

## Research Article

# Contribution of the Types of Tropical Coastal Aquaculture Ponds to the Differential Distribution of the Family Bacillaceae Bacterial Community

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Received 27 February 2023; Revised 20 May 2023; Accepted 23 May 2023; Published 2 June 2023

Academic Editor: Hisham Abdelrahman

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The family Bacillaceae bacteria, particularly *Bacillus* communities, are useful and attractive probiotics with desirable features to fulfill the vast demand for aquaculture. In Hainan Island, the coastal aquaculture ponds hold about 1% of the total area in China, whereas the knowledge of Bacillaceae bacterial communities was limited. Herein, the Bacillaceae bacterial communities in the coastal aquaculture ponds of the artificial ponds (lined with high-density polyethylene plastic sheets) and earthen ponds were investigated by the culturable method. The characteristics of culturable Bacillaceae communities in these two types of coastal aquaculture ponds showed a massive difference. All the culturable Bacillaceae bacteria were identified as *Bacillus* in the artificial ponds, whereas the earthen ponds were dominated by *Bacillus* followed by *Halobacillus*. It is worth noting that the artificial ponds with a low Bacillaceae diversity displayed a high Bacillaceae concentration with a better water quality, whereas the earthen ponds with a higher Bacillaceae diversity and concentration displayed a higher risk of *Vibrio* infection, indicating that the Bacillaceae concentration than its diversity made a greater contribution to water purification and *Vibrio* control. This work besides providing a promising strategy for healthy coastal aquaculture by balancing the relationship between Bacillaceae concentration and its diversity also will lay a foundation for the development of probiotic Bacillaceae agents for wastewater purification and disease control.

## 1. Introduction

Coastal aquaculture ponds play an important role in the development of the Hainan fisheries economy, accounting for about 1% of the total area in China [1]. Among the aquaculture species along the coast of Hainan Island, shrimp aquaculture, particularly penaeid shrimp as a marine species, is important due to its easy-to-farm feature and remarkable

commercial interest in the international fisheries trade, which has met a large proportion of China's shrimp market demand [2]. However, intensive aquaculture in Hainan has been suffering from long-term disease threats caused by the eutrophication of aquaculture water due to the over-throwing of baits [3, 4]. To control and prevent the outbreak of diseases rapidly and effectively in aquaculture, nonpathogenic probiotic microbes instead of antibiotic

chemicals give a safe and efficient strategy for disease prevention [5]. Reportedly, some nonpathogenic microbes as probiotics were employed to purify the aquaculture water and to control the pathogenic bacteria through the principle of competitive exclusion [2, 6].

The strains belonging to the family Bacillaceae, particularly *Bacillus*, well known as the more useful and attractive probiotics are frequently applied in the industry due to their advantage characteristics such as ease of cultivation, high growth rate, no side effects, available enzyme production capacity, and strong environment resistance. [3, 5, 7]. In addition, numerous *Bacillus* species are reported for their real-world applications of water purification and pathogenic bacteria control by preventing organic matter accumulation and mitigating nitrogen and phosphate pollution [3, 4, 8–10], i.e., *Bacillus acidopullulyticus*, *Bacillus cereus*, *Bacillus flexus*, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus vallismortis*, and *Bacillus velezensis* [4, 11–15]. In the aquaculture system, the Bacillaceae genera, particularly *Bacillus*, as dominant members of Gram-positive bacteria, could minimize the deposits of dissolved and particulate organic carbon by converting organic matter back to carbon dioxide [16], which besides improving the water quality for sustainable aquaculture, also make a great contribution to the immunity and nutrient digestion of farmed animals. We reason that the Bacillaceae communities must play an imperative role in the aquaculture system.

Currently, in the farm-led shrimp farming industry, there are mainly two types of ponds for shrimp aquaculture along the coast of Hainan Island, the earthen ponds with sediment and the artificial ponds lined with high-density polyethylene plastic sheets instead of sediment, whereas the knowledge of the diversity of Bacillaceae communities in these two types of ponds was limited. In this work, we completely investigated the distribution of the Bacillaceae communities, their capacity of producing amylase, and the successions of water quality and *Vibrio* in these two shrimp aquaculture systems, to provide excellent probiotic Bacillaceae candidates for the aquaculture industry.

## 2. Materials and Methods

**2.1. Study Site Description and Sample Collection.** Six ponds lined with high-density polyethylene plastic sheets instead of sediment (19°26'39"N, 108°50'11"E; Haiwei, Changjiang, Hainan, China) defined as artificial ponds and six ponds with sediment (19°27'28"N, 110°45'13"E; Huiwen, Wenchang, China) defined as earthen ponds were selected to investigate the differential diversity of Bacillaceae communities in these two types of shrimp (*Litopenaeus vannamei*) aquaculture systems. These ponds were pretreated with quicklime sterilization and sunlight exposure for 15 days before breeding shrimp. The special geographical position is shown in Figure 1, and the experimental ponds are summarized in Table 1.

**2.2. Physicochemical Properties.** The physicochemical property of breeding water at a depth of 1.0 m from each pond was investigated. The pH, salinity, and dissolved

oxygen (DO) were measured by a multiparameter water quality detector (YSI 6600 V2, YSI Inc., Yellow Springs, USA). The concentrations of chemical oxygen demand (COD),  $\text{NO}_2^-$ -N,  $\text{NH}_4^+$ -N, and  $\text{NO}_3^-$ -N were measured by auto discrete analyzers (CleverchemAnna, DeChem-Tech, Germany), respectively, referring to the standard methods of alkaline potassium permanganate, hydrochloric acid naphthyl ethylenediamine spectrophotometry, hypobromite chlorination, and phospho-molybdenum blue.

**2.3. Determination of the Concentration of Bacillaceae.** The samples of surface sediment (0.5 g) with a depth of 1 cm and water (100  $\mu\text{L}$ ) at a depth of 1.0 m were serially diluted with a sterile normal saline solution (0.85% NaCl) within 24 h of collection to obtain 1:10, 1:100, and 1:1,000 dilutions. For detecting the concentration of *Vibrio*, 100  $\mu\text{L}$  of each diluted sample was spread-plated on TCBS agar. For detecting the concentration of Bacillaceae, each diluted sample was heated in a water bath at 80°C for 20 min, and then 100  $\mu\text{L}$  of each sample was spread-plated on marine 2216E agar and incubated at 30°C. After the incubation at 30°C for 24 h, the plates with individual colonies of 30~300 for each sample were selected for the determination of the concentration of *Bacillus* and *Vibrio*.

**2.4. Isolation and Identification of Bacillaceae Genera.** The individual colonies on the 2216E agar plate were collected for the identification of the Bacillaceae genera by the 16S rDNA sequencing approach. The bacterial genomic DNA was extracted using the DNA Purification Kit (Tiangen Biotech Co., Ltd., Beijing, China). The 16S rDNA universal primers, 27F (5'-AGAGTTTGATCCTGGCTCA-3') and 1492R (5'-GGTTACCTTGTACGACTT-3'), were employed for the PCR amplification [4, 17]. PCR amplifications were performed by using the following conditions: initial denaturation of template DNA (95°C for 2 min), then 1 cycle consisting of denaturation (30 s at 95°C), annealing (30 s at 60°C), and extension (1 min at 72°C), with 25 cycles, and a final extension at 72°C for 5 min. After the amplification, the sequencing work was performed at Bosheng Biotechnology (Shanghai) Co., Ltd., Shanghai, China. The BLAST algorithm was used for 16S rDNA sequence comparison in the Korean Standard Strain Database (<https://www.ezbiocloud.net/>) and analyzed.

**2.5. Screening of Amylase-Producing Bacillaceae Strains.** The amylase-producing Bacillaceae strains were screened by the hydrolytic capacity of starch. 1  $\mu\text{L}$  culture broth with  $\text{OD}_{600}$  of 0.8 was dropped on a starch-enriched agar plate in duplicates. Diameters of bacterial colonies and related-clearance zones were measured by the I-KI staining method after 30 h incubation at 40°C. The hydrolytic capacity value determined the amylase activity was calculated from the ratio between the diameter of the clearance zone and the diameter of the bacterial colony. The bacterial colony with a ratio greater than one was identified as an amylase-producing Bacillaceae strain.



FIGURE 1: (a) Location of the study areas, Changjiang (19°26'39"N and 108°50'11"E) and Wenchang (19°27'28"N and 110°45'13"E), Hainan Island, China. (b) Artificial pond in Changjiang. (c) Earthen pond in Wenchang. The location is shown in Google Earth web (<https://earth.google.com/>).

TABLE 1: Experimental ponds and management.

Earthen ponds in Wenchang						
Ponds	E1	E2	E3	E4	E5	E6
Size (m <sup>2</sup> )	2,000	2,000	2,000	2,000	2,000	666.67
Water depth (m)	1.5	1.5	1.5	1.5	1.5	1.5
Healthy condition of <i>L. vannamei</i> (50-day)	Healthy	Healthy	Healthy	Healthy	Healthy	Healthy
Stocking density (tails/m <sup>3</sup> )	80	80	80	80	80	80
Shrimp length (cm)	7~8	7~8	7~8	7~8	7~8	7~8
Aerator	2	2	2	2	2	2
Feed intake (kg/d; 3 times)	6	6	6	6	6	6
Artificial ponds in Changjiang						
Ponds	A1	A2	A3	A4	A5	A6
Size (m <sup>2</sup> )	2,000	2,000	2,000	2,000	2,000	2,000
Water depth (m)	1.5	1.5	1.5	1.5	1.5	1.5
Healthy condition of <i>L. vannamei</i> (50-day)	Healthy	Healthy	Healthy	Healthy	Healthy	Healthy
Stocking density (tails/m <sup>3</sup> )	80	80	80	80	80	80
Shrimp length (cm)	8~9	8~9	8~9	8~9	8~9	8~9
Aerator	2	2	2	2	2	2
Feed intake (kg/d; 3 times)	6	6	6	6	6	6

### 3. Results

**3.1. Physicochemical Traits.** The physicochemical traits of the two types of shrimp ponds are shown in Table 2. The pH, salinity, and DO of the artificial ponds were mostly higher than those of the earthen ponds, whereas the concentrations of COD, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and NO<sub>2</sub><sup>-</sup>-N of the artificial ponds were mostly lower than those of the earthen ponds.

**3.2. Diversity and Distribution of the Family Bacillaceae Genera.** The concentrations of Bacillaceae and *Vibrio* were detected in the artificial ponds and earthen ponds by the culturable approach. The artificial ponds showed a high concentration of Bacillaceae, and no observed *Vibrio* was detected in artificial ponds but was detected in A6 ponds (Figure 2(a)). However, relatively high concentrations of *Vibrio* were observed in each earthen pond due to the lower concentration of Bacillaceae (Figure 2(b)). A total of 276 and 1,151 Bacillaceae strains from the artificial ponds and earthen ponds were isolated and identified (Figures 2(c) and 2(d)). Of the Bacillaceae strains, all Bacillaceae strains from

the water samples of artificial ponds were divided into *Bacillus* genus, whereas 557 and 594 Bacillaceae strains were, respectively, screened from the water samples and sediment samples of the earthen ponds. In addition, it is worth noting that the concentrations of Bacillaceae in the earthen ponds were lower than that in the artificial ponds, but the diversities of Bacillaceae in earthen ponds were higher than that in the artificial ponds.

The diversities of Bacillaceae genera in two types of shrimp aquaculture systems, artificial ponds and earthen ponds, were further analyzed in this work (Figure 3). All the Bacillaceae isolates were divided into 11 genera, i.e., *Bacillus*, *Halobacillus*, *Thalassobacillus*, *Paenibacillus*, *Fictibacillus*, *Ornithinibacillus*, *Oceanobacillus*, *Solibacillus*, *Lysinibacillus*, *Brevibacillus*, and *Virgibacillus*.

In the artificial ponds, most Bacillaceae strains were identified as *Bacillus* genus and the dominant species was *Bacillus paralicheniformis*, whereas only 2 strains were divided into the *Halobacillus* genus. In the earthen ponds, 557 strains from water were divided into 7 genera, namely, *Bacillus*, *Halobacillus*, *Thalassobacillus*, *Oceanobacillus*,

TABLE 2: Physicochemical parameters of sampling stations in the artificial ponds and earthen ponds.

No.	pH	Salinity (‰)	DO (mg/L)	COD (mg/L)	NH <sub>4</sub> <sup>+</sup> -N (mg/L)	NO <sub>3</sub> <sup>-</sup> -N (mg/L)	NO <sub>2</sub> <sup>-</sup> -N (mg/L)
<i>Artificial ponds in Changjiang</i>							
A1	8.270	34.50	6.44	34.5	0.003	0.003	0.001
A2	8.410	34.67	5.75	83.0	0.000	0.000	0.012
A3	8.740	34.62	6.41	80.0	0.006	0.000	0.046
A4	7.966	34.87	6.27	86.0	0.000	0.008	0.024
A5	7.540	34.14	6.03	74.5	0.003	0.003	0.001
A6	8.110	34.00	7.03	74.5	0.011	0.012	0.010
<i>Earthen ponds in Wenchang</i>							
E1	7.770	25.68	3.12	129.6	0.265	0.025	0.906
E2	7.660	21.87	2.82	74.8	3.009	0.074	0.740
E3	7.410	26.58	2.95	141.0	1.267	0.027	0.250
E4	7.525	29.88	2.42	140.0	0.918	0.025	0.907
E5	7.494	26.30	2.61	82.0	2.203	0.073	0.196
E6	7.280	26.00	3.01	147.0	2.362	0.448	0.987

*Lysinibacillus*, *Virgibacillus*, and *Brevibacillus*, while 594 strains from sediments were divided into 10 genera, i.e., *Bacillus*, *Halobacillus*, *Thalassobacillus*, *Paenibacillus*, *Fictibacillus*, *Ornithinibacillus*, *Oceanobacillus*, *Solibacillus*, *Lysinibacillus*, and *Brevibacillus*. No matter in water or sediment of the earthen ponds, *Bacillus* exhibited the highest abundance, followed by *Halobacillus* (Figure 3(a)), and the dominant species were *Bacillus hwajinpoensis* and *Halobacillus trueperi*.

As shown in Figure 3(b), 2 Bacillaceae genera were shared by the artificial ponds and earthen ponds, namely, *Bacillus* and *Halobacillus*. Nine unique Bacillaceae genera were detected with low abundance in the earthen ponds, *Thalassobacillus*, *Paenibacillus*, *Fictibacillus*, *Ornithinibacillus*, *Oceanobacillus*, *Solibacillus*, *Lysinibacillus*, *Virgibacillus*, and *Brevibacillus*. Of which, 6 genera were shared by the water and sediment of earthen ponds, namely, *Bacillus*, *Halobacillus*, *Oceanobacillus*, *Lysinibacillus*, *Thalassobacillus*, and *Brevibacillus*, while 4 unique genera (*Paenibacillus*, *Fictibacillus*, *Ornithinibacillus*, and *Solibacillus*) in sediment and one unique genus (*Virgibacillus*) in water were presented in the earthen ponds.

**3.3. Diversity of Amylase-Producing Bacillaceae Strains.** As shown in Figure 4, 248 of 276 Bacillaceae strains were identified as amylase-producing bacteria in the artificial ponds, while 651 of 1,151 Bacillaceae strains were identified as amylase-producing bacteria in the earthen ponds. In the artificial ponds, all the amylase-producing strains belonging to the *Bacillus* genus were ubiquitous in every shrimp pond. In the earthen ponds, the dominant amylase-producing Bacillaceae strains belonged to two genera, *Bacillus* and *Halobacillus*. In the water of earthen ponds, 318 amylase-producing Bacillaceae strains were divided into 5 genera, *Bacillus*, *Halobacillus*, *Thalassobacillus*, *Lysinibacillus*, and *Brevibacillus*, and the dominant Bacillaceae genera belonging to *Bacillus* (79.28%) and *Halobacillus* (19.22%) were accounted for more than 98.5% of the total abundance. In the sediment of earthen ponds, 333 amylase-producing Bacillaceae strains were divided into 5 genera, *Bacillus*, *Halobacillus*, *Thalassobacillus*, *Lysinibacillus*, and *Paenibacillus*, and the dominant genera belonging to *Bacillus*

(66.36%) and *Halobacillus* (32.10%) were accounted for more than 98.46% of the total abundance.

**3.4. Correlation Analysis between the Bacillaceae Communities and Environmental Factors.** As shown in Figure 5, all the detected environmental factors made some contributions to the distribution of the family Bacillaceae in the artificial ponds and earthen ponds. The dominant genera of *Bacillus* and *Halobacillus* were significantly affected by the most detected environmental factors, such as DO, pH, salinity, NO<sub>2</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, and NO<sub>3</sub><sup>-</sup>-N, but these influences on *Bacillus* and *Halobacillus* were opposite. For instance, DO, pH, and salinity as the main factors were positively related to the distribution of *Halobacillus*, whereas these three factors negatively affected the distribution of *Bacillus*, similarly, NO<sub>2</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, and NO<sub>3</sub><sup>-</sup>-N were the main factors positively affecting the distribution of *Bacillus*, whereas these three factors made negative contributions to the distribution of *Halobacillus*. The relationship between *Thalassobacillus* and environmental factors resembled that of *Halobacillus* and environmental factors. Although *Thalassobacillus* displayed less distribution, which was still significantly affected by the detected factors, for instance, COD, NO<sub>2</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, and NO<sub>3</sub><sup>-</sup>-N as the main factors negatively affected this type of strain, while DO, pH, and salinity made negative influences on the distribution of *Thalassobacillus*. In addition, the distribution of *Oceanobacillus* was affected by the factors of NO<sub>3</sub><sup>-</sup>-N (negative) and pH (positive).

## 4. Discussion

Coastal aquaculture production has continued to grow steadily over the past four decades, particularly in coastal Southeast Asia where large-scale coastal land was expropriated for the development of aquaculture [18]. Hainan is a member of this region which holds about 1% of the total area covered by coastal aquaculture ponds in China [1]. It is therefore conceivable of the irreplaceable role of these ponds in the development of the aquaculture industry in Hainan Island of China. These ponds were mainly divided into two types: the artificial pond without sediment and earthen

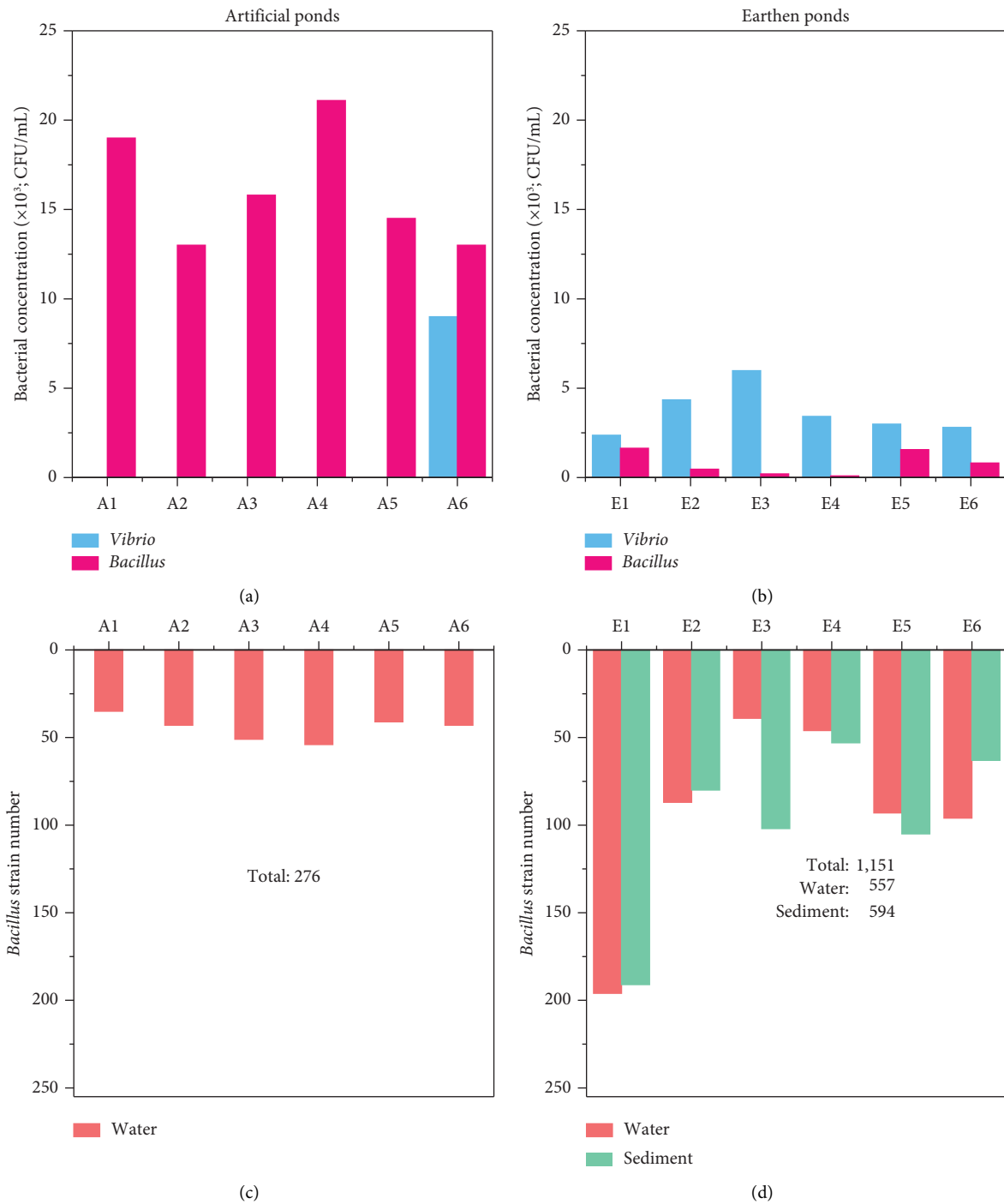


FIGURE 2: The concentrations of Bacillaceae and *Vibrio* in the artificial ponds (a) and earthen ponds (b). The number of Bacillaceae strains in the artificial ponds (c) and the earthen ponds (d).

ponds with sediment. The family Bacillaceae genera, particularly *Bacillus* strains, are commonly employed as candidate probiotics instead of antibiotics/chemicals to improve the aquaculture water quality, and have no side effects on aquaculture animals by reducing the contents of COD, ammonia nitrogen, nitrite, and other substances that cause water eutrophication [3, 16]. We reason that the Bacillaceae communities besides playing a critical role in disease

prevention and water purification, also are considered as a treasure trove of attractive Bacillaceae strains, particularly *Bacillus*, due to their cultural properties, high growth rate, capacity to excrete protein into cultivation medium, and security for various industrial applications [4, 19], such as *B. subtilis*, *B. licheniformis*, *B. vallismortis*, *B. acidopullulyticus*, *B. cereus*, and *B. flexus* [4, 11, 13, 15, 20]. Therefore, in the present work, we completely investigated

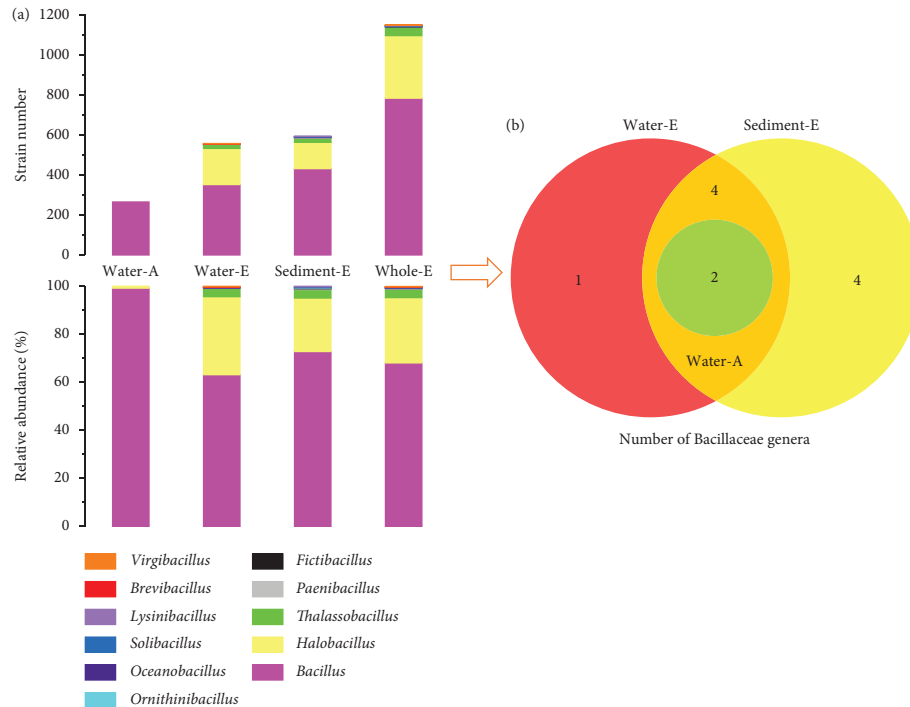


FIGURE 3: (a) Distribution of Bacillaceae in the artificial ponds and earthen ponds at the genus level. (b) Proportional Venn diagram showing the number of Bacillaceae genera of the detected strains found in three types of samples, namely, water from artificial ponds: water-A, water from earthen ponds: water-E, and sediment from earthen ponds: sediment-E.

the Bacillaceae communities in two types of coastal aquaculture ponds of the artificial ponds without sediment and earthen ponds with sediment, which besides enriching the understanding of Bacillaceae community structures in these two types of coastal aquaculture ponds, also lays a foundation for the isolation and utilization of the probiotic Bacillaceae strains in aquaculture and/or other various industries.

There was a negative correlation between the content of DO and the concentration of Bacillaceae in the artificial ponds. Bacteria communities are generally sensitive to the fluctuations of DO, while the growth of a large number of aerobic bacteria usually leads to a decrease in dissolved oxygen. In addition, it is worth noting that the contents of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ , and COD in the artificial ponds were wholly significantly lower than those in the earthen ponds due to much higher concentrations of Bacillaceae in the artificial ponds than those in the earthen ponds, thus validating the consensus that Bacillaceae strains could effectively improve wastewater quality. In addition, the concentrations of *Vibrio* in the artificial ponds were significantly lower than those in the earthen ponds still due to much higher concentrations of Bacillaceae in the artificial ponds than those in the earthen ponds, confirming that Bacillaceae strains could effectively prevent *Vibrio*. Reportedly, Bacillaceae strains, particularly *Bacillus* species, well-known as consumers and decomposers in aquaculture can improve water quality and prevent pathogens due to their nitrifying and denitrifying actions which subsequently convert ammonia-nitrogen to nitrite, nitrite to nitrate, and then nitrate to nitrogen gas, their capacity of converting organic matter back to  $\text{CO}_2$ , and their ability to produce antibiotics/metabolites which

have antagonistic effects against pathogenic microbes [6, 8–10, 16, 21–26].

For the artificial ponds, only two Bacillaceae genera were detected in the artificial ponds, but the genus of *Bacillus* occupied absolute predominance with a relative abundance of about 99%, among which the dominant species was *B. paralicheniformis*. *B. paralicheniformis* was first noticed because of its high enzyme-producing ability, such as amylase, gelatinase, and caseinase [27]. In addition, *B. paralicheniformis* was reported to have high nitrite reduction ability and can live in the intestine of aquatic animals for a long time [28, 29]. In this study, *B. paralicheniformis* as the dominant *Bacillus* species with a relatively high concentration exhibited excellent water purification ability, such as COD,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , and  $\text{NO}_2^-\text{-N}$ . For the earthen ponds, the dominant Bacillaceae genus was *Bacillus*, followed by *Halobacillus*, and the Bacillaceae genera diversity of this type of aquaculture ponds was relatively high compared with that of the artificial ponds, whereas the earthen ponds had a higher risk of *Vibrio* infection than artificial ponds due to the low concentration of Bacillaceae. Of the dominant genera, the dominant species were *B. hwajinpoensis* and *H. trueperii*. *B. hwajinpoensis* was first isolated from sea water from Hwajinpo Beach, East Sea in Korea [30], and its heterotrophic nitrification-aerobic denitrification effects on water purification by removing inorganic nitrogen and total nitrogen were subsequently reported [31]. *Halobacillus* as a major ecosystem-adapted microbial component could survive from extreme environments due to its stronger survivability and flexible adaptability, such as salt lakes,

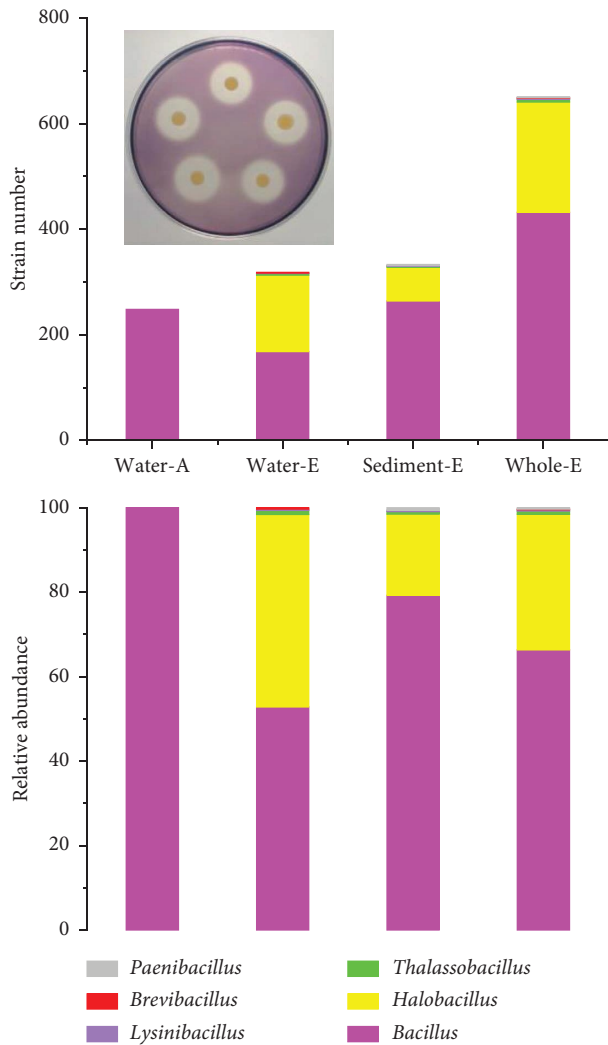


FIGURE 4: Distribution of amylase-producing Bacillaceae strains in the artificial ponds and earthen ponds at the genus level.

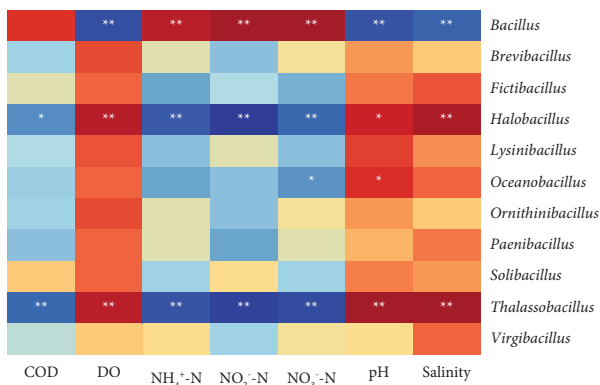


FIGURE 5: Correlation of environmental physicochemical properties and Bacillaceae communities across two types of shrimp aquaculture ponds, i.e., artificial ponds and earthen ponds. \* $p < 0.05$  and \*\* $p < 0.01$  respectively indicate a significant correlation level and an extremely significant correlation level.

hypersaline sediments and soils [32–34]. Some reporters have reported its outstanding capacity of inhibiting fungi activity thereby promoting the growth of plants [34, 35], while some reporters have reported that *Halobacillus* from a shrimp pond exhibited antibacterial potencies against the opportunistic marine pathogens of *Vibrio parahaemolyticus* and *Vibrio cholerae* by producing antimicrobial compounds [32]. Meanwhile, although some unique Bacillaceae genera was presented in the earthen ponds, such as *Thalassobacillus*, *Paenibacillus*, *Fictibacillus*, *Ornithinibacillus*, *Oceanobacillus*, *Solibacillus*, *Lysinibacillus*, *Virgibacillus*, and *Brevibacillus*, the concentration of these bacteria was not high. Overall, although the diversity of the family Bacillaceae of artificial ponds was significantly lower than that of the earthen ponds, but the Bacillaceae concentration of artificial ponds was higher than that of the earthen ponds. Reportedly, microbial interactions make a great contribution to the survival of organisms as well as for the functioning of an ecosystem as a whole by the transformation of organic carbon, sulfur, nitrogenous compounds, and metals, and maintaining the physical stability of a certain ecosystem, while the microbial interactions are significantly related to the microbial species diversity [36–38]. Therefore, we reason that the earthen ponds have higher system stability due to the high bacterial diversity, while the artificial ponds have a higher water purification capacity due to higher Bacillaceae concentrations.

According to the standards of the Food and Agriculture Organization of the United Nations, shrimp feed is typically composed of 20%–25% carbohydrates [39]. The intensive aquaculture model often adds excessive bait, resulting in an excess of bait in the aquaculture systems and depositing at the bottom of the ponds. This not only worsens the water quality of the aquaculture, but also poses a threat to the growth and health of aquaculture aquatic organisms. In the process of aquaculture, amylase serves as a catalyst to degrade the starch nutrients of the feed, allowing it to be fully absorbed and utilized by the aquatic animals. In addition, it can also decompose excess starch feed in the event of excessive feeding, thereby reducing the environmental load of aquaculture. Given this, we focused on investigating the amylase-producing bacteria. Amylases ( $\alpha$ -1,4-glucan 4-glucano-hydrolase, EC 3.2.1.1), which hydrolyze starch to dextrin and different monomeric products at  $\alpha$ -1,4 glycosidic bond, are of tremendous value due to the characteristics such as bulk production and easy genetic manipulation [40]. In this work, we found that most of the Bacillaceae strains displayed the amylase-producing ability in the artificial ponds and earthen ponds, particularly the genera of *Bacillus* and *Halobacillus*. Numerous *Bacillus* species were identified as the producer of amylases, such as *B. licheniformis* [41], *B. amyloliquefaciens* [42], *B. subtilis* [43], *B. cereus* [44], *B. paramycoides* [45], *B. koreensis* [46], and *B. velezensis* [47]. At the same time, there were relatively few reports on *Halobacillus* species with the capacity of producing

amylases, such as *Halobacillus* sp. strain MA-2 [48] and *Halobacillus* sp. LY9 [49]. We reason that the coastal aquaculture ponds must be the candidates for screening novel amylase-producing bacteria with special structure and catalytic activity that is meant for specific industrial desires.

The shaping of the bacterial community in aquaculture is generally sensitive to the differentiated concentrations of salinity, nitrate, organic matter, and phosphates, and to a lesser extent to variations of DO, temperature, and pH [3, 4, 50], which provides more information on the relationships between the distribution of bacterial community and complex environmental conditions that is important for aquaculture-ecosystem protection/remediation and resource utilization/development. In this work, according to the physiochemical characteristics of the two types of shrimp aquaculture ponds, i.e., artificial ponds and earthen ponds, the influence of environmental factors on the Bacillaceae community in shrimp aquaculture system was revealed by the Spearman rank correlation coefficient at the genus level. We found that all detected environmental factors made immense contributions to the Bacillaceae community mainly due to the two types of coastal aquaculture ponds of the artificial ponds and the earthen ponds that led to the immense differentiated environmental factors. The types of coastal aquaculture ponds in Hainan Island made a great contribution to the Bacillaceae community.

## 5. Conclusion

In this work, the Bacillaceae communities across two types of coastal aquaculture ponds of the artificial ponds (lined with high-density polyethylene plastic sheets) and earthen ponds in Hainan Island of China were investigated by the culturable method. The dominant Bacillaceae genus was *Bacillus* dominated by *B. paralicheniformis* in the artificial ponds, while the dominant Bacillaceae genus was *Bacillus* followed by *Halobacillus* in the earthen ponds. In addition, the artificial ponds with a high concentration of Bacillaceae displayed a significantly lower diversity of Bacillaceae, whereas the earthen ponds with a low concentration of Bacillaceae exhibited higher diversity of Bacillaceae and a higher risk of *Vibrio* infection than the artificial ponds. We reason that the concentration of Bacillaceae plays a more important role in the process of water purification than the diversity of Bacillaceae in the coastal aquaculture ponds, while the diversity of Bacillaceae is more related to the stability of the aquaculture system. Therefore, how to balance the relationship between Bacillaceae concentration and its diversity to promote the development of aquaculture is an important research topic and direction in the future.

## Data Availability

The data used to support the findings of the study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Wei Ren and Zhenyu Xie conceptualized, designed, and supervised this study. Ziqiao Feng and Ying Li prepared the samples, performed the experiments, and analyzed the data. Wei Ren and Ziqiao Feng wrote the manuscript. Xinyi Wang, Hao Long, Xiaoni Cai, and Aiyu Huang analyzed the data. All authors contributed to the article and approved the submitted version.

## Acknowledgments

This work was financially supported by the Hainan Provincial Natural Science Foundation of China (Grant no. 2019RC106 and 321QN176) and Hainan Province Science and Technology Special Fund (Grant no. ZDYF2022XDNY216 and ZDYF2022XDNY173).

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