Assessment of total bacterial diversity in whiteleg shrimps and its aquaculture environment in Pangkajene and Banyuwangi, Indonesia

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Abstract. Detection of bacterial diversity in whiteleg shrimps and its rearing water is a vital first step in monitoring aquaculture activities. Bacterial community imbalance in whiteleg shrimps and its rearing water influences the quality and quantity of shrimp production. Identifying the bacterial community provides basic information related to dominant bacterial groups in whiteleg shrimps and environments, providing recommendations for proper environmental monitoring and management. In this study, we investigated bacterial community structure in the rearing water and intestinal tract of whiteleg shrimp (*Litopenaeus vannamei*) collected from two sites, i.e., Pangkajene, South Sulawesi (SU) and Banyuwangi, East Java (BW), Indonesia. The bacterial community was analyzed using amplicon sequencing with Illumina sequencing platform based on the V3-V4 region of the 16S rRNA genes. Bacterial diversity and composition were found differed between the rearing water and the shrimps' intestines. Bacterial diversity in the rearing water of Banyuwangi (W.BW) was higher than that of Pangkajene (W.SU). Proteobacteria, Bacteroidetes, and Firmicutes were found as the most dominant phyla in rearing water from both farms, while distinct bacterial composition was observed in the shrimps' intestines. The shrimp intestine from Banyuwangi (U.BW) was dominated by Firmicutes (22.36%), Proteobacteria (22.33%), and Verrucomicrobia (21.11%). In contrast, the shrimp intestine from Pangkajene (U.SU) was highly dominated by Tenericutes (88.54%), followed by Proteobacteria (4.66%), and Firmicutes (2.27%). The difference in bacterial community structure between the rearing water and shrimps' intestines suggested that the host intestinal environment might have greater selective pressure for bacterial composition inhabiting *L.vannamei* intestines. Our observations suggest that the shrimps cultured in the rearing water with the similar dominant bacterial group have specific intestinal bacterial diversity.

Keywords: aquaculture water, bacterial composition, *Litopenaeus vannamei*, metagenomic analysis, shrimp culture

INTRODUCTION

Whiteleg shrimp is one of the mainstay aquaculture products in Indonesia and other several countries in the world. The production of whiteleg shrimp through aquaculture activities has

undoubtedly been able to meet the needs of protein foods globally. Food and Agriculture Organization (FAO) has identified that Indonesia is one of the pilot countries for shrimp farming

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(Immink *et al.*, 2017). However, along with the continuing increase in demand for shrimp products throughout the world, farmers face many challenges in producing high quality and quantity products. Shrimp farming in Indonesia has spread in a variety of regions, including Lampung province, Java, and Sulawesi Island. In Java Island, some areas with high shrimp production are Gresik, Situbondo, Banyuwangi, and Probolinggo. Meanwhile, in Sulawesi, shrimp producers are located along the coast in South Sulawesi.

The success of shrimp farming activities should not be separated from appropriate and sustainable environmental monitoring and management activities. Environmental conditions affect the condition of cultured animals. Basic information on environmental conditions, including physical, chemical, and biological characteristics, is required to produce comprehensive information related to further environmental management techniques, such as bioremediation techniques and probiotics applications in aquaculture (Hastuti, 2011; Hastuti *et al.*, 2020). Bacterial diversity as a biological parameter is significantly influenced by the dynamic of physical and chemical parameters in aquaculture environments and cultured animals. Previous studies reported that the bacterial community structure in the aquatic environment is correlated with nutrients (Biddanda *et al.*, 2001), viral lysis (Suttle, 1994), phytoplankton (Pinhassi *et al.*, 2004), pH and water clarity (Yannarell & Triplett, 2005), environmental temperature (Tang *et al.*, 2014), marine sediment texture (Zheng *et al.*, 2014), salinity (Bouvier & del Giorgio 2002), ammonia and nitrite stress (Duan *et al.*, 2018), and microbial aerosols in atmospheric ecosystems (Dueker *et al.*, 2018). And vice versa, the environment could be affected by microbial diversity, which further resulted in the dynamics of other biogeochemical parameters that are constantly moving. The environment is one of the factors that is able to have a double effect on shrimp stress responses, both directly and indirectly (Snieszko, 1974). Aquatic microbiota in an aquaculture environment is composed of microbes that are beneficial, neutral, harmful obligate, and opportunistic pathogenic to the cultured animals. The microbiota inhabiting the

aquatic environment is usually stable in composition (Schulze *et al.*, 2006).

In shrimp aquaculture, the bacterial diversity and composition within the digestive tract must be in equilibrium. Several factors influence the intestinal microbial community, such as host developmental stage (Rungrassamee *et al.*, 2013; Zeng *et al.*, 2017; Cornejo-Granados *et al.*, 2018), host's internal environment (Rungrassamee *et al.*, 2014), diet and dietary supplementation (Sha *et al.*, 2016; Cornejo-Granados *et al.*, 2018), lifestyle and organ (Cornejo-Granados *et al.*, 2018), and exposure to pathogens (Wang *et al.*, 2019). Bacterial diversity is linked to the microbial community's functional stability in the shrimp intestines (Duan *et al.*, 2018). Intestinal microbiota contributes to promoting their host health, participating in nutrient absorption, immune response, and disease resistance, including against pathogens, such as *Vibrio harveyi* in black tiger shrimp and whiteleg shrimp (Rungrassamee *et al.*, 2016). Dysbiosis in the intestinal microbial community might cause diseases to its host. Intestinal microbiota management could be suggested to play an essential role in host development and disease prevention. Thus, maintaining healthy balanced microbial communities in the shrimp rearing environment and digestive tract could be an appropriate strategy for disease prevention and yield improvement in shrimp culture. Aquaculture environment dominated by pathogenic microbes would lead to some diseases in shrimp, such as White Spot Syndrome Virus (WSSV) (Wang *et al.*, 2018), and Early Mortality Syndrome (EMS)/Acute Hepatopancreatic Necrosis Disease (AHPND) (Cornejo-Granados *et al.*, 2017; Dong *et al.*, 2021). Also, the previous study reported that White Feces Shrimp (WFS) may be caused by the shift in bacterial community inhabiting shrimp infected by pathogens (Hou *et al.*, 2018).

Bacteria are considered the main actor in the continuity of varying biogeochemical cycles on Earth, including in the shrimp farming environment. Total bacterial diversity in the aquaculture environment as part of biological parameters is still not common in the primary parameter analysis. Identification of total bacterial diversity in shrimp culture environment should be the main concern as basic information for

recommendations regarding further cultivation management based on microbial activity. It is not only for the sustainability of the cultivation process but also for the sustainability of the aquatic environment in general. Identifying bacterial diversity is carried out to obtain information related to bacterial diversity at different taxonomic ranks. According to a new estimate, more than 99% of the whole microbial species on the Earth remain unobserved (Locey & Lennon, 2016). Bacterial diversity in the aquaculture environment might affect the bacterial diversity within the farmed shrimp, which presumably differs from one location to another. In order to answer the hypotheses, experiments are needed to determine the total bacterial diversity in cultured shrimp and their environment. This present study was conducted to identify the total bacterial diversity inhabiting the shrimp's intestines and its rearing water in Pangkajene, South Sulawesi and Banyuwangi, East Java using Illumina-based 16S rRNA gene amplicon sequencing.

MATERIALS AND METHODS

Water quality measurement

Water quality parameters measured in this study were pH, dissolved oxygen (DO), and salinity using pH meter, DO meter, and refractometer, respectively.

Samples collection

Pond water and 50-day-old shrimp samples were aseptically collected from three aquaculture ponds in the coastal areas of Pangkajene and Islands Regency, South Sulawesi (Pond 1: 4°46.273'S 119°31.794'E; Pond 2: 3°58.912'S 119°34.986'E; and Pond 3: 3°29.239'S 119°8.828'E) and Banyuwangi Regency, East Java (Pond 1: 7°41'53.5"S 114°21'12.8"E; Pond 2: 7°41'59.3"S 113°54'12.5"E; and Pond 3: 8°28'32.9"S 114°21'12.8"E). Shrimp samples were randomly taken from each pond in both locations. We collected water and shrimp samples from shrimp ponds in Pangkajene and Banyuwangi that applied a bioremediation-based environmental management system. The probiotics used in these ponds were commercial probiotics freely sold in

Indonesia. Its aquaculture system tends to intensive and semi-intensive farming with a density of >100 shrimps per m². The bottom of the ponds was high-density polyethylene (HDPE) layer and concrete. Water samples from Pangkajene, South Sulawesi and Banyuwangi, East Java, were coded as W.SU and W.BW, respectively. Meanwhile, shrimp samples from Pangkajene Regency, South Sulawesi and Banyuwangi Regency, East Java, were marked as U.SU and U.BW, respectively. An equal amount of three water samples were pooled based on the same location into a composite sample, so do the shrimp intestine samples.

DNA extraction and PCR amplification

Prior to extracting the total genomic DNA, a total of 200 mL rearing water samples from each location was filtered through a polycarbonate membrane filter with a pore size of 0.22 µm. DNA from the rearing water entrapped in the filter was extracted using PowerWater DNA Isolation Kit (Mobio Laboratories, Carlsbad, CA, US) following the manufacturer's recommendation. Meanwhile, shrimps' intestine samples were aseptically collected from 5 individuals in each location (50-day-old shrimp), then were pooled into one sample for each location. DNA from the shrimp intestine samples was extracted using PowerFood DNA Isolation Kit (Mobio Laboratories, Carlsbad, CA, US) based on the supplier's manual. The quality and quantity of gDNA were assessed using electrophoresis on an agarose gel and Nanodrop 2000c (NanoDrop Technologies, Wilmington, US). DNA samples were sent to NovogeneAIT Genomics Singapore Pte. Ltd. (Singapore) for bacterial community analysis performed using the Illumina-based sequencing technique.

PCR amplification was conducted using a universal primer set targeting the V3-V4 hypervariable region of the bacterial 16S rRNA gene, i.e., 341F (5'-CCTACGGGAGGCAGCAG-3') (Muyzer *et al.*, 1993) and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso *et al.*, 2011) added with unique barcode sequences. The PCR reaction was performed using Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, US). PCR products were confirmed using electrophoresis

on 2% agarose gel, and distinct bands with a size of 400-450 bp were picked up for further analysis. Prior to library generation, PCR products were purified using Qiagen Gel Extraction Kit (Qiagen, Hilden, DE). The sequencing library was generated with NEBNext® Ultra™ DNA Library Prep Kit (NEB, Ipswich, MA, US) for the Illumina platform. The DNA library's quality and quantity were determined by Qubit® dsDNA assays (Thermo Fisher Scientific, Waltham, MA, US) and qPCR, then sequenced by Illumina HiSeq 2500 PE250.

Analysis of sequences data

Illumina HiSeq platform generates the paired-end reads. Barcode and primers sequence of the paired-end reads were cut off, and then the paired-end reads were merged using FLASH v1.2.7 (<http://ccb.jhu.edu/software/FLASH/>) (Magoč & Salzberg, 2011). The merging process yielded raw tags. Subsequently, quality filtering of the raw tags was conducted under specific filtering conditions to obtain high-quality clean tags using Quantitative Insights Into Microbial Ecology (QIIME) v1.7.0 (http://qiime.org/scripts/split_libraries_fastq.html) (Bokulich *et al.*, 2013; Caporaso *et al.*, 2010). Obtained tags were compared to a reference database (GOLD database, http://drive5.com/uchime/uchime_download.html) using UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html) (Edgar *et al.*, 2011). This step was conducted to detect and remove chimera sequences (Haas *et al.*, 2011). Finally, the effective tags were obtained from the steps.

For OTU clustering and species annotation, the effective tags were analyzed using UPARSE software v7.0.1001 (<http://drive5.com/uparse/>) (Edgar, 2013). Sequences exhibiting $\geq 97\%$ similarity were assigned to the same operational taxonomic unit (OTUs). Each OTU in the sample represented a unique phylotype. The highest frequency sequences in the OTU (the representative sequences) were screened and chosen for each OTU for further analysis. Each representative sequence was aligned against the SSU rRNA database of the SILVA database (<http://www.arb-silva.de/>) (Wang *et al.*, 2007) using MOTHUR software to obtain species annotation at each taxonomic level with Threshold 0.8~1 (Quast *et al.*, 2013). The

phylogenetic relationship of all OTUs representative sequence was obtained using the MUSCLE v.3.8.31 (<http://www.drive5.com/muscle/>) (Edgar, 2004). OTUs abundance information was normalized using a standard of the sequence number corresponding to the sample with the fewest sequences. Normalized data were used to analyze alpha diversity and beta diversity.

Bacterial diversity within a sample (alpha diversity) was calculated using QIIME v.1.7.0 and displayed by R software v.2.15.3. Alpha diversity was showed through 6 indices, including observed species, Chao1, ACE, Shannon, Simpson, and Good's coverage. Meanwhile, bacterial diversity between samples (beta diversity) on both weighted and unweighted UniFrac was determined using QIIME software v.1.7.0. Cluster analysis using the Unweighted Pair-Group Method with Arithmetic Means (UPGMA) method was performed using QIIME software v1.7.0 and visualized through cladogram.

RESULTS

Pond water quality

Pond water quality was measured in each location. Table 1 shows pH, DO, and salinity of pond water in both locations. Water quality during sample collection was within an optimal range for shrimp farming according to the Indonesian National Standard (Standar Nasional Indonesia, SNI) 8008:2014 (SNI 2014).

Bacterial diversity in the rearing water and shrimp intestines

A total of 888,588 raw tags was obtained from all samples with an average length of 412 bp. OTUs at a sequence similarity of $\geq 97\%$ were used to determine alpha diversity and rarefaction curves (Table 2; Figure 1). Analysis of Illumina-based sequencing showed that bacterial species' distribution differed between locations, both in shrimp pond environment and shrimp intestine. Observed species ranged from 944 to 1,794 species. Among these four samples, W.SU had the most observed species, i.e., 1,794 species. Chao1 and ACE are nonparametric estimators of species richness and indicate the total number of species.

Richness indices of Chao1 and ACE showed that W.SU had the highest richness (Chao1 = 2,069, ACE = 1,879), whereas U.SU had the lowest richness (Chao1 = 1,379, ACE = 1,396). Chao1 and ACE analysis showed that the bacterial richness was underestimated than that of actual or observed species. W.SU samples also harbored the highest Shannon and Simpson diversity index (8.9 and 0.994, respectively). At the same time, U.SU had the lowest Shannon and Simpson diversity index occupying 1.5 and 0.216, respectively. The result of alpha diversity analysis suggests that W.SU was more diverse than that of other samples. Good's coverage estimates the proportion of OTUs in the sample. Good's coverage of the samples ranged from 0.991 to 0.996. In addition, the rarefaction curve approached the plateau for the four samples (Figure 1).

OTUs obtained from the rearing water and intestinal tract of *L. vannamei* were visualized in the Venn diagram to study the relationships between microbial communities in the rearing water and shrimp intestine (Figure 2). A Venn diagram showed the specific OTUs in a sample and the shared OTUs between samples. The diagram showed that there were 284 OTUs commonly found in all samples (Figure 2A). In Pangkajene and Banyuwangi, it was clear that the rearing water samples have many OTUs that did not exist in the shrimp intestines samples (Figure 2B, Figure 2C). The bacterial OTUs in the rearing water in Pangkajene (W.SU) were more diverse than those of Banyuwangi (W.BW) (Figure 2D). The bacterial OTUs in the shrimp intestinal tract in Pangkajene (U.SU) was fewer compared to Banyuwangi (U.BW) (Figure 2E).

Bacterial composition in the rearing water and shrimp intestines

These four samples had distinct bacterial communities at the taxonomic levels, as summarized in Figure 3. The top 10 of each taxonomic level were displayed through a relative abundance graph. The relative abundance showed the species sequences percentage compared to total sequences in each sample. The result showed that the bacterial composition was different between the rearing water and the shrimp intestine. At the phylum level, Bacteroidetes, Proteobacteria, and Firmicutes dominated the

rearing water (W.SU and W.BW) (Figure 3A). In W.SU, a total of 69.12% of OTUs belong to Proteobacteria (24.09%), Bacteroidetes (21.61%), and Firmicutes (22.36%). In W.BW, 98.78% of OTUs consisted of the most abundant phyla, i.e., Bacteroidetes (65.09%), Proteobacteria (10.16%), and Firmicutes (23.42%). The intestinal tract of U.BW shrimp was dominated by Firmicutes (22.36%), Proteobacteria (22.33%), and Verrucomicrobia (21.11%), added up 65.80% of the total identified sequences. The bacterial composition of the shrimp intestines U.SU sample was distinct from the U.BW sample. U.SU sample was highly dominated by Tenericutes (88.54% of the total identified sequences), followed by Proteobacteria (4.66%) and Firmicutes (2.27%).

Bacterial composition at family and genus levels were varied among the samples (Figure 3B; 3C). Bacteroidales S24-7 group was the most dominant family in W.SU and W.BW (8.80% and 31.62%, respectively), yet the least was found in U.SU and U.BW (0.28% and 0.72%, respectively). U.SU and U.BW were mainly dominated by Mycoplasmataceae and LD29 (order Chthoniobacterales) with 88.54% and 19.84% of relative abundance, respectively. At the genus level, *Streptococcus*, *Prevotella*, and *Alloprevotella* were three major genera associated with W.SU with a relative abundance of 3.74%, 3.25%, and 1.57%, respectively. *Alloprevotella* (7.99%), *Prevotella* (7.88%), and *Bacterioidetes* (4.92%) were relatively higher in abundance in W.BW compared to other groups. For shrimp intestine samples, *Candidatus Bacilloplasma* (88.53%) and *Streptococcus* (0.46%) were the most prevalent group in U.SU, whereas *Lactobacillus* (14.97%) and *Ruegeria* (2.10%) were dominant in the U.BW sample.

Bacterial diversity between the rearing water and shrimp intestines

Beta diversity compared the similarity and dissimilarity of bacterial diversity between samples. UPGMA with a weighted UniFrac clustering method was used to determine the similarity and dissimilarity of sequences among these samples. It showed that these samples were clustered by sample type, i.e., rearing water and shrimp intestine, rather than the sample location (Figure 4).

Table 1. pH, DO, and salinity range of shrimp pond water in Pangkajene, South Sulawesi and Banyuwangi, East Java.

Locations	pH	DO (mg/L)	Salinity (ppt)
Pangkajene, South Sulawesi	7-7.7	4.3-5.5	24-25
Banyuwangi, East Java	7.5-8	5-5.6	26-27
Optimal range (SNI 8008:2014)	7.5-8.5	>4	10-32

Table 2. The summary of bacterial diversity in the rearing water and the shrimp intestine, consisted of bacterial richness (OTUs), the diversity index (Shannon and Simpson), estimated OTUs richness (Chao1 and ACE), and sample coverage (Good's coverage).

Sample name	Raw Tags	High-quality tags	OTUs number	Observed species	Chao1	ACE	Shannon	Simpson	Good's coverage
W.SU	398,392	53,966	1,794	1,794	2,069	1,879	8.9	0.994	0.996
W.BW	272,122	104,002	1,598	1,450	1,790	1,852	7.4	0.984	0.991
U.SU	153,678	123,731	1,287	944	1,379	1,396	1.5	0.216	0.992
U.BW	154,396	110,555	1,259	1,321	1,448	1,450	6.3	0.936	0.995

W.SU: the rearing water sample in Pangkajene, South Sulawesi; W.BW: rearing water sample in Banyuwangi, East Java; U.SU: shrimp intestine samples collected from Pangkajene, South Sulawesi; U.BW: shrimp intestine samples collected from Banyuwangi, East Java.

Figure 1. The rarefaction curves of bacterial diversity from four samples. OTUs were defined by $\geq 97\%$ similarity.

Figure 2. Venn diagram of the bacterial diversity at the OTU level between the rearing water and shrimp intestine in two sites (A), rearing water and shrimp intestines in Pangkajene, South Sulawesi (B) and Banyuwangi, East Java (C), rearing water in two sites (D), and shrimp intestines in two sites (E).

DISCUSSION

Analysis of bacterial diversity in the aquaculture environment is expected to provide basic information regarding its bacterial profile, supporting further aquaculture management based on bacterial activity. Bacterial diversity can affect microbial activities in the aquaculture environment, including the activity of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria (Hastuti *et al.*, 2017). Bacterial community structure in the rearing water and intestinal tract of whiteleg shrimp can be detected by Illumina-based high-throughput sequencing platform, a powerful technique for documenting the microbial community from the environment. In this study, we investigated the bacterial communities in the rearing water and the shrimp intestine of *L. vannamei* originating from two sites in Indonesia, i.e., Pangkajene, South Sulawesi and

Banyuwangi, East Java, where high fisheries products are produced (KKP & JICA, 2017).

In the two locations, water pH, DO, and salinity values inside the acceptable ranges for shrimp farming according to SNI 8008:2014 (Table 1) (SNI, 2014). In this present study, we did not determine the effect of environmental parameters on the bacterial diversity inhabiting the rearing water and shrimp intestines. However, it is suggested that there are direct and indirect effects of the bacterial diversity on the water quality of the aquaculture environment and shrimps' growth. The quality of rearing water influences shrimp's survival. Bacteria and phytoplankton in the rearing water environment may rapidly absorb the excessive nutrients, leading to no significant increase in inorganic nutrients in the shrimp pond water. These microorganisms contribute to removing organic matter in the water, which is able to improve the water quality to avoid rearing failure (Alfiansah *et al.*, 2018).

Figure 3. Bacterial composition of the rearing water from Pangkajene, South Sulawesi (W.SU), and Banyuwangi, East Java (W.BW), and the shrimp intestines from Pangkajene, South Sulawesi (U.SU) and Banyuwangi, East Java (U.BW) at phylum (A), family (B), and genus (C) level of taxonomy. Others group reflects the bacterial sequences affiliated to the other groups with low relative abundance.

Figure 4. Clustering analysis of the bacterial community in the rearing water and shrimp intestines of *L. vannamei* from two different sites in Indonesia, i.e., the rearing water from Pangkajene, South Sulawesi (W.SU) and Banyuwangi, East Java (W.BW), and the shrimp intestine from Pangkajene, South Sulawesi (W.SU) and Banyuwangi, East Java (W.BW).

Alpha diversity describes the species diversity found in certain habitats on the ecosystem's local scale (Whittaker, 1972). In this current work, Good's coverage value of approximately 99% revealed that the majority of bacterial species from each sample had been recovered (Table 2). The rarefaction curves showed that the OTUs number obtained from each sample was adequate for reflecting the total bacterial community of each sample (Figure 1). In this present study, we found the difference between bacterial distributions in the pond water and the shrimp intestines between the two locations. Generally, bacterial diversity inhabiting the rearing water in Pangkajene, South Sulawesi (W.SU) was the highest than those of the rearing water in Banyuwangi, East Java (W.BW) and shrimp intestines in two sites (U.SU and U.BW) (Figure 2). It was observed that bacteria in the rearing water (W.SU and W.BW) were more diverse compared to the shrimp intestine (U.SU and U.BW). This result was presumably caused by the difference in environmental conditions supporting the bacterial growth and survival in each habitat. Also, the bacterial diversity in the rearing water of two different sites was found more similar to each other. However, high bacterial diversity in the rearing environment did not necessarily affect the bacterial diversity that

existed in the shrimp intestines. We found that the high bacterial diversity in the pond water of Pangkajene, South Sulawesi (W.SU) did not influence the bacterial diversity in the shrimp intestines obtaining from the same site. In contrast, the bacterial diversity inhabiting the shrimp intestines was different among the two sites. Regarding this analysis, U.SU had a lower diversity index than that of U.BW (Table 2; Figure 2). Through meta-analysis, Cornejo-Granados *et al.*, (2018) reported that diseased shrimps had a lower phylogenetic diversity index compared to the healthy shrimps. The difference in microbial composition in the shrimps' intestine is also strongly influenced by the cultivation technology applied (Zeng *et al.*, 2017). Nevertheless, alpha diversity in this study indicated that the bacterial groups inhabited their certain habitat according to environmental characteristics.

Bacterial compositions in the shrimp rearing water of the two sites were dominated by Bacteroidetes, Proteobacteria, and Firmicutes (Figure 3A). A previous investigation reported that the bacterial community in the shrimp rearing water was dominated by Proteobacteria and Bacteroidetes (Huang *et al.*, 2018). Meanwhile, we observed the difference in the shrimp intestinal microbiota among the two sites (Figure 3).

Our investigation showed that bacterial composition differed between U.BW and U.SU (Figure 3A). The bacterial community in the shrimp intestine of U.BW was dominated by Firmicutes, Proteobacteria, and Verrucomicrobia (Figure 3A). Proteobacteria have been previously reported as the most dominant phylum in the intestinal tract of *L. vannamei* (Huang *et al.*, 2014) and *Penaeus monodon* (Rungrassamee *et al.*, 2013). Our study showed that Firmicutes is one of the abundant phyla in the shrimp intestine of the U.BW sample with a similar relative abundance to Proteobacteria (~22.3%) (Figure 3A). In contrast, Huang *et al.*, (2014) reported that Firmicutes is the minority phylum in *L. vannamei* intestines. This study also reported Verrucomicrobia as a dominant phylum in the shrimp intestine of U.BW. As the previous investigation, Verrucomicrobia, Proteobacteria, Actinobacteriales, and Flavobacteria were the major bacterial group in shrimp intestines of *L. vannamei* fed with basal diet and probiotic-supplemented diet (Sha *et al.*, 2016). According to these results, Proteobacteria and Firmicutes were bacterial phylum commonly found with almost similar proportions and suggested to be adaptable in the two distinct environments, both the rearing water and the shrimp intestinal tract.

According to information obtained from the farmers, WFS and IMNV frequently infect the shrimps in the two locations. In our study, the results showed that *Candidatus Bacillioplasma* affiliated to Tenericutes extremely dominated microbiota in U.SU with the relative abundance of 88.53%, suggesting that the shrimp intestinal microbiota might be changed in U.SU compared to U.BW (Figure 3C). The previous study reported that *Candidatus Bacillioplasma*, *Phascolarctobacterium*, and *Ruminococcus* were overgrowth in the WFS compared with those of the healthy shrimp. In WFS shrimp, an increase in those bacterial groups and a decrease in *Paracoccus* and *Lactococcus* might contribute to the disease. Tenericutes and Firmicutes were higher, yet Proteobacteria was lower in the WFS shrimp than healthy shrimp (Hou *et al.*, 2018).

We also observed the bacterial community at the genus level. In the U.BW, we found that the shrimp intestine microbiota was dominated by *Lactobacillus* affiliated to Firmicutes (Figure 3C). This genus had been found as one of the most

dominant groups in the shrimp intestines (Hou *et al.*, 2018). The proportion of lactic acid bacteria, such as *Lactobacillus*, was higher in the shrimps fed with the *L. plantarum*-supplemented diet, suggesting they contribute to improving resistance to pathogens infection, such as through producing antimicrobial compound (Sha *et al.*, 2016) and immunostimulating agents (Vieira *et al.*, 2010). *Lactobacillus* was found at a high proportion in wild *P. monodon* (Rungrassamee *et al.*, 2014) and aquatic animals (Lee *et al.*, 2010). *Lactobacillus* plays an essential role as probiotics, and their population in the digestive tract might be increased by ingested food. Surprisingly, we did not find *Vibrio*, affiliated to Proteobacteria, as a large group in the digestive tract of *L. vannamei*, as previously reported in the intestine of wild and domesticated *P. monodon* (Rungrassamee *et al.*, 2014), healthy and WSSV-infected *L. vannamei* (Wang *et al.*, 2019). In this study, *Vibrio* was found at a low relative abundance of 2.80% in U.SU and 2.35% in U.BW (data not shown).

Beta diversity reflects the dissimilarity of bacterial diversity in different samples. Clustering analysis indicated the difference in the bacterial community between these samples, which was divided into two clusters according to sample type rather than sampling site (Figure 4). As shown in the dendrogram, the bacterial community in the rearing water had similarities between sample sites. In Pangkajene (W.SU), the abundant phylum was evenly distributed in varying groups. Whereas, in Banyuwangi (W.BW), the bacterial assemblage was highly dominated by Bacteroidetes. However, the bacterial community in the rearing water was different from the shrimp intestinal tract, in which Tenericutes largely dominated the shrimp intestinal microbiota in Pangkajene (U.SU). This result suggests that different environmental variables between rearing water and shrimp intestine led to their distinct bacterial community. Environmental variables within the host body might have stronger effects on the intestinal bacterial community shift (Rungrassamee *et al.*, 2014). Furthermore, in the future, many studies are expected to be performed to produce such information regarding bacterial diversity in the aquaculture environment and cultured animals, which is able to be developed as a basic knowledge for the sustainable

management of shrimp cultivation according to microbial diversity and activity.

CONCLUSION

Bacterial community structure in the rearing water differs with shrimp intestines of *L. vannamei*, either in the samples from Pangkajene, South Sulawesi, or Banyuwangi, East Java. The specific environmental characteristics might influence this discrepancy in each sample type. Our study suggests that the host intestinal environment might have greater selective pressure for the bacterial composition in *L. vannamei* intestines than that of the rearing water environment. Understanding the correlation of bacterial community between the rearing water and the shrimp intestines related to environmental parameters and the bacterial diversity composition's stability need to be further explored.

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CONFLICT OF INTERESTS

The authors have declared that no conflict of interest exists.

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