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Profiling of microbiome biodiversity and structure associated with the gastrointestinal tract of Pacific white shrimp (*Penaeus vannamei*) using high-throughput sequencing

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Abstract. *Amin M, Musdalifah L, Lewaru MW, Alimuddin, Alim S, Martin MB. 2024. Profiling of microbiome biodiversity and structure associated with the gastrointestinal tract of Pacific white shrimp (*Penaeus vannamei*) using high-throughput sequencing. Biodiversitas 25: 1984-1992.* The Pacific white shrimp (*Penaeus vannamei*) holds significant importance as an aquaculture commodity in Indonesia, where the government actively promotes the expansion of brackish water pond intensification and extensification. Despite its global cultured prominence, there remains a research gap regarding the biodiversity of the microbiome within the gastrointestinal tracts (GITs) of cultured *P. vannamei* and the implications of aquaculture practices on microbiota dynamics. This study focuses on investigating the diversity and structure of the GIT microbiome in *P. vannamei* from selective intensive aquaculture ponds in East Java, Indonesia. Sampling from three distinct intensive rearing ponds, shrimp GITs were meticulously dissected to analyze the bacterial composition using high-throughput sequencing. The microbial diversity within the gastrointestinal tracts of these shrimps from commercial intensive aquaculture farms was evaluated through 16S rRNA amplicon sequencing, targeting the V3-V4 region, and the utilization of Operational Taxonomic Units (OTUs) for bacterial categorization. The results revealed that the gastrointestinal tract of shrimp reared in an intensive aquaculture system was dominated by nine bacterial phyla, namely Proteobacteria (53.95%), followed by Actinobacteria (26.78%), Bacteroidetes (3.95%), Firmicutes (2.41%), Tenericutes (1.98%), Chloroflexi (1.22%), Verrucomicrobia (1.03%), Cyanobacteria (0.71%) and Planctomycetes (0.65%). High levels of microbial diversity were indicated by the diversity indices, reflecting both richness (Simpson's index) and evenness (Shannon index). The findings contribute to a deeper understanding of microbiota dynamics in aquaculture systems and underline the significance of preserving microbial diversity for sustainable shrimp production.

Keywords: 16S rRNA amplicon, biodiversity, microbiota, operational taxonomic unit, shrimp

INTRODUCTION

Indonesia has gained recognition for its extensive range of biodiversity, encompassing a spectrum that spans from single-cell microorganisms to complex eukaryotic organisms (Amin et al. 2022a; Barus et al. 2017; Liu et al. 2020; Von Rintelen et al. 2017). Numerous studies have shown that the presence of high biodiversity may serve as an indicator of favorable ecological health and environmental conditions (Guajardo 2015; Roberts 2019). Moreover, an environment rich in biodiversity implies the coexistence of diverse organisms, including microorganisms that could potentially confer benefits to humans or other cultivated organisms. Notably, various bacterial species possess the capacity to produce a wide array of advantageous compounds, such as bacteria generating digestive enzymes, antimicrobial agents, or immunostimulants. These compounds have the potential to enhance the growth and disease resistance of cultured aquatic species like fish and shrimp (Amin 2016; Amin et al. 2023a; Amin et al. 2020). Therefore, the thorough exploration of microbiome biodiversity within specific environmental niches or habitats holds significant importance. Driven by these considerations, the current study embarks on an exploration of the microbiota within the Gastrointestinal Tract (GIT) of *Penaeus vannamei* (Boone 1931), commonly known as Pacific white shrimp or white leg shrimp.

Penaeus vannamei is one of the predominant shrimp species in global aquaculture, constituting over 70% of the total cultured shrimp. Its significance extends to Indonesia, where it holds a pivotal position as a key aquaculture commodity, contributing significantly to the socioeconomic advancement of coastal wetland regions (Camacho-Valdez et al. 2013). Notably, the year 2015 witnessed white leg shrimp production securing the eighth position within Indonesia's production spectrum (Ministry of Marine Affairs and Fisheries 2015). In light of this, the Ministry of

Marine Affairs and Fisheries of the Republic of Indonesia has outlined ambitious goals, targeting a substantial 250% surge in shrimp production and export value by the year 2024. This objective necessitates the expansion and intensification of brackish water pond culture within the Indonesian context (Ministry of Marine Affairs and Fisheries 2020; Gusmawati et al. 2018). It is, however, crucial to acknowledge that the progression of this technology holds the potential to engender escalated environmental pollution, primarily attributed to heightened feed consumption (Fakhri et al. 2015; Khan 2018).

Within aquaculture systems, the delicate balance of nutrient inputs emerges as a decisive factor; insufficient inputs impede shrimp growth, while excessive inputs invite ecological deterioration and unwarranted financial investments (Chaikaew et al. 2019; Iber and Kasan 2021). Prior research conducted in Kaur District, Bengkulu Province, brought to light an array of challenges associated with intensive technology adoption, encompassing disease outbreaks and a dwindling environmental carrying capacity within white leg shrimp cultivation (Nardiyanto et al. 2019). Similarly, in Barru District, South Sulawesi Province, a gamut of issues including disease susceptibility, climate variations, pollution, and land transformation were identified (Husain et al. 2020). Consequently, the intensification of shrimp ponds is posited to yield both adverse and favorable outcomes, precipitating an array of environmental and socioeconomic quandaries (Macusi et al. 2022). However, an avenue of potential resolution emerges through the comprehensive exploration of animal nutrition, specifically by delving into the intricate roles played by microbiota in the shrimp's biological processes. By attaining a nuanced understanding of these dynamics, there exists the prospect of enhancing aquaculture systems, thereby bolstering the health, growth, and survival prospects of *P. vannamei*.

While limited studies on the structure of the microbiome in shrimp have been completed for the GITs of Pacific white shrimp (Amin et al. 2022b; Amin et al. 2023b; Amin et al. 2023c). Few studies have explored the biodiversity of microbiome associated with the GITs of cultured Pacific white shrimp, *P. vannamei* using a high-throughput sequencing technique. So, the aim of this study was to investigate the biodiversity of microbiome in farmed shrimp as a proxy for the health of these cultured species. Nonetheless, there exists a dearth of comprehensive investigations regarding the diversity of microbiota residing within the gastrointestinal tracts (GITs) of *P. vannamei*.

MATERIALS AND METHODS

Sampling

Pacific white shrimp were collected from three commercial shrimp ponds situated in the East Java Province of Indonesia according to a protocol previously elucidated by Amin et al. (2023c), albeit with minor adjustments. A total of 30 shrimp, each with an average weight of 5.23±1.86 g, were randomly collected from three commercial shrimp ponds (approximately 10 shrimps from each pond) using a scope net. These specimens were then promptly transported within one hour to the Laboratory of Microbiology, Faculty of Fisheries and Marine, Universitas Airlangga, Indonesia. Subsequently, gastrointestinal tracts were meticulously dissected out of aseptic condition and immediately preserved in 15 mL sterile falcon tubes. These tubes were subsequently stored within a freezer set at -20°C, where they were maintained until further analysis was conducted.

DNA extraction, PCR and sequencing

Microbial DNA extraction was executed in alignment with a previous methodology outlined by Amin et al. (2023b), with slight adaptations. Genomic DNA sourced from shrimp gastrointestinal tract (GIT) samples was extracted by leveraging Zymo Research, Irvine, CA, USA, and following the directives stipulated by the manufacturer. Subsequently, the 16S rRNA gene's target region (V3-V4) was subjected to amplification through the utilization of forward primers (515F: CCTAYGGGRBGCASCAG, and 806R: GGACTACNNGGGTATCTAAT) that encompassed partial barcodes. The process encompassed PCR reactions and barcode integration, closely mirroring the procedures expounded by Amin et al. (2022b). In brief, the amplification of targeted (V3-V4) 16sRNA gene was performed in a total of 50-μL reaction consisting of PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and 10 ng DNA template. The mixture was run under the following protocol: 98°C for 2 min for initial denaturation, 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s and final extension at 72°C for 10 min (35 cycles). Thereafter, the resultant libraries were quantified, standardized, combined, denatured, and subsequently subjected to sequencing employing the Illumina MiSeq platform, administered by Novogene, situated in China.

Bioinformatics analysis

The examination of microbiome 16S rRNA gene sequences adhered to the procedure detailed by Amin et al. (2022b), incorporating minor adjustments. Diversity assessment was carried out by computing the Simpson's index to gauge bacterial species richness, alongside the Shannon diversity indices for assessing bacterial species evenness. These computations were executed utilizing the 'core_diversity.py' script in Python, implemented through QIIME 1.9.1. Subsequently, core genera constituting the shrimp gut microbiome were investigated and delineated by genera. The visualization of prevalence and proportional representation of these shrimp gut core genera were visualized using the R ggplot2 package.

RESULTS AND DISCUSSION

Diversity of microbiome associated with the GITs of Pacific white shrimps

The Simpson's index revealed species richness values of 0.72, 0.92, and 0.91 in the context of Pond 1, Pond 2, and Pond 3, respectively. Species richness pertains to the total count of distinct species within an ecological community, signifying the diversity of species present. Conversely, species evenness, which measures the uniformity of species distribution in the ecosystem, was reflected by the Shannon index values of 3.08 for Pond 1, 5.15 for Pond 2, and 4.89 for Pond 3. In addition, the Chao1 index, representative of species count, manifested as 439.08, 646.48, and 468.85 for Pond 1, Pond 2, and Pond 3, correspondingly.

Structure of GIT microbiomes

Upon meticulous optimization and stringent quality control measures, the current investigation yielded a total of 306,859 successfully merged reads, which were subsequently adeptly mapped to encompass 1,750 Operational Taxonomic Units (OTUs). In specific terms, Pond 1 contributed 112,775 sequences, categorically clustered into 453 OTUs, whereas Pond 2 produced 101,593 sequences resulting in 640 OTUs. Similarly, Pond 3 contributed 102,491 sequences, aggregating into 477 OTUs.

The analysis of these sequences extracted from the shrimp intestine predominantly revealed affiliations with specific phyla. Most notably, Proteobacteria emerged as the dominant phylum, constituting 53.95% of the identified reads, trailed by Actinobacteria at 26.78%, Bacteriodetes at 3.95%, Firmicutes at 2.41%, Tenericutes at 1.98%, Chloroflexi at 1.22%, Verrucomicrobia at 1.03%, Cyanobacteria at 0.71%, and Planctomycetes at 0.65%. Furthermore, a collective 5.17% of reads were attributed to various other phyla, whereas a subset remained unclassified, accounting for 2.15% of the dataset (Figure 1). Data representing more specific identify and the configuration of microbiomes within the GIT of Pacific white shrimp are presented in Figure 2.

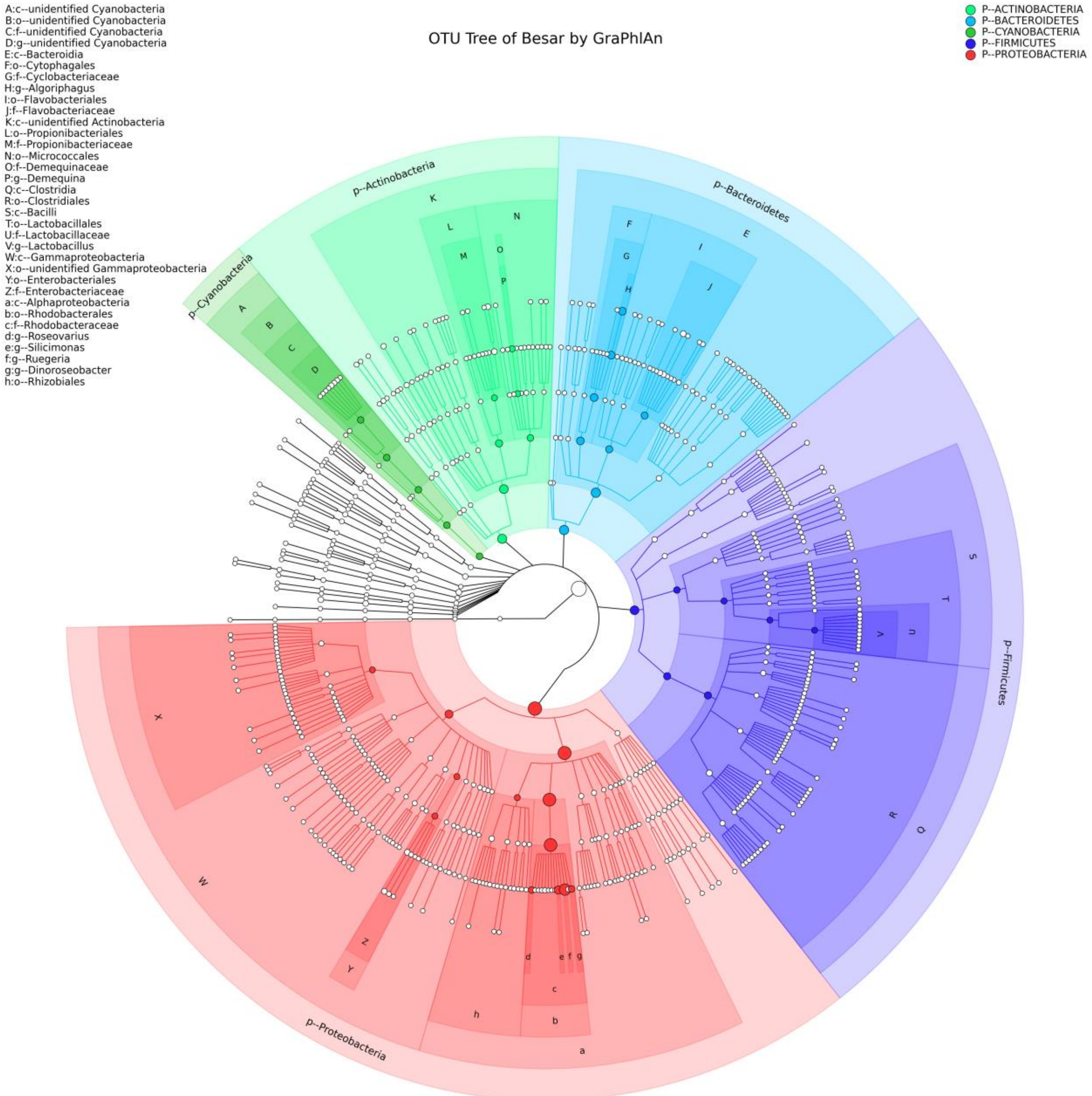

Figure 1. Structure of microbiome associated with the gastrointestinal tract of Pacific white shrimp reared in intensive ponds

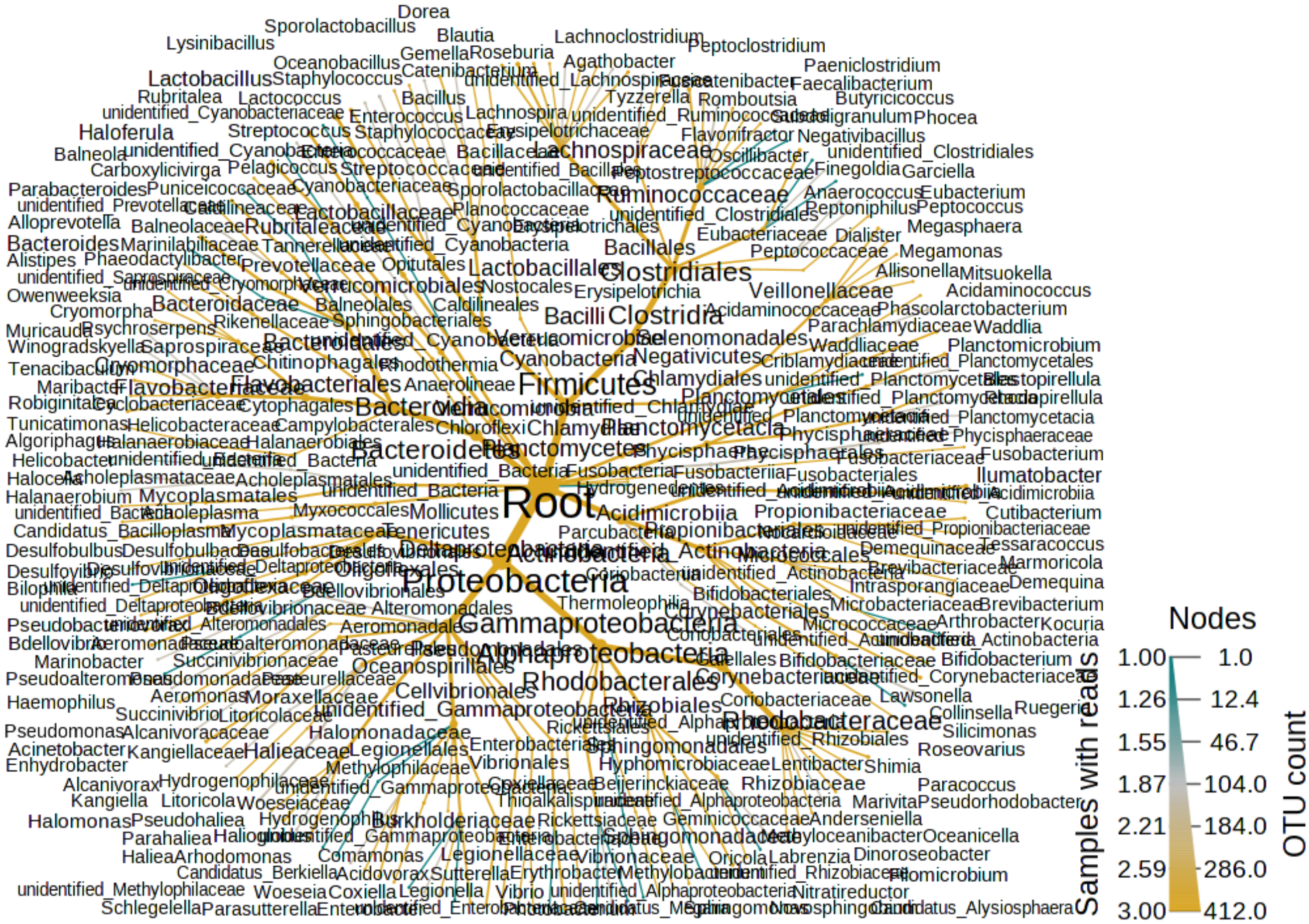

Figure 2. A heat tree representing the configuration of microbiomes within the GIT of Pacific white shrimp

At the class taxonomic level, a comprehensive tally of 22 classes was successfully distinguished within the gastrointestinal tracts (GITs) of Pacific white shrimps. The preeminent ten classes, in terms of abundance, were led by Alphaproteobacteria, which constituted 49.60% of the total, trailed by Acidimicrobiia at 24.65%. Additionally, Bacteroidia accounted for 3.84%, Gammaproteobacteria contributed 3.42%, and Mollicutes represented 1.99% of the composition. Further inclusions consisted of Anaerolineae at 1.23%, Deltraproteobacteria at 1.11%, Verrucomicrobiae at 1.03%, Clostridia at 1.02%, and Bacilli at 0.88% (Figure 3.A).

Furthermore, at order taxonomic level, the GITs of Pacific white shrimps exhibited a total of 34 distinct bacterial orders. Notably, the uppermost ten orders in terms of prevalence encompassed Rhodobacterales at 47.96%, trailed by Flavobacteriales at 2.17%. Mycoplasmatales contributed 1.98%, while Cytophagales stood at 1.29%, and Caldilineales was at 1.23%. Verrucomicrobiales made up 1.03%, with Clostridiales accounting for 1.02%, and Probionibacteriales at 0.97%. Additionally, Rhizobiales and Lactobacillales concluded the top ten orders with proportions of 0.92% and 0.73%, respectively (Figure 3.B).

At the family taxonomic level, an assemblage of 86 bacterial families was observed to inhabit the gastrointestinal tracts (GITs) of Pacific white shrimps. Among these, the leading ten families in terms of prevalence encompassed Rhodobacteraceae, constituting 47.96% of the total composition. Flavobacteriaceae followed with a representation of 2.07%, while Mycoplasmataceae contributed 1.98%. Further entries comprised Cyclobacteriaceae at 1.29%, Caldilineaceae at 1.23%, Rubritaleaceae at 0.92%, Lactobacillaceae and Bdellovibrionaceae at 0.65% each. Demequinaceae and Lachnospiraceae concluded as the top ten families with proportions of 0.51 and 0.46% respectively (Figure 3.C).

Delving into the genus taxonomic level, the GITs of Pacific white shrimps exhibited the presence of a total of 131 distinctive bacterial genera. The top ten most abundant genera included *Ilmatobacter* at 24.44%, followed by *Ruegeria* at 6.24%. Subsequently, *Roseovarius* constituted 2.48%, *Candidatus Bacilloplasma* stood at 1.97%, and *Silicimonas* held 1.61% of the composition. The list further included *Algoriphagus* at 1.29%, *Haloferula* at 0.91%, *Dinoroseobacter* at 0.70%, *Lactobacillus* at 0.65%, alongside an unidentified_cyanobacteria, also at 0.65% (Figure 3.D).

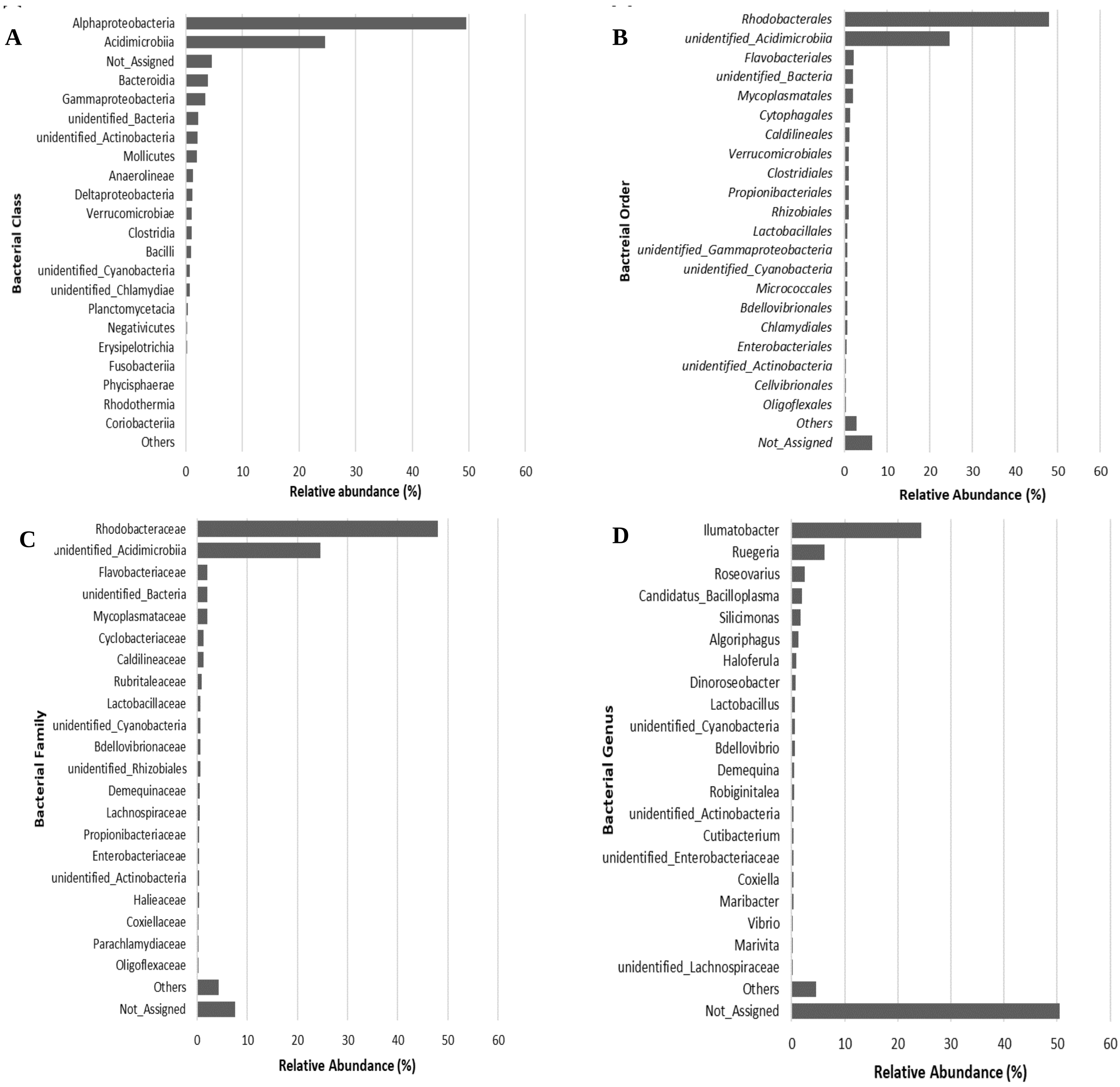

Figure 3. The most prevalent microbiomes within the gastrointestinal tract (GIT) of Pacific white shrimp in terms of: A. Class; B. Order; C. Family; D. Genus

Ternary plot for microbiome inhabiting GITs of shrimps

A ternary plot representing phylum distribution across distinct samples was generated to visualize the relative prevalence of phyla in various samples. Each vertex of the triangle corresponds to the ponds from which the shrimp samples were collected. The magnitude of dots plotted on the graph was indicative of the abundance of phyla, class, order, family, and genus, as demonstrated in Figure 3. Among the phyla, Proteobacteria emerged as the most dominant, closely pursued by Actinobacteria (Figure 3.A). Analyzing the class distribution, Alphaproteobacteria and Acidimicrobiia emerged as the predominant classes (Figure 3.B). At the order level, Rhodobacteriales and an unidentified Acidimicrobiia representative occupied prominent positions (Figure 3.C). Within the family classification, Rodobacteriaceae and unidentified Acidimicrobiia demonstrated noteworthy prevalence (Figure 3.D). Moving to the genus level, the gastrointestinal tracts (GITs) predominantly featured *Illumobacter* and *Ruegeria* (Figure 3.E), while the species taxa level was marked by *Algoriphagus* marincola as the dominant species (Figure 3.F).

Core microbiome in terms of genus

Regarding the genus classification, the present investigation unveiled that the fundamental microbiomes within the gastrointestinal tracts (GITs) of Pacific white shrimps comprised Illumobacter, Ruegeria, Silicomonas, Roseovarius, Candidatus Bacilloplasma, Algoriphagus, Haloferula, Dinoroseobacter, Robiginitalea, and Bdellovibrio (Figure 4).

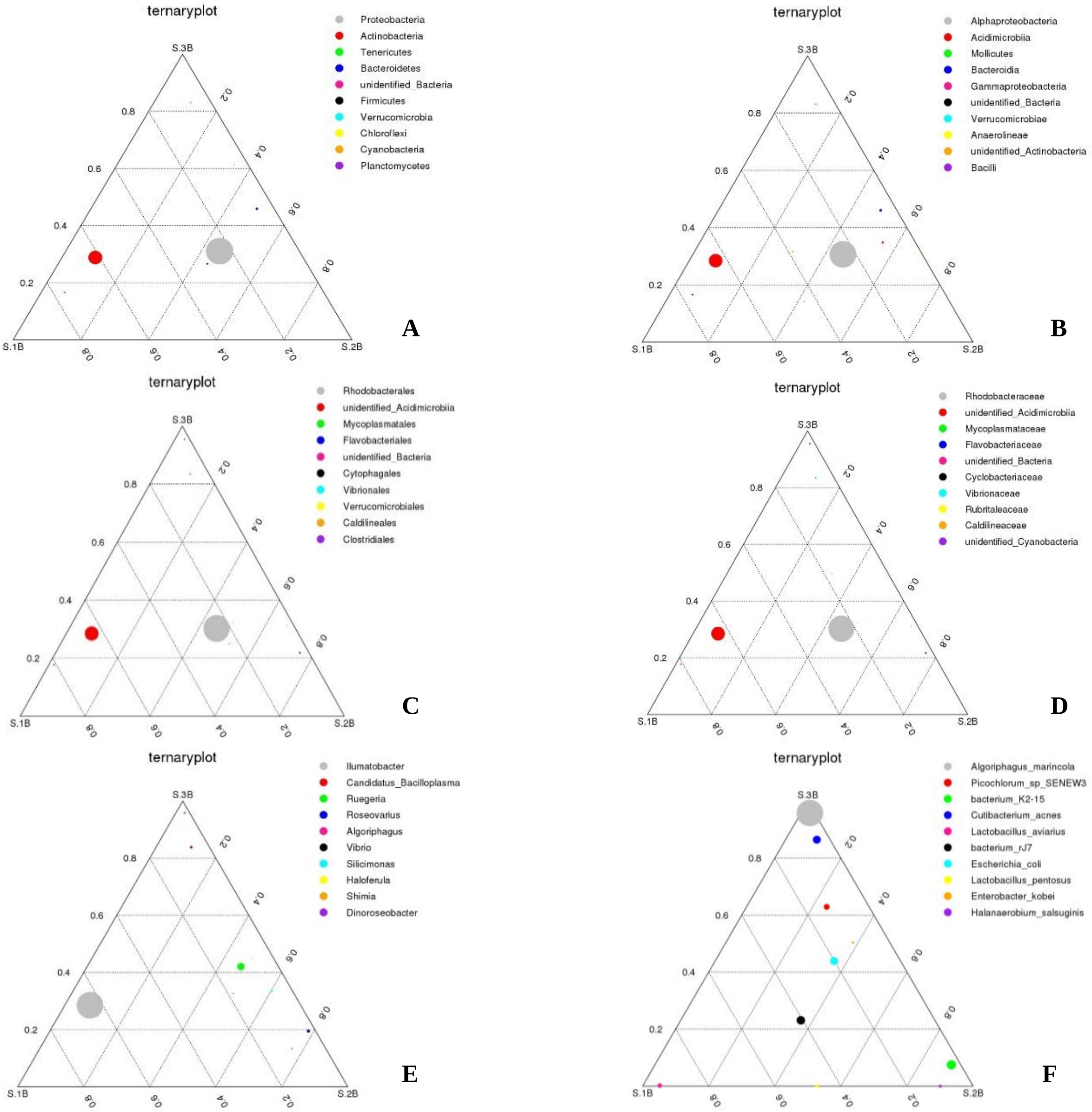

Figure 3. A ternary plot depicting the dispersion of bacterial taxonomic categories across various sample types in the subsequent experiment

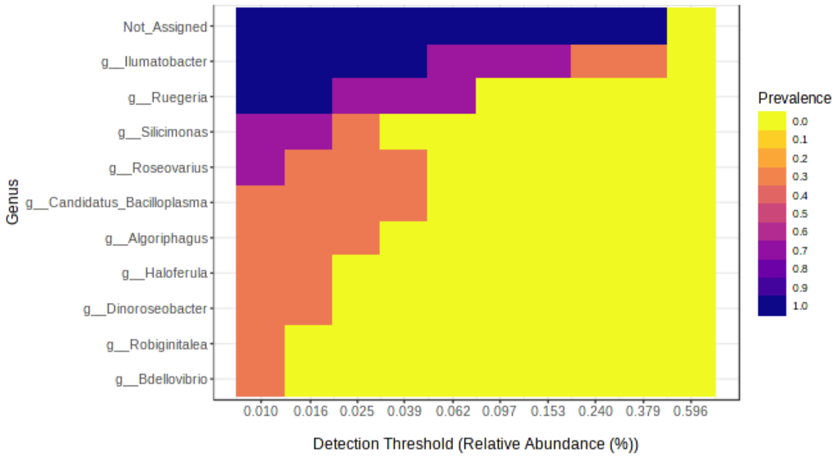

Figure 4. Dominant microbial communities within the gastrointestinal tracts of Pacific white shrimps cultured in high-intensity aquaculture ponds

Discussion

The current investigation centered on exploring the diversity and composition of the microbiome within the gastrointestinal tract of Pacific white shrimp, cultivated in intensive aquaculture ponds within a commercial shrimp farm in Indonesia. This exploration was facilitated through the utilization of the Illumina MiSeq sequencing technique. The association between diversity and ecosystem health has been frequently established (Guajardo 2015). A common approach for gauging biodiversity involves assessing richness, representing the total number of species within the ecosystem, and evenness, indicating the equitable distribution of these species (Roberts 2019). The assessment of richness and evenness typically employs indices such as Simpson's and Shannon's.

The results revealed a spectrum of Simpson's index values spanning from 0.72 to 0.92. These values lie between the range of 0.61 to 0.99, it can be inferred that the microbial species residing within the GITs of Pacific white shrimp across Indonesian farms exhibit a moderate to high diversity level. These findings were consistent with the number of species identified in the present study, amounting to 416, 448, and 606 bacterial species for Pond 1, Pond 3, and Pond 2, respectively. Interestingly, the Simpson index values observed were closely align with previous findings from other Indonesian shrimp farms, which reported an approximate value of 0.99 (Hastutia et al. 2021). However, the values derived from the present study are exceeded the Simpson index values recorded for microbiomes within Pacific white shrimp from Vietnam, Malaysia (Zoqratt et al. 2018), and French farms, which ranged from 0.15 to 0.18 (Cardona et al. 2016). These observations collectively suggest that the GITs of Pacific white shrimp nurtured in Indonesian farms harbor greater species richness when compared to their counterparts in other countries.

Furthermore, the evenness, was observed to be 3.08, 5.15, and 4.89 for Pond 1, Pond 2, and Pond 3, respectively. All the Shannon index values surpass 3.5, it can be deduced that the diversity of microbiome linked with the gastrointestinal tracts (GITs) of Pacific white shrimps reared within intensive aquaculture systems exhibits a markedly elevated level (Baliton et al. 2020). This observation contrasts with the Shannon index value previously documented for Pacific white shrimp raised in various Indonesian farms, which averaged around 1.5 (Hastutia et al. 2021). Additionally, the values derived from current study outperformed the Shannon index values reported for Pacific white shrimp cultivated in French farms, which ranged from 2.66 to 2.86 (Cardona et al. 2016). A separate study by Zeng et al. (2017) unveiled a similarly lower Shannon index value of approximately 1.936 for microbiomes inhabiting the GITs of Pacific white shrimp reared in Chinese farms.

Moreover, the Chao1 index, signifying species count, manifested as 439.08, 646.48, and 468.85 for Pond 1, Pond 2, and Pond 3 correspondingly. Evidently, all Chao1 values surpassed the number of observed Operational Taxonomic Units (OTUs), implying that there was a potential for the identification of more OTUs across all samples. Furthermore, these species counts exceeded those reported by Cardona et al. (2016) which reported 322 species. These findings collectively accentuate the heightened evenness of microbiome within the GITs of Pacific white shrimps in Indonesia. Given the concurrent elevation in both richness and evenness, it can be reasonably inferred that the microbiome within the GITs of Pacific white shrimp nurtured within Indonesia's commercial farms boasts greater diversity than that observed in shrimp cultivated in other nations.

The composition of microbiome within the gastrointestinal tracts (GITs) of Pacific white shrimps exhibits considerable similarity in terms of predominant phyla. This study identified nine principal phyla found in the GITs of shrimp samples, namely Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Tenericutes, Chloroflexi, Verrucomicrobia, Cyanobacteria, and Planctomycetes. Comparable dominant phyla were also documented by various researchers investigating the GITs of the same shrimp species (Cornejo-Granados et al. 2017; Tepaamorndech et al. 2020; Wang et al. 2020; Zoqratt et al. 2018). Nevertheless, discrepancies arise concerning the prevalent sequences. In this study, Proteobacteria (53.95%) was found as the prevalent phylum, followed by Actinobacteria (26.78%). In contrast, Kurniawinata et al. (2022), reported that Actinobacteria was the prevailing phylum (79.88%), followed by Proteobacteria (12.52%). Delving into the family-level analysis, the result revealed the presence of 86 bacterial families within the GITs of Pacific white shrimps. Among these, the top ten most abundant families comprised Rhodobacteraceae, Flavobacteriaceae, Mycoplasmataceae, Cyclobacteriaceae, Caldilineaceae, Rubritaleaceae, Lactobacillaceae, Bdellovibrionaceae, Demequinaceae, and Lachnospiraceae. Notably, these bacterial families diverge significantly from the composition reported by Zoqratt et al. (2018) in Vietnam, where Alcaligenaceae, Flavobacteriaceae, Microbacteriaceae, Acidimicrobiaceae, and Rhodobacteraceae featured prominently. Furthermore, distinct family taxa were documented in the GITs of Pacific white shrimp cultured in French shrimp farms, where Vibrionaceae and Enterobacteriaceae emerged as the most prevalent families (Cornejo-Granados et al. 2017).

At the genus taxonomic level, 131 distinct bacterial genera were successfully identified within the gastrointestinal tract of Pacific white shrimps. The uppermost ten abundant genera comprised *Ilmatobacter*, *Ruegeria*, *Roseovarius*, *Candidatus Bacilloplasma*, *Silicimonas*, *Algoriphagus*, *Haloferula*, *Dinoroseobacter*, *Lactobacillus*, and an unidentified *Cyanobacteria*. Diverse genera were documented by Cornejo-Granados et al. (2017) including *Vibrio*, *Photobacterium*, and *Paracoccus*. Similarly, Wang et al. (2020) reported *Vibrio*, *Rhodobacter*, and *Flavobacterium* as the most abundant genera. Moreover, Tepaamorndech et al. (2020) identified *Pseudalteromonas*, *Photobacterium*, *Shewanella*, *Alteromonas*, *Bacillus*, *Lactobacillus*, *Acinetobacter*, *Clostridium*, *Marinifilum*, *Pseudomonas*, *Granulosicoccus*, and *Ruegeria* as the dominant genera. Several studies concluded the dominant bacterial genera may also represent health status of cultured animals including Pacific white shrimps. For instance, a study by Wang et al. (2020) reported that the gastrointestinal tract of Pacific white shrimp dominated by Vibrio, Rhodobacter and Flavobacterium was infected with white feces syndrome. Meanwhile, Amin et al. 2022b reported that *Ilmatobacter*, *Ruegeria*, and *Candidatus Bacilloplasma* were associated with fast-growing Pacific white shrimps.

In the investigation conducted by Zoqratt et al. (2018), Imaizumi et al. (2021), and Shen et al. (2022), a relatively higher prevalence of *Vibrio* bacteria was observed in the midgut of white leg shrimp in comparison to the stomach. Conversely, our study reveals a low occurrence of *Vibrio* throughout the gastrointestinal tract (GIT). It is worth noting that the shrimp's GIT is a lengthy digestive structure encompassing the Foregut (stomach), Midgut (hepatopancreas), and Hindgut (intestine), each serving distinct roles in terms of structure, immunity, and digestion. Imaizumi et al. (2021) found a higher bacterial diversity in the stomach compared to the midgut for both species. Shen et al. (2022), documented a substantial rise in the prevalence of *Vibrio* correlating with the severity of *Enterocytozoon hepatopenaei* (EHP) infection ($P<0.05$). However, the present study did not provide an in-depth elucidation of the diverse populations or changes of various infections within the GIT. This underscores the significance of investigating microbiota not solely within the midgut but also within the stomach of shrimp, a factor that warrants consideration in forthcoming endeavors. The role of intestinal microbiota in shrimp health has been posited (Li et al. 2018). To unravel the bacterial colonization in the shrimp's stomach, more extensive analyses including histological investigations employing specialized staining techniques are indispensable.

In essence, the findings of this study underscore the notable variability in the structure of the microbiome associated with the gastrointestinal tracts of Pacific white shrimp across different studies and researchers. These disparities could likely be attributed to variations in both biotic and abiotic environmental conditions in which the Pacific white shrimps were cultivated (Amin 2010; Amin et al. 2022b; Infante-Villamil et al. 2021; Kurniawinata et al. 2022; Zeng et al. 2017).

The investigation into the microbiome of the gastrointestinal tracts (GITs) of Pacific white shrimps within intensive aquaculture ponds has yielded insightful findings concerning microbiota diversity. This is the first study of an operational taxonomic unit (OTU) focused examination of the gut microbiota within aquaculture shrimp coming from East Java, Indonesia, using a standardized Illumina 16S rRNA amplicons sequencing protocol. The result highlighted the presence of a diverse array of bacterial phyla, classes, orders, families, and genera within the GITs. The foremost nine phyla in terms of abundance encompass Proteobacteria, succeeded by Actinobacteria, Bacteroidetes, Firmicutes, Tenericutes, Chloroflexi, Verrucomicrobia, Cyanobacteria, and Planctomycetes. In tandem, the diversity indices, as inferred from the richness denoted by Simpson's index and evenness indicated by Shannon's index, were notably elevated.

In summary, the present study revealed the top five dominant members of bacteria associated with the GI tract of Pacific white shrimp were Proteobacteria, followed by Actinobacteria, Bacteroidetes, Firmicutes, and Tenericutes. In addition, high diversity of microbiome was observed indicated by richness (Simpson's index) and evenness (Shannon index). The comprehensive examination of the microbiome within the GITs of Pacific white shrimp in this study has provided invaluable insights into its diversity, structure, implications, and future research directions.

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