INTRODUCTION

Coastal aquatic systems are highly susceptible and can be directly or indirectly affected by adjacent terrestrial ecosystems, anthropogenic activities and climate change. Yamagawa Bay is a coastal basin located in Ibusuki, Kagoshima Prefecture, Japan with a central depth of up to 50 m with a shallow entrance of 8 m. The geographical feature gives rise to reduction of the water exchange and makes the water stagnant for a long time, allowing deposition of organic matters and resulting in bottom-water hypoxia and sulfide accumulation in the sediment (Ide, 2012).

Aquatic microbiota vary spatio-temporarily due to changes of their surrounding environment, and composition and dominancy of the bacterial assemblages are highly correlated to the environmental conditions; thus, elucidation of the community structure enable us to understand physicochemical status of the environments, especially of the sediments.

Anoxygenic photosynthetic bacteria (AnPBs) are Gram-negative prokaryotes, performing anoxygenic photosynthesis with pigments such as bacteriochlorophylls (Bchl) and carotenoids. They convert light energy into chemical energy and grow autotrophically by using...
carbon dioxide as a sole source of carbon. Major groups are purple non-sulfur bacteria, purple sulfur bacteria, green sulfur bacteria and green non-sulfur bacteria (Kobilížek et al., 2006). Aerobic anoxygenic phototrophic bacteria, accounting for up to 10% of bacterial communities in the marine euphotic zones (Yutin et al., 2007), also produce Bchl a and complement their energy requirements by harvesting light under an aerobic condition. Habitats of AnPBs are restricted by availability of light and electron donors including reduced sulfur or organic compounds for their phototrophic growth as well as redox potential (van Gemerden and Mas, 1995; Guyoneaud et al., 1996). Therefore, community structure of the phototrophs will be a good bioindicator reflecting their ambient pollution levels, especially in the organically polluted marine sediments, resulting in eutrophication, oxygen depletion and high sulfide concentration.

The aim of this study is to gain insight into diversity of AnPBs in the Yamagawa Bay sediments. Three approaches were adopted: isolation and identification of pigmented anaerobic microorganisms; polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) of 16S ribosomal RNA (16S rRNA) genes (16S rDNA); and PCR-DGGE of puM encoding the M subunit of the reaction center complex.

MATERIALS AND METHODS

Sample collection
Sediment samples were collected from Yamagawa Bay, Kagoshima, Japan from May to November 2016 and May 2017 with a G.S. type core sampler (Ashura). Surface sediments within a depth of 10 mm were used for bacterial isolation and environmental DNA preparation.

Enrichment and isolation of photosynthetic bacteria
Portions of the collected sediments were transferred into tightly-sealed test tubes filled with 30 mL of Basic I medium (Hoshino and Kitamura, 1984) and cultivated at 20˚C under 12:12 light:dark cycling condition in order to enrich photosynthetic bacteria. Composition of the medium is as follows (concentrations are given as grams per 100 mL except as otherwise noted): KH₂PO₄, 0.5; K₂HPO₄, 0.6; (NH₄)₂SO₄, 1.0; MgSO₄·7H₂O, 0.2; NaCl, 0.2; CaCl₂·2H₂O, 0.05; Na₂S₂O₄·5H₂O, 0.1; yeast extract, 0.1; malate, 0.5. The following supplements were also added: the growth factor solution (thiamin-HCl, 0.05; nicotinic acid, 0.05; p-aminobenzoic acid, 0.03; vitamin B₂, 0.01; pyridoxine-HCl, 0.01; D-biotin, 0.005; shown as grams per 100 mL), 1 mL/L; the trace element solution (EDTA·2Na, 2.0; FeSO₄·7H₂O, 2.0; H₂BO₃, 0.1; CoCl₂·6H₂O, 0.1; ZnCl₂, 0.1; MnCl₂·4H₂O, 0.1; Na₂MoO₄·2H₂O, 0.02; shown as grams per 100 mL), 1 mL/L.

For AnPB isolation, a double layer agar technique was used by spreading the enriched cultures on Basic I plates with 1.5% of agar and then covering the plates with 1.2% agar. The agar plates were incubated anaerobically with Anaeropack Kenki system (Mitsubishi Gas Chemical, Tokyo, Japan) under the same condition as above. Pure isolates were obtained by sequential isolation from colonies with different morphology and maintained in Basic I liquid or agar plate media for further application.

Determination of the 16S rDNA nucleotide sequences
Liquid cultures of the isolates were centrifuged to obtain cell pellets, from which the bacterial DNA were extracted with DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Genes of 16S rRNA were amplified by PCR using a universal primer set 27F and 1525R (TABLE 1). A reaction mixture of PCR consisted of 1 x ExTaq Buffer (Takara Bio, Otsu, Japan), 100 µM dNTP Mixture (Takara Bio, Otsu, Japan), 0.5 µM primers and 0.025 units/µL ExTaq DNA Polymerase (Hot Start Version, Takara Bio, Otsu, Japan), and 5 µL of the bacterial DNA solutions were added to 100 µL of the mixture. Thermal cycling was conducted at 94˚C for 1 min, followed by 25 cycles of denaturation at 94˚C for 30 s, annealing at 58˚C for 30 s and extension at 72˚C for 90 s, and final extension at 72˚C was performed for 7 min. Specific amplification of the target gene was confirmed by subjecting the PCR products to 1.5% agarose gel electrophoresis in TAE buffer (40 mM Tris-acetate, pH 8.3, 1 mM ethylenediaminetetraacetic acid).

The amplified 16S rDNA fragments were cleaned up with ExoSAP-IT (Affymetrix, Santa Clara, CA, USA), and their nucleotide sequences were determined using a set of universal primers, 27F, PrSSU.2F, 1525R and 531R (TABLE 1), with ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA). The obtained sequences were assembled with the program GENETYX-MAC Ver. 19 (Genetyx, Tokyo, Japan). Their most homologous sequences were retrieved from the GenBank DNA database with the program of Basic Local Alignment Search Tool (BLAST; Altschul et al., 1990).

Bacterial community analyses by 16S rDNA PCR-DGGE
Microbial DNAs in the sediments were extracted using PowerSoil DNA Isolation Kit (MOBio, Carlsbad, CA, USA). Amplification of their 16S rDNAs was conducted by PCR with the primers 341F-GC and 907R (TABLE 1); composition of the reaction mixtures was the same as above. After initial denaturation at 95˚C for 1 min,
thermal cycling was performed as follows: 20 cycles of denaturation at 95°C for 1 min, 62°C for 1 min with a decrement of 0.8°C at every cycle and extension at 72°C for 1 min, followed by 10 cycles of 95°C for 1 min, 52°C for 1 min and 72°C for 1 min with final extension at 72°C for 10 min.

The 16S rDNA amplicons were applied to DGGE with DCode System (Bio-Rad, Hercules, CA, USA). The reaction mixtures were applied onto 6% polyacrylamide gel with 25-55% denaturant, in which 100% denaturant contained 40% deionized formamide and 7 M urea. Bands were visualized on a blue light transilluminator Safe Imager 2.0 (Thermo Fisher Scientific, Waltham, MA, USA) by staining the gel with SYBR Gold (Thermo Fisher Scientific, Waltham, MA, USA). Representative bands were excised using 1 mL pipette tips. The gel pieces were suspended in 100 µL of TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM ethylenediaminetetraacetic acid), and frozen and thawed. The eluted DNA fragments were re-amplified and subjected to nucleotide sequencing with the primer 907R (TABLE 1) and homology searches as mentioned above.

**Bacterial community analyses by pufM PCR-DGGE**

The _pufM_ genes were amplified from the sediment DNAs by nested PCR. The first-round amplification was done with an outer primer pair _pufM557F_ and _pufM.1R_ (TABLE 1), whose thermal cycling was as follows: 30 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 30 s. The amplified products were further subjected to the second run with inner primer sets _pufM557F_ and _pufM750R_, or _pufM557Fencing_ and _pufM750R_ (TABLE 1). The same thermal setting as above was adopted, except for the primer set with a GC clamp: annealing temperature and cycle numbers were 58-63°C and 10-12 cycles, respectively.

The amplified products were applied to DGGE as shown above, except for 10% of polyacrylamide and 30-60% or 40-60% of the denaturant. Representative bands were excised, re-amplified with the same primers, and subjected to DGGE. This procedure was repeated until homogeneous sequences were obtained; bands whose sequences were still heterogeneous were excluded for further analyses. Nucleotide sequences were determined using the primer 907F (TABLE 1) and their homology searches were performed.

**Results**

**Isolation and identification of photosynthetic bacteria**

Enrichment of AnPBs from the Yamagawa Bay sediments in the Basic I liquid medium under semi-anaerobic or anaerobic condition showed growth of microbial consortia with the color of green, pink or yellow. Totally

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**TABLE 1. Oligonucleotide primers used for PCR amplification, PCR-DGGE and nucleotide sequencing**

| Primers | Application | Target genes | Target organisms | Nucleotide sequences (5' -> 3') |
|---------|-------------|--------------|------------------|-------------------------------|
| 27F     | PCR, sequencing | 16S rDNA | Bacteria | AGAGTTTGATCCTGGCTCAG |
| 1525R   | PCR, sequencing | 16S rDNA | Bacteria | AAAGGAGGTGATCCAGCC |
| PrSSU.2F | Sequencing | 16S rDNA | Bacteria | TCCTACGGGAGGCAGCA |
| 531R    | Sequencing | 16S rDNA | Bacteria | TACCGCGGCTGCTGGCA |
| 341F-GC | PCR-DGGE and nucleotide sequencing | 16S rDNA | Bacteria | CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGC |
| 907R    | PCR-DGGE and nucleotide sequencing | 16S rDNA | Bacteria | CCGTCAATTCCTTTGAGTTT |
| 341F-GC | PCR-DGGE and nucleotide sequencing | 16S rDNA | Bacteria | CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGC |
| pufM557F | PCR-DGGE and nucleotide sequencing | 16S rDNA | Purple photosynthetic bacteria | CGCACCTGGACTGGAC |
| pufM557FGC | PCR-DGGE and nucleotide sequencing | 16S rDNA | Purple photosynthetic bacteria | CGCACCTGGACTGGAC |
| pufM.1R  | PCR-DGGE and nucleotide sequencing | 16S rDNA | Aerobic anoxygenic phototrophic bacteria | GCAAAACCAAGCCCA |
| pufM750R | PCR-DGGE and nucleotide sequencing | 16S rDNA | Purple photosynthetic bacteria | CCGTCAATTCCTTTGAGTTT |
| pufMR   | PCR-DGGE and nucleotide sequencing | 16S rDNA | Aerobic anoxygenic phototrophic bacteria | CCGTCAATTCCTTTGAGTTT |
| pufM557F | PCR-DGGE and nucleotide sequencing | 16S rDNA | Purple photosynthetic bacteria | CCGTCAATTCCTTTGAGTTT |

*a* GC clamps added to 5' termini of the PCR-DGGE primers are underlined.  
*b* The International Union of Pure and Applied Chemistry (IUPAC) codes are used for mixed nucleotides.

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36 bacterial isolates were obtained in the agar plates and their 16S rDNA sequences were determined. They showed identities to known species with 99% or above with one exception (TABLE 2). *Rhodobacter sphaeroides*, an anoxygenic, photosynthetic purple non-sulfur bacterium with a freshwater origin (Pfennig and Trüper, 1971), was most prevalent (16 isolates) in the sediments. The remaining bacterial isolates were primarily chemoorganotrophic and showed no close relationship to AnPBs. Eight isolates possessed homologous sequences to plastidal 16S rDNAs of a chlorophyte *Chlorella* spp.

**Bacterial community analyzed by 16S rDNA and pufM PCR-DGGE**

Environmental DNAs extracted from microbial communities in the sediments of Yamagawa Bay were applied to 16S rDNA PCR-DGGE. Resultant band profiles were shown in FIG. 1. One major band prevailing among all the sediment was observed with some faint bands, whose profiles were indistinguishable among the sampling periods. The nucleotide sequences were identical to *Chlorobium phaeobacteroides* (Chlorobia - Chlorobiales - Chlorobiaceae), an anoxygenic photosynthetic green sulfur bacterium with a freshwater origin (Pfennig, 1968; Pfennig and Overmann, 2001).

Band profiles of *pufM* DGGE were similar among the sampling periods and the triplicated samples with some differences (FIG. 2). In order to determine their nucleotide sequences, representative bands were collected, re-amplified, and electrophoresed on DGGE gel. Bands whose mobilities were not identical to the originals were also sequenced, but bands whose sequences had been still heterogeneous were excluded from further analyses. All the bands showed close relationships to known AnPBs or potential Bchl a-producing AnPBs, including *Rhodopseudomonas lichen*, *Rhodovibrio sodomensis*, *Porphyrobacter tepidarius*, *Thiorhodococcus bheemlicus*, *Dinoroseobacter shibae*, *Sulfitobacter gutiformis* and *Roseobacter litoralis* (TABLE 3). The band A1 was also homologous to *Sphingomonas lacus* (TABLE 3): this species is not possibly a phototroph, since Bchl a was not detected, although harboring structural genes, *putL* and *pufM*, of the type II photosynthetic reaction center (Kim et al., 2015).

Band profiles of *pufM* DGGE with the GC clump-added primer also showed almost identical patterns with slight variation (FIG. 3). All the sequences obtained from the bands 1-4, commonly detected in all the sediments, showed 92% of identities to *Thiocapsa marina* and *Allochromatium phaeobacterium* (Gammaproteobacteria - Chromatiales - Chromatiaceae), both of which are purple sulfur bacteria.

**Discussion**

In order to elucidate compositions of bacterial populations in the organically polluted, anoxic, reductive sediments in the geographically enclosed coastal marine inlet, approaches of AnPB isolation and PCR-DGGE of 16S rRNA and *pufM* genes were adopted.

Basic I medium employing the AnPB isolation contains a low concentration of yeast extract as a carbon source and lacks sulfides. Under an anaerobic condition, the medium is selective for purple non-sulfur bacteria among AnPBs; therefore, green sulfur bacteria, which dominantly detected in 16S rDNA PCR-DGGE (mentioned below), were not detected, due to their requirement of sulfur as an electron donor. Among the AnPB isolates
from the Yamagawa Bay sediments, relatives of *Rhodob. sphaeroides* were most dominant. This species is known as an anoxygenic purple non-sulfur bacterium (Pfennig and Trüper, 1971) with a freshwater origin. In view of their diverse metabolic systems and multiple uses (e.g., Lu et al., 2011; Subudhi et al., 2016), the Rhodob. sphaeroides isolates may be associated with its rapid growth and the possibility of using a variety of organic pollutant substances as their nutrients.

One isolate, PSBYam1608St4-1 belonged to the same family Rhodobacteraceae as *Rhodob. sphaeroides*. However, the most relative species *Thioclava pacifica* is not phototrophic: it grows chemooautrophically with thiosulfate, heterotrophically with simple organic compounds, or methylotrophically utilizing hydrogen as an electron donor (Sorokin et al., 2005). The remaining bacterial clones showed no close relationship to AnPBs: all of them are heterotrophic, and no photoautotrophic growth has been reported. An eukaryotic green microalga *Chlorella sorokiniana* was also found in the anaerobically enriched culture, attributable to its capacity of anaerobic growth with photosynthesis and internally produced oxygen (Qiao et al., 2009).

The band profiles of PCR-DGGE were similar throughout the sampling period with slight differences in faint

**FIG. 2.** Band profiles of *pufM* PCR-DGGE. Amplicons of *pufM*, amplified from environmental DNAs of Yamagawa Bay sediments with secondary primers *pufM*557F/*pufM*750R, were applied. Bands excised for further analyses are shown (A1-A12), and the bands whose nucleotide sequences were determined are underlined. Years/months of sediment sampling and serial numbers (#) of the samples are as follows: lane 1, 2016/May, #1; lane 2, 2016/May, #2; lane 3, 2016/May, #3; lane 4, 2016/June, #1; lane 5, 2016/June, #2; lane 6, 2016/June, #3; lane 7, 2016/July, #1; lane 8, 2016/August, #1; lane 9, 2016/November, #2; lane 10, 2016/November, #3.

**FIG. 3.** Band profiles of *pufM* PCR-DGGE with a GC-clamp primer. Amplicons of *pufM*, amplified from environmental DNAs of Yamagawa Bay sediments with secondary primers *pufM*557FGC/*pufM*750R, were applied. Bands excised for further analyses are shown (1-8), and the bands whose nucleotide sequences were determined are underlined. Years/months of sediment sampling and serial numbers (#) of the samples are as follows: lanes 1, 4, 5, 8, 2016/May, #1; lanes 2, 6, 9, 2016/May, #2; lane 3, 7, 10, 2016/May, #3. Annealing temperatures/thermal cycling to obtain the *pufM* amplicons with the secondary primers were 60˚C/10 cycles (lanes 1-4), 58˚C/12 cycles (lanes 5-7), and 63˚C/12 cycles (lanes 8-10).
| PSB isolates     | Isolation years/months | Sequence length (nts) | Most homologous relatives                                      | Taxonomic groups                                      | Sequence identity (%) |
|------------------|------------------------|-----------------------|----------------------------------------------------------------|-------------------------------------------------------|-----------------------|
| PSBYam1606St1-1  | 2016/June              | 1412                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 100                   |
| PSBYam1606St3-1-1| 2016/June              | 787                   | *Chlorella sorokiniana/variabilis/thermophila*                  | Trebouxiophyceae - Chlorellales - Chlorellaceae        | 99                    |
| PSBYam1606St3-1-2| 2016/June              | 798                   | *Chlorella sorokiniana/variabilis/thermophila*                  | Trebouxiophyceae - Chlorellales - Chlorellaceae        | 99                    |
| PSBYam1606St3-1-3| 2016/June              | 817                   | *Chlorella sorokiniana/variabilis/thermophila*                  | Trebouxiophyceae - Chlorellales - Chlorellaceae        | 99                    |
| PSBYam1606St3-1-4| 2016/June              | 829                   | *Chlorella sorokiniana/variabilis/thermophila*                  | Trebouxiophyceae - Chlorellales - Chlorellaceae        | 99                    |
| PSBYam1606St3-1-5| 2016/June              | 793                   | *Chlorella sorokiniana/variabilis/thermophila*                  | Trebouxiophyceae - Chlorellales - Chlorellaceae        | 99                    |
| PSBYam1606St3-1-6| 2016/June              | 830                   | *Chlorella sorokiniana/variabilis/thermophila*                  | Trebouxiophyceae - Chlorellales - Chlorellaceae        | 99                    |
| PSBYam1606St3-3-1| 2016/June              | 898                   | *Acinetobacter venetianus*                                      | Gammaproteobacteria - Pseudomonadales - Moraxellacea  | 100                   |
| PSBYam1606St3-5  | 2016/June              | 1405                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 100                   |
| PSBYam1606St3-7-1| 2016/June              | 857                   | *Pseudomonas putida/pseudoalcaligenes*                           | Gammaproteobacteria - Pseudomonadales - Pseudomonadaceae | 100                   |
| PSBYam1606St3-9  | 2016/June              | 1414                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1606St5-1  | 2016/June              | 1412                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 100                   |
| PSBYam1606St5-3  | 2016/June              | 1389                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1607St1-1  | 2016/July              | 1426                  | *Bosea vestrisii/eneae/thiocidans*                              | Alphaproteobacteria - Rhizobiales - Bradyrhizobiaceae   | 99                    |
| PSBYam1607St1-5  | 2016/July              | 1401                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1607St1-7  | 2016/July              | 1414                  | *Rhodobacter sphaeroides/johrii*                                | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1607St1-8  | 2016/July              | 1405                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1607St2-4  | 2016/July              | 1404                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 100                   |
| PSBYam1607St2-5  | 2016/July              | 1398                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1607St2-7  | 2016/July              | 1404                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 100                   |
| PSBYam1607St3-1  | 2016/July              | 1414                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1607St3-3  | 2016/July              | 1413                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1607St3-5  | 2016/July              | 1405                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1607St4-2  | 2016/July              | 1340                  | *Mycoplana ramosa*                                              | Alphaproteobacteria - Rhizobiales - Brucellaceae        | 98                    |
| PSBYam1608St1-1  | 2016/August            | 1402                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1608St2-1  | 2016/August            | 1407                  | *Rhodobacter sphaeroides*                                       | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1608St4-1  | 2016/August            | 756                   | *Thioclava pacifica*                                            | Alphaproteobacteria - Rhodobacterales - Rhodobacteraceae | 99                    |
| PSBYam1705St.2-4-A1| 2017/May              | 701                   | *Acidovorax delafieldii*                                       | Betaproteobacteria - Burkholderiales - Comamonadaceae   | 99                    |
| PSBYam1705St.2-4-A2| 2017/May              | 703                   | *Pseudomonas knackmussii/nitroreducens*                         | Gammaproteobacteria - Pseudomonadales - Pseudomonadaceae | 100                   |
| PSBYam1705St.2-4-A3| 2017/May              | 700                   | *Pseudomonas knackmussii/nitroreducens*                         | Gammaproteobacteria - Pseudomonadales - Pseudomonadaceae | 99                    |
| PSBYam1705St.3-1-A4| 2017/May              | 700                   | *Pseudomonas knackmussii/nitroreducens*                         | Gammaproteobacteria - Pseudomonadales - Pseudomonadaceae | 100                   |
| PSBYam1705St.3-2-A8| 2017/May              | 703                   | *Pseudomonas knackmussii/nitroreducens*                         | Gammaproteobacteria - Pseudomonadales - Pseudomonadaceae | 100                   |
| PSBYam1705St.3-3-A13| 2017/May              | 671                   | *Chlorella sorokiniana/variabilis/thermophila*                  | Trebouxiophyceae - Chlorellales - Chlorellaceae        | 99                    |
| PSBYam1705St.4-1-A14| 2017/May              | 701                   | *Acidovorax delafieldii*                                       | Betaproteobacteria - Burkholderiales - Comamonadaceae   | 99                    |
| PSBYam1705St.4-1-A16| 2017/May              | 703                   | *Pseudomonas knackmussii/nitroreducens*                         | Gammaproteobacteria - Pseudomonadales - Pseudomonadaceae | 100                   |
| PSBYam1705St.4-2-A18| 2017/May              | 666                   | *Chlorella sorokiniana*                                         | Trebouxiophyceae - Chlorellales - Chlorellaceae        | 99                    |

*a* Lengths of the nucleotide sequences subjected to BLASTN searches were shown.

*b* A sequencing primer PrSSU.2F was used for sequencing.

*c* Sequencing primers PrSSU.2F and 531R were used for sequencing.

*d* Identity to 16S rDNA encoded in their plastidal genomes.
bands, regardless of the primer sets used. Due to its high water depth, the water column of Yamagawa Bay was stratified throughout the year, resulting in poor vertical mixing. Consequently, with loading and accumulation of organic pollutants, the sedimentary environments were continuously in a deleterious condition: poor dissolved oxygen and high sulfide content in the pore water (Ide, 2012). The persistence of the microbial community structures during the experimental period could account for such temporal stability of the benthic environments.

The primers universal to the Domain I of 16S rDNAs including the hypervariable regions V1-V4 produced one major band in DGGE whose nucleotide sequence was identical to a green sulfur bacterium Chlorob. phaeobacteroides BS1. The continual dominance of this bacterial species, which is a strictly anaerobic photolithotroph oxidizing reduced sulfur (Overmann, 2001; Frigaard et al., 2003), was also reported in a brackish lake with oyster aquaculture (Santander-de Leon et al., 2013), reflecting the reductive condition observed in the seabed as mentioned above. However, it should be noted that the strain BS1 was phylogenetically reclassified into the genus Prosthocochloris (Imhoff and Thiel, 2010), suggesting its diverse characteristics from Chlorob. phaeobacteroides. In fact, monospecific abundance of Chlorobium sp. BS1 was also reported in the Black Sea (Marschall et al., 2010) whose water body is characterized by oxic-anoxic transition zone and sulfidic chemocline. The bacterium was inhabited at the depth of upper limit of sulfide-containing water layers with lower limit of downwelling irradiance for photosynthetic carbon fixation. The bacterial assemblage was deposited at the flocculent surface layer of the sea bottom and could survive under the extreme low-light conditions (Marschall et al., 2010). Predominance of the green sulfur bacterium in the Yamagawa Bay sediments could be explained by the similar process to the Black Sea, although its microbial ecophysiology should be elucidated.

A protein PufM, a gene product of pufM, is a component of the type II (pheophytin-quinone type) photosynthetic reaction center (RC; Cardona, 2015). The protein is known as an accurate tool for assessing phylogeny and diversity of bacteria employing the photosystem II in nature (Imhoff et al., 2018). Distribution of pufM is limited to purple bacteria including aerobic anoxygenic photosynthetic bacteria, and green sulfur bacteria including the genus Chlorobium lack the pufM gene since their photoenergy capturing depends on the type I (iron-sulfur type) RC with a protein PscA (Cardona, 2015). Thus, PCR-DGGE with the pufM primer sets was carried out to clarify temporal diversity of the type II RC-harboring bacteria, eliminating preferred amplification from a limited number of predominant populations, such as Chlorob. phaeobacteroides BS1, in 16S rDNA-targeted PCR. As a result, three out of eleven bands sequenced showed high identity to a pufM gene of Rhodop. lichen (TABLE 3); however, its bacterial characteristics have not been reported. The closest relative to this uncharacterized species is Rhodopseudomonas palustris, a purple non-sulfur phototroph, whose pufM possessed 92% identity at a nucleotide level and 95% at an amino acid level. In addition, diverse comparables to photosynthetic sulfur oxidizers were also found. Rhodov. sodomensis, Thiorn. bheemicus, Thiorn. marina and A. phaeobacterium are members of purple sulfur bacteria, oxidizing sulfide, thiosulfate or elemental sulfur as electron donors for photolithotrophic growth under an anoxic condition (Imhoff et al., 1998; Caurnette et al., 2004; Kumar et al. 2007; Srinivas et al., 2009). This finding is supported by the study of Mukkata et al. (2016), in which high concentration of H2S was assumed by the detection of anaerobic purple sulfur bacteria Allochromatium sp. in shrimp pond. Further, Imhoff et al. (2018) clearly recognized anaerobic purple non-sulfur bacteria Rhodovibrio sp. with PuLM sequences. The existence of Rhodovibrio sp. with Allochromatium sp. refers to the ability of Rhodovibrio to tolerate high levels of sulfides.

It is noticeable that pufM sequences closely related to aerobic anoxygenic phototrophs were also detected, such as Ros. litoralis and D. shibae. Both the species were originally isolated from micro- and macroalgal phycosphere (Shiba, 1991; Biebl et al., 2005). The former species belongs to the Roseobacter clade based on the 16S rDNA phylogeny, and the latter species is one of the closest sister taxa (Biebl et al., 2005). This lineage is one of the major marine bacterial groups, representing diverse marine habitats from coastal to open oceans as well as of sediments (Buchan et al., 2005). Moreover, several Roseobacter isolates harbor abilities to transform inorganic sulfur compounds (González et al., 1999; Buchan et al., 2005). Lenk et al. (2012) also revealed abundance of Roseobacter clade bacteria in marine surface sediments of tidal flats. They reported up to 9.6% of relative bacterial abundance of this clade and succeeded in its own enrichment under an anoxic, sulfidic condition. They also detected gene components of the SOX and reverse dissimilatory sulfite reductase (rDSR) pathways for inorganic sulfur oxidation. Taken together, Roseobacter is likely to be one of the sulfur oxidizers in highly eutrophicated marine coastal sediments, including Yamagawa Bay.

The present study suggested prevalence of AnPBs under the deteriorating sediment condition. The sulfide-rich reductive environments considered, colonization of the photosynthetic sulfur oxidizers is very relevant. It is also conceivable that purple non-sulfur phototrophs
have a potential role of degrading the organic pollutants. However, distribution and metabolism of the AnPBs must be regulated by the environmental factors in their sedimental habitat, such as oxygen availability, redox potential, types and concentrations of sulfur compounds, and solar illuminance. Hence, more detailed research on relationship between physicochemical factors of the sediments and population dynamics and biological activity of the AnPB communities is expected to improve our understanding of how the AnPBs contribute to cycling of the organic pollutants and their organic or inorganic sulfur derivatives.

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