



Microbiome analysis and detection of pathogenic bacteria of *Penaeus monodon* from Jakarta Bay and Bali



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ABSTRACT

Penaeus monodon, the Asian black tiger shrimp is one of the most widely consumed marine crustaceans worldwide. In this study, we examine and compare the fecal microbiota of *P. monodon* from highly polluted waters around Jakarta Bay, with those of less polluted waters of Bali. Using next generation sequencing techniques, we identified potential bacterial pathogens and common viral diseases of shrimp. *Proteobacteria* (96.08%) was found to be the most predominant phylum, followed by *Bacteroidetes* (2.32%), *Fusobacteria* (0.96%), and *Firmicutes* (0.53%). On the order level, *Vibrionales* (66.20%) and *Pseudaltermonadales* (24.81%) were detected as predominant taxa. qPCR profiling was used as a confirmatory step and further revealed *Vibrio alginolyticus* and *Photobacterium damsela* as two potential pathogenic species present in most of the samples. In addition, viral diseases for shrimp were discovered among the samples, WSSV in Jakarta free-living samples, YHV in Bali free-living samples and IHNV in both.

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1. Introduction

The black tiger shrimp, *Penaeus monodon* is one of the most widely traded marine crustaceans over the last few decades (Liu et al., 2011; FAO: Food Agric Organ United Nations, 2014). Due to its high economic value, the number of *P. monodon* aquaculture farms has rapidly increased. But several problems have emerged which have resulted in decreasing shrimp production (Acre and Moss, 2003; Venkateswara-Rao, 1998; Lightner, 2003). Low quality control of feed and an ineffective disease management for shrimp represents a major problem. Previous studies have reported the importance of bacterial communities in intestinal tract to maintain the metabolism and immunity of their host, as well as to evade viral and bacterial diseases (Zhang et al., 2014; Chaiyapechara et al., 2012). Due to its aquatic environment, *P. monodon* is highly exposed to potential pathogens (Rungrassamee et al.). In order to support the innate immunity of the host, fecal microbiota has evolved an established mechanism for host defense (Xia et al., 2014). Previous studies have shown, that the composition of bacterial communities is influenced by host phylogeny and diet (Zhang et al., 2014; Xia et al., 2014). Furthermore, ecological and environmental interaction also has an influence on alteration of bacterial diversity

(Sullam et al., 2012; Asplund, 2013). A comprehensive understanding of bacterial community composition and alteration factors is essential for the enhancement of aquaculture quality. Currently, studies of fecal microbiota in shrimp have been done (Liu et al., 2011; Zhang et al., 2014; Luis-Villasenor et al., 2012), particularly in *P. monodon* (Chaiyapechara et al., 2012; Rungrassamee et al.). Comparative analyses between wild and domesticated *P. monodon* will provide a solid base for the development of probiotics. As previously reported (Partida-Arangure et al., 2013; Balcazar et al., 2006; Luis-Villasenor et al., 2012), probiotics have the ability to reshape bacterial communities and act as a host defense mechanism or in the improvement of surrounding conditions. Rungrassamee et al. showed that *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, and *Actinobacteria* represent the predominant phyla in free-living *P. monodon*, as well as in aquaculture ones (Rungrassamee et al.). The bacterial community composition differs in each phylum under different conditions. Moreover, predominant genus that have been discovered are potential pathogens for shrimp and human, for instance, *Vibrio* sp. and *Photobacterium* sp. (Rungrassamee et al.; Vaseeharan et al., 2007). Furthermore, several common viral diseases in shrimp also play an important role for aquaculture improvement. They could cause a great loss, either by reducing the production or collapsing the whole aquaculture (Xia et al., 2014; Wegner et al., 2013; Guarner and Malagelada, 2003; O.L. et al., 2007). In this study, we use a 16S rRNA approach on an Illumina MiSeq

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sequencing platform to elucidate the fecal microbiome of 24 *P. monodon* samples from three different locations, representing three different environments, polluted water around Jakarta Bay, non-polluted water of Bali Bay and cultured *P. monodon* from a traditional aquaculture farm in Bali. We focus on this three way comparison in order to determine differences in the bacterial community composition between these three environments. In addition, we also reveal the presence of several potential bacterial and viral pathogens that could have an impact on shrimp and human health using qPCR (quantitative Polymerase Chain Reaction).

2. Results

2.1. Bacterial community analysis

The Illumina MiSeq sequencing platform yielded in total 6,705,305 reads over all 22 samples. We had to exclude two free-living samples from Jakarta (Jk1: 2096 reads, Jk7: 159 reads) due to low number of reads. On average 304,800 mapped reads were obtained, ranging from 166,400 reads up to 581,400 reads (Supplementary Fig. 1), which were assigned to 935 Operational Taxonomic Units (OTUs) in order to assess the bacterial communities of the fecal samples. Selecting only phyla with an abundance level at above 0.1%, the bacterial communities revealed four bacterial phyla appearing predominantly: *Proteobacteria* (96.08%), *Bacteroidetes* (2.32%), *Fusobacteria* (0.96%), and *Firmicutes* (0.53%). These four phyla spread over twelve bacteria on the order level, which also appeared predominantly in all samples (Fig. 1a). On average 66.20% of *Vibrionales* accounted for the bacterial community

composition, followed by 24.81% of *Alteromonadales*. These two dominant orders were relatively similar distributed in each sample, except in Jk3, Jk4, and Jk6 which showed higher numbers of *Vibrionales* and absence of *Alteromonadales*. Ba7 pointed out a different pattern, showing *Alteromonadales* (68.42%) as predominant order, followed by *Vibrionales* (28.83%). Aquaculture samples exhibited a significant number of bacteria at the order level beyond *Vibrionales* and *Alteromonadales*. For instance, Aq4 showed one unclassified bacteria, which we were unable to annotate on order level, but belonging to *Alphaproteobacteria* (21.64%), whereas *Sphingobacteriales* (15.01%) appeared in high numbers in Aq9. Bali free-living samples, particularly Ba4, showed a large portion of *Fusobacteriales* (20.21%). Rare phyla, showing abundance below 0.1% were also assessed. *Actinobacteria* (0.0623%), followed by *Cyanobacteria* (0.0070%), *Verrucomicrobia* (0.0039%), *Deinococcus-Thermus* (0.0013%), *Planctomycetes* (0.0006%), *Spirochaetes* (0.0005%), *Chloroflexi* (0.0004%), *Tenericutes* (0.0002%), *Acidobacteria* (0.0001%) and *Ignavibacteria* (<0.0001%) were found among all samples (Fig. 1b). Samples derived from Bali aquaculture showed *Actinobacteria* as the most prevalent rare phyla, specifically in Aq4 (0.30%) and Aq7 (0.07%). A similar trend was observed in Jakarta free-living samples, particularly in Jk2 (0.25%) whereas *Cyanobacteria* tended to be the most prevalent rare phylum in Bali free-living samples, especially in Ba6 (0.21%).

2.2. Diversity of the microbiome

Three different approaches were employed to investigate bacterial diversity between the samples. First, observed OTU richness, number of unique OTUs, revealed a high median number of observed OTUs for

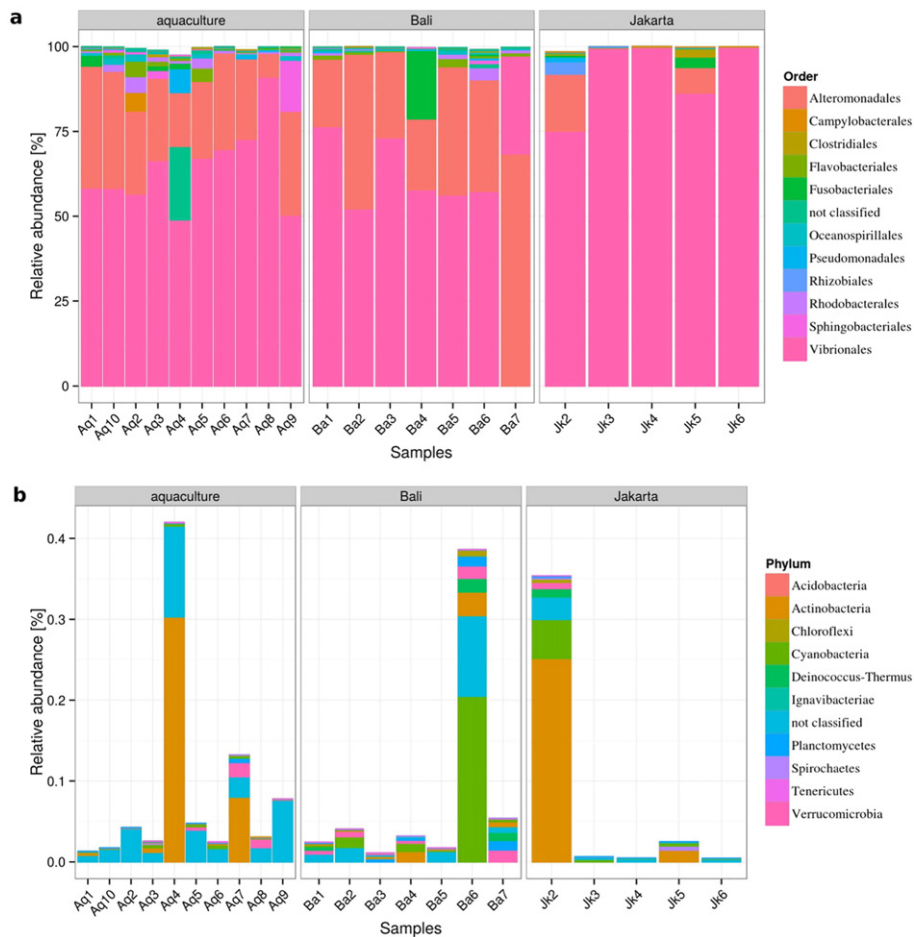


Fig. 1. Bacterial community composition of shrimp samples under Bali traditional aquaculture, Bali free-living, and Jakarta free-living condition. (a) corresponds to the predominantly appearing bacterial taxa on order level of taxonomy. Only bacterial taxa with an abundance over 0.1% were selected. (b) shows the rare phyla appearing in each sample under the different conditions.

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