OTU A42 Sulfuricella denitrificans skB26 (BAI66427) 92 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 83 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 85 1 0 0 OTU A44 Sulfuricella denitrificans skB26 (BAI66427) 85 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 Uncertain Unclassified ^e OTU A13 N.A. 1 0 0 Uncertain Unclassified ^e OTU A20 N.A. NA. 1 0		Lineage 2 ^b	OTU A40	Sulfuricella denitrificans skB26 (BAI66427)	97	1	0	0
Lineage 2* OTU A42 Sulfuricella denitrificans skB26 (BAI66427) 92 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 83 1 0 0 OTU A43 Sulfuricella denitrificans skB26 (BAI66427) 85 1 0 0 OTU A44 Sulfuricella denitrificans skB26 (BAI66427) 85 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 Uncertain Unclassified ^e OTU A13 N.A. 3 0 0 Uncertain Unclassified ^e OTU A20 N.A. N.A. 1 0 OTU A30 N.A. N.A. 0 1 0			OTU A41	Sulfuricella denitrificans skB26 (BAI66427)	91	1	0	0
Uncertain Unclassified ^e OTU A43 OTU A44 Sulfuricella denitrificans skB26 (BAI66427) 83 1 0 0 Uncertain Unclassified ^e OTU A44 Sulfuricella denitrificans skB26 (BAI66427) 85 1 0 0 Uncertain OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 Uncertain Unclassified ^e OTU A13 N.A. 100 1 0 0 Uncertain Unclassified ^e OTU A13 N.A. N.A. 1 0 0			OTU A42	Sulfuricella denitrificans skB26 (BAI66427)	92	1	0	0
Uncertain Unclassified ^e OTU A44 OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 85 1 0 0 Uncertain Unclassified ^e OTU A45 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 Uncertain Unclassified ^e OTU A46 Thiothrix sp. CT3 (ABV80023) 100 1 0 0 Uncertain Unclassified ^e OTU A20 N.A. N.A. 3 0 0 Total number of cloper OTU A30 N.A. N.A. 0 1 0			OTU A43	Sulfuricella denitrificans skB26 (BAI66427)	83	1	0	0
Uncertain Unclassified ^e OTU A45 OTU A46 Sulfuricella denitrificans skB26 (BAI66427) 87 1 0 0 Uncertain Unclassified ^e OTU A13 N.A. 1 0 0 Uncertain Unclassified ^e OTU A20 N.A. N.A. 3 0 0 Total auxiliary OTU A30 N.A. N.A. 1 0 0			OTU A44	Sulfuricella denitrificans skB26 (BAI66427)	85	1	0	0
Uncertain Unclassified ^c OTU A46 Thiothrix sp. CT3 (ABV80023) 100 1 0 0 Uncertain Unclassified ^c OTU A20 N.A. N.A. 3 0 0 OTU A20 N.A. N.A. 1 0 0 OTU A30 N.A. N.A. 1 0 0			OTU A45	Sulfuricella denitrificans skB26 (BAI66427)	87	1	0	0
Uncertain Unclassified ^c OTU A13 N.A. N.A. 3 0 0 Uncertain Unclassified ^c OTU A13 N.A. N.A. 1 0 0 OTU A20 N.A. N.A. 1 0 0 OTU A30 N.A. N.A. 0 1 0			OTU A46	Thiothrix sp. CT3 (ABV80023)	100	1	0	0
Uncertain Unclassified ^e OTU A20 N.A. N.A. 1 0 0 OTU A30 N.A. N.A. 1 0			OTUA13	N.A.	N A	3	0	0
OTU A30 N.A. N.A. 0 1 0 Total number of clopes 62 44 62	Uncertain	Unclassified ^c	OTU 420	NA	ΝΔ	1	0	0
Tatal number of elense 62 44 62		C.IOROGINEU .	OTU A20	N A	N A	0	1	0
			010 A30	Total number of ala	IN.A.	62	1	62

^a Closest cultured strains were determined according to BLASTP search result. GenBank accession number of AprA protein is in parentheses.
 ^b Classification of the lineages of sulfur-oxidizing bacteria is according to Meyer and Kuever [24].
 ^c Sequences have less than 70% sequence identity to any known sequences were grouped as Unclassified.
 N.A., Not available