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Bacterial Community Structure of Aquaculture and Non-aquaculture Sediments of Taal Lake (Philippines) using PCR-DGGE of 16S rDNA

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RESEARCH ARTICLE

Abstract

Background and Objective: Microorganisms, including bacteria, serve as major players in various processes affecting both the quality of aquatic sediment as well as the fate of pollutants released into such matrix. This study, evaluated the similarity in bacterial community structure between sediments collected from aquaculture and non-aquaculture sites of a tropical lake. Describing and comparing the bacterial community present in each site may provide clues on the impact of aquaculture practices on aquatic ecosystems.
Methodology: Microbial DNA was extracted using PowerSoil® DNA Isolation Kit for all sediment samples. DNA isolates were used as template in the analysis of the hypervariable region of 16S rDNA through nested polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE). Excised representative 16S rDNA DGGE bands were sequenced and identified through BLAST analysis.
Results: Based on the generated mean Dice similarity coefficient of 57.77%, the bacterial community structure between aquaculture and non-aquaculture sediments was highly similar but certain taxa were found unique for each site. Bacteria belonging to *Proteobacteria* and *Firmicutes* dominated the aquaculture sediments while *Proteobacteria, Firmicutes,* and *Chloroflexi* dominated the non-aquaculture sediments. Certain physico-chemical parameters operating in the two sites may have influenced the shift in representative microbes. *Shewanella baltica* and *Trichococcus* sp. were found only in aquaculture sediment owing to their ability to tolerate quantities of ammonia and high organic matter from their environment.
Conclusions: This study described the applicability of 16S rDNA PCR-DGGE as a culture-independent technique for describing and comparing the similarity between bacterial communities in sediment. Based on the generated similarity index, the bacterial community between aquaculture and non-aquaculture sediments of Taal Lake was highly similar but interestingly, harbored unique bacterial populations as seen in the DGGE profiles. The shift in dominant taxa and unique representatives per site may have been influenced by certain differences between each site's physico-chemical parameters.

Keywords: *sediment bacterial community, aquaculture, 16S rDNA, Dice coefficient*

Introduction

Microorganisms, including bacteria, numerically and biochemically dominate inland waters, such as lakes, ponds, and rivers. Most of these microorganisms are key players in the biogeochemical processes (*i.e.* metabolism of dissolved organic carbon and/or nitrogen cycling), which are crucial processes for entire ecosystems [1]. Furthermore, microorganisms have critical roles in processes controlling the water quality of aquatic habitats and are crucially involved in the fate of pollution released to the environment.

Microbial communities in lakes and other aquatic ecosystems are constantly subjected to disturbances. Aquaculture, the farming of aquatic organisms including fish, mollusks, crustaceans, and aquatic plants, is a common practice that brings about changes in the aquatic ecosystem.

Aquaculture practices in lakes involve introduction of chemicals through the use of feeds and accumulation of fish wastes causing a significant impact on the current ecosystem particularly the accumulation of organic matter. Previous studies have clearly demonstrated that disturbances induced by increasing organic loads in coastal areas regulates community structure and biodiversity changes of biotic assemblages both in soil and water. Due to altered conditions in aquaculture environment, the presence of pathogenic bacteria, antibiotic-resistant strains, or new bacterial species was also observed [2,3,4].

A number of genomic approaches has greatly advanced the understanding of the ecology and diversity of microbial communities in aquatic environments. Together with polymerase chain reaction (PCR), fingerprinting methods like denaturing-gradient gel electrophoresis (DGGE), obtain a qualitative representation of the presence and abundant phylotypes in a given sample [5]. By profiling the composition and structure of microbial communities, these techniques are valuable for tracking genotypic community changes over time, as well as for comparative analysis of microbial community profiles inhabiting different environments [5]. Changes in microbial communities reflect changes in the over-all aquatic ecosystem, thus, monitoring genotypic community shift is significant to assess the effects and impacts of various disturbances in the environment.

In this study, bacterial community profiles of sediments obtained from aquaculture and non-aquaculture sites of Taal Lake in Batangas, Philippines were investigated through PCR-DGGE and compared using Dice similarity coefficient. Given the biogeochemical roles that microorganisms play, describing the bacterial populations present in the two sites may provide clues as to the impact of aquaculture on inland water ecosystems.

Methodology

Sampling and Sample Processing

Composite sampling was conducted at Taal Lake; specifically, at Barangay Gonzales, Tanauan City (N 14°3.838' E 120°56.266'), and Barangay Balakilong, Laurel (N 14°4.069' E 121°4.342'). The first area, which is an open water fishing site, served as the non-aquaculture site, whereas Balakilong served as the study site with aquaculture activities. Local divers collected surface sediments using a grab sampler at ten random sampling points in each of the study sites. For the aquaculture site, the sampling points were set around the perimeter of fish cages. The discrete sediment samples were then manually mixed using a hand trowel inside a plastic pail to create a composite sample. One kilogram of the composite sample was stored inside double airtight, polyethylene zip lock bags and kept dark and cold (about 1-4°C) inside a portable ice cooler following the guidelines listed by the U.S. Environmental Protection Agency and the Resources Information Standards Committee of the British Columbia Integrated Land Management Bureau. Aliquots of the samples were subjected to physical and chemical analysis. The rest of the samples were eventually stored at -20°C in the laboratory until further use. Sampling was done on two occasions under the same good weather conditions.

Identification of Sediment Bacteria

Lake sediment microbial DNA was extracted using PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc). The extracted genomic DNA was subjected to nested PCR. The first PCR was performed to generate a 1.5 kilobase pair (kbp) 16S rDNA amplicon, while the second PCR was conducted to amplify the 585 base pair (bp) 16S rDNA internal variable region. A pair of universal 16S rDNA PCR primers, 8f (5' AGA GTT TGA TCC TGG CTC AG 3') and 1492r (5' GGT TAC CTT GTT ACG ACT T 3'), was utilized to generate the 1.5 kbp amplicon. In the second PCR, the 585 bp 16S rDNA internal variable region was amplified using the 1.5 kbp PCR product as template and 341f-GC (5' CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GCC TAC GGG AGG CAG CAG 3') and 926r (5' CCG TCA ATT CCT TTG AGT TT 3') as primers.

For the DGGE proper, a gradient of 30% to 60% denaturants was used to separate the sediment bacterial community amplicons. The DGGE profile images of the aquaculture and non-aquaculture samples were analyzed using the Quantity One Software (Biorad) to assess bacterial diversity and obtain similarity coefficients. The Dice similarity coefficient generated for each DGGE gel was averaged. Similarity coefficient values range from 0 to 100. A value of 0 indicates total difference between the two species assemblages. In contrast, a value of 100 indicates that they are identical. Index values greater than 75 suggests very high similarity; values in the range of 51 and 75 implies high similarity; 26-50 signifies moderate similarity; and values 25 and below imply low similarity [6].

Excised bands from DGGE profiles were reamplified and sent to Macrogen, Inc. in Korea for purification and sequencing. The resulting sequences were compared with those available in GenBank via Basic Local Alignment Search

Tool (BLAST) program to determine their approximate phylogenetic affiliation and 16S rRNA gene sequence similarities. The 16S ribosomal RNA sequences database was selected instead of the Nucleotide collection to account for the DNA source as being from environmental samples. BLAST has been done three times for all sequences to ensure that the identified bacterial species were up-to-date with the GenBank sequence database.

Results and Discussion

Bacterial Community Structure of Aquaculture and Non-aquaculture Sediments

Selected physico-chemical parameters obtained from both sites revealed differences that may influence presence of certain nutrients that microbial groups require for growth or chemical that may influence decrease in density (Table 1). For instance, higher levels of organic carbon, organic matter, and total ammonia were recorded for the aquaculture site.

Based on the DGGE profile, sediments from aquaculture and non-aquaculture sites exhibited banding patterns with comparable number of bands, indicating similarity between sediment types (Plate 1 and Plate 2). Some bands were present in both aquaculture and non-aquaculture groups but occurred more frequently in one site. These ubiquitous bands represent bacterial species shared by the aquaculture and non-aquaculture sites and potentially represent the inherent species thriving in the Taal Lake sediments. On the other hand, site-specific yellow bands represent bacterial species that prefer one site as its niche due to the presence or absence of certain nutrients or conditions necessary for its colonization in that given site. Though the obtained DGGE profiles only represent the dominant bacterial populations in the community and may not reflect the complete bacterial community richness in the aquaculture and non-aquaculture sites, the band intensity can still mirror the relative density of a PCR product from a sample [7]. Thus, it gives an overall picture of microorganisms that predominate a given niche.

The mean Dice similarity coefficient between aquaculture and non-aquaculture sediments was 57.77% (71.76% for gel 1 and 43.79% for gel 2) indicating that the bacterial communities between the two sites are highly similar [6]. This may be attributed to the predominant core bacterial groups that are present in both aquaculture and non-aquaculture sediments, as shown by the several recurring (matched common) bands in the DGGE profiles. The high similarity may also be attributed to the low number of distinct bands as shown in Table 2.

Comparison of 16S rDNA sequences with sequences available in the GenBank database revealed that aquaculture sediments harbor bacteria under the *Proteobacteria* and *Firmicutes* phyla, whereas non-aquaculture sediments harbor members of the *Proteobacteria, Firmicutes,* and *Chloroflexi* phyla. Despite the observed similarity in the bacterial populations, a shift in *Chloroflexi* may be due to the marked differences in the physicochemical conditions of the two types of sediments (Table 1) favoring the growth of this microbial group. Recently, a 16S rRNA gene profiling reported that members of the bacterial phylum *Chloroflexi* are common in sediment and have potential roles in sediment carbon cycling beyond organohalide respiration to include respiration of sugars, fermentation, CO2 fixation, and acetogenesis with ATP formation by substrate-level phosphorylation [8]. It was also previously reported that there was an observed decrease in

Table 1. *Selected physicochemical parameters of the sampled aquaculture and non-aquaculture sediments from Barangay Balakilong and Barangay Gonzales, respectively (as analyzed by the Natural Sciences Research Institute-University of the Philippines and Bureau of Soils and Water Management, Department of Agriculture).*

Parameters	Aquaculture	Non-aquaculture
	Barangay Balakilong, Laurel	Barangay Gonzales, Tanauan City
Organic Carbon (%)	1.36	0.21
Organic Matter (%)	2.34	0.36
Moisture, %	1.97	0.76
NH4-N (ppm)	22.97	3.53
NO3-N (ppm)	1.84	1.77
Total ammonia	59.85	8.29
Cadmium, (mg/kg) b	<1	<1
Copper, (mg/kg) b	29	25.9
Lead, (mg/kg) b	<5	<5
Zinc, (mg/kg) b	45.2	55

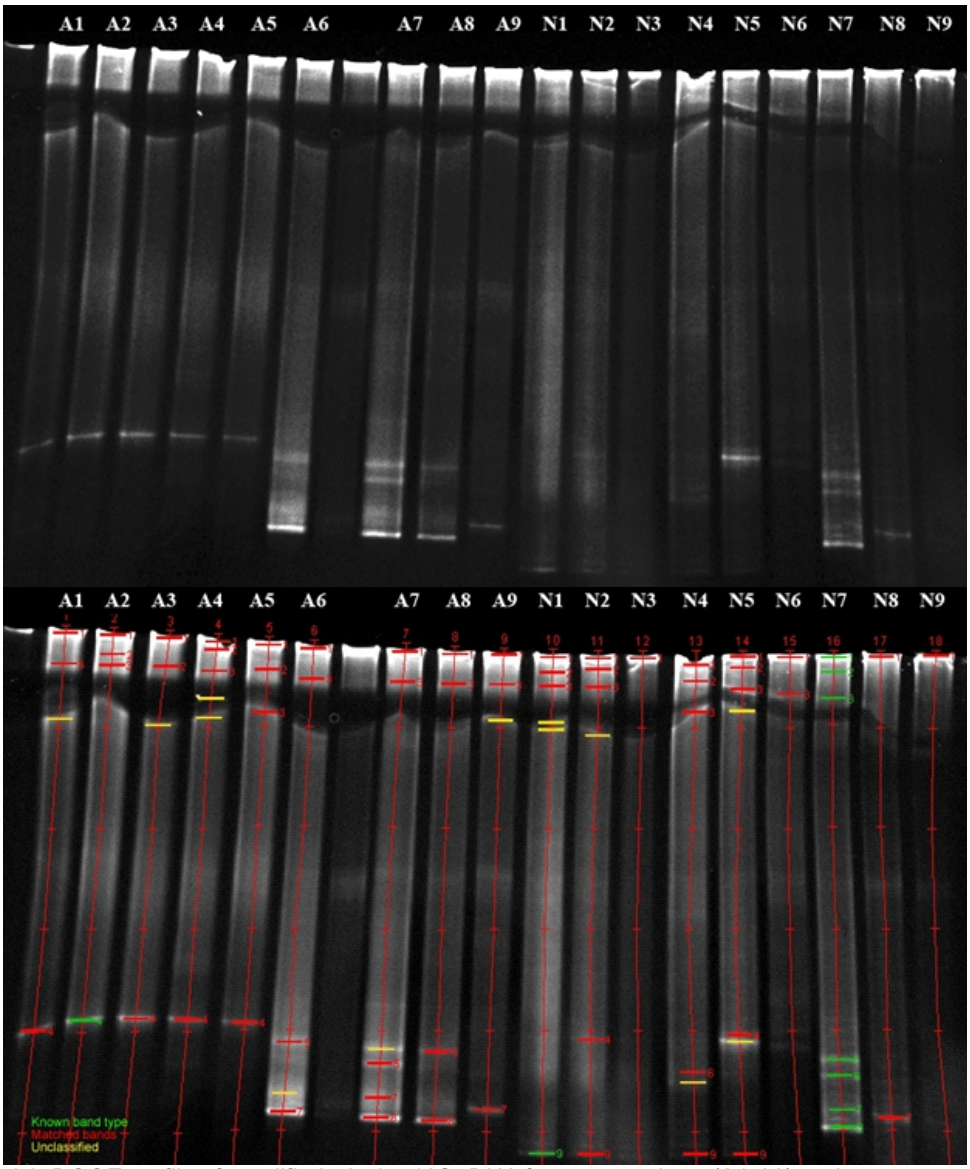

Plate 1. *Gel 1. DGGE profile of amplified 585 bp 16S rDNA from aquaculture (A1-A9) and non-aquaculture (N1-N9) sediment.*

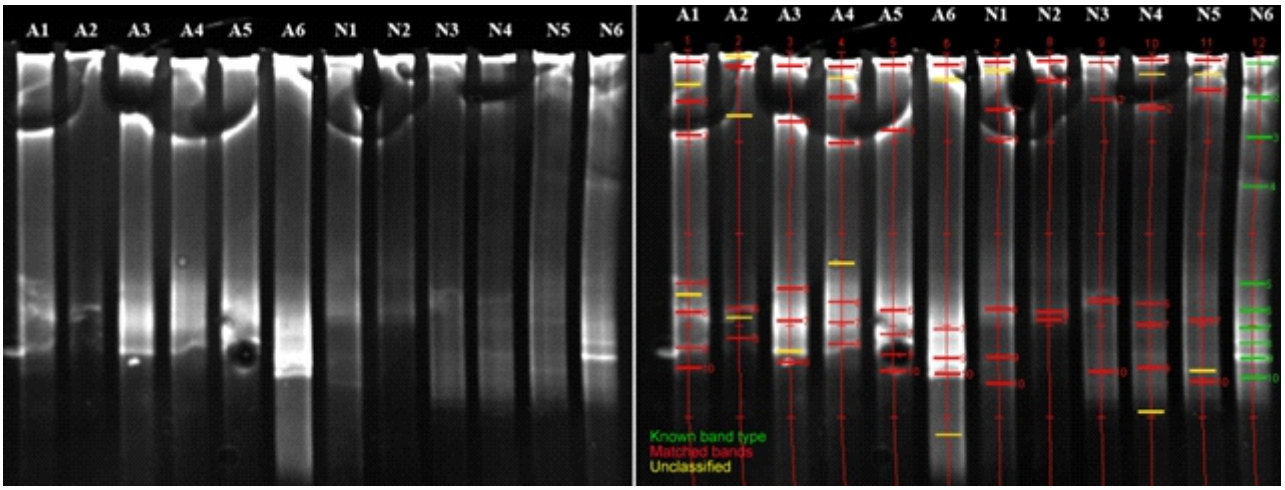

Plate 2. *Gel 2. DGGE profile of amplified 585 bp 16S rDNA from aquaculture (A1-A9) and non-aquaculture (N1-N9) sediment.*

Table 2. *Summary of the DGGE gel profiles of sediments in terms of band types.*

Parameters	Sampling Site	Number of matched distinct bands	Number of matched common bands	Number of unique Bands	Total number of bands
DGGE Gel 1	Aquaculture Non-aquaculture	0 2	7 7	7 6	14 15
DGGE Gel 2	Aquaculture Non-aquaculture	0 1	9 10	10 5	19 16

Matched distinct bands (red) = classified site-specific bands (i.e., present in only one group)
Matched common bands (red) = present in both groups
Unique bands (yellow) = unclassified, unmatched bands

overall abundance for *Chloroflexi* in sediments with high inorganic matter [9].

Influence of Physico-Chemical Parameters on Dominant Taxa and Unique Taxa

Bands representing *Janthinobacterium agaricidamnosum, Psychrobacter alimentarius*, and *Massilia aerilata* were found from both sites (Table 3). *Shewanella baltica* and *Trichococcus* sp. bands were found predominantly from the aquaculture site while *Sporacetigenium mesophilum, Herpetosiphon geysericola, Ammonifex thiophilus*, and *Planomicrobium okeanokoites* were observed predominantly from the non-aquaculture site.

Band 4 in Lanes A2, A3, A4, N2, and N5 of Gel 1 represent *Janthinobacterium agaricidamnosum,* an aerobic Gram-negative

Table 3. *Bacterial species identification after sequencing of 585 bp 16S rDNA sediment samples from DGGE.*

Sediment samples	Band	Identification	Accession No. in GenBank	Max Identity (%)
Aquaculture	4 (Gel 1)	*Janthinobacterium agaricidamnosum*	Y08845	99%
	5 (Gel 1)	*Psychrobacter alimentarius*	AY513645	90%
	7 (Gel 1)	*Shewanella baltica*	AJ000214	98%
	8 (Gel 1)	*Trichococcus spp.*	AJ306611	99%
	9 (Gel 2)	*Massilia aerilata*	EF688526	95%
Non-aquaculture	4 (Gel 1)	*Janthinobacterium agaricidamnosum*	Y08846	99%
	5 (Gel 1)	*Psychrobacter alimentarius*	AY513645	90%
	7 (Gel 1)	*Sporacetigenium mesophilum*	AY682207	89%
	8 (Gel 1)	*Herpetosiphon geysericola*	AF039293	100%
		Ammonifex thiophilus	EF554597	100%
	9 (Gel 1)	*Planomicrobium okeanokoites*	D55729	99%
	9 (Gel 2)	*Massilia aerilata*	Ef688526	95%

rod originally described to cause a soft rot disease of the cultivated mushroom, *Agaricus bisporus* [10]. These species, which are found in both sites, may be considered ubiquitous in aquatic ecosystem as they have been reported to thrive in river basins [11]; freshwater sediments [12]; coral mucus [13]; estuaries [14] as well as soil and water from temperate regions [15]. Band 5 in Lane A7 of Gel 1 was identified as *Psychrobacter alimentarius,* an aerobic, Gram-negative, non-motile, moderately halophilic bacterium previously isolated from squid jeotgal, a traditional Korean fermented seafood, [16] and was described to be found in various habitats, including fish, poultry, food, clinical specimens, sea water, and even Antarctic ornithogenic soils [17]. *Massilia aerilata* represents band 9 in gels 1 and 2, a novel aerobic, Gram-negative, rod-shaped, motile bacterium, previously reported to be found in air samples [18].

Band 7 in Lane A9 of Gel 1 was identified as *Shewanella baltica* (formerly classified as *S. putrefaciens),* a fish spoilage bacterium widely distributed in marine and freshwater environments hypothesized to have an important role in the turnover of organic matter coupled to anaerobic respiration electron acceptors [19, 20, 21, 22] previously described that *S. baltica* may produce substances that can suppress the growth of other bacterium. Band 8 in Lanes A7 and A8 of Gel 1 was identified as *Trichococcus spp*., a mesophillic, Gram-variable, psychrotolerant, facultative anaerobic bacteria. This species has been previously isolated from penguin guano in Chile [23], a hydrocarbon spill site in USA, [24] and an activated sludge in Germany [25]. More importantly, these two taxa were reportedly capable of thriving in the low oxic waters of the Baltic sea [26] and ammonia-rich environments [27], respectively.

Meanwhile, Band 7 in Lane N8 of Gel 1 was identified as *Sporacetigenium mesophilum,* a Gram-positive bacterium of the *Firmicutes* phyla, which was first isolated from the sludge of an anaerobic digester treating municipal solid waste and sewage in Fujian province, China [28]. *S. mesophilum* is spore-forming and motile. *Herpetosiphon geysericola* is a thermotolerant, strictly aerobic and organotrophic species from the phylum *Chloroflexi,* but can be cultivated on media with very low organic constituents [29]. *Herpetosiphon* is known to occur in freshwater and sewage systems, near hot springs and in various types of soil systems in many countries such as Africa, America, Australia, Europe, India, Japan, and Mexico [29]. *Ammonifex thiophilus* is a novel anaerobic, extremely thermophilic, facultatively chemolithoautotrophic bacterium isolated from a terrestrial hot spring in Kamchatka, Russia [30] while *Planomicrobium okeanokoites* were previously reported to be isolated from coastal sediments [31].

Bacterial responses to sediment pollution (eg. aquaculture wastes) may follow two trends. Some bacterial species may exhibit metabolic inhibition leading to either cell lysis or decrease in cell growth. Others may become passively tolerant or can actively degrade and solubilize the pollutants [32]. Pollutants, themselves, can act as potent evolutionary factors, favoring bacterial species that can degrade anthropogenic compounds and inhibit potentially competitive species within the community. The higher organic matter content (150% higher) in the aquaculture site can be attributed to the high waste production associated with fish net-pens in the area. In a study by to Torsvik, Sorheim, and Goksoyr, genomic diversity in heavily polluted fish farm sediments was lower compared to pristine sediments [33]. Although greater bacterial density in sediments nearer to fish cages is observed due to organic enrichment and a propensity for aerobic heterotrophic bacteria, vibrio, sulfur reducers, and methanogens to thrive there, an inhibition of some microbes is also possible resulting to lower bacterial diversity or a shift in dominant species [34,35,36,37,38,39,40,41] which was observed in this study. Li *et al.* added that excess nutrients from aquaculture facilities may lead to a reduction of bacterial diversity in nearby sediments and the emergence of opportunistic species [42].

Localized high inputs of organic carbon are also implicated in the increase of benthic oxygen demand [43]. The sediments became anoxic and consequently led to switching of microbial oxidation of carbon to other electron acceptors such as sulfate [44]. This allows for the presence of sulfate reducers, such as *Shewanella baltica,* in aquaculture sediments [45]. Moisture content was also higher in the aquaculture sediments than in the non-aquaculture sediments. A desert stream sediments study by Zeglin *et al*. reported that there is no obvious correlation between sediment water content and bacterial diversity [46]. However, it was noted that moist sediments harbor a bacterial community distinctive from dry sediments, possibly because water stimulates microbial activity and can act as a dispersal vector for aquatic species [46,47]. As observed on *Janthinobacterium agaricidamnosum,* its activity is stimulated by high moisture [10].

Since bacteria are directly and indirectly involved in crucial environmental processes, shifts in bacterial species composition may have an impact on the functionality of a given ecosystem [32,48] although this impact will not be further discussed in this paper. It is important to note that freshwater ecosystems, such as Taal Lake, are adjacent to other aquatic and terrestrial ecosystems and the interactions among these various ecosystems may influence the composition of microbial communities in freshwater sediments [49] and any bacterial

community analysis as a pollution assessment should be used in conjunction with chemical analyses, bioassays, and community studies in the field [50]. These ecological parameters and their effect on the bacterial community composition mirror how aquaculture and non-aquaculture sites have bacterial profiles of low similarity.

Conclusion and Recommendations

This study exhibited the applicability of 16S rDNA PCR-DGGE as a culture-independent technique of describing the diversity of sediment bacterial communities. Based on the generated similarity index, the bacterial community between aquaculture and non-aquaculture sediments of Taal Lake was highly similar due to the presence of same bacterial species in both sites. Corollary, the bacterial communities between the two types of sediments were less diverse. Nonetheless, both aquaculture and non-aquaculture sediments harbored unique bacterial populations as seen in the DGGE profiles although the number of unique species was not enough to affect the overall diversity. Based on sequencing data, aquaculture sediment bacterial communities were composed mainly of *Proteobacteria* and *Firmicutes,* whereas non-aquaculture sediments contained members belonging to *Proteobacteria, Firmicutes*, and *Chloroflexi.*

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