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Assessment of Bacterial Community Profile in the Rearing Pond Environment and the Intestinal Tract of Pacific White Shrimp *Litopenaeus vannamei* in Lampung Province, Indonesia using 16S rRNA Gene Amplicon Sequencing: A Short Research Investigation

Yuni Puji Hastuti[1,*], Yuli Siti Fatma[2] and Siska Tridesianti[3]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor 16680, West Java, Indonesia
[2]Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Bogor 16911, West Java, Indonesia
[3]Department of Biology, Faculty of Science and Technology, UIN Sunan Gunung Djati Bandung, Bandung 40614, West Java, Indonesia

([*]Corresponding author's e-mail: yuniha@apps.ipb.ac.id)

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Abstract

Along with the high demand for shrimp, the production challenges faced by practitioners are increasing in Indonesia, one of which is in Lampung region. Various basic monitoring techniques regarding the environment and farmed shrimp are needed to control production sustainability. This present study aimed to identify the bacterial community profile in the rearing pond water and intestinal tract of Pacific white shrimp *Litopenaeus vannamei*. Illumina-based sequencing was chosen to determine the bacterial community using the V3-V4 region of the bacterial 16S rRNA gene. Sequence data revealed the differences in bacterial community structure between the rearing water and shrimp intestines. Proteobacteria was the most prevalent phylum in the rearing water (W.B), accounting for 45.29 %, followed by Cyanobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Fusobacteria. In the shrimp intestinal tract (S. B), Cyanobacteria (35.15 %) dominated the microbiota, followed by Proteobacteria, Saccharibacteria, Actinobacteria, Bacteroidetes, Verrucomicrobia, TM6 (Dependentiae), and Firmicutes. Cyanobacteria were higher in the shrimp intestines (35.15 %) than in the rearing water (26.63 %). In addition, *Escherichia-Shigella* was the most common genera in the rearing water and shrimp intestines with different relative abundance. Cyanobacteria and *Escherichia-Shigella* highly detected in the rearing water and shrimp's intestines might indicate that pond water had been polluted. Further investigation is necessary on the correlation of Cyanobacteria in the shrimp intestines with water pollution, proven by the dominance of Cyanobacteria and *Escherichia-Shigella.* These findings provide basic information to enhance our understanding of the microbial community and their roles in the shrimp culture environment to improve the quantity and quality of the yield and support its sustainability.

Keywords: Aquaculture environment, Bacterial diversity, Intestinal tract, Metagenomic, Whiteleg shrimp

Introduction

Indonesia contributes as the 4th largest Pacific white shrimp *Litopenaeus vannamei*-producing country worldwide after China, Thailand, and Vietnam [1]. Lampung has been one of the largest shrimp-producing regions in Indonesia and Asia since 2006. Until 2017, it was confirmed that the distribution of Lampung shrimp fry was the second largest number in Indonesia subsequent to East Java [2]. Along with the increasing demand for shrimp globally, farmers' challenges in producing shrimp were also increasing. Maintaining the sustainability of shrimp production in Indonesia has become an important step in monitoring aquaculture activities from the beginning to the end of the rearing. The increasing age of Pacific white shrimp increases waste in aquaculture media [3].

Snieszko [4] stated that the environment could have a dual effect on the survival of culture biota. The environment is able to affect other biotic and abiotic parameters. Environmental variables, such as temperature, pH, groundwater capacity, and indigenous bacterial community and activity, strongly influence organic matter degradation in aquaculture environments [5]. Microorganisms play major roles in biogeochemical cycling in aquaculture environments. The diversity and activity of microorganisms control varying nutrient cycles in cultivation environments, including in biogeochemical cycling [6]. Biomonitoring activities that are essential to be performed in aquaculture are identifying microbial community profiles inhabiting culture media and biota. Shaping the bacterial composition in the shrimp intestines, such as prebiotics and probiotics supplementation, needs to consider the bacterial community both in the rearing water and the host's internal environment. Preventing pathogenic invasions from the rearing environment to shrimp could be performed by determining the environment parameters using bioecological-based monitoring, one of which is identifying the indigenous bacterial community. Venail *et al.* [7] reported a positive effect of the bacterial diversity in the ecosystem on other parameters in bioremediation activities. In a bioremediation context of aquaculture waste, bacterial diversity might affect bacterial enzyme activity applied in this environment. Furthermore, information obtained from the analysis of the bacterial community in the rearing water and the host's internal environment is essential for bioaugmentation sustainability using bacteria as a bioremediation agent, which in turn produces good water quality and high survival farmed shrimp. Nitrification and denitrification reactions involve bacterial activity, and their balance must be maintained in the aquaculture environment [8].

The total bacterial diversity can be identified through community, population, and species analysis based on environmental DNA. This approach is able to describe the bacterial distribution in the water environment and the shrimp body. High-throughput sequencing (HTS) based on the bacterial 16S rRNA gene has been widely used as a comprehensive technique to identify the bacterial diversity in aquaculture pond water, sediment, and intestinal tract of farmed shrimp. Southern sea waters such as in Nampu and Sembukan waters, Wonogiri Regency, showed that Actinobacteria and Firmicutes are the dominant bacteria followed by Bacteroidetes and Cyanobacteria groups [9]. The 16S rRNA bacterial analysis of the shrimp rearing water in Banyuwangi proved the dominance of Proteobacteria, Bacteroidetes and Firmicutes, and the shrimp's intestinal tract also had almost similar bacterial diversity [10]. Shrimp intestinal microbiota in Banyuwangi, East Java is dominated by Firmicutes, Proteobacteria and Verrucomicrobia, while in Pangkajene, South Sulawesi it is dominated by Tenericutes, Proteobacteria and Firmicutes [10]. Huang *et al.* [11] proved using HTS that the bacterial community structure is significantly different between water, sediment, and intestinal shrimp of *L. vannamei*. During 90 days rearing period, bacterial diversity indices in the shrimp intestinal tract strongly correlated with those in sediment. In addition, bacterial diversity in the environment would affect bacterial diversity in shrimp intestines, leading to the alteration of physiological response and disease resistance of shrimp. Some bacterial species inhabiting aquaculture environments and shrimp intestines are pathogenic to farmed shrimp and even influential on the morphology and physiology of the host body, affecting nutrients absorption ability and immune responses [12]. The abundance of bacteria in the aquaculture environment could contribute to the stress level of shrimps and significantly interfere with the shrimp intestinal microbiota of Asian tiger shrimp *Penaeus monodon* [13]. Decreased water quality, including physical, chemical, and biological characteristics, are able to indirectly affect the resident microbial community in shrimp intestines [14].

The bacterial diversity profile provides complete information related to the taxonomy, distribution, and abundance of indigenous bacteria in the environment. Identification of bacterial diversity in the aquaculture environment and shrimp intestinal tract is expected to provide a specific description of the total bacterial distribution. The dominance of bacterial phylum, family and genus in the aquaculture environment and shrimp intestine can describe the actual condition of shrimp production. The higher the dominance of the toxin-producing microorganism group in the environment, the higher the habitat expansion and toxicity impact on the cultivated biota. Therefore, environmental management could be controlled from the beginning of the cultivation period. The purpose of this study is to determine the profile of total bacterial diversity in the aquaculture pond water and the intestinal tract of shrimp *L. vannamei* originated from Lampung, Indonesia, based on the 16S rRNA gene high-throughput sequencing. The finding of this study provides essential information on the shrimp cultures to build better management. Bacterial community structure in shrimp culture environment needs to be explored to understand their functions in order to improve the quantity and quality of the yield.

Materials and methods

Pond water and shrimp sample collection

Pond water and Pacific white shrimp samples were taken from the Kalianda area, the Southern region of Lampung Province, Indonesia. Sample collection was performed on November 16, 2018, when the day of culture (DOC) was 45 of a 120-day rearing period. Water and shrimp samples were collected from 2 different sites. Each site consisted of 4 rearing ponds with an intensive aquaculture system. We collected the samples from one rearing pond at each site (Pond at Site 1: 5.670385 S, 105.548605 E and Pond at Site

2: 5.692115 S, 105.563646 E). A total of 600 mL pond water and 12 individual shrimps were collected from those 2 shrimp rearing ponds (300 mL-pond water and 6 individual shrimps per rearing pond). Sample collection, either pond water or shrimp, was carried out using aseptic procedures to prevent contamination. Pond water and shrimp samples were marked as W.B and S.B, respectively. The samples were transported to the laboratory using a cold and dark container for immediate analysis. According to sample type, the pond water and shrimp samples were separately pooled with an equal number to obtain a composite sample.

DNA extraction

We pooled all water samples to form one composite water sample. Microbial biomass was collected by filtering a 600-mL rearing water sample (W.B) with a 0.2 µm-pore size polycarbonate membrane (Whatman, Buckinghamshire, UK). Subsequently, total genomic DNA was extracted from the filter membrane using the PowerWater DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer’s instructions. Among all shrimp samples, 5 shrimps were aseptically dissected to obtain the intestines, and the intestines were pooled into one sample tube to minimize the individual differences. Genomic DNA from the shrimp intestines was extracted using the PowerFood DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) based on the manufacturer’s recommendations. The concentration and purity of the extracted DNA were assessed by electrophoresis on 1 % ($^{w}/_{v}$) agarose gel and spectrophotometer using Nanodrop 2000c (NanoDrop Technologies, Wilmington, DE, USA). DNA samples were delivered to NovogeneAIT Genomics Singapore Pte. Ltd. (Singapore) to determine bacterial community using Illumina-based sequencing technology.

The 16S rRNA gene amplification and sequencing

The bacterial 16S rRNA gene in DNA sample of pond water and shrimp was amplified using the primer set targeting the V3-V4 hypervariable region, i.e., 341F (5’-CCTAYGGGRBGCASCAG-3’) and 806R (5’-GGACTACNNGGGTATCTAAT-3’) [15]. Each primer was added with an 8-bp unique barcode for the Illumina sequencing platform. PCR reaction was carried out using Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA) following the manufacturer’s instructions. The PCR cycling was run using the PCR condition described by Yu *et al.* [15]. PCR products were assessed by electrophoresis on 2 % ($^{w}/_{v}$) agarose gel. Positive DNA bands with a size of 400 - 450 bp were chosen for the subsequent process. PCR fragment was purified using the Qiagen Gel Extraction Kit (Qiagen, Hilden, DE). DNA library was generated using NEBNext® Ultra^{TM} DNA Library Prep Kit (NEB, Ipswich, MA, USA) for Illumina platform and assessed by qPCR and Qubit® dsDNA assays (Thermo Fisher Scientific, Waltham, MA, USA). According to the manufacturer’s manual, the DNA library was sequenced on the Illumina HiSeq 2500 PE250/Ion S5 XL platform.

Analysis of DNA sequences data

Illumina-based sequencing generates the paired-end reads. The barcode and primer sequence of the paired-end reads were truncated to obtain the targeted DNA sequence. Then, the paired-end reads were merged using FLASH v1.2.7 to generate the raw tags [16]. The raw tags quality was filtered under specific filtering conditions using the QIIME v1.7.0 to obtain high-quality clean tags [17,18]. The clean tags were compared to the reference database (GOLD database) to detect chimera sequences using the UCHIME algorithm [19]. The chimera sequences were removed according to Haas *et al.* [20]. This process generated effective tags.

All effective tags were analyzed using UPARSE software [21]. Sequences with ≥ 97 % similarity were clustered into one operational taxonomic unit (OTUs). The highest frequency sequences in each OTU were assigned as representative sequences. Taxonomical annotation of the OTU-representative sequence was conducted by MOTHUR software using the SILVA database [22] at each taxonomic rank with a threshold of 0.8 ~ 1 [23]. OTUs abundance data were normalized using a standard sequence number corresponding to the least sequences. In further analysis, the normalized data were used to determine alpha diversity, such as observed species, richness indices (Chao1 and abundance-based coverage estimator (ACE)), diversity indices (Shannon and Simpson), and Good’s coverage. Alpha diversity was analyzed using QIIME v1.7.0 and displayed using R software v2.15.3. A Venn diagram was constructed to determine specific and common bacterial OTUs between rearing pond water and shrimp intestines samples.

Results and discussion

Understanding the microbial ecology of shrimp cultures improves aquaculture management leading to greater quality and quantity of shrimp production. Balanced microbial community in the aquaculture environments and the shrimp intestinal tract is the main issue in shrimp cultivation. This study explored bacterial community profile in the rearing pond water and the shrimp intestines obtained from the shrimp cultivation in the South region of Lampung, Indonesia. Despite a few production failures due to poor water quality and diseases, Lampung has successfully produced high shrimp production in Indonesia. The sustainability of shrimp production in Lampung is relatively higher in comparison with other regions in Indonesia. Therefore, it is important to study the bacterial diversity profile as a biological parameter for supporting better aquaculture and environmental management. We used Illumina-based next-generation sequencing to draw the whole bacterial community in the rearing water and the shrimp intestines samples.

Bacterial diversity in the rearing pond water and the shrimp intestines

A total of 310,079 high-quality sequences were obtained from the two samples. The average length of the obtained sequences was 409 bp. The alpha diversity of all samples is shown in **Table 1**. The result revealed that the observed species, Chao1, ACE, Shannon, and Simpson of bacterial communities in rearing pond water were higher than shrimp intestines. The two samples had a different number of observed species (species richness in a sample), in which W.B (2,084 observed species) has more observed species compared to S.B (1,592 observed species). Chao1 and ACE, the species richness estimators, showed that W.B was expected to possess more diverse observed species than S.B. According to the estimators, the observed species of these two samples were underestimated. Diversity indices consisting of Shannon and Simpson indices were performed to compare bacterial richness and evenness within a sample. W.B had higher Shannon and Simpson indices than those in S.B, indicating the bacterial diversity in W.B was more diverse compared with S.B. Good's coverage of both samples were more than 0.99 (99 % of bacterial species were successfully sequenced in each sample), which means that the sequencing sufficiently represents the total bacterial community within the sample. The rarefaction curve revealed that both samples have different sequencing depths, in which both samples reach the saturation at different OTUs numbers (**Figure 1**).

Table 1 Alpha diversity of the bacterial community in the rearing pond water (W.B) and shrimp intestines of *L. vannamei* (S.B) according to Illumina-based sequencing.

Sample name	Observed species	Chao1	ACE	Shannon index	Simpson index	Good's Coverage
W.B	2,084	2,092	2,105	6.30	0.90	0.999
S.B	1,592	1,714	1,723	5.71	0.87	0.997

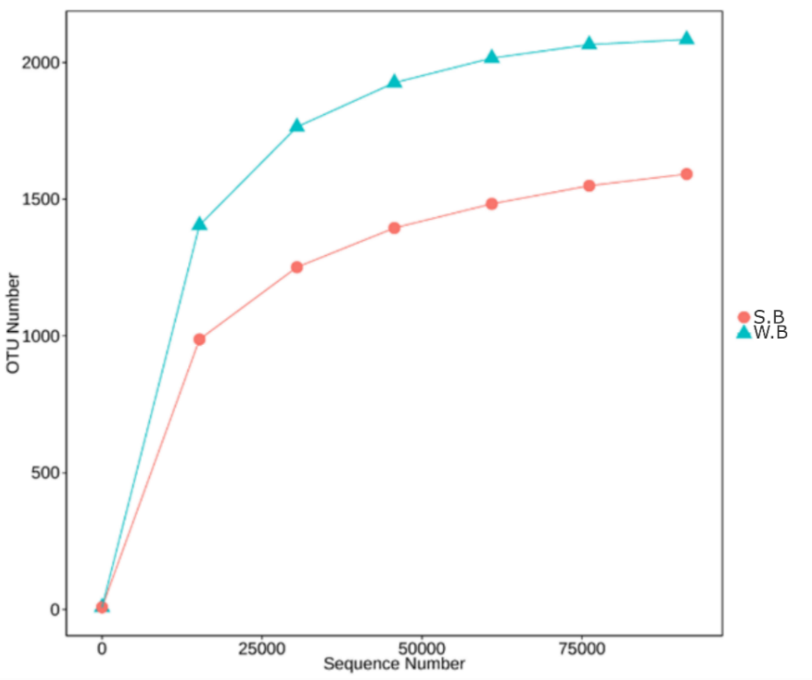

Figure 1 The rarefaction curve obtained from bacterial sequence data of the rearing pond water (W.B) and the shrimp intestines (S.B).

Bacterial OTUs in W.B and S.B are shown in the Venn diagram (**Figure 2**). This diagram shows the relationship of bacterial communities between the two samples. There were 1,095 OTUs shared between both samples. Rearing pond water has many bacterial OTUs that were not found in the shrimp intestines (989 OTUs), while there were 497 OTUs that only existed in the shrimp intestines. This diagram revealed that the total bacteria in the rearing water was more diverse than that of the shrimp intestines.

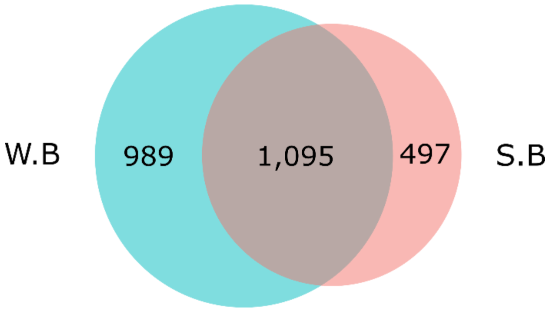

Figure 2 Venn diagram of bacterial diversity at the OTU level among the rearing pond water (W.B) and the shrimp intestinal tract (S.B).

Alpha diversity analysis showed that the rearing pond water has more diverse bacteria than that of the shrimp intestines (**Table 1**, **Figure 1**). This result was in agreement with a previous study conducted by Cardona *et al.* [24]. Higher bacterial diversity in the rearing pond water might be due to the discrepancies in environmental conditions between the rearing pond water and the shrimp intestines. The environmental characteristics may shape the bacterial composition and function and the stability of the microbial community. The aquaculture environment is a large area containing complex nutrients from varying sources, whereas the shrimp intestines have specific environmental characteristics and are presumably influenced by surrounding environmental conditions. As shown in **Figure 2**, the Venn diagram showed that the bacterial diversity might be affected by the environmental conditions of their habitat.

Bacterial composition in the rearing pond water and the shrimp intestines

The bacterial community in the rearing pond water and the shrimp intestines had diverse profiles (**Figure 3**). Across all taxonomic ranks, the bacterial composition in the rearing pond water had similarities with the shrimp's intestines, either richness or dominance. At the phyla level, five phyla were considered predominant phyla with a relative abundance of more than 1 % of the total sequences (**Figure 3**). Proteobacteria was the most dominant phylum in the rearing pond water (W.B) occupying 45.29 %, followed by Cyanobacteria (26.63 %), Firmicutes (10.59 %), Bacteroidetes (6.10 %), Actinobacteria (5.77 %), and Fusobacteria (1.32 %). The bacterial community in the shrimp intestines (S.B) was dominated by Cyanobacteria (35.15 %), Proteobacteria (31.36 %), Saccharibacteria (8.39 %), Actinobacteria (8.16 %), Bacteroidetes (3.63 %), Verrucomicrobia (3.08 %), TM6 (Dependentiae) (2.35 %), and Firmicutes (2.27 %). Proteobacteria and Cyanobacteria represented more than 65 % of the total bacteria in each sample.

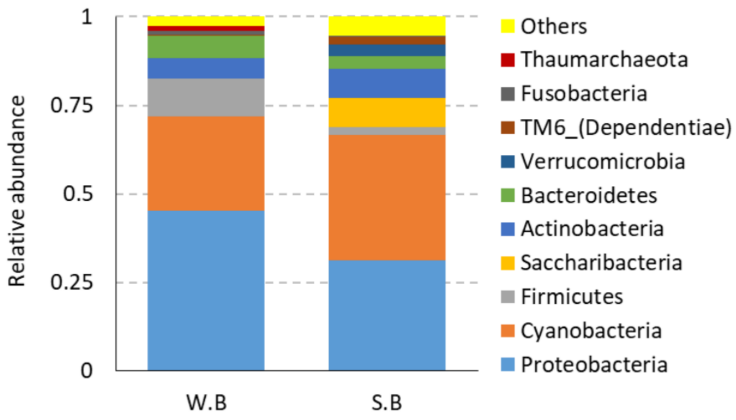

Figure 3 Relative abundance of bacterial OTUs in the rearing pond water (W.B) and the shrimp intestines (S.B) at phylum level of taxonomy.

A distinct bacterial community structure was observed between the rearing water and the shrimp gut. We identified that the rearing pond water and the shrimp intestines had a similar predominant phylum, namely Proteobacteria (**Figure 3**). However, the abundance of Proteobacteria in the rearing pond water (45.29 %) was higher than that in the intestinal tract of shrimp *L. vannamei* (31.36 %) (**Figure 3**). This phylum has been reported as a dominant group in the marine aquaculture environment and the intestinal microbiota of the shrimp [25,26]. Proteobacteria, including Enterobacteriaceae and Xanthomonadaceae, are widely distributed in marine ecosystems and contribute to the nutrient cycle in the environment [24]. Healthy shrimp had primary bacterial diversity, dominated by Proteobacteria [25]. The dominance of Proteobacteria in the shrimp intestines might be caused by water environmental exposure. The microbial community has a relationship between these two environments [27]. In an aquatic environment, shrimp can be exposed naturally to microbes in their culture media, both water and sediment [25]. Another previous investigation reported that Alphaproteobacteria, Bacteroidetes, and Actinobacteria dominate the bacterial community in aquaculture ponds. In contrast, bacteria in the bottom layer of ponds (sediment) have similar diversity to bacterial diversity in the shrimp gut, dominated by Gammaproteobacteria and Tenericutes [11].

The composition of bacteria in *L. vannamei* shrimp shifted at different growth stages, including when the shrimp in the nursery and harvest phase from larval to adult stage [26,28]. Gainza *et al.* [28] reported that CKC4, Proteobacteria, Actinobacteria, and Firmicutes were the dominant phyla during the nursery phase, while Proteobacteria, Chloroflexi, and Actinobacteria were most abundant in the harvest phase. Zeng *et al.* [26] revealed that Proteobacteria, Tenericutes and Fusobacteria were the dominant phyla in the shrimp intestine. Huang *et al.* [29] also reported that Proteobacteria, Bacteroidetes, and Actinobacteria were the 4 dominant phyla in the intestines at all growth stages of *L.vannamei*, i.e., 14 days postlarvae (L14) and 1-, 2- and 3-month-old juveniles (J1, J2 and J3). As shown in previous investigations, Proteobacteria is a major group throughout the developmental stage of the shrimp, which was also observed in this present study in DOC of 45 days (**Figure 3**). Proteobacteria was also the predominant phylum in the intestine of black tiger shrimp (*Penaeus monodon*) [13,30].

Pond water and shrimp intestines obtained from Lampung were also dominated by Cyanobacteria, accounting for 26.63 % and 35.15 %, respectively (**Figure 3**). Interestingly, Cyanobacteria in the shrimp intestines was more abundant than in the rearing pond water. A high abundance of Cyanobacteria, also known as blue-green algae (BGA), within the shrimp gut is probably linked with the rearing water environment. A previous report also revealed that Cyanobacteria is observed in Pacific whiter shrimp intestines [26]. Unfortunately, BGA is one of the microalgae that is unwanted in high numbers in the aquaculture environment due to producing a high level of nitrogenous waste in the water from nitrogen fixation activity [31], and BGA-blooms is able to generate hypoxia condition in water, increasing shrimp mortality [32]. An increase in the abundance of BGA would cause unstable environmental variables, such as nitrogen, oxygen, and turbidity. BGA is also capable of producing toxins that decrease the survival of Pacific white shrimp [33]. Aside from Proteobacteria and Cyanobacteria, the rearing pond water was dominated by Firmicutes (10.59 %), Bacteroidetes (6.10 %), Actinobacteria (5.77 %), and Fusobacteria (1.32 %). In comparison, the shrimp intestinal microbiota was also dominated by Saccharibacteria (8.39 %), Actinobacteria (8.16 %), Bacteroidetes (3.63 %), Verrucomicrobia (3.08 %), TM6 (Dependentiae) (2.35 %), and Firmicutes (2.27 %). Thaumarchaeota, belonging to Archaea, was also detected in these two samples (**Figure 3**). These results revealed the difference in the bacterial community structure among both environments. These prevalent groups may contribute to nutrient cycles in each habitat.

At the family level, Enterobacteriaceae, Xanthomonadaceae, and Lachnospiraceae belonging to Proteobacteria were the most abundant group in W.B, accounting for 19.12, 7.7 and 2.37 %, respectively (**Figure 4**). S.B has a distinct bacterial community structure, in which Enterobacteriaceae, Rhodobacteraceae, and Flavobacteriaceae, belonging to Proteobacteria, were the predominant group, constitutively occupying 7.54, 7.61 and 2.35 % (**Figure 4**). Enterobacteriaceae and Rhodobacteraceae, belonging to Alphaproteobacteria phylum, were most abundant in the shrimp gut of *L. vannamei*, which is in accordance with Sha *et al.* [34]. Rhodobacteraceae was detected across all growth stages, and they probably form the core microbiome with Flavobacteriaceae in the intestinal of *L. vannamei* [29]. Enterobacteriaceae and Rhodobacteriaceae have previously been reported as the enriched group in shrimp larvae and juveniles, respectively [35]. Nevertheless, in this present study, we also observed Enterobacteriaceae and Rhodobacteriacea within the intestinal tract of shrimp *L. vannamei* at DOC 45 (**Figure 4**). A high abundance of Enterobacteriaceae could be from fecal matter in the intestinal tract [35]. Rhodobacteraceae has a diverse range of metabolic activity and may contribute to maintaining the health of culture systems [24].

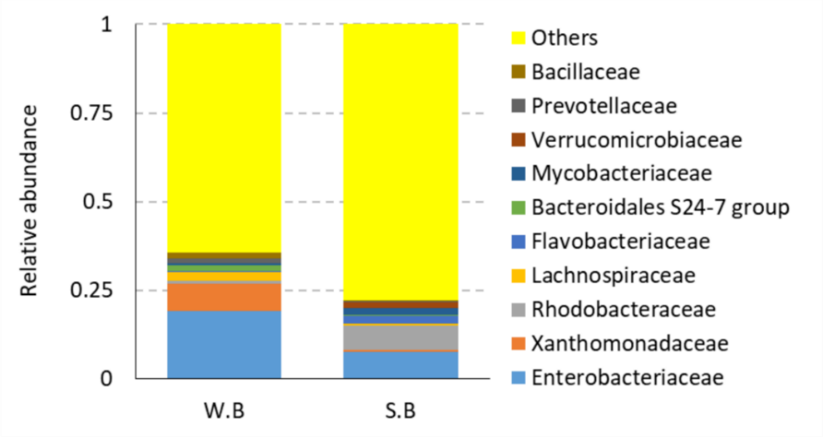

Figure 4 Relative abundance of bacterial OTUs in the rearing pond water (W.B) and the shrimp intestines (S.B) at family level of taxonomy.

At the genus level, *Escherichia-Shigella*, *Stenotrophomonas*, and *Bifidobacterium* were the most abundant group in W.B with a relative abundance of 17.32, 7.44 and 1.23 %, respectively (**Figure 5**). Meanwhile, S.B was dominated by Escherichia-Shigella, Pantoea, and Mycobacterium reached 5.12, 1.85, and 1.81 %, respectively (**Figure 5**). *Escherichia-Shigella* was frequently found in the rearing pond water and the shrimp intestines (**Figure 5**). *Escherichia-Shigella*, belonging to Enterobacteriaceae, was most abundant in *L. vannamei* intestines at the nursery phase [28]. *Escherichia-shigella* was also observed in the shrimp digestive tract of *P. monodon* [30]. This genus has been reported as a common group in the intestine, yet it could be a potential pathogen infecting the shrimp [36]. According to the results, a high abundance of *Escherichia-Shigella* in the rearing pond water should need more attention related to further management.

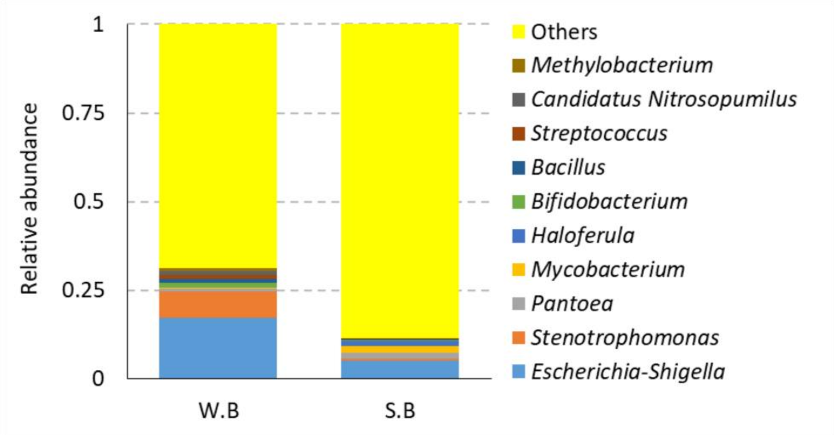

Figure 5 Relative abundance of bacterial OTUs in the rearing pond water (W.B) and the shrimp intestines (S.B) at genus level of taxonomy.

The dominance of Cyanobacteria in the rearing water and shrimp’s intestines is quite worrying in shrimp culture. Rienzi *et al.* [37] reported that Melainabacteria, a new candidate of Cyanobacteria, has been reported to be able to fix nitrogen and live in the human intestine and other aquatic environments. Soo *et al.* [38] reported that the Melainabacteria genome does not have the ability to photosynthesis but produces energy through the anaerobic fermentation process. Cyanobacteria in the aquatic environments have been proven to delay the growth of white leg shrimp, suppress haemocyte concentration, and increase phagocytosis of haemocytes, affecting the shrimp immune system [39]. This result showed that there might be a close relationship between Cyanobacteria in the shrimp intestinal tract and those commonly found in mammalian and human guts. Also, this present study indicated that the water quality in Kalianda waters is getting worse, as revealed by the increase in the dominance of *Escherichia-Shigella* in the rearing water and the shrimp intestines (**Figure 5**). *Shigella* has been known as gram-negative, non-motile, rod-shaped bacteria related to *Escherichia coli* and *Salmonella*. The higher the dominance of *E. coli* and *Shigella* in water, the higher the threat of disease and environmental damage. According to this result, pond water in Kalianda area, Lampung, Indonesia, might have been polluted. However, further investigation is needed on the waste sources in that aquatic environment.

The dominance of bacterial groups in the rearing pond water was similar to the shrimp intestines, yet their proportion differed among the two samples (**Figure 5**). Bacteria and other microorganisms in the rearing water may influence the microbial structure and function in the shrimp gut [40]. Intestinal bacterial communities in the larva as early developmental stage mainly comes from water [41]. Dynamics of the microbial community in shrimp intestines occurred during the host's growth stage [29]. Microbial composition in the shrimp gut might be fluctuated and contribute to the host's health and development. Shrimp pathogens, such as bacteria and viruses, inhabiting the rearing water may enter the digestive tract, then the pathogens trigger the immune systems of the shrimp *P. monodon* [42]. In *L. vannamei*, the intestinal microbiota is associated with the shrimp's body weight by influencing nutrient absorption in the intestines [43]. In this study, the bacterial community varied between the shrimp rearing water and the intestinal tract of *L. vannamei*. The close interaction of the bacterial community would need to be understood to implement their function in shrimp cultivation.

Conclusions

This study describes the bacterial community in the rearing water environment and the shrimp intestinal tract of *L. vannamei*. Illumina-based sequencing revealed the difference in bacterial diversity and composition between these two samples. The diversity of bacteria in the rearing water and the intestinal tract of shrimp have a high similarity, yet the bacterial composition differed. The bacterial diversity in the rearing water was higher compared to the shrimp intestines. Proteobacteria is the most dominant phylum in the rearing pond water and the shrimp intestines, in which the abundance of Proteobacteria in the water environment shows a higher number than that in the shrimp intestine. Rearing pond water and shrimp intestines from Lampung are also dominated by Cyanobacteria or blue-green algae (BGA). Cyanobacteria were higher in the shrimp intestines than in the rearing water, suggesting that the shrimp might consume a high number of BGA. At the genus level, the bacterial community inhabiting the rearing pond water is dominated by *Escherichia- Shigella*, *Stenotrophomonas,* and *Bifidobacterium*. While, in the shrimp intestines, the bacterial community is dominated by *Escherichia-Shigella*, *Pantoea*, and *Mycobacterium*. The analysis of the bacterial community and their metabolic activity in the rearing water according to the 16S rRNA (metatranscriptomics) would be future research as a base knowledge to manipulate the bacterial community through biostimulation or bioaugmentation in order to improve the quality and quantity of farmed shrimp.

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