Coastal ecosystems, play critical ecological roles of which tidal flats are a significant component of coastal wetlands, such as habitat and nutrient cycling in aquatic biology. Microbial communities in tidal flats are known to play vital roles of self-purification. And the microbial ecology of the sediment is easily affected by human activities and pollution. In this paper, we applied pyrosequencing technology to investigate microbial communities in three different tidal flats (Ganghwa Island, Ongnyeon land region and Yeongjong Island) on the Incheon, Korea peninsula. A total of 16,906 sequences were obtained. We used these sequences to identify the dominant phyla in the three tidal flats: Proteobacteria, Chloroflexi, Actinobacteria, and Bacteroidetes. The composition of the bacterial community of Ganghwa Island and the Ongnyeon region were more similar to each other than they were to the bacterial community of Yeongjong Island. Simpson’s dominance index of Yeongjong Island was higher than that of the other regions, and the Shannon diversity index of this region was the lowest. Previous research of samples in these regions indicated that the three tidal flats had similar geochemical characteristics. However, their bacterial communities were rather distinct. This might be because the analysis of microbial communities and physiochemical analysis have different perspectives. Therefore, the pyrosequencing of a bacterial community with physiochemical analysis is recommended as an effective monitoring tool for the comprehensive management of tidal flats.

Key Words: bacterial diversity; microbial communities; next generation sequencing; tidal flats

Introduction

Tidal flats are located between marine ecosystems and land environments. Such regions are unique compared with marine sediments as they are flooded and exposed periodically to sea water (Wilms et al., 2006). These regions usually form several hundred meters along the coast line, and are affected by soil properties, vegetation types, and sea currents (Mariotti and Fagherazzi, 2013). They play vital ecological roles, such as promoting nutrient cycling to adjacent rivers and the seaside, and providing a habitat for aquatic organisms and migratory birds. Moreover, they also defend against flash floods, prevent erosion of coastal lines, treat environmental pollutants, and are used for recreation (Doi, 2009; Goodwin et al., 2001). Therefore, the sediment of tidal flats is easily affected by climate change, habitat destruction, and various types of pollution. In particular, due to intensive human activities, such as sewage discharge, excessive fertilizer use, land reclamation...
projects, and concentration of human civilization near coastal regions, coastal areas face serious ecosystem pollution which is reflected by over-nutrition and lower amounts of dissolved oxygen (Lee et al., 2011; Waycott et al., 2009). Tidal flats in the west coast wetlands of the Korean peninsula are known to be the fifth largest in the world, after the north coast of Europe, the wetlands of the Amazon, the east coast of Canada, and the eastern wetlands of America (Cho et al., 2004; Wilms et al., 2006; Won et al., 2017). The tidal flats of Incheon in Korea are known for their variety of geographical types. The Ganghwa Island region is close to the Han River, which flows from Seoul, the capital of Korea. Moreover, the region’s tidal flats are near tourist areas. In previous studies, this region was a typical tidal flat with a high bacterial productive capacity and total number of bacteria (Cho et al., 2004). The Ongnyeon region is located near a residential area and the public is permitted to enter it. The Yeongjong Island is famous for its huge land reclamation project for the Incheon National Airport. The ocean current of the Yeongjong Island flows from the Han Rivers as in the case of Ganghwa. However, the tidal flat of Yeongjong Island is off limits to the public (Oh et al., 2006).

The microorganisms of a tidal flat play important ecological roles in biogeochemical cycles in terrestrial ecosystems. The unusual environment factors of a tidal flat, such flooding and the salinity, results in the formation of unique bacterial communities (Duarte et al., 2008). Because of the shallow seawater, the sediment accumulates particular biogenic materials and the bacteria of a tidal flat is responsible for the biogeochemical processes in a number of ecological processes, such as mineralization of organic matter, sulfate reduction, and carbon fixation (Huber et al., 2007; Yang et al., 2003). They also serve as the diet of invertebrates and fish that live in the coastal regions. The microbial communities in tidal flats are complex and highly diverse, and interact with coastal ecosystems. There has been little research on the correlation between several physiochemical environmental factors and microbial communities in different physiographic tidal flats.

To protect the coastal ecosystem, many nations have rigorous regulations to control pollution sources which involve taking measurements of geochemical factors: chemical oxygen demand (COD), total organic carbon (TOC), total nitrogen (TN), acid volatile sulfide (AVS) etc. (Park et al., 2013). The chemical analysis has much scientific merit, such as the direct measurement of pollution components. However, the chemical analysis alone may not be suitable to predict potential risk of new chemical components (Bertold and Martin, 2003). The microorganisms respond to slight ecological changes and the community structures adapt to the new environments. However, 99.8% of the microbes on Earth could not be unculturable, it is hard to use biomonitoring tools (Amann et al., 1995). With the development of biotechnology, the rise of culture-independent techniques has allowed us to further research the microbial ecology of tidal flats. Molecular approaches that are well suited for high-resolution descriptions of the microbial communities of tidal flats includes denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), terminal restriction fragment length polymorphism (T-RFLP), etc. These methods can be used to investigate microorganism communities directly and accurately (Abdo et al., 2006; Dar et al., 2005). The recently-developed next generation sequencing technology is more efficient in terms of high throughput. Such methods are advantageous for researching genetic diversity, population structure, and the ecological role of microbiology in the environment (Petersen and Dahllof, 2005). In addition, this information can determine the taxonomic composition and relative abundances of genes (Martinez et al., 2006).

In this study, the microbial communities of three tidal flats (Ganghwa Island, Ongnyeon land region and Yeongjong Island) near Incheon on the Korean peninsula have been analyzed by pyrosequencing and the results compared with the previous geochemical assessments of these regions. This research will provide foundational data for the analysis of bacterial communities and the management of the ecosystems of tidal flats.

### Material and Methods

#### Sediment samples collection and analysis

The sediment samples were collected at three different sites in each region. The sampling sites of the Ganghwa Island region were site G_1 (N 37°35′27.90″, E 126°27′26.80″), site G_2 (N 37°35′18.18″, E 126°27′20.61″), and site G_3 (N 37°35′03.65″, E 126°27′11.36″). The sampling sites of the Ongnyeon land region were site O_1 (N 37°35′42.62″, E 126°38′03.14″), site O_2 (N 37°35′40.36″, E 126°38′03.11″), and site O_3 (N 37°35′39.10″, E 126°38′03.88″). The sampling sites of the Yeongjong Island region were site Y_1 (N 37°26′01.58″, E 126°28′09.55″), site Y_2 (N 37°25′54.43″, E 126°28′14.83″), and site Y_3 (N 37°25′39.38″, E 126°28′26.50″) (Fig. 1). At each site, 5 g of sediment was

![Fig. 1. Map of the sampling sites of tidal flats in Incheon. Sampling sites are marked with circle on the map.](image-url)
collected using a hand core sampler (Wildco, Yulee, FL, USA) at 30 cm below the surface layer and transported back to the laboratory on dry ice. The samples were stored at –70°C until ready for further analysis. Three site samples from each region were homogenized for experiments in the laboratory.

**DNA extraction and pyrosequencing.** For the pyrosequencing analysis, the DNA of the microbial communities was extracted from the total 15 g tidal flat sediment sample using Fast DNA SPIN Kit (MP Biomedicals, Santa Ana, CA, USA), according to manufacturer’s instructions. The DNA concentration was quantified using Santa Ana, CA, USA, according to manufacturer’s instructions. The DNA concentration was quantified using NanoQuant Infinite M200 PRO (Tecan, Switzerland). The DNA extraction and pyrosequencing.

**Results**

**Sequencing statistics and diversity estimates**

A total of 16,906 qualified pyrosequencing reads were obtained from tidal flat samples from the three different regions. The numbers of validated sequence reads were 5,847 (Ganghwa Island), 5,055 (Ongnyeon land region), and 6,004 (Yeongjong Island). Using a 3% sequence cut-off value, the OTU number ranged from 1,659 to 1,924 across all samples, with the Ongnyeon region harboring the fewest OTUs among the sediments. The Good’s coverage of microbial communities in tidal flat samples was calculated at the OTU level. The Shannon index, Simpson index, and Rarefaction curve were calculated using the Mothur platform (Heck et al., 1975; Schloss et al., 2009). The beta diversity, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was performed for comparing species diversity in each tidal sample using the Fast UniFrac (http://www.r-project.org/). The Fast UniFrac distance was the variant of the original UniFrac algorithm using the Eztaxone (http://www.ezbiocloud.net/taxonomy) structure as the backbone phylogenetic tree. (Altschul et al., 1990; Kim et al., 2012). The following cutoff values were used for taxonomic assignment: species (X ≥ 97%), genus (94 > X ≥ 97%), family (94% > X ≥ 90%), order (90% X ≥ 85%), class (85% X ≥ 80%), and phylum (80 % X ≥ 75%) (Chun et al., 2007; Park et al., 2012).

All statistical analyses of microbial communities were performed using CLcommunity software (Chunlab, Inc., Seoul, South Korea). The operational taxonomic units (OTUs) were defined with the CD-Hit programs at 3% sequence dissimilarity (Li and Godzik, 2006). The alpha diversity of microbial communities in tidal flat samples was calculated at the OTU level. The Shannon index, Simpson index, and Rarefaction curve were calculated using the Mothur platform (Heck et al., 1975; Schloss et al., 2009). The beta diversity, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was performed for comparing species diversity in each tidal sample using the Fast UniFrac (http://www.r-project.org/). The Fast UniFrac distance was the variant of the original UniFrac algorithm using the Eztaxone (http://www.ezbiocloud.net/taxonomy) structure as the backbone phylogenetic tree. (Altschul et al., 1990; Kim et al., 2012).

**Table 1.** Statistical analyses.

|                      | Ganghwa Island | Ongnyeon | Yeongjong Island |
|----------------------|----------------|----------|------------------|
| Number of validated reads | 5847           | 5055     | 6004             |
| Number of OTUsc      | 1924           | 1659     | 1763             |
| Shannon              | 6.79 ± 0.03    | 6.68 ± 0.03| 6.45 ± 0.03      |
| Simpson              | 0.0028 ± 0.0002| 0.0029 ± 0.0002| 0.0055 ± 0.0004  |
| Good’s coveraged     | 0.81           | 0.83     | 0.81             |

aOTUs: Operational Taxonomic units.
bShannon: Shannon diversity index (>=0, higher, more, diverse).
cSimpson: Simpson diversity index (0-1, 0 = most simple).
dGood’s coverage: 1–(number of singleton OTUs/number of sequences), 1 = 100% coverage.
communities. The structures of bacterial communities in Ganghwa Island and Ongnyeon were more similar than those of Yeongjong Island (Fig. 3).

**Bacterial community composition from the three different regional sediments**

Relative abundance analysis showed that the phylum **Proteobacteria** was the most dominant phyla, covering 41.16% to 49.08% of the total sequence reads (Fig. 4). The relative abundance of most phyla was quite similar across the three samples. The exceptions were **Chloroflexi** and **Bacteroidetes**, in which the Yeongjong Island sample was very different from the other two site samples. In the **Proteobacteria** phyla, the **DeltaProteobacteria** including various sulfate reducing bacteria was the most abundant class in the three tidal flats. The **AlphaProteobacteria** and **GammaProteobacteria** were also abundant phylum. The **EpsilonProteobacteria**, which including various sulfur oxidizing bacterium, was comparatively more abundant at the Ongnyeon region (4.36%) than at Ganghwa Island (0.73%) and at Yeongjong Island (0.18%).

At the class level, the Ganghwa Island sample consisted predominantly of **DeltaProteobacteria** (16.22%), **GammaProteobacteria** (15.34%), **Anaerolineae** (10.00%), **AlphaProteobacteria** (8.18%) and **Caldilineae** (5.28%). The Ongnyeon region sample showed predominance of **DeltaProteobacteria** (18.42%), **Anaerolineae** (10.62%), **GammaProteobacteria** (10.33%), **Caldilineae** (8.14%), and **AlphaProteobacteria** (7.66%) classes, similarly. The Yeongjong Island sample showed predominance of **DeltaProteobacteria** (20.86%), **GammaProteobacteria** (18.23%), **Flavobacteria** (10.95%), and **AlphaProteobacteria** (9.40%) classes. The bacterial communities of the Ganghwa Island and the Ongnyeon region had more similar patterns than those of Yeongjong Island at the class level. In particular, the proportion of

![Rarefaction curves for Operational Taxonomic Units (OTUs) from Ganghwa Island, Ongnyeon and Yeongjong Island. OUTs were clustered 3% dissimilarity using CD-Hit.](image)

**Fig. 2.** Rarefaction curves for Operational Taxonomic Units (OTUs) from Ganghwa Island, Ongnyeon and Yeongjong Island. OUTs were clustered 3% dissimilarity using CD-Hit.

![UPGMA phylogenetic tree.](image)

**Fig. 3.** UPGMA phylogenetic tree.

![Taxonomic composition of phyla from Ganghwa Island, Ongnyeon region and Yeongjong Island. The bar graph means the percentage of the phylum and the circle graph express the composition of the Proteobacteria phylum.](image)

**Fig. 4.** Taxonomic composition of phyla from Ganghwa Island, Ongnyeon region and Yeongjong Island. The bar graph means the percentage of the phylum and the circle graph express the composition of the Proteobacteria phylum.
Flavobacteria class in Yeongjong Island (10.95%) was approximately two times higher than Ganghwa Island (4.38%) and the Ongnyeon region (5.39%).

The two classes, Anaerolineae and Caldilineae of the phylum Chloroflexi, in Yeongjong Island (3.63% and 1.67%, respectively) were significantly less than those of Ganghwa Island (10.00% and 5.28%, respectively) and the Ongnyeon region (10.62% and 8.14%, respectively). The Actinobacteria of the phylum Actinobacteria (3.97%), Clostridia of the phylum Firmicutes (5.33%), and EpsilonProteobacteria of the phylum Proteobacteria (4.36%) in the Ongnyeon region were more abundant than those in the other two regions, while the Acidimicrobiota of phylum Actinobacteria was exceptionally low (Table 2).

At the order level, the microbe at Ganghwa Island consisted predominantly of the Anaerolineae (9.41%), Xanthomonadales (8.33%), Caldilineae (5.21%), Rhizobiales (4.52%), and Flavobacteriales (4.30%). The bacterial community in the Ongnyeon region comprised the Anaerolineae (9.72%), Caldilineae (8.04%), Desulfobacteriales (6.00%), Flavobacteriales (5.37%), and Clostridiales (4.98%) orders. The microbiology of Yeongjong Island was composed predominantly of the Flavobacteriales (10.87%), Desulfuromonadales (9.63%), Xanthomonadales (9.05%), and Rhizobiales (6.34%) orders. The order Campylobacterales (4.36%) in sediment from the Ongnyeon region was more plentiful than those of Ganghwa Island (0.73%), and Yeongjong Island (0.18%). While, the Acidimicrobiales (0.99%) order in the Ongnyeon region was significantly low of the three regions (Table 3).

At the family level, the Ganghwa Island sample showed predominance of the Anaerolinaceae (7.46%), Flavobacteriaceae (4.01%), and Hyphomicrobiaceae (3.25%) families. The Ongnyeon region was dominated by the Anaerolinaceae (8.29%), Flavobacteriaceae (5.11%) and Helicobacteraceae (4.36%) families. The Yeongjong Island samples were predominated by Flavobacteriaceae (10.59%), Desulfuromonadales (9.12%), and Hyphomicrobiaceae (3.64%) families. The family of Desulfuromonadales in Yeongjong Island was more abundant, and the family of Anaerolineae in Yeongjong Island was significantly less abundant than the other two regions. On the other hand, the abundance of Helicobacteraceae family in the Ongnyeon region was 40 times higher than the other two regions (Table 4).

At the genus level, the taxonomic compositions of only 391 genera that have a scientific name were analyzed. The microbial community at Ganghwa Island was composed of uncultured genera of Anaerolineae (1.4%), Chloroflexi phylum and of Flavobacteriaceae (0.4%), Bacteroidetes phylum. The microbial community in Ongnyeon region predominated by unidentified genera of Anaerolineae (1.5%) and the genus Robiginitalea (0.4%), Bacteroidetes phylum. The bacterial community of Yeongjong Island

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**Table 2.** Taxonomic composition of bacterial class level from Ganghwa Island, Ongnyeon land, and Yeongjong Island.

| Taxonomy | Ganghwa Island | Ongnyeon | Yeongjong Island |
|----------|----------------|-----------|------------------|
|          | % Fr            | % Fr      | % Fr             |
| Actinobacteria_Acidimicrobia | 3.03 179 | 0.99 50 | 3.69 230 |
| Actinobacteria_Acidimicrobia | 9.46 57 | 3.97 201 | 0.50 31 |
| Acidobacteria_Holophagae | 4.93 291 | 3.44 174 | 3.32 207 |
| Bacteroidetes_Flavobacteria | 4.38 259 | 5.39 273 | 10.95 682 |
| Chloroflexi_Anaerolineae | 10.00 591 | 10.62 538 | 3.63 226 |
| Chloroflexi_Caldilineae | 5.28 312 | 8.14 412 | 1.67 104 |
| Firmicutes_Clostridium | 2.29 135 | 5.33 270 | 2.26 141 |
| Proteobacteria_AlphaProteobacteria | 8.18 483 | 7.66 388 | 9.40 586 |
| Proteobacteria_EpsilonProteobacteria | 0.73 43 | 4.36 22 | 0.18 11 |
| Proteobacteria_DeltaProteobacteria | 16.22 938 | 18.42 933 | 20.86 1300 |
| Proteobacteria_GammaProteobacteria | 15.34 906 | 10.33 523 | 18.23 1136 |
| Total | 71.33 462 | 78.65 406 | 74.69 492 |

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**Table 3.** Taxonomic composition of bacterial order levels from Ganghwa Island, Ongnyeon land, and Yeongjong Island.

| Taxonomy | Ganghwa Island | Ongnyeon | Yeongjong Island |
|----------|----------------|-----------|------------------|
|          | % Fr            | % Fr      | % Fr             |
| Actinobacteria_Acidimicrobiota_Acidimicrobia | 3.03 179 | 0.99 50 | 3.66 228 |
| Bacteroidetes_Flavobacteria_Flavobacteriales | 4.30 254 | 5.37 272 | 10.87 677 |
| Chloroflexi_Anaerolineae_Anaerolineae | 9.41 556 | 9.72 492 | 3.45 215 |
| Chloroflexi_Caldilineae | 5.21 308 | 8.04 407 | 1.65 103 |
| Firmicutes_Clostridium_Clostridiales | 2.22 131 | 4.98 252 | 2.15 134 |
| Proteobacteria_AlphaProteobacteria_Rhizobiales | 4.52 267 | 3.87 196 | 6.34 395 |
| Proteobacteria_AlphaProteobacteria_Rhodobacteriales | 2.49 147 | 3.28 166 | 2.34 146 |
| Proteobacteria_DeltaProteobacteria_Desulfobacteriales | 3.54 209 | 6.00 304 | 3.31 206 |
| Proteobacteria_DeltaProteobacteria_Desulfuromonadales | 3.05 180 | 4.01 203 | 9.63 600 |
| Proteobacteria_EpsilonProteobacteria_Campylobacteriales | 0.73 43 | 4.36 22 | 0.18 11 |
| Proteobacteria_GammaProteobacteria_Xanthomonadales | 8.33 492 | 4.36 221 | 9.05 564 |
| Total | 46.82 271 | 54.98 278 | 52.62 318 |
Table 4. Relative abundance of family level from Ganghwa Island, Ongnyeon and Yeongjong Island.

| Taxonomy                                      | Ganghwa Island | Ongnyeon | Yeongjong Island |
|-----------------------------------------------|----------------|----------|-----------------|
|                                               | % Fr           | % Fr     | % Fr            |
| Bacteroidetes_Flavobacteria_Flavobacteriales  | 4.01 237       | 5.11 259 | 10.59 660       |
| Chloroflexi_Anaerolineae_Anaerolineae         | 7.46 441       | 8.29 420 | 2.28 142        |
| Proteobacteria_AlphaProteobacteria_Rhodobacterales | 3.25 192   | 2.69 136 | 3.64 227        |
| Proteobacteria_DeltaProteobacteria_Desulfurimonadales | 2.49 147 | 3.28 166 | 2.31 144        |
| Proteobacteria_EpsilonProteobacteria_Campylobacteriales_Helicobacteriales | 0.73 43   | 4.36 221 | 0.14 9          |
| Total                                         | 20.70          | 27.43    | 28.09           |

Table 5. The number of bacterial taxa observed in Ganghwa Island, Ongnyeon and Yeongjong Island.

| Pylum | Ganghwa Island | Ongnyeon | Yeongjong Island |
|-------|----------------|----------|-----------------|
|       | C* UC**        | C* UC**  | C* UC**         |
| Pylum |                |          |                 |
| Class | 5844 3         | 5055 0   | 6001 3          |
| Order | 4775 1072      | 4290 765 | 5234 770        |
| Family| 3715 2132      | 3654 1401| 4308 6004       |
| Genus | 1997 3850      | 2258 2979| 2710 3294       |
| Species| 310 5537      | 413 4642 | 397 5607        |
|       | 273 5574      | 379 4676 | 339 5665        |

was also predominated by uncultured genera of Flavobacteriaceae (0.8%) and Anaerolineae (0.4%).

Discussion

In the present study, bacterial communities in the sediments of three different regions (Ganghwa Island, the Ongnyeon region and Yeongjong Island) were examined using high throughput DNA pyrosequencing of the V1–V3 region of the 16S rRNA gene. The numerical values of Good’s coverage mean in three tidal flats were 0.81 (Ganghwa Island and Yeongjong Island) and 0.83 (Ongnyeon region). This means that the data’s effectiveness was over 80%. The Shannon index and rarefaction curve indicated that bacterial diversity in Ganghwa Island was higher than that of the other two regions. In contrast, Yeongjong Island was the least diverse according to the Simpson dominance index and rarefaction curves. There were fewer dominant bacterial taxa in the Yeongjong tidal flats. The bacterial community structures in Ganghwa Island and the Ongnyeon region were more similar to each other than those of Yeongjong Island, by UPGMA. The dominant phyla were Proteobacteria, Chloroflexi, Actinobacteria, and Bacteroidetes in all three tidal flats. In the previous studies, the dominant phyla in the coastal region (Sichang Island of Thailand and Dokdo Island of Korea) were also Proteobacteria, Actinobacteria, and Bacteroidetes (Kim et al., 2014; Shokralla et al., 2012). The Proteobacteria, especially DeltaProteobacteria and GammaProteobacteria, were found to be the most abundant in all three sediments. Won et al. (2017) also showed that the Delta- and GammaProteobacteria were dominant in the sediment and deep sea samples from Korea. Two abundant orders of Deproteobacteria, Desulfbacterales in the Ongnyeon region and Desulfurimonadales in Yeongjong Island, include sulfate-reducing bacteria and sulfur-oxidizing bacteria, respectively. Peng et al. (2017) showed that the Desulfobacterales from sediments played a key role in sulfur cycling (Wu et al., 2016; Yin and Kwang, 2016). The sulfate-reducing bacteria decompose sulfate to sulfides to obtain energy. Because the EpsilonProteobacteria and Desulfobacteriales were abundant in the Ongnyeon region, suggesting active sulfur metabolism occurred in the site. The order Xanthomonadales of GammaProteobacteria in Ganghwa Island and Yeongjong Island was more abundant than in the Ongnyeon region. The order Rhodobacteriales in the phylum Proteobacteria existed in the three tidal flat at similar levels. Several species in Rhodobacteriales is known for performing the reduction of nitrite to nitrous oxide and using cbb3-type terminal oxidase, which has high affinity to oxygen, for aerobic respiration in tidal cycling in a laboratory chemostat (Chen et al., 2017). The environments of tidal sediments, in which aerobic, microaerobic and anaerobic regions can exist, may be advantageous for microorganisms using several respiratory mechanisms. The relative abundance of phylum Chloroflexi was much higher in the Ongnyeon region and Ganghwa Island. The phylum Chloroflexi widely existed in deep sea sediments, including coastal regions in southern Korea and this phylum is critical in the decomposition of organic matter (Hug et al., 2013). The two orders, Anaerolineae and Caldilineae, in the phylum Chloroflexi were prevalent in this study. The phylum Bacteroidetes was much more abundant in Yeongjong Island than the other two sites. In particular, the order Flavobacteriales in Yeongjong Island was more than twice as in the abundant as other regions. The order
Flavobacteriales was found to be associated with phytoplankton in marine environments and contributed to organic matter degradation (Abbasian et al., 2015; Fernández et al., 2013).

Previously, a study by Park et al. (2013) assessed the environmental health of samples from these three tidal flats with environmental chemical factors such as AVS, COD, and TOC. In their researches, the AVS values of Ganghwa and Yeongjong Island were 0.04 ± 0.01/g identically and the value of the Ongnyeon region was 0.06 ± 0.02/g. The AVS of sediment represented the operational pool of sedimentary substances generating H2S gas upon the addition of HCl and indicated the oxygen deficiency in the sediment. The contents of AVS in a sediment might be related to the abundance of anaerobic sulfate reducing bacteria such as those in the order Desulfobacterales in the Ongnyeon region. In addition, EpsilonProteobacteria including various sulfur oxidizing bacteria, are also particularly more abundant in the Ongnyeon region than in the other two regions. These results may suggest that the sulfur metabolism is one of key pathways in the microbial community of Ongnyeon region. While, the Desulfuromonadales order, which was known for the capability of anaerobic respiration by various compounds as an electron acceptor, including sulfur, was more plentiful in Yeongjong Island than in the Ganghwa region (Garrity et al., 2006).

All the organic concentration indices of the three tidal flats in their study were similar values or were much lower than those of the environmental pollution standards of Korea (Park et al., 2013). Therefore, they concluded that all three regions had similarly good benthic ecological environments and were protected from environmental pollutants by chemical analysis. However, as we have seen above, the bacterial composition was clearly distinct, in particular between Ganghwa Island and the Ongnyeon region versus Yeongjong Island. It is known that microorganisms have demonstrated a variety of evolutionary adaptations and physiological acclimations in the face of ecological stress and change (Schimel et al., 2007). For instance, human disturbances such as soil mining and land reclamation, can cause a remarkable loss of microbial physiological responses and changes in the community composition (Bertold and Martin, 2003; Prosser et al., 2007). Most of the sequences were classified at the phylum level in the three tidal flats, but the classified reads considerably decreased in number from the phylum down to the species. Therefore, the pyrosequencing method must be actively applied when researching tidal flats with environmental chemical factors. Further studies are required which examine the specific correlation between chemical analysis and microbial analysis. Microbial analysis, together with geochemical analysis, will provide more accurate and comprehensive data to assist in tidal flat management.

Conclusion

In this study, we used pyrosequencing to analyze the bacterial communities in three tidal flat regions in Incheon. The dominant phyla of the three tidal flats were Proteobacteria, Chloroflexi, Actinobacteria, and Bacteroidetes. Physicochemical profiles were not very distinctive among the tidal flat regions, but the bacterial communities of Ganghwa Island and the Orkyun region were distinct from those of Yeongjong Island. Considering the importance of comprehensive monitoring and assessment of the status of tidal flats, next-generation sequencing of bacterial communities may prove to be an effective monitoring tool.

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