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Accumulation of microcystins, bacterial community composition and *mlrA* gene abundance in shrimp culture ponds

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ABSTRACT: Cyanobacteria blooms occur frequently in low-salinity shrimp culture ponds. To provide useful information about the potential harm and *in situ* biodegradation pathway of microcystins (MCs) in these ponds, we investigated accumulation of MCs in shrimp hepatopancreas and sediments, bacterial community composition and *mlrA* gene abundance in intestines and sediments in 11 *Litopenaeus vannamei* culture ponds from 9 cities in China. MCs (MC-LR, MC-RR and MC-YR) accumulated in shrimp hepatopancreas and sediments in each sampled pond. Higher bacterial richness and diversity were observed in sediments than in intestines. Two MC-degrading genera (*Sphingobium* and *Roseomonas*) were detected in the intestine bacterial community, and 3 MC-degrading genera (*Sphingobium*, *Rhizobium* and *Acinetobacter*) were detected in the sediment bacterial community. The *mlrA* gene was easier to detect and more abundant in shrimp intestines than in sediments. These results suggest that there was a potential MC hazard in shrimp culture ponds, and the biodegradation pathway in shrimp intestines seemed to be more dependent on the *mlr* pathway than that in the sediments.

KEY WORDS: Microcystins · Accumulation · *mlrA* gene · Biodegradation · *Litopenaeus vannamei* · Pond

1. INTRODUCTION

Pond aquaculture is an important part of freshwater aquaculture worldwide including China (Cao & Wang 2010). In 2021, pond aquaculture in China covered 2604630 hm^2, accounting for 52.26% of the total freshwater aquaculture area. In intensive aquaculture pond practices, water eutrophication from pond fertilization, uneaten feed, feces and organic matter often occurs (Santhana Kumar et al. 2017), leading to the increased occurrence of cyanobacteria blooms (Rastogi et al. 2014, Huisman et al. 2018). Some cyanobacteria species can produce cyanotoxins, which pose a threat to the growth, reproduction, survival and food safety of cultured animals, and ultimately endanger human health through the food chain (Codd et al. 2005, Greer et al. 2017). Microcystins (MCs), a family of cyclic heptapeptides, are the most frequently occurring and widespread cyanotoxins (Wood et al. 2011). Due to their cyclic structure, MCs are chemically stable and resistant to many abiotic factors (Buratti et al. 2017, Massey et al. 2018). There is growing concern about the fate of MCs in aquaculture ponds (Zimba et al. 2006, Bi et al. 2019).

MCs can accumulate in water, sediment and organisms in aquaculture ponds (Ni et al. 2017, Bi et al. 2019). Accumulated MCs can be degraded in different ways, and biodegradation is considered to be the main process of natural elimination of MCs (Bourne et al. 2006, Bukowska et al. 2018). Since the first MC-degrading bacteria strain was isolated from the Murrumbidgee River in Australia (Jones et al. 1994), more and more bacteria strains with the ability to degrade MCs have been isolated (Massey &

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Yang 2020). There is still only 1 fully described metabolic pathway responsible for the biodegradation of MCs, which is encoded by the *mlr* gene cluster (Bukowska et al. 2018). Within the *mlr* gene cluster, the *mlrA* gene encodes microcystinase (MlrA), and MlrA hydrolysis occurs between the ADDA sequence and the 4th position amino acid in MCs (Xu et al. 2019). MlrA catalyzes the most important step in the biodegradation of MCs (Bourne et al. 2001, Xu et al. 2019), and the *mlrA* gene is commonly used as a marker gene (Dexter et al. 2018). Targeting this gene in polymerase chain reaction (PCR) could qualitatively detect indigenous MC-degrading bacteria (Saito et al. 2003, Bourne et al. 2006, Ho et al. 2006). Hoefel et al. (2009) developed a quantitative real-time PCR (qPCR) assay targeting the *mlrA* gene, which provided a powerful tool for the detection of MC-degrading bacteria populations. The *mlrA* gene has been detected in sediments from Mikoajskie, a eutrophic freshwater lake in Poland (Bukowska et al. 2018). However, there is little information on the distribution of the *mlrA* gene in aquaculture ponds.

Litopenaeus vannamei, also known as white shrimp, is one of the most popularly cultured shrimp species worldwide. Pond culture of *L. vannamei* in low salinity water (LSW) is widespread in many countries (Santa & Vinatea 2007, Zha et al. 2007, Roy et al. 2009, Castillo-Soriano et al. 2011, Gunalan et al. 2012). Cyanobacteria blooms often occur in these shrimp ponds (Zimba et al. 2006). As a benthic omnivorous feeder, *L. vannamei* can ingest not only cyanobacteria suspended in pond water, but also organic debris containing both MCs and cyanobacteria in pond sediment. However, few studies have focused on the accumulation and biodegradation pathway of MCs in shrimp ponds. More than 300 MC variants have been found in natural waters, of which MC-LR, MC-RR and MC-YR are the most frequently detected and predominant variants (Jones 2019, Miles et al. 2024). In this study, we collected shrimp hepatopancreas, intestine and sediment samples from 11 *L. vannamei* culture ponds in China in August 2022, and analyzed the accumulation of MCs (MC-LR, MC-RR and MC-YR), *mlrA* gene abundance and microbial community structure in these samples. The results will provide useful information on the potential harm of MCs and *in situ* biodegradation pathways in shrimp culture ponds.

2. MATERIALS AND METHODS

2.1. Sampled ponds

Samples were collected from 11 *Litopenaeus vannamei* earthen culture ponds located between 20 and 40° N, and 103 and 122° E in China during August 2022 (Table 1). In these ponds, *Microsystis* blooms have occurred frequently in recent years. Three ponds were sampled in Binhai, Baodi and Xiqing Districts in Tianjin, and 1 pond was sampled from each of the other cities.

2.2. Sampling

Sixty shrimp were randomly collected from each pond, and the body length and weight of 30 out of every 60 shrimp were measured (Table 1). Hepatopancreas and intestines of collected shrimp were dissected and immediately frozen at −20°C at the field stations. Surface sediment samples (0 to 10 cm) were

Table 1. Locations of the sampled ponds and size of the sampled shrimp. Values are presented as mean ± SD, n = 30

Pond	Location	Longitude	Latitude	Size of shrimp	
				Body weight (g)	Body length (mm)
MS	Meishan, Sichuan Province	103° 75′ 39.92″ E	30° 15′ 68.19″ N	2.83 ± 0.57	67.59 ± 2.64
BY	Baiyin, Gansu Province	104° 35′ 13.70″ E	37° 20′ 27.90″ N	9.64 ± 0.55	96.78 ± 3.63
YJ	Yangjiang, Guangdong Province	112° 10′ 84.14″ E	21° 82′ 81.71″ N	3.12 ± 0.86	68.65 ± 11.21
ZH	Zhuhai, Guangdong Province	113° 24′ 54.24″ E	22° 36′ 74.12″ N	14.61 ± 1.69	131.42 ± 4.32
XQ	Xiqing District, Tianjin	117° 24′ 88.16″ E	38° 87′ 28.18″ N	21.43 ± 3.90	131.09 ± 4.23
BD	Baodi District, Tianjin	117° 38′ 26.42″ E	39° 44′ 83.45″ N	24.90 ± 3.65	131.28 ± 5.68
BH	Binhai District, Tianjin	117° 48′ 36.95″ E	38° 87′ 37.87″ N	24.23 ± 3.18	135.07 ± 5.50
BZ	Binzhou, Shandong Province	118° 15′ 62.90″ E	37° 35′ 64.77″ N	2.96 ± 0.57	62.62 ± 3.40
TS	Tangshan, Hebei Province	118° 51′ 38.66″ E	39° 25′ 89.14″ N	10.10 ± 2.35	93.09 ± 5.61
NB	Ningbo, Zhejiang Province	121° 63′ 75.57″ E	29° 88′ 61.17″ N	9.67 ± 0.36	95.84 ± 1.95
PJ	Panjin, Liaoning Province	122 °24 ′21.14 ″E	40 °92 ′34.05 ″N	8.48 ± 1.57	93.44 ± 4.35

collected from 3 sites in each pond using a columnar sediment sampler and frozen immediately. In the laboratory, the collected shrimp tissues and sediments were stored at −80°C prior to the analysis of accumulated MCs, microbial community composition and the abundance of the *mlrA* gene.

2.3. MC extraction and determination

For each pond, the hepatopancreas of every 20 shrimp were pooled together, and 3 replicate hepatopancreas samples were obtained. Each hepatopancreas sample was lyophilized and ground, and then extracted 3 times with 90% aqueous methanol solution at a 1:20 mass:volume ratio. Each sediment sample was freeze-thawed 3 times, lyophilized and ground, then extracted with 35 ml of 0.1% trifluoroacetic acid (TFA) with a solid:liquid ratio of 1:35. The mixtures obtained were ultrasonicated 3 times (1 min each time, 10 s interval) at 60% amplitude and then centrifuged at 12000 × *g* for 10 min. Extraction of MCs from each sediment sample was repeated 3 times. The 3 supernatants obtained from each sample were pooled and passed through a C_{18} solid phase extraction cartridge (Sep-Pak, Waters) pretreated with 10 ml of methanol and 10 ml of deionized water at a flow rate of 8 ml min^{-1}. The cartridge was rinsed with 20% methanol solution, then the bound MCs were eluted using 10 ml of methanol solution containing 0.1% TFA. The MC-containing elution was evaporated to dry under a stream of nitrogen gas, and the residue was dissolved in 1 ml of 50% methanol and then filtered through a 0.22 µm nylon membrane (COMYBION). Finally, the solution was analyzed using a HPLC System (SPD-M20A, Shimadzu) equipped with a Shim-Pack VP-ODS column (250 × 4.6 mm) and DAD detector. According to the relationship between analyte concentration and chromatographic peak area, the linear calibration curves of MC-LR, MC-RR and MC-YR were obtained using serially diluted solutions of corresponding standard products (Pushi). The concentrations of MC-LR, MC-RR and MC-YR were calculated by comparing the peak area of the sample with its linear calibration curve.

2.4. High throughput sequencing and data analysis

The sediment samples were precipitated at 12000 × *g* for 10 min. DNA was extracted from shrimp intestines and precipitated sediments using a soil DNA isolation kit (Omega) according to the manufacturer's instructions. The V3-4 hypervariable region of the bacterial 16S rRNA gene in sediments and intestines was amplified using the primers presented in Table 2. The PCR was performed on a Mastercycler Gradient (Eppendorf) with a 25 µl reaction volume containing 12.5 µl of 2× *Taq* PCR MasterMix, 3 µl of BSA (2 ng μl^{-1}), 1 µl of each primer (5 µM), 2 µl of template DNA and 5.5 µl of double-distilled water. The cycling parameters were 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 60 s, with a final extension at 72°C for 7 min. The PCR products were purified using an Agencourt AMPure XP Kit. Deep sequencing was performed on a Miseq platform at Overson Gene Technology (Beijing, China). The data analysis was performed according to the method of Dai et al. (2022).

2.5. Cloning and sequencing of the *mlrA* gene

The sequence of the *mlrA* gene was amplified from DNA extracted from sediment samples using the primers presented in Table 2. The PCR reaction mixture consisted of 12.5 µl of Premix Taq™ (TaKaRa), 0.5 µl of each primer (10 µM) and 2 µl of template DNA. The PCR program included 30 cycles of 94°C for 20 s, 57°C for 15 s and 72°C for 40 s. An initial step of denaturation at 94°C for 5 min and a final step of

Table 2. Primers and TaqMan probes used in PCR and qPCR analysis of the bacterial 16S rRNA gene and *mlrA* gene

Primer/probe	Sequence (5'−3')	Reference
338F	ACT CCT ACG GGA GGC AGC AG	Munyaka et al. (2015)
806R	GGA CTA CNN GGG TAT CTA AT	
mlrAtf	ACG CAC GCT CAC CTC AAC	Zhu et al. (2016)
mlrAtr	CAA GGC TCC TCC CAC AAA T	
qmlrA-F	AGC CCK GGC CRC TGC	Hoefel et al. (2009)
qmlrA-R	ATG CCA RGC CCA CCA CAT	
Probe *qmlrA*-tm	FAM-TGC CSC AGC TSC TCA AGA AGT TTG-BHQ	

10 min extension at 72°C were also included. The secondary PCR conditions were the same. Purified PCR products were cloned into the pMD19-T vector (TaKaRa) and then amplified in *Escherichia coli* DH5α. Recombinant plasmids were purified using the TaKaRa MiniBEST Plasmid Purification Kit Ver.4.0. Presence of the target DNA insert was confirmed by PCR amplification and sequenced at Sangon Biotech (Shanghai, China). The positive recombinant plasmid was extracted using the MiniBEST Plasmid Purification Kit Ver.4.0, and the *mlrA* gene copy number (copies μl^{-1}) was calculated as:

$$mlrA \text{ copy number} = (6.02 \times 10^{23} \times P_c \times 10^{-9}) / (P_L \times 660) \quad (1)$$

where P_c is the plasmid concentration (ng μl^{-1}) and P_L is the plasmid length (bp).

2.6. Quantitative analysis of the *mlrA* gene using TaqMan qPCR

The standard curve showing the relationship between the *mlrA* gene copy number and its quantification cycle (Cq) value was prepared using continuous dilutions of purified plasmid DNA from 4.98×10^8 to 4.98×10^2 copies μl^{-1} in triplicate. Quantification of the *mlrA* gene was performed on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). The primers and TaqMan probe are shown in Table 2. qPCR reactions were performed in triplicate in a 20 µl reaction mixture containing 10 µl of Probe qPCR Mix (TaKaRa), 0.5 µl of each primer and probe (10 µM) and 5 µl of template DNA (10 ng μl^{-1}). The PCR conditions were 95°C for 1 min followed by 45 cycles of 95°C for 15 s and 62°C for 45 s.

3. RESULTS

3.1. Accumulation of MCs in shrimp hepatopancreas and sediments

As shown in Table 3, the concentration of MCs (MC-RR, MC-LR and MC-YR) in hepatopancreas ranged from 0.02 to 4.83 µg g^{-1} dry weight (DW). Among the 3 variants detected, MC-RR was most commonly found accumulated in shrimp hepatopancreas. The concentration of MC-RR detected in hepatopancreas ranged from 0.02 to 3.54 µg g^{-1} DW. MC-LR and MC-YR were detected in shrimp hepatopancreas in 6 and 2 ponds, respectively. When MC-LR and MC-YR were identified in the hepatopancreas, they were also found to be the dominant variant in most ponds. The highest accumulation of MC-RR and total MCs were all observed in Pond YJ in Guangdong Province.

MCs were detected in all sampled pond sediments (Table 4). Consistent with the accumulation in shrimp hepatopancreas, MC-RR was the variant most frequently detected in sediments. The detected concentration range was 0.01 to 0.30 µg g^{-1} DW. MC-LR was detected in only 1 pond sediment (Pond BH). MC-YR was detected in the sediments of 3 ponds (Ponds MS, BY and ZH) and was the dominant variant in these ponds. MC content in the sediments from different sampling sites in the same pond varied greatly, suggesting the distribution of MCs in the sediments was not uniform.

Table 3. MC accumulation in hepatopancreas of shrimp from sampled ponds (µg g^{-1} DW). Values are presented as mean ± SD, n = 3. Total MCs: sum of MC-RR, MC-LR and MC-YR; nd: not detected. See Table 1 for pond abbreviations

Pond	MC-RR	MC-LR	MC-YR	Total MCs
MS	0.82 ± 0.37	2.14 ± 1.15	nd	2.96 ± 1.51
BY	0.06 ± 0.05	0.36 ± 0.31	nd	0.46 ± 0.22
YJ	3.54 ± 1.78	nd	1.29 ± 0.15	4.83 ± 1.92
ZH	0.59 ± 0.03	nd	2.95 ± 0.90	3.54 ± 0.89
XQ	0.11 ± 0.01	0.92 ± 0.15	nd	1.03 ± 0.15
BD	0.13 ± 0.11	0.57 ± 0.13	nd	0.70 ± 0.20
BH	0.38 ± 0.22	nd	nd	0.38 ± 0.22
BZ	0.16 ± 0.06	nd	nd	0.16 ± 0.06
TS	1.05 ± 0.06	0.74 ± 0.65	nd	1.79 ± 0.60
NB	0.02 ± 0.02	nd	nd	0.02 ± 0.02
PJ	0.58 ± 0.16	0.34 ± 0.30	nd	0.92 ± 0.14

Table 4. MC accumulation in sediment of sampled ponds (µg g^{-1} DW). Values are presented as mean ± SD, n = 3. Total MCs: sum of MC-RR, MC-LR and MC-YR; nd: not detected. See Table 1 for pond abbreviations

Pond	MC-RR	MC-LR	MC-YR	Total MCs
MS	nd	nd	0.37 ± 0.38	0.37 ± 0.38
BY	0.01 ± 0.01	nd	0.66 ± 0.06	0.67 ± 0.06
YJ	0.05 ± 0.09	nd	nd	0.05 ± 0.09
ZH	0.07 ± 0.03	nd	0.29 ± 0.51	0.36 ± 0.52
XQ	0.08 ± 0.14	nd	nd	0.08 ± 0.14
BD	0.08 ± 0.04	nd	nd	0.08 ± 0.04
BH	0.30 ± 0.27	0.20 ± 0.04	nd	0.50 ± 0.31
BZ	0.03 ± 0.04	nd	nd	0.03 ± 0.04
TS	0.05 ± 0.09	nd	nd	0.05 ± 0.09
NB	0.03 ± 0.02	nd	nd	0.03 ± 0.02
PJ	0.05 ± 0.09	nd	nd	0.05 ± 0.09

3.2. Microbial richness and diversity in shrimp intestines and sediments

The number of observed operational taxonomic units (OTUs) in shrimp intestines and sediments ranged from 228 to 3539 and 4342 to 9226, respectively. As shown in Fig. 1, the Chao1 and Shannon indices for intestines and sediments varied widely among different ponds. Both showed that intestine from Pond NB and sediment from Pond XQ had the highest species diversity, while intestine from Pond TS and sediment from Pond MS had the lowest species diversity. The bacterial richness and diversity indices (observed OTUs, Chao1 and Shannon indexes) in intestinal samples were much lower than those of sediment samples.

3.3. Microbial community composition in shrimp intestines and sediments

A total of 761 microbial genera were identified in shrimp intestines (Fig. 2). The abundances of the top 30 dominant genera varied greatly in different ponds. *Cetobacterium* and *Sphingobium* showed relatively high abundances in 5 ponds. *Cetobacterium* was the most dominant genus in 3 ponds (Ponds BZ, TS and PJ) and the second most dominant genus in 2 ponds (Ponds BD and MS). The relative abundance of *Cetobacterium* in Pond BZ was the highest, accounting for 34.24%. *Sphingobium* was the most dominant genus in 3 ponds (Ponds BD, NB and BY) and the second most dominant genus in 2 ponds (Ponds BH and PJ), with a relative abundance of 16.65 to 27.02%. Another genus with a relatively high abundance in most ponds was uncultured. The most dominant genus in Ponds ZH and YJ was *Paraclostridium*, accounting for 20.99 and 30.86%, respectively. *Lactococcus* was the predominant genus in Pond MS, with a relative abundance of 39.26%.

There were 1191 microbial genera identified in pond sediments. As shown in Fig. 3, the most dominant genera in Ponds MS and BY were *Acetobacteroides* (with a relative abundance of 43.77%) and *Rhodobacter* (with a relative abundance of 39.66%). *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* was the second most dominant genus in Pond ZH. The second and third most dominant genera in Ponds MS and BY were uncultured or could not be unidentified, and the dominant genus in Pond ZH could not be identified. The top 3 or top 2 most dominant genera in the sediments from the other 8 ponds could also not be identified or were uncultured. Among the other identified genera in sediments, *Thiobacillus* showed a relatively high abundance in 5 ponds (Ponds BD, YJ, NB, XQ and ZH), and its abundance varied greatly in these ponds. The relative abundance in Pond NB was the highest (18.97%), while that in Pond BZ was only 2.27%. In addition to *Thiobacillus*, the relative abundance of *Cyanobium* in most ponds was also high, ranging from 0.15 to 13.84%, indicating cyanobacteria deposited in the sediments.

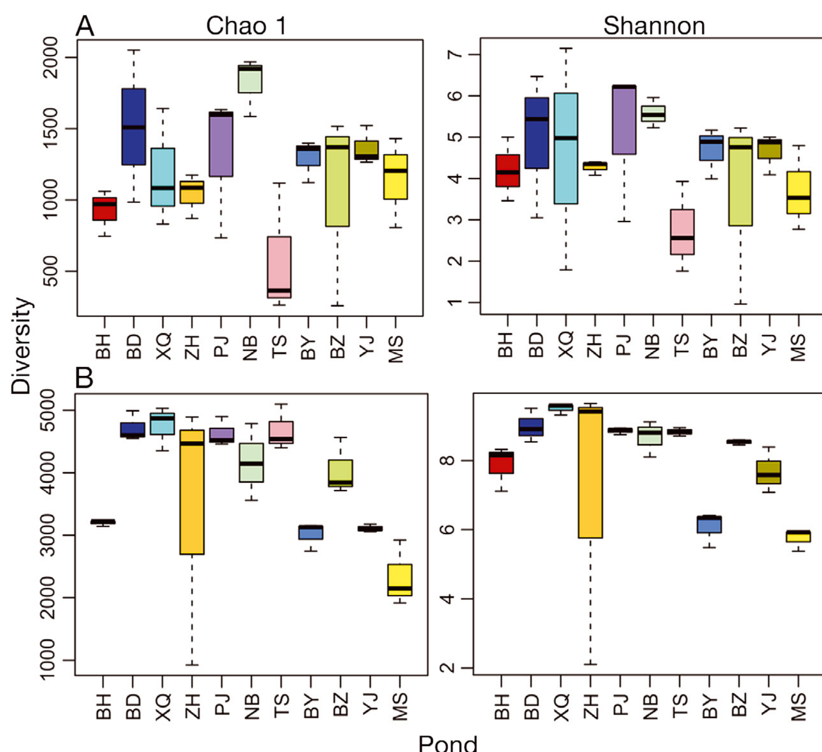

Fig. 1. Microbial diversity indexes in (A) shrimp intestines and (B) sediments of sampled ponds. Horizontal line, boxes and whiskers represent the median, number located at 50% position in data sequence and mild outliers, respectively. See Table 1 for pond abbreviations

3.4. Standard curve for the quantification of the *mlrA* gene

The sequence of the *mlrA* gene fragment amplified from the sediment is shown in Fig. A1 in the Appendix. The sequence showed 99% identity with the *mlrA* gene fragments of *Sphingosinicella microcystinivorans* B9 (AP018711.1), *Rhizobium* sp.

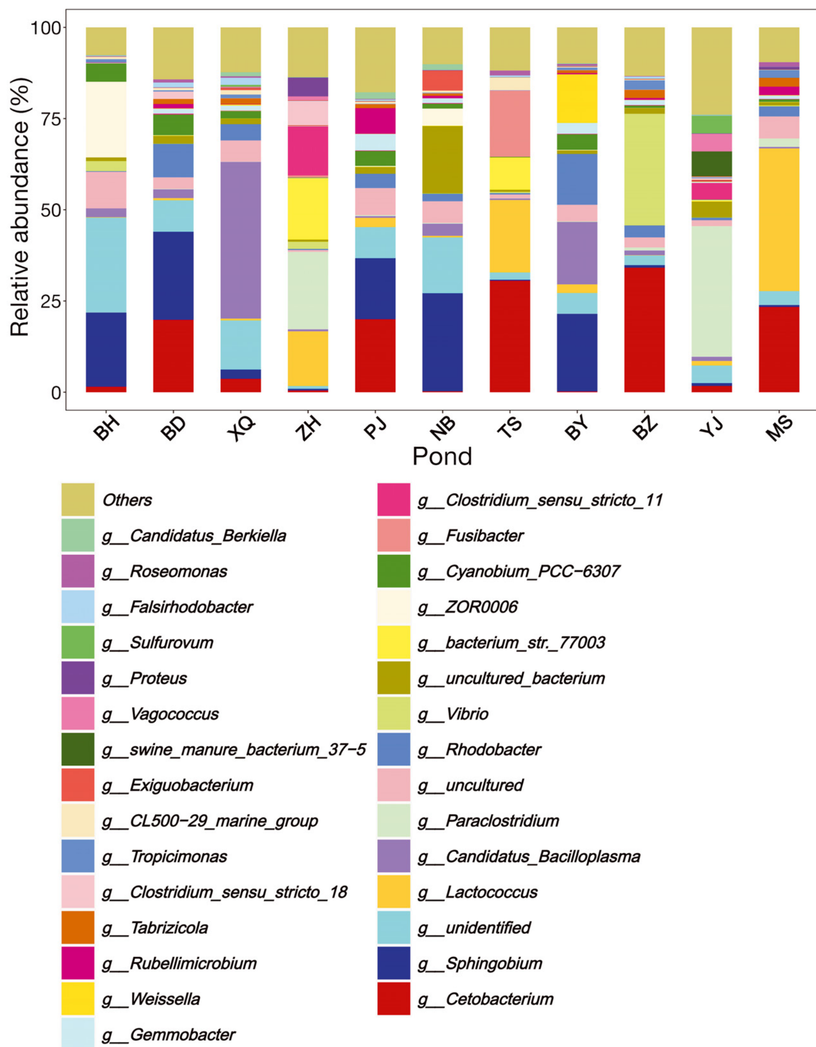

Fig. 2. Microbial community composition in the intestines of shrimp from sampled ponds. See Table 1 for pond abbreviations. *g_*: at genus level

TH (KX371892.1) and *Sphingomonas* sp. Y2 (T AB114203.1). As shown in Fig. A2 in the Appendix, in the established standard curve, the Cq value had a very good linear relationship with the log values of plasmid DNA copy number: $y = -3.380 \times \lg(x) + 33.299$ ($R^2 = 0.999$, E = 97.6%).

3.5. Abundance of the *mlrA* gene in shrimp intestines and sediments

As shown in Table 5, the *mlrA* gene could be detected in all shrimp intestines, and its abundance ranged from 679.12 to 8581.98 copies g^{-1}, in Ponds YJ and BZ, respectively. The *mlrA* gene was detected only in sediments from ponds in Guangdong Province (Ponds ZH and YJ), Tianjin City (Ponds BH, BD, XQ) and Hebei Province (Pond TS), with detected abundances ranging from 3.68 to 291.31 copies g^{-1}. No *mlrA* gene was detected in sediments from the ponds in the other 5 provinces. The variation trend of MC concentration and *mlrA* gene abundance in sediments was not consistent. The *mlrA* gene was more easily detected in intestines than in sediments, and the abundance of the *mlrA* gene in intestines was much higher than that in sediments.

4. DISCUSSION

Previous studies mainly focused on MC distribution in shrimp and water in shrimp culture ponds (Zimba et al. 2006, Zhang et al. 2018). In addition to their distribution in water, MCs can also accumulate in sediments through direct adsorption (Mohamed et al. 2007), chemical coagulation and flocculation (Drikas et al. 2001), co-precipitation with clay or particulate matter, and precipitation of feces from cyanobacteria-consuming organisms (Wu et al. 2011). MCs were detected in all sampled pond sediments, indicating that shrimp ponds suffering from cyanobacteria blooms were often contaminated with varying degrees of MCs. Once absorbed by the intestinal epithelia or gills, MCs can be distributed in various organs or tissues of animals, and the hepatopancreas or liver is the primary target organ for MC accumulation (Cazenave et al. 2005, Bi et al. 2019). There was a low negative linear correlation between MC concentration in the hepatopancreas and sediment (r = −0.062). One of the reasons might be the fact that the primary intake source of MCs for shrimp is water rather than sediment. In 3 ponds (Ponds MS, YJ and BZ) where the shrimp were similar in size (about 3 g in body weight), the highest and lowest accumulation of MCs in the hepatopancreas were 4.83 and 0.16 μg g^{-1} DW, respectively. In Ponds BH and BD, the shrimp were larger, reaching more than 24 g in body weight. However, the accumulation of MCs in the hepatopancreas of these shrimp was only 0.38 and 0.70 μg g^{-1} DW. Consumption of market-size shrimp from these sampled ponds would pose different potential health

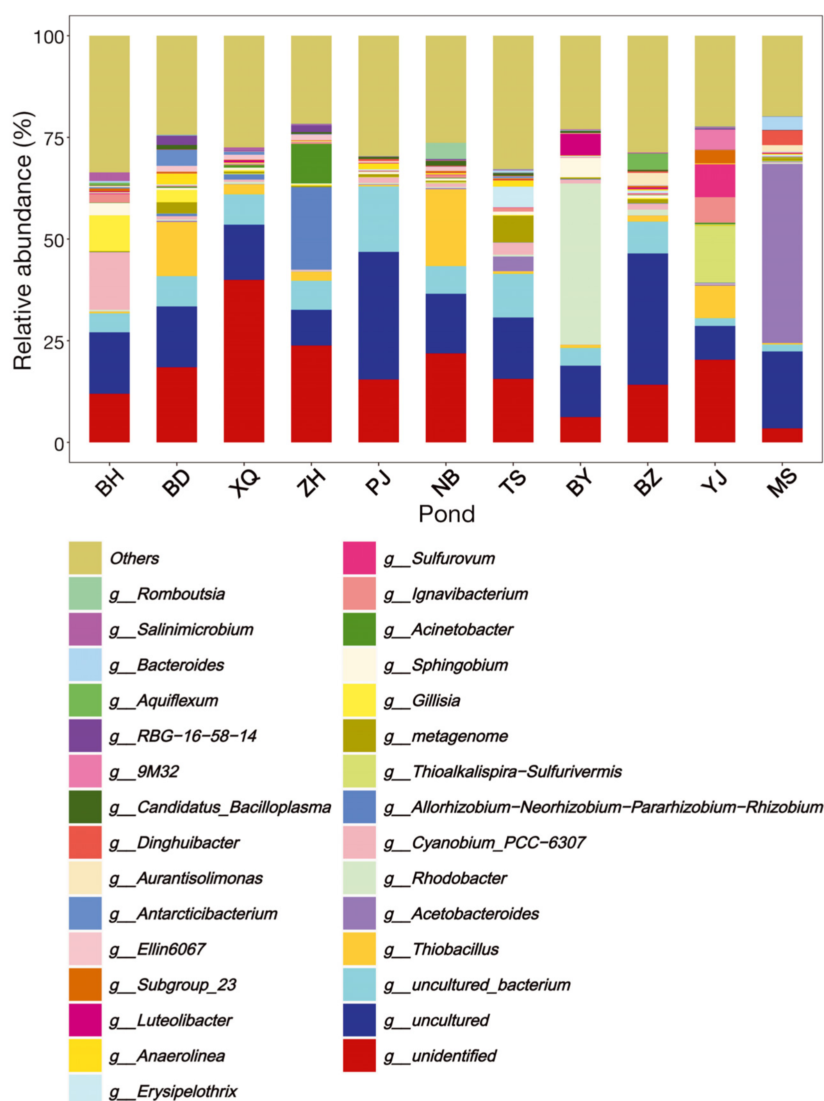

Fig. 3. Microbial community composition in the sediments of shrimp ponds. See Table 1 for pond abbreviations. *g*_: at genus level

risks. In other water systems, it has often been observed that MC-RR and MC-LR are more prevalent than MC-YR (Sivonen et al. 1994, Sivonen 2005), and MC-RR or MC-LR are often the dominant variants (Ozawa et al. 2005). Among the 3 variants detected, MC-RR was the most frequent and MC-YR was the dominant variant if detected in the sampled sediments. MC-LR was only detected in sediment from 1 pond, but in shrimp hepatopancreas from 6 ponds. MCs are not uniformly distributed in sediments and MC-LR might be present in unsampled sediments. Moreover, the MC-LR accumulated in shrimp came not only from the sediment but also from the water column.

Bacteria that degrade MCs have been reported from multiple phyla, including *Proteobacteria* (α, β and γ), *Actinobacteria*, *Bacilli* and *Flavobacteriia* (Massey & Yang 2020). *Sphingomonas* was the first and most commonly reported MC-degrading bacteria genus (Park et al. 2001, Ishii et al. 2004, Amé et al. 2006), followed by *Sphingopyxis* (Ding et al. 2018, Qin et al. 2019, Yang et al. 2020). Bacteria species belonging to other genera, such as *Pseudomonas* (Li & Pan 2014, Krishnan et al. 2018), *Arthrobacter* (Manage et al. 2009, Lawton et al. 2011), *Bacillus* (Alamri 2012, Zhang et al. 2015), *Stenotrophomonas* (Chen et al. 2010, Yang et al. 2014), *Neosphingobium* (Jiang et al. 2011, Phujomjai et al. 2016), *Paucibacter* (Rapala et al. 2005, Lezcano et al. 2016), *Acinetobacter* (Li & Pan 2014, 2016), *Rhizobium* (Zhu et al. 2016) and *Methylobacillus* (Hu et al. 2009), have also been reported to degrade MCs. No MC-degrading bacteria isolates were observed in the top 30 most dominant genera in shrimp intestines, but 2 genera (*Sphingobium* and *Roseomonas*) belonging to the MC-degrading bacterial community were found. *Sphingobium* was the most or second most dominant bacteria in 5 ponds. *Sphingomonas*, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis* are the hydrocarbon-degrading members of the *Sphingomonadaceae* capable of degrading a broad range of mono- and polycyclic aromatic compounds (Ker-

Table 5. Abundance of the *mlrA* gene in shrimp intestine and sediment of sampled ponds (copies g^{-1}). Values are presented as mean ± SD, n = 3. nd: not detected. See Table 1 for pond abbreviations

Pond	Intestine	Sediment
MS	1753.38 ± 530.09	nd
BY	1188.58 ± 156.72	nd
YJ	679.12 ± 263.59	291.31 ± 41.13
ZH	1605.75 ± 312.53	90.89 ± 40.08
XQ	2793.45 ± 1031.00	48.63 ± 5.29
BD	1632.43 ± 1052.86	58.89 ± 13.82
BH	2042.50 ± 169.78	116.57 ± 83.70
BZ	8581.98 ± 7424.76	nd
TS	4384.25 ± 737.16	3.68 ± 1.44
NB	1141.09 ± 446.08	nd
PJ	2143.39 ± 304.48	nd

tesz & Kawasaki 2010). Tsao et al. (2017) found that a mixed culture of indigenous bacteria isolated from a reservoir could remove >99% of MC-LR, and *Sphingobium* sp. was one of the species identified in this mixed culture. *Sphingobium* identified in shrimp intestines might play an important role in the degradation of MCs. In addition to *Sphingobium*, *Roseomonas* was also one of the top 30 most dominant genera in shrimp intestines. This genus has been found in a natural bacterial community that completely degraded MC-LR and desmethyl MC-LR (Des-MCLR) (Briand et al. 2016). As in sediment, MC-degrading bacteria are also present in shrimp intestine and may be one of the main defense strategies against the harm of MCs. In order to reduce accumulation of MCs in shrimp, efficient MC-degrading bacterial communities or specific MC-degrading strains could be isolated and developed as feed supplements, which would ultimately minimize the risk of MC intoxication in consumers and benefit human safety. *Rhizobium* and *Acinetobacter* were also observed in the top 30 most dominant bacteria in sediments. *Rhizobium* and *Acinetobacter* have been identified in MC-degrading bacteria isolates (Li et al. 2016, Zhu et al. 2016), and also in MC-degrading bacterial communities (Tsao et al. 2017, Manheim et al. 2018). To date, the number of MC-degrading bacteria isolated from the natural environment is much lower than that thought to be present (Mankiewicz-Boczek et al. 2015). There might also be other unreported genera with MC-degrading capacity in the bacterial communities of intestines and sediments.

In the natural environment, there are 2 ways to degrade MCs: through photodegradation and microbial decomposition (Bukowska et al. 2018). In natural waters, the degradation of MCs cannot be mediated by sunlight alone because the absorption spectrum of MCs does not overlap with the solar spectrum (Tsuji et al. 1994). MCs can be degraded by sunlight in the presence of photosensitizers, such as humic substances or algal pigments (Tsuji et al. 1994, Welker & Steinberg 2000). In sediments, photosensitized degradation of MCs cannot occur due to the lack of light. In the absence of photodegradation conditions in sediments, biodegradation might be the main pathway for MC degradation. Previous studies have found sediments contain MC-degrading bacteria (Dziga et al. 2013, Li et al. 2017, Bukowska et al. 2018, Santos et al. 2020). In this study, 3 reported MC-degrading genera were detected in the sediments of all shrimp ponds, while the *mlrA* gene was detected in the sediments of only 6. The total relative abundances of the 3 MC-degrading genera were 5.08% in Pond BZ and 0.71% in Pond YJ. However, no *mlrA* gene was detected in Pond BZ, and *mlrA* gene abundance was highest in Pond YJ (291.31 copies g^{-1}), which was much higher than that in Pond ZH. The total relative abundance of the 3 MC-degrading genera was highest in Pond ZH (30.36%). Apart from the biodegradation pathway encoded by the *mlr* gene cluster, other mechanisms have not been fully elucidated. In some higher plants, invertebrates and vertebrates, the glutathione S-transferases (GSTs) may be involved in MC detoxification by catalyzing the conjugation of glutathione (GSH) with MCs (Pflugmacher et al. 1998, Campos & Vasconcelos 2010, Antas et al. 2018). In Lake Erie, GSTs were more abundant where MCs were degraded (Mou et al. 2013). Alkaline proteases from *Pseudomonas aeruginosa* play a role in the biodegradation of MCs, but the specific alkaline proteases have not been well described (Takenaka & Watanabe 1997). Krausfeldt et al. (2019) found that transcripts of *mlr* genes were absent in samples collected from Lake Erie and Lake Taihu, and detected temporal and spatial expression of glutathione S35 transferase and alkaline protease genes in both lakes, suggesting they might be individually or collectively more important than *mlr* genes in the natural environment. MC-degrading bacteria were present in all sampled sediments, but the *mlrA* gene was not detected in some samples. In addition to the *mlr* pathway, other alternative pathways, such as GSTs and alkaline proteases, might biodegrade MCs in sediments. As in sediment, the degradation of MCs in intestines might mainly depend on biodegradation since the light required for photodegradation is not present in the intestine. In the Xiangang reservoir of Huizhou, China, the *mlrA* gene could be amplified from the intestines of 5 fish species (*Cirrhinus molitorela*, *Cyprinus carpio*, *Hypophthalmichthys molitrix*, *Carassius auratus* and *Xenocypri davidi*) during cyanobacteria blooms, but not in the absence of blooms (Liang et al. 2013). In this study, the *mlrA* gene was more easily detected and more abundant in intestines than in sediments. Biodegradation via the *mlr* pathway played a more important role in intestine than in sediment.

5. CONCLUSION

MCs were detected in shrimp hepatopancreas and sediments from all shrimp ponds, suggesting that MC pollution occurs frequently in shrimp ponds. Reported MC-degrading genera were detected in intestinal and sediment bacterial communities. In addition, the *mlrA* gene was more easily detected and more abundant in

intestines than in sediments. The biodegradation pathway in intestines seemed to be more dependent on the *mlr* pathway. As in sediment, MC-degrading bacteria were also present in shrimp intestine and may be one of the main defense strategies against the harm of MCs. In order to reduce accumulation of MCs in shrimp, efficient MC-degrading bacterial communities or specific MC-degrading strains could be isolated and developed as feed supplements, which would ultimately minimize the risk of MC intoxication in consumers and benefit human safety.

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Appendix. Supplementary statistics

GGG GTG CCC GGT TCG ATT ATC CAA CAG CCT ATA CGT TCC TGT TGT TCC CGG CTG CAC CAA TGC TTG CGG CCC
TGA TTG TGA CCG GAA TAG GCT ACG GTC GCG CAG GAT TTC GTG AGC TGC TAA GCC GCT GCG CCC CTT GGC GAG
ATC CTG TTT CGT GGC GGC AGG GCG TCA CTG TCA TTG CGG TGT GCT TCC TTG TCT TCT TCG CGC TCA CCG GGA
TGA TGT GGG TTC AGA CCT ACC TAT ACG CTC CGT CAG GTA CGC TCG ATC GCG CAT TCC TGC GCT ATG GGT CAG
ATC CGC TCT CCA TTT ACG CGA TGC TGG CAG CAT CGC TTC TTA TCA GCC CGG GCC CGC TGC TCG AGG AGC TTG
GCT GGC GCG GGT TCG CAC TGC CCC AGC TGC TCA AGA AGT TTG ACC CCC TGA CGG CGG CGG TCA TCC TCG
GCA CCA TGT GGT GGG CCT GGC ATT TGC CAC GCG ACT TGC CGG CAA TGT TCT CCG GCG AGC CTG GTG CCC TCT
GGG GGG TTA TCG TCA AGC AGT TCG TTA TCG CGC CCG GAA TGA TCG CCA GTA CGA TCA TCG CTG TCT TTG TTT
GCA ACA AAC TGG GTG GAT CGC TGT GGG GCG GAT TGC TTA CTC ACG CGA TCC ATA ACG AGC TGG GCG TAA ACG
TAA TGG CCG AAT GGT CGC CCG CGG CTG CAG GAC TCG GGT GGC GCC CTT GGG ATT TCA TCG AAT TCG CCG TGG
CCA TTG GGC TCG TCC TGA TTT GTG GGA GGA GCC TTG AAT CGT CGA CCT GCA GGC ATG CAA GCT TGG CGT AAT
CAT GGT CAT AGC TGT TTC CTG TGT GAA TTG TTA TCC GCT CAC ATT CAC ACA CAT ACG AGC CGG AAG CAT AAA
GTG TAA AGC TGG GGT GCT ATG AGT GAG CTA CTC ACA TTA TTG CGT GCG CTC ACT G

Fig. A1. Sequence of the *mlrA* gene fragment amplified from the sediment

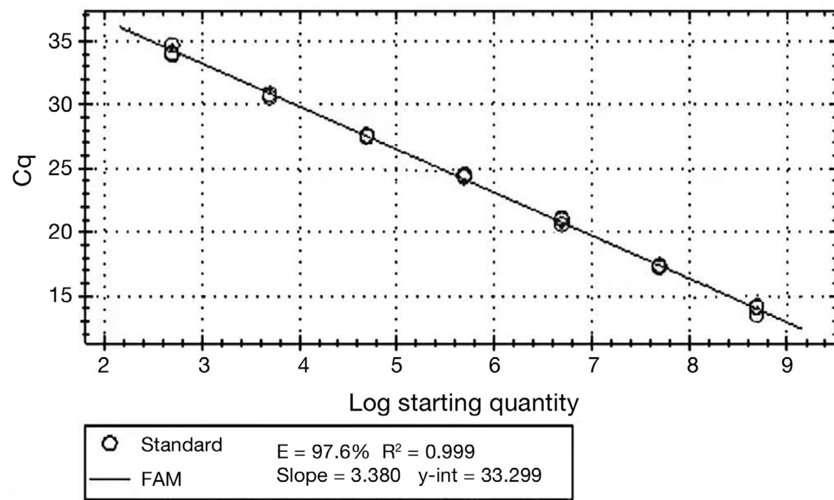

Fig. A2. Standard curve for the quantification cycle (Cq) of *mlrA*

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