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EXPERIMENTAL ARTICLES

Phylogenetic Analysis of Culturable Marine Bacteria in Sediments from South Korean Yellow Sea[1]

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Abstract—Biogeochemical and microbiological characterization of marine sediments taken from the Yellow Sea of South Korea was carried out. One hundred and thirty six bacterial strains were isolated, characterized and phylogenetic relationship was evaluated. The gene sequences of 16S rDNA regions were examined to study the phylogenetic analysis of bacterial community in the marine sediments. Among 136 isolates, 5 bacterial isolates were identified as novel members, remaining 131 isolates were fall into 5 major linkages of bacterial phyla represented as follows: *Firmicutes*, , -@*Proteobacteria*, High G + C and *Bacteroidetes*. Bacterial community in sediments mainly dominated by *Firmicute* (58.77%) and followed by @-*Proteobacteria* (38.16%). @-*Proteobacteria* domain highly diverged and mainly consists of the genera *Vibrio, Marinobacterium, Photobacterium, Pseudoalteromonas, Oceanisphaera, Halomonas, Alteromonas, Stenotrophomas* and *Pseudomonas*. Total N and Organic matter content in Yellow Sea of South Korea were relatively high. The Total-N content in the sediments was varied from 177.31 to 1974.96 (mg/kg) and organic matter ranged from 0.82 to 4.23 (g/100 g^{-1}). The current research work provides clear explanation obtained for the phylogenetic affiliation of the culturable bacterial community in sediments of South Korean Yellow Sea and revealed the relationship with biogeochemical characteristics of the sediments.

Key words: marine sediments, biogeochemical characterizations, marine bacterial diversity, phylogenetic relationship.

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Abundant microbial resources are available in various marine environments. More than 85 to 95% of marine bacteria are not yet cultivable [1]. For the better understanding of marine bacterial community, the researchers are using traditional or conventional culturing and identification method with various molecular techniques, including PCR based (16s rDNA region sequencing, DGGE, RFLP and ARDRA) [2] and non PCR based tools, such as FISH [3–5]. Modern molecular tools provides a clear evidence to construct the phylogenetic tree and this phylogenetic technique reflecting the results obtained from conventional culture method or supporting the data of morphological observations.

Marine sediments are predominantly occupied by bacterial community. Marine microbes, especially, bacteria and archae are directly influenced by the physico-chemical processes in aquatic sediments [6, 7]. Thus, understanding the bacterial community in sediment is essential in order to relate with physico-chemical properties of marine sediment. Suzuki et al. (1997) [8] distinguished the bacterial diversity between the sequences of culturable isolates in marine water and sequences of rDNA from direct marine water samples. In fact, it's not easy to cultivate the entire marine bacterial community in commercially available microbial growth media, since most of bacterial communities are composed of unknown species. Some reports currently exist regarding the differences between culturable marine bacterial diversity and direct sequencing of various marine environmental substrates [5, 8]. Dunbar et al. (1999) [9] reported same kind of difference in bacterial community in arid soils. Cetecioglu et al. (2009) [6] reported the biogeographical distribution and diversity of bacteria with archaeal communities in polluted marine sediments. The majority of the marine bacterial communities have been described based on the rRNA sequences in the literatures. However, the direct sequencing of environmental samples has been representing the rDNA sequences of bacterial communities and these rDNA sequences are long to undescribed marine bacterial species [8]. Thus, its necessary to carry out the

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Table 1. Details of sample sites and depths

Sample reference number	Collection point	Latitude (N)	Longitude (E)	Depth (cm)
1	Sunyu-Island	36°30′04″	126°23′16″	100
2	Sunyu- Island	36°30′04″	126°23′16″	300
3	Sunyu-Island	35°48′06″	126°24′32″	100
4	Sunyu-Island	35°48′06″	126°24′32″	300
5	Sunyu-Island	35°48′13″	126°24′45″	100
6	Sunyu-Island	35°48′13″	126°24′45″	300
7	Buan	35°41′34″	126°32′46″	100
8	Buan	35°41′34″	126°32′46″	300
9	Buan	35°41′20″	126°32′52″	100
10	Buan	35°41′20″	126°32′52″	300
11	Buan	35°41′28″	126°32′41″	100
12	Buan	35°41′28″	126°32′41″	300
13	Anmyeon-Island	36°30′04″	126°20′02″	100
14	Anmyeon-Island	36°30′04″	126°20′02″	300
15	Anmyeon-Island	36°30′10″	126°20′23″	100
16	Anmyeon-Island	36°30′10″	126°20′23″	300
17	Anmyeon-Island	36°30′18″	126°20′42″	100
18	Anmyeon-Island	36°30′18″	126°20′42″	300
19	Anmyeon-Island	36°30′34″	126°20′52″	100
20	Anmyeon-Island	36°30′34″	126°20′52″	300

traditional microbial culture method for the better understanding of bacterial physiology with account of molecular identity. Even though some references exist for culturable bacterial diversity in marine substrates, there are very few works reported on the culturable bacterial diversity in marine sediments. And, comparisons of biogeochemical properties of marine sediments with culturable marine bacterial diversity are rare.

Few researchers studied the biogeochemical properties of the marine sediments [6, 10–13]. The organic contents in the sediments are main source of oxygen for microbial community in the marine sediments [13] and those compounds were linked with the global organic cycles. Simple and easily decomposable organic compounds in sediments are first consumed by microbial community in the sediments [13].

Based on the phylogenetic analysis, bacterial communities in the marine sediments are highly diverged and linked together by various family domains: α, β, γ-*Proteobacteria*, *Planctomyces*, *Clostridia*, Gram positive high G + C bacteria, *Firmicute, Bacteroidetes* and *Actinobacteria* [3, 14, 15]. Recently, many researches had been focused on bacterial diversity in marine sediments in order to find the novel bioactive compounds. Some researchers already reported the segregation of biologically active secondary metabolites and industrially important enzymes from the sediment bacteria [16, 17]. In this work, we have attempted to made culture of marine bacteria from the sediments collected from Yellow Sea of South Korea and the cultured bacterial communities were characterized based on morphological and molecular features. We have successfully isolated 136 different marine bacterial strains with different morphological appearance among them 5 strains are identified as novel strains (manuscript in preparation) and those strains were classified into five major groups based on molecular sequencing. In addition, the sediments were characterized in order to elucidate the relationship between the biogeochemical properties of marine sediments with bacterial diversity.

MATERIALS AND METHODS

Sampling sites and sample collection. Samples were collected during August 2008. Sediment samples were taken from twenty different sites of Yellow Sea of South Korea. The detailed description of locations of sampling sites and depth of samples were given in Fig. 1 and Table 1. Triplicate samples were obtained at every sampling point. Each sediment samples were subdivided into two parts, one part was used for biogeochemical analysis of sediments and another part was used for isolation of marine bacteria. Three points were selected randomly in first two locations (Sunyu Island and Buan) and four points were selected randomly in third location (Anmyeon Island). Sediment samples were taken at two depths (1 and 3 meters) in each point (Table 2). Obtained samples were placed in 50 ml sterile falcon tubes and were immediately transferred to laboratory in ice box and stored at −20°C.

Characterization of sediments. Sediment characterization was done by using the samples divided for biogeochemical analysis. Sediments were dissolved in triple distilled water in ratio 1 : 5 (H_2O) for pH determination (Mettler Toledo S20 Meter, USA). EC were determined by paste saturation method (LF-538, Germany). Total nitrogen content was measured by Kjeldahl method and organic matter of the sediments was determined by Walkley and Black method (1934) [18].

Isolation and characterization of marine bacteria. Filtered and sterilized sea water obtained at the sample collection points was used for entire isolation experiment, including media preparation and sample preparation for inoculation experiments. One gram of sediment sample was suspended in 10 mL sterile sea water and vortex for 3 min. Finally 0.1 mL of suspension was spread onto petri dishes containing 0.5× strength marine agar (Difco 2216). Triplicates were maintained for each sample. Control plate was maintained by inoculating the sterilized sea water onto pertri dishes. Plates were incubated at 25°C. Different individual colonies were picked for further characterizations based on following properties: colony form, size, color and surface. Pure cultures were maintained on same

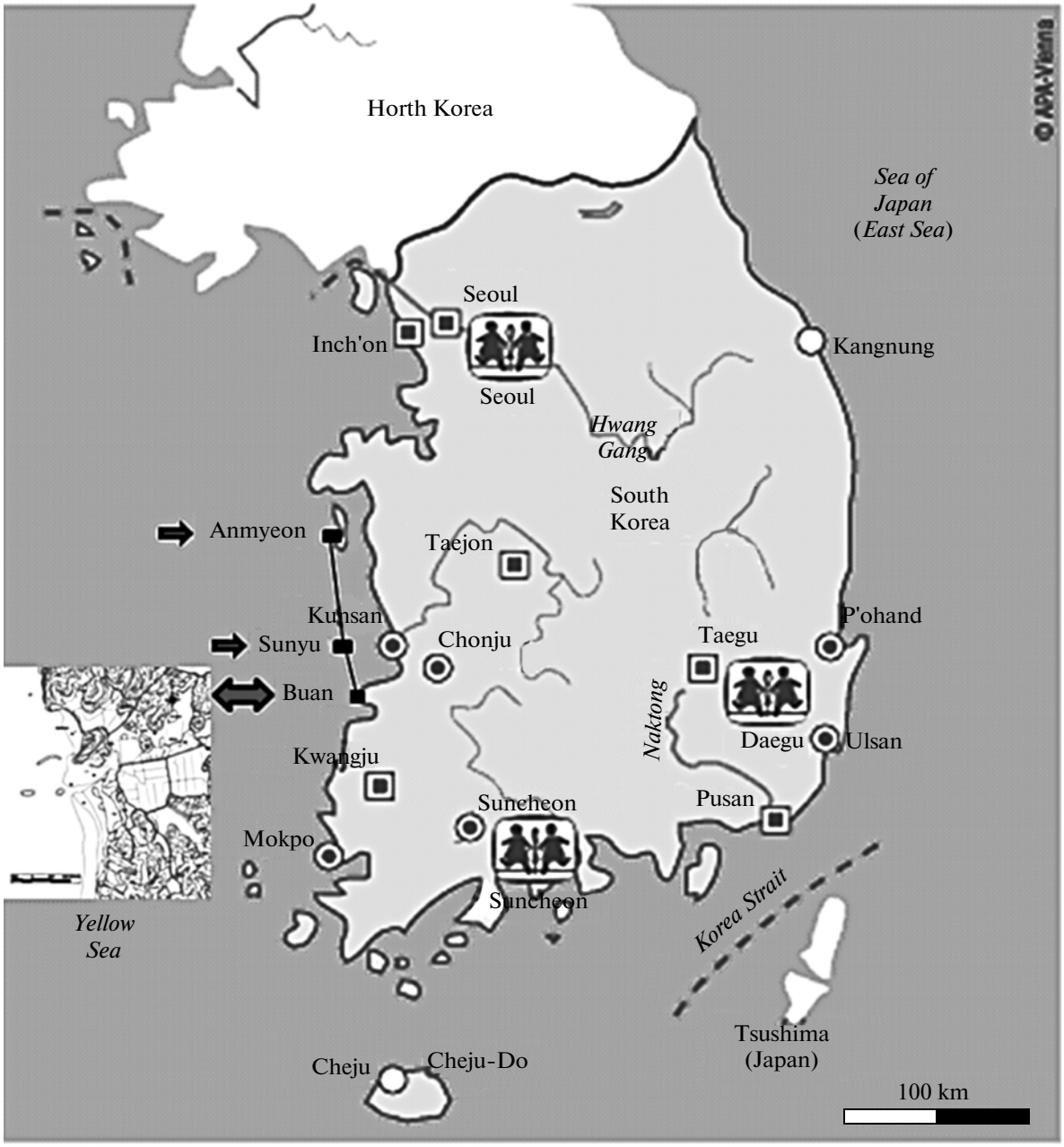

Fig. 1. Geographical map of sampling sites.

0.5× strength marine agar supplement with sterilized sea water.

The total genomic DNA of each isolated pure marine bacterial strains were extracted using the modified method from Experimental Techniques in Bacterial Genetics, Jones and Bartlet (1990) [19]. Isolated DNA fragments were observed on a 0.8% agarose gel with a 100 bp ladder (TaKaRa, Japan). Genomic DNA bands were observed under UV light after EtBr staining. Individually extracted fragment of each bacterial isolates was amplified by using primers forward 27F (5'AGAGTTTGATCMTGGCTCAG3') and reverse 1492R (5'TACGGYTACCTTGTTACGACTT3'). Both primers represent the rDNA genomic region of *E. coli*. PCR reaction mixture consisting of 10× PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3); 0.5 mM dNTPs; 27F and 1492R primers, each 0.5 mM; 2.0 mM $MgCl_2$; IU AmpliTaq Gold DNA polymerase (Perkin-Elmer); 2 μL DNA sample; each mixture was made up to a final volume of 30 μL using deionized sterile distilled water. PCR amplification was carried out in the thermocycler (ESCO, Swift Maxi Thermal Cycler) with following reaction conditions: initial denaturation at 94°C for 5 mins, and 30 cycles of denaturation at 94°C for

Table 2. Physico-chemical characteristics of marine sediments

Sample reference number	pH	Temp. (°C)	EC (mS/cm)	Total-N (mg/kg)	Organic matter (g/100 g)	OM/N ratio
1	8.4	23.1	3.89	1531.79	3.57	23.8
2	8.56	22	3.98	1974.96	4.23	21.2
3	8.23	24.1	3.51	1454.85	1.32	8.8
4	8.07	22.3	2.68	821.08	2.48	31.0
5	8.39	22.5	3.05	771.92	2.19	27.4
6	8.28	23.8	3.85	585.68	1.96	32.7
7	8.71	22.7	3.62	545.57	3.79	75.8
8	8.65	24.3	3.63	419.06	2.19	54.8
9	8.45	23.4	3.07	469.19	0.82	16.4
10	7.87	22.7	2.73	332.80	0.94	31.3
11	8.8	23.7	2.25	220.5	1.5	75
12	8.89	23.5	3.19	259.15	1.9	76
13	8.74	26	2.95	395.54	2.9	74.4
14	8.49	26.7	2.87	395.54	2.9	74.4
15	8.77	24.5	3.08	272.78	2	74.1
16	8.65	24	3.17	327.34	2.4	75
17	8.66	24	3.11	436.46	3.2	74.4
18	8.7	24	2.96	463.73	3.4	74
19	8.41	24.1	2.81	177.31	1.3	76.5
20	8.40	24	2.79	245.51	1.8	75

1 min, annealing at 58.6°C for 1 min and extension at 72°C for 2 min, and followed by final extension at 72°C for 10 mins. After amplification, amplified bacterial genomic DNA fragments were visualized on 1.5% agarose gel under UV light after EtBr staining.

DNA sequencing, analysis and phylogenetic tree construction. The amplified PCR products of bacterial gene fragments were sequenced at MACROGEN sequencing company, S. Korea using automated DNA sequencer. Primers 27F (5'AGAGTTTGATCMTGGCTCAG3') and 1492R (5'TACGGYTACCTTGTTACGACTT3') were used for automated sequencing. The obtained sequences of bacterial genomic fragments were analyzed by using CLUSTALW, DNASIS and GENETYX software and the sequence arrangements were carefully checked manually at individual base level. GenBank BLASTn search was used to confirm the sequence identity of each isolates [20]. CLUSTALW Version 1.83 was used to align the regions between synchronized sequences and sequences obtained from the GenBank [21]. GenBank sequence data and current experimental data were used for Neighbor-Joining (NJ) phylogenetic tree construction, analysis was conducted by CLUSTALW.

Accession numbers of nucleotide sequence. The 16S rDNA of marine bacterial isolates have been deposited in GenBank and accession numbers were obtained. Accession numbers are AB490784 to AB490789, AB518926 to AB519012 and AB536935 to AB536977.

RESULTS

Biogeochemical properties of sediments. The biogeochemical characteristics of the sediments were showed in Table 2. Biogeochemical characteristics of the sediments were described by measuring the temperature, pH, EC values, total nitrogen, organic matter and OM/N ratio (Table 2). The chemical analysis of sediments showed that all the samples were alkaline-saline in nature and their pH varied from 7.87 to 8.89 (Table 2). Higher values were observed for the total nitrogen and organic matter content in the sediments. The distribution of total nitrogen content ranged from 177.31 to 1974.96 mg/kg and simultaneously the percentage ranged from 0.01 to 0.20% (Table 2). The EC content of the sediments was differing from 2.25 to 3.98 mS/cm (Table 2). The distribution of total organic matter varies from 0.82 to 4.23%.

Characterization and phylogenetic analysis of marine sediment bacteria. A total number of 136 bacterial isolates were primarily sorted out based on following colony morphological properties: colony form, size, color, texture, and surface. Strains were selected for molecular analysis based on the morphological differentiations. Approximately 1400 bp of 16S rDNA region of bacterial strains were cloned and sequenced for phylogenetic analysis. The genomic region of 16S rDNA segment of bacteria is generally used to distinguish the phylotypes among their relatives and reveal the phylogenetic relationships between them [22]. Based on the molecular characterization, 131 strains were affiliated to 5 major phyla. The families are γ-*Proteobacteria*, *Firmicutes*, α-*Proteobacteria*, High G + C content bacteria, *Bacteroidetes*. Among the 5 families, *Firmicutes* was dominant by having 58.77% and followed by γ-*Proteobacteria* (38.16%). The phylogenetic placements of 5 families were shown in Fig. 2 to Fig. 6.

γ-Proteobacteria. Gammaproteobacteria were found to be one of major predominant group among the 5 total communities. Eleven different bacterial genera were identified within the γ-*Proteobacteria* group. Among 11 genera, *Pseudoalteromonas* was found to be predominant and followed by *Photobacterium*, *Halomonas*, *Marinobacterium*, *Vibrio*, *Gammaproteobacterium*, *Oceanisphaera*, *Pseudomonas*, *Stenotrophomonas*, *Alteromonas* and *Marinomonas*. Fig. 2 showed the phylogenetic tree constructed by using γ-*Proteobacteria* sequences obtained from this study and reference γ-*Proteobacteria* sequences obtained from the GenBank. From the phylogenetic tree (Fig. 2), among 19 *Pseudoalteromonas* strains isolated in current study, most of the *Pseudoalteromonas* 16S rDNA sequences were appeared to be very close and clustered together. Surprisingly, *Photobacterium* was found to be a second largest community within the γ-*Proteobacteria* in the current study. Seven isolates of *Photobacterium* formed a

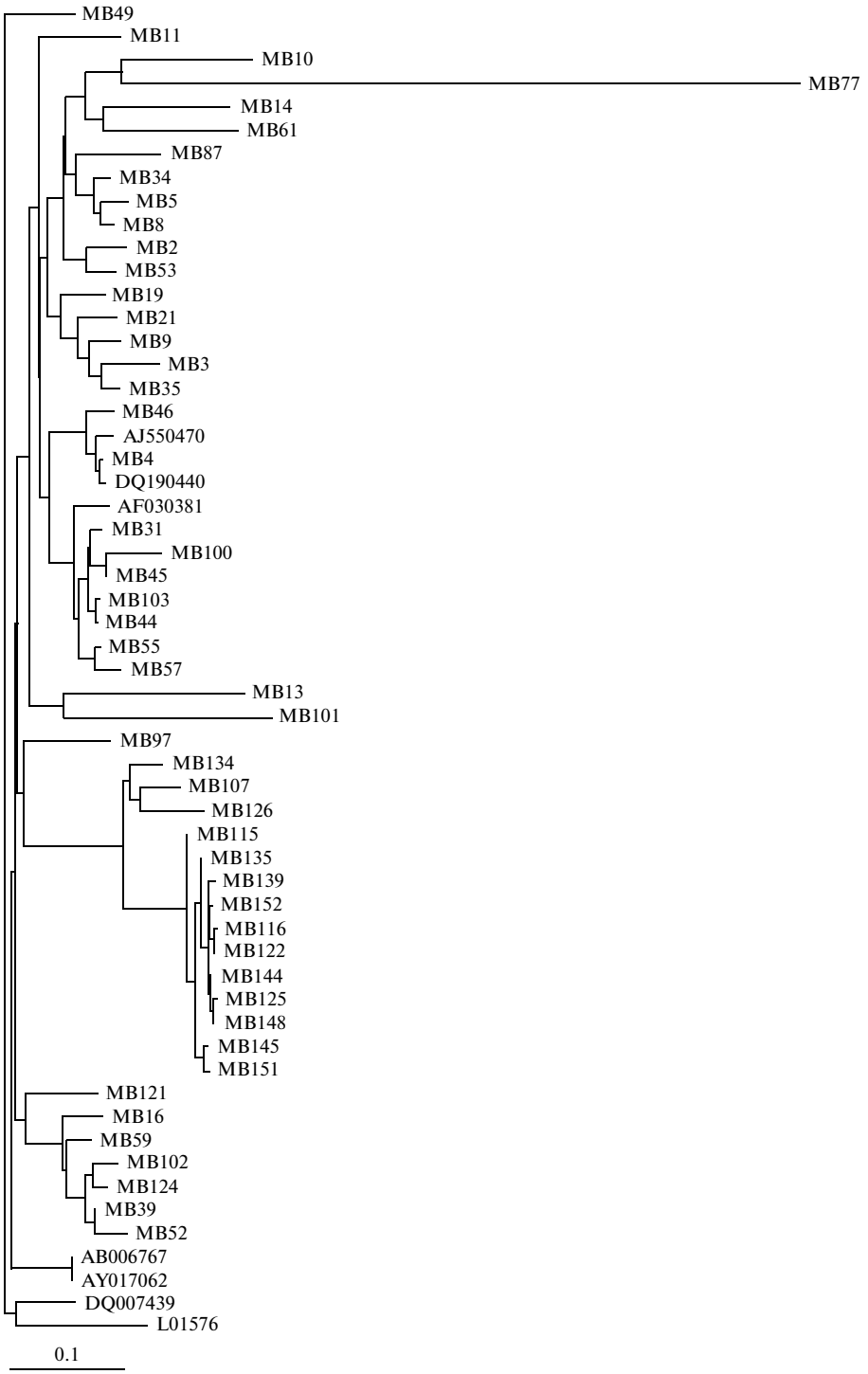

Fig. 2. Phylogenetic tree of 16S rDNA sequences of marine bacteria that are belong to γ-*Proteobacteria* family, with reference strains obtained from GenBank. Reference strains are as follows: *Oceanisphaera litoralis* (AJ550470), *Oceanisphaera* sp. (DQ190440), *Pseudomonas* sp. (AY017062), *Pseudoalteromonas* sp. (AF030381), *Oceanisphaera* sp. (AB006767), *Marinomonas* sp. (DQ007439), *Thiomicrospira* sp. (L01576).

separate cluster and shares close relationship with *Vibrio* (Fig. 2).

Firmicutes. Among 5 genera identified in *Firmicutes*, low G + C content microbe *Bacillus* was the most dominant and followed by *Sporosarcina*, *Jeotgalibacillus*, *Halobacillus* and *Caryophanon*. Fig. 3 showed the phylogenetic tree of firmicute family. *Halobacillus* and *Jeotgalibacillus* isolated in the current study, shared a clade together, separately. However, one strain of *Sporosarcina* (MB6) was separated from the *Sporosarcina* clade (MB26 & 27) (Fig. 3).

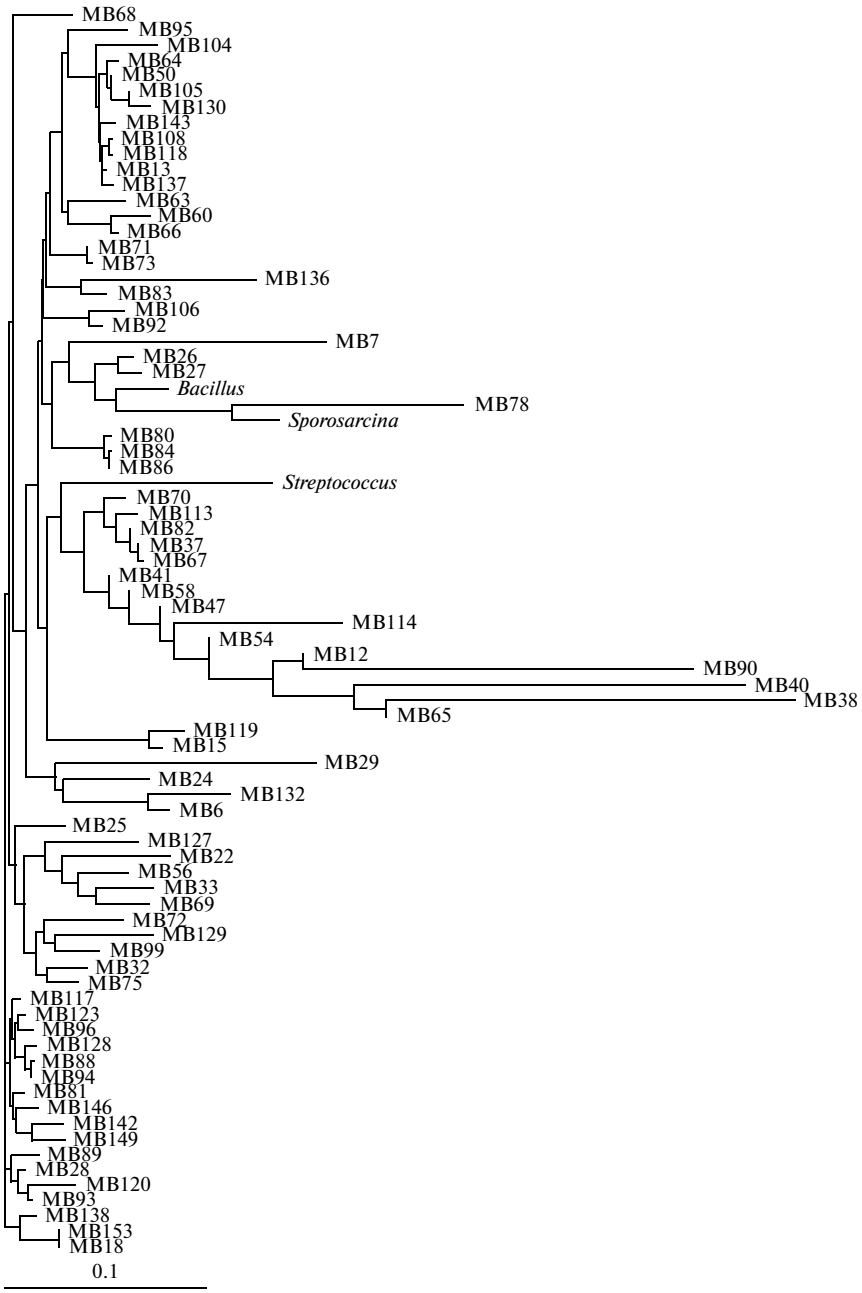

Fig. 3. Phylogenetic analysis of 16S rDNA sequencing of marine bacteria belong to *Firmicutes*, with reference sequences obtained from GenBank. Details of reference strains are as follows: *Bacillus psychrophilus* (D16277), *Sporosarcina mitis* (AF003929), *Streptococcus* sp. (AY439261).

This strain (MB6) may form a novel species. *Sporosarcina* clade shared close relationship with *Caryophanon* (MB7). *Sporosarcina* clade in phylogenetic tree (Fig. 3) was closely related with *Bacillus psychrophilus* (D16277). *Jeotgalibacillus* in this study (MB40 & 38) formed a distinctive clade and having affinity with *Marinebacillus* (MB65, 90 & 12).

High G + C content bacteria. The phylogenetic placement for high G + C content bacteria was separately showed in Fig. 4. Two strains of *Brevibacterium* were identified in this study as high G + C content bacteria and those two strains (MB140 & 150) were identical to each other and sequences were mostly similar (Fig. 4). The identical strains clade shares a strong affinity with other high G + C content bacteria *Rhodococcus erithropolis* (AM236137) and *Nocardia* sp. (DQ112024). Identical *Brevibacterium* strains were closely related with well known high G + C bacterial strains *Micrococcus* (AM403127, AM235879, AM403126), and *Kocuria* (AM778702) [15].

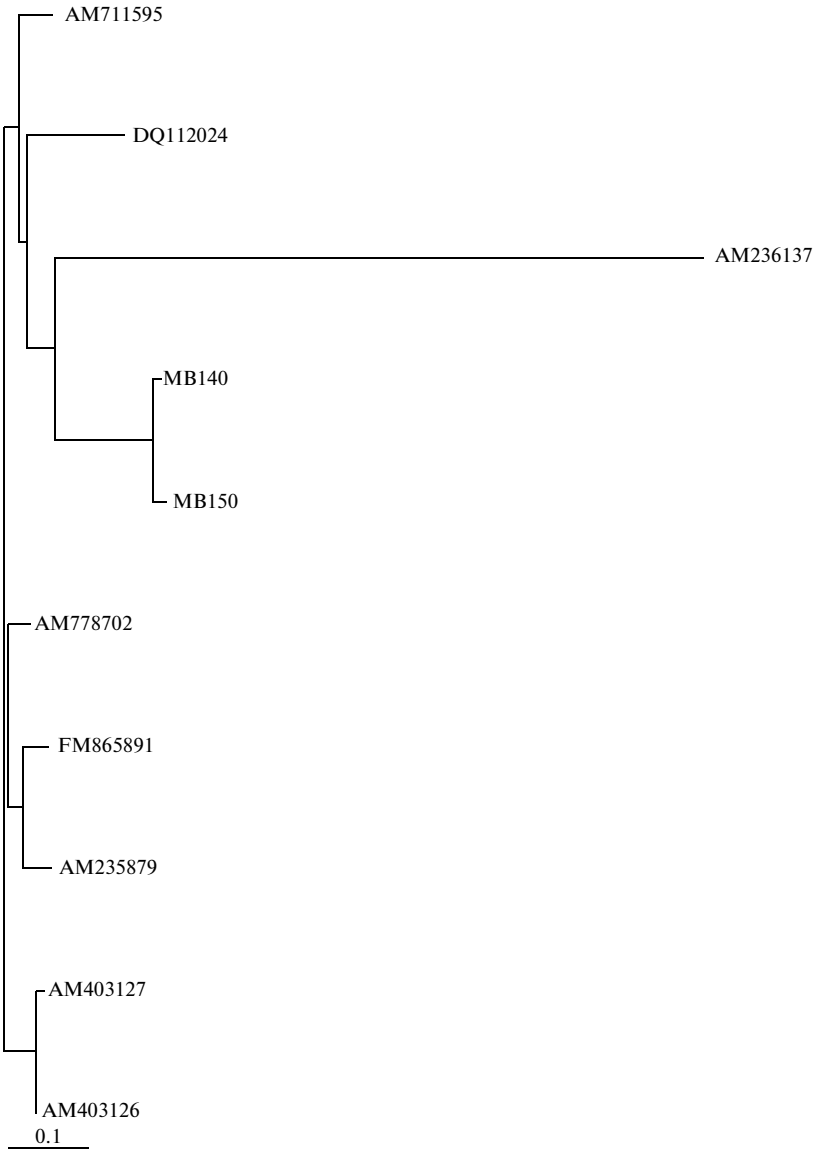

Fig. 4. Phylogenetic analysis of 16S rDNA sequencing of marine bacteria that are belong to High G + C content bacteria, with the reference sequences obtained from GenBank. The references are as follow: *Brevibacterium* sp. (AM711595), *Kocuria* sp. (AM778702), *Micrococcus* sp. (FM865891), *Micrococcus* sp. (AM403127), *Nocardia* sp. (DQ112024), *Micrococcus* sp. (AM235879), *Micrococcus* sp. (AM403126), *Rhodococcus erithropolis* (AM236137).

α-*Proteobacteria* and *Bacteroidetes*. Fig. 5 shows the phylogenetic tree of the strains belonging to the subgroup α-*Proteobacteria*. *Sulfitobacter* MB20 strain was isolated during this study and it showed strong phylogenetic affinity with *Sulfitobacter* Iso 3^T (Fig. 5). Cultured genera within this group include the species of *Sulfitobacter* and *Roseobacter*. The strain MB51 isolated was identified as *Joostella* sp. and found to be a member of *Bacteroidetes*. The strain MB51 was affiliated with *Bacteroidetes* subdivision of the marine bacterial community (Fig. 6) and may be representative of a new branch. The nearest relatives to strain MB51 were *Bizionia paragorgiae* (AY651070), *Idiomarina zobelli* (AF052741), *Tenacibaculum discolor* (AM411030), symbiont cf. *Flavobacterium* of *Tetraponera binghami* (AF459795), D-clone 128 (DQ274150), D-clone 79 (DQ274144), Uncultured sponge symbiont RSWS18 (AF434946) (Fig. 6). Quan et al. (2008) [23] described the *Joostella* sp. as a member of *Flavobacteriaceae* family.

DISCUSSION

The comparative values of total organic matter and nitrogen ratio were also given in Table 1. The organic matter and nitrogen ratio was very high in Anmyeon Island samples than Sunyu and Buan samples. This may arise due to the tideland formation or/and industrial contamination in Anmyeon Island. A large num-

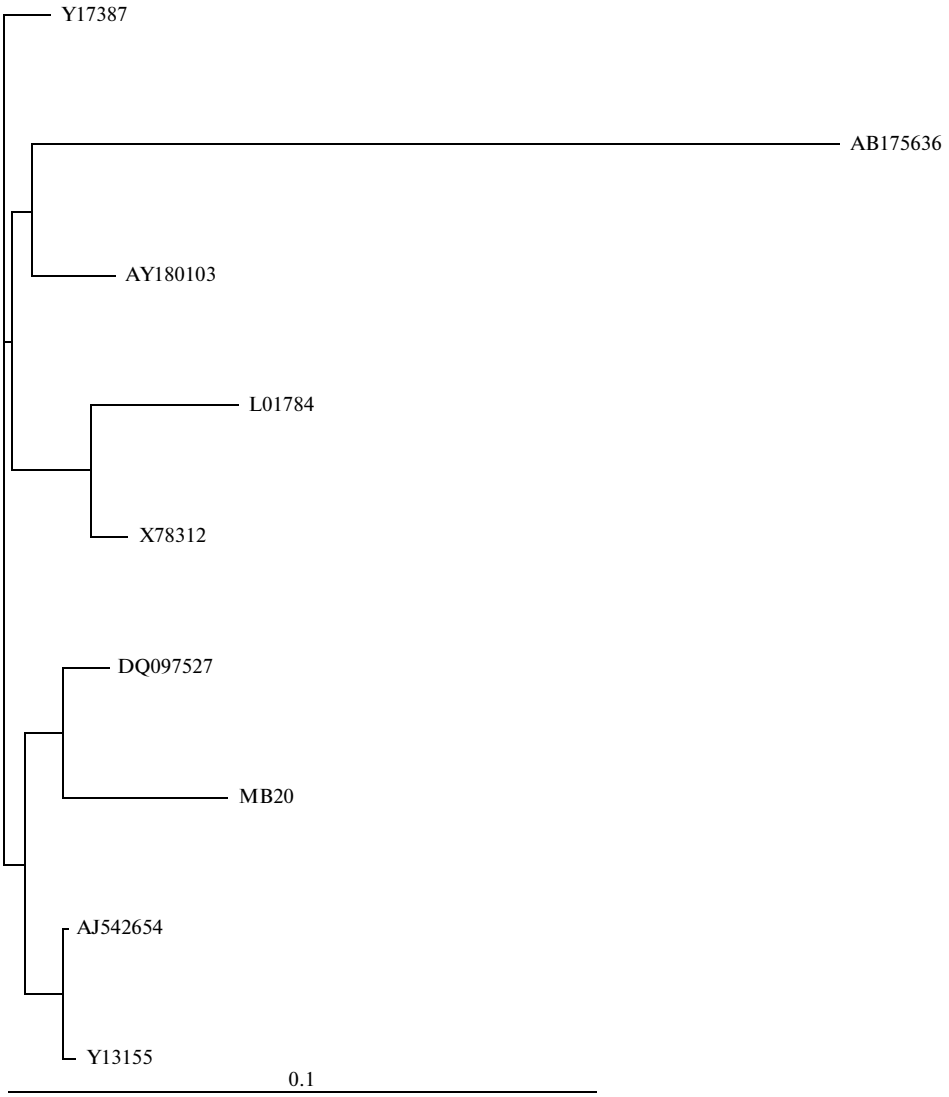

Fig. 5. Phylogenetic analysis of 16S rDNA sequencing of marine bacteria that are belong to α-*Proteobacteria*, with the reference sequences obtained from GenBank. The references are as follow: *Methylobacterium mesophilicum* (AB175636), *Sulfitobacter* Iso 3^T (DQ097527), *Sulfitobacter pontiacus* (AJ542654), *Sulfitobacter delicates* (AY180103), *Roseobacter denitrificans* (L01784), *Roseobacter litoralis* (X78312), *Sulfitobacter mediterraneus* (Y17387), *Sulfitobacter pontiacus* (Y13155).

ber of firmicutes were found in Anmyeon Island samples than other region samples. Cetecioglu et al. (2009) [6] previously reported that pH values of sediments are directly affected by the redox potential in marine sediments. The variations in pH values were showed similarity with earlier reports [6, 24]. They explained that increasing in pH (around 8) may be due to the reduction of Mn and Fe oxides and decreasing of pH in sediments may be caused by sulfate reduction, denitrification and methanogenesis processes. Sediment electron conductivity EC (ms/cm) was measured in order to understand the ion concentration of the sediment, since, marine sediments are dominated by sodium and chloride and precipitate form of these salts are contributing to the ion concentration of the sediment [25]. The overall OM/N ratio of the marine sediment is low compared to normal ratio (Table 2). Sediment aerobic bacterial community are having strong relationship to POC and PON content of sediment while weaker relationship to C/N ratio [26]. Zhou et al. (2009) [16] reported that variations among the content of organic matter, total nitrogen and C/N ratio of marine sediments collected in different points are not influencing the riches of cultivable protease-producing marine bacteria. Koster and Meyer-Reil (2001) [27] already reported that major properties of marine sediments such as, physicochemical and biological properties, could serve as a resource of adsorbed nutrients. The concentration of total nitrogen and total organic matter gives the information on the potential of microbial decomposition of organic materials in sediments and on redox condi-

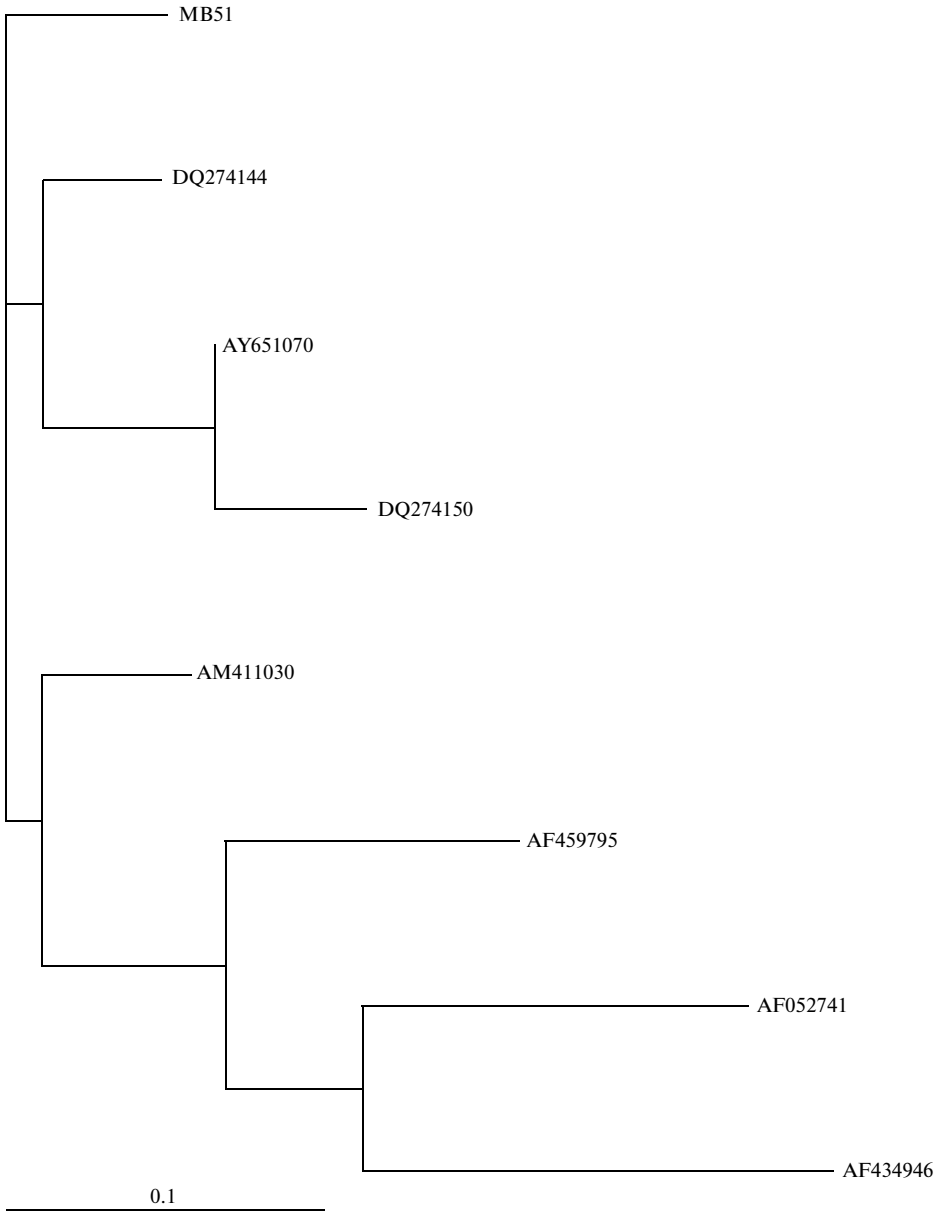

Fig. 6. Phylogenetic analysis of 16S rDNA sequencing of marine bacteria that are belong to *Bacteroidetes*, with the reference sequences obtained from GenBank. The references are as follow: *Bizionia paragorgiae* (AY651070), *Idiomarina zobelli* (AF052741), *Tenacibaculum discolor* (AM411030), symbiont cf. *Flavobacterium* of *Tetraponera binghami* (AF459795), D-clone 128 (DQ2741150), D-clone 79 (DQ274144), Uncultured sponge symbiont RSWS18 (AF434946).

tions of the sediments [6]. These results give a preliminary account on the available nutrient cycle in the marine sediments for the microbial metabolism.

Phylogenetic tree of 16S rDNA sequences from entire isolates showed that most of the described species fall under the same phylogenetic domain, however, some strains of described species were branched separately from the domain, which indicated that molecular data's of described marine bacterial strains are not fair enough to determine the taxonomy [5]. Results of molecular analysis suggested that firmicute diversity was respectively, more diverse than γ-*Proteobacteria* in sediments of Yellow Sea of South Korea. Lee et al. (1999) [5] reported that culturable bacterial isolates collected from seawater and marine coral from S. Korea were mainly predominated by high G + C content bacteria and followed by γ-*Proteobacteria*, low G + C content gram positive bacteria. It was found that molecular profile of *Firmicutes* collected at different stations was similar whereas γ-*Proteobacteria* community exhibited diverged molecular profile at every station.

Ast et al. (2007) [28], Ast and Dunlap (2005) [29] were already reported the symbiotic relationship of *Photobacterium* strain with marine substrate luminous and non-luminous deep sea fishes. The genera *Oceanisphaera*

was found to be very close with the genera *Pseudoalteromonas* in the phylogenetic tree within the γ-*Proteobacteria* community (Fig. 2). The sequence diversity of *Marinomonas* was found to be high and deeply branched within the γ-*Proteobacteria* community (Fig. 2), and most closely related with *Oceanisphaera* cluster (Gray and Herwig 1996). However, the current study γ-*Proteobacteria*, are distinguished from free living, obligately chemolithotrophic microbes *Thiomicrospira* (L01576). Our report strongly supported the previous report of Urakawa et al. (1999) [2]. Gray and Herwig (1996) [3] reported that the phylogenetic relationship between the γ-*Proteobacteria* community appeared to be very close, but these are very different from each other in phenotypical characteristics. The cold adapted genera *Pseudoalteromonas* and *Alteromonas* are well known for biological important enzyme production, such as, bacteriolytic enzymes, hemolysins and protease [16, 30, 31]. The production of bacteriolytic enzymes by *Pseudoalteromonas* could be the possible reason for their dominance among the microbial community [32, 33]. γ-*Proteobacteria* community was found common among all the sampling sites. Thus, no clear relationships between the γ-*Proteobacteria* community and physico-chemical properties of the sediments were found among the sites. This result supports Zhou et al. (2009) [16] report regarding the relationship between bacterial community and geochemical properties of the sediments. Among the genera within γ-*Proteobacteria*, *Pseudoalteromonas*, *Vibrio* and *Halomonas* were found to be pathogenic microbes [31] and *Pseudomonas*, *Stenotrophomas*, *Halomonas* and *Marinobacterium* were highly resistance to UV radiation [15].

The most frequently collected strains were *Firmicutes* in this study. In general, *Firmicutes* and γ-*Proteobacteria* are major predominant communities in all kind of marine environment. Ordonez et al. (2009) [15] reported the abundance of *Firmicutes* in extreme conditions. *Firmicutes* family in the current study comprised of *Bacillus*, *Sporosarcina*, *Jeotgalibacillus*, *Caryophanon* and *Halobacillus*. Recently, a wide range of *Firmicutes* were characterized from various sources of marine sediments and marine substrates [15, 22, 34]. It is well known that the genus *Bacillus* is phenotypically highly diverged and having a very considerable heterogeneity within genus [35]. Recently, Yoon et al. (2009) [36] isolated *Jeotgalibacillus* from marine saltern in Yellow Sea of S. Korea. Yoon et al. (2009) [36] proposed to transfer the *Marinibacillus marinus* and *Marinibacillus campisalis* into *Jeotgalibacillus* based on the rDNA sequencing and chemotaxonomic characters. *Caryophanon* (MB7) isolated in this study having phylogenetic affinity with *Sporosarcina* (MB26 & 27) strains (Fig. 3). Farrow et al. (1994) [35] reported the phylogenetic relationship between the *Bacillus* and *Caryophanon*. The marine bacteria genera's *Bacillus*, *Caryophanon* and *Sporosarcina* were sharing very close affinity in phylogenetic relationship [35]. The genus *Sporosarcina* physiologically differed from *Bacillaceae* by their following properties: coccoid or rodshaped morphology, motility and sporulation [37]. *Bacillus psychrophilus* [38] has recently been transferred to the *Sporosarcina psychrophila* [39].

Some researchers reported the presence of HGC gram positive bacteria in marine environment [40–42]. Jensen and Fenical (1995) [43] reported the abundance of gram positive bacteria in marine samples and they concluded that the growth of most of the gram positive bacteria obtained from marine environments required the supplement of marine water. Indigenous presence of gram positive high G + C content bacterial population in marine sediments was confirmed by Urakawa et al. 1999 [2] and Moran et al. 1995 [44]. Since, earlier it was not clear that either the presence of high G + C content gram positive bacteria obtained from marine sediments are indigenous or those are derived from terrestrial samples by water flow [2].

Lee et al. (1999) [5] and Park et al. (2007) [45] were already demonstrated the abundant presence of α-*Proteobacteria* in marine samples collected in S. Korea. Sorokin (1995) [46] first reported the presence of *Sulfitobacter* in Black Sea. Ivanova et al. (2004) [47] described the *Sulfitobacter* as unique marine bacteria and a member of the *Roseobacter* family. The genus *Sulfitobacter* occurs in marine and extremely saline environments containing sulfate. Sulfate reducing bacterial strains in marine sediments are metabolically diverged [48], since relative abundance of sulfate in the marine environment and those could serve as possible terminal electron acceptor [3].

CONCLUSIONS

The study shows the expended knowledge of culturable bacterial diversity in marine sediments of South Korean Yellow Sea. The results of this paper revealed the relationship between the biogeochemical properties of sediments and bacterial diversity and concluded marine bacterial consortium was highly diverged and evenly distributed among the sediments of Yellow Sea. The significances of the study were as follows: Total N and organic matter content in Yellow Sea of South Korea were relatively high and those are not affected the distribution of bacterial diversity within the sediments. *Gammaproteobacteria* and *Firmicutes* are predominant bacterial communities. Phylogenetic studies of culturable isolates are providing a better knowledge to understand the microbial diversity and community structure in marine sediments. Further research investigations are necessary in order to find out the novel bacterial species.

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