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CHARACTERIZATION OF BACTERIAL COMMUNITY IN THE GUT OF AQUACULTURED SHRIMP *PENAEUS MONODON*

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ABSTRACT

The knowledge of bacterial communities in the brackish shrimp farming in Kerala is still insufficient.16S rRNA gene-based high-throughput sequencing revealed distinct and diverse microbial communities in the analyzed sample. Analysis of the results showed a high abundance of Betaproteobacteria, followed by Alphaproteobacteria, Clostridia, Actinobacteria, Gammaproteobacteria and Bacilli in the metagenome retrieved from the gut sample. Unclassified bacteria also contributed a significant portion of the metagenome. Microbes that play essential roles in nutrient cycling and mineralization of organic compounds such as Bacteroidetes, Planctomycetes, Gammaproteobacteria, Firmicutes, Cyanobacteria, and Actinobacteria could also be identified. Due to the strong influence of the gut microbiota on fish health, dominant bacterial species in the gut are strong candidates for probiotics. This study aimed to characterize the gut microbiota of giant tiger shrimp, Penaeus monodon. These findings provide valuable information on the microbial community and contribute to control the diseases in shrimp farms.

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INTRODUCTION

The shortage of wild fishery resources and the rising demand for human nutrition has driven a great expansion in aquaculture during the last decades in terms of production and economic value. However, the intensification of seafood farming has resulted in higher risks of disease outbreaks and in the increased use of antimicrobials to control them. The selective pressure exerted by these drugs provides the ideal conditions for the emergence of antimicrobial resistance hotspots in aquaculture facilities. Aquaculture is the fastest growing animal food-producing sector and is set to overtake capture fisheries as a source of food fish (Subasighe 2005). Currently, one of the main factors limiting expansion and profitability of aquaculture is lack of disease control (FDA 2012). However, the gut microbiota strongly influences fish health in other ways such as assisting in the development of the gut epithelium, providing essential nutrients and stimulating the innate immune system (Nayak 2010). The use of microorganisms in aquaculture as environmental biomarkers, bioremediators, probiotics, and as a direct food source for the cultured species has expanded further in the last few decades. However, we are still unaware of the various microbial species thriving within the aquaculture systems and their specific roles. Evidence has revealed that the diversity of microorganisms in aquaculture systems is far from being elucidated. Metagenomics is the study of genetic material recovered directly from the environmental sample.

It is a culture-independent approach that provides an ample opportunity to discover the unexplored microbial community Metagenomics undoubtedly can provide additional information regarding the understanding of the microbial diversity that thrives within the aquaculture systems. The present study reports metagenomic sequencing and analysis of the sediment samples of a semi-intensive penaeid shrimp culture system to explore its microbial diversity. 16S rRNA gene-based highthroughput sequencing was employed to reveal distinct and diverse microbial communities present in the sample.

MATERIAL AND METHODS

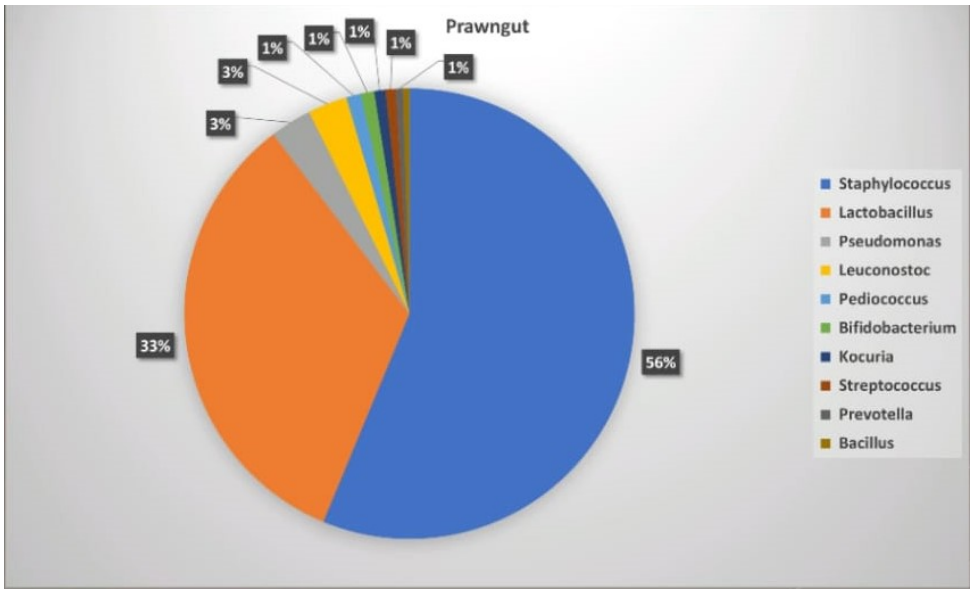
Sample collection and processing: The present study was carried out in a semi-intensive aquaculture system for Penaeus monodon production, located at Munroe Inland, Kerala, India .The aquaculture system operates under semi-intensive management, receiving natural water from the Munroe Inland estuary. Approximately shrimp samples were collected from the culture pond from a depth of 70 cm by using a sterile grab. The samples were transferred to ice baskets. The shrimp gut were isolated with proper care. The isolated gutwere immediately transferred to ethanol. Gut DNA extraction were DNA extraction was done as per the manufacturer's recommendation. Extracted DNA from the samples was subjected to Nano Drop and GEL Check before being taken for further steps. The Nano Drop

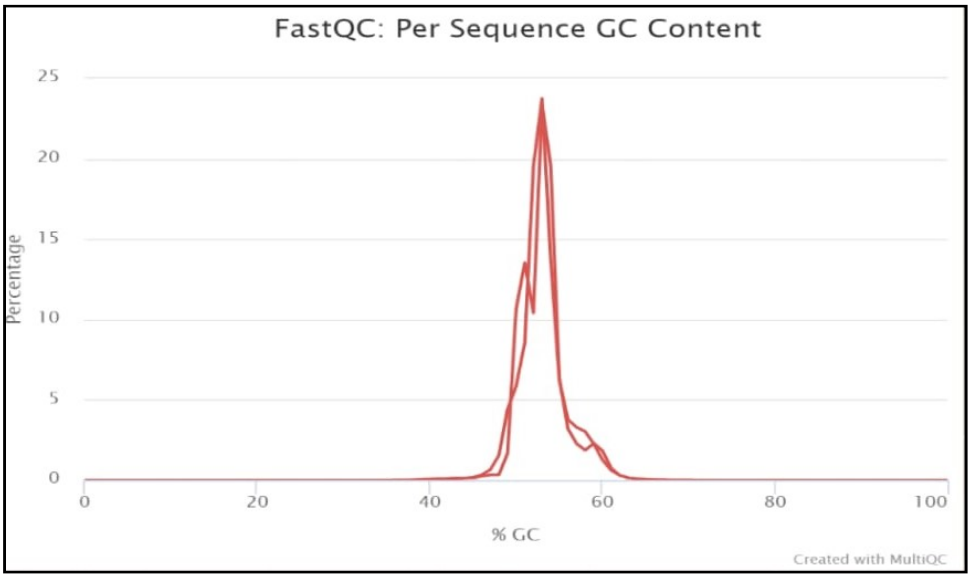
readings of 260/280 at an ~ value of 1.8 to 2 is used to determine the DNA's quality.

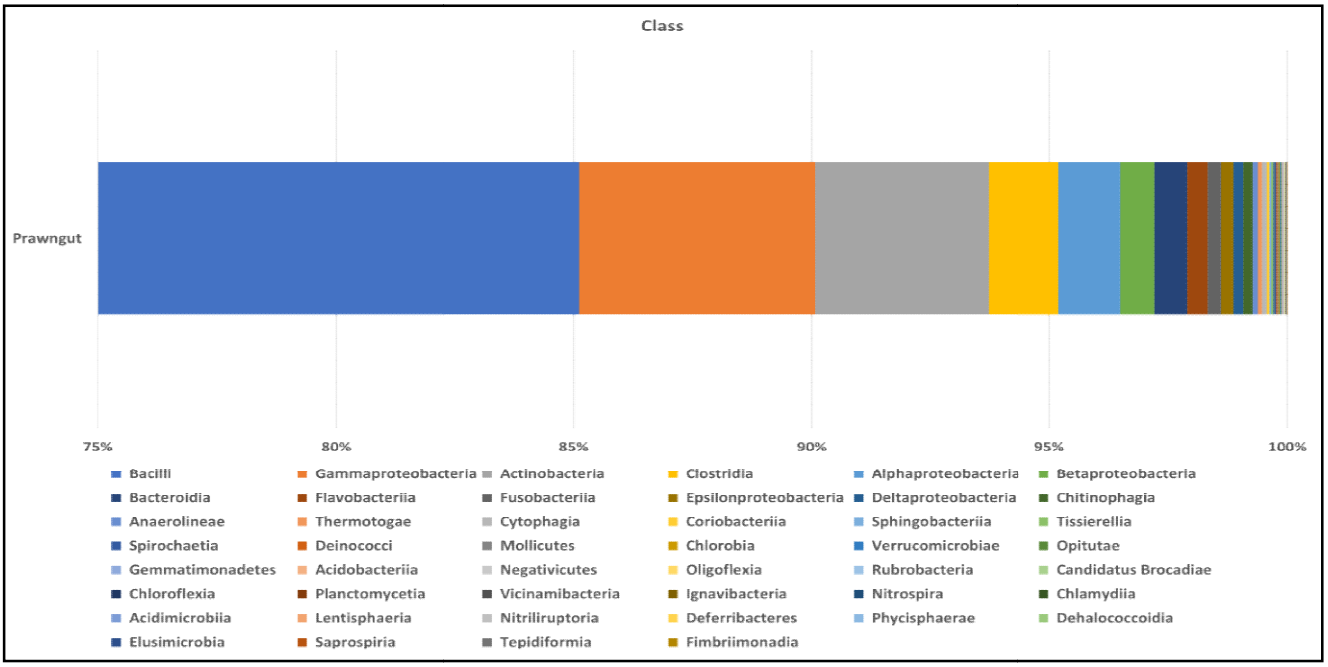
Next generation sequencing Analysis: Metagenomic nucleic acid extracted from the gut were subjected to 16S rRNA gene-based high throughput sequencing and analysis at Phytocom Pharmaceuticals (P) Ltd. Kalamassery, Kerala, India. Briefly, 455ng of DNA was used to amplify 16S rRNA hyper variable region V3– V4. The DNA extracted samples are fragmented using KAPPA fragmentation method to fragment the DNA into 600 bp length. The fragmented samples were processed for end repair and A-tailing with Hypa peep plus ERAT enzyme mix. Immediately after the end repair and A-tailing the adapter were added and ligated to the end repaired DNA fragments using DNA ligase. Library amplification was done to the adapter ligated samples with Illumina primers. Libraries were purified using Ampure beads and quantitated using Qubit dsDNA High Sensitivity assay kit. Sequencing was performed using Illumina Hiseq 4000. The size selection of product for the sequencing was based on 0.7x (>450bp). QC was done to the sequenced raw data. Deep analysis were made using Kraken2 software is used for this process. -build parameter was used to build algal databases for the analysis. The source to build the database was downloaded from NCBI. The raw data is trimmed to remove the adapter sequences using the tool Trimgalore version 0.4.5.The trimmed raw data was used as the input for Kraken2 analysis.

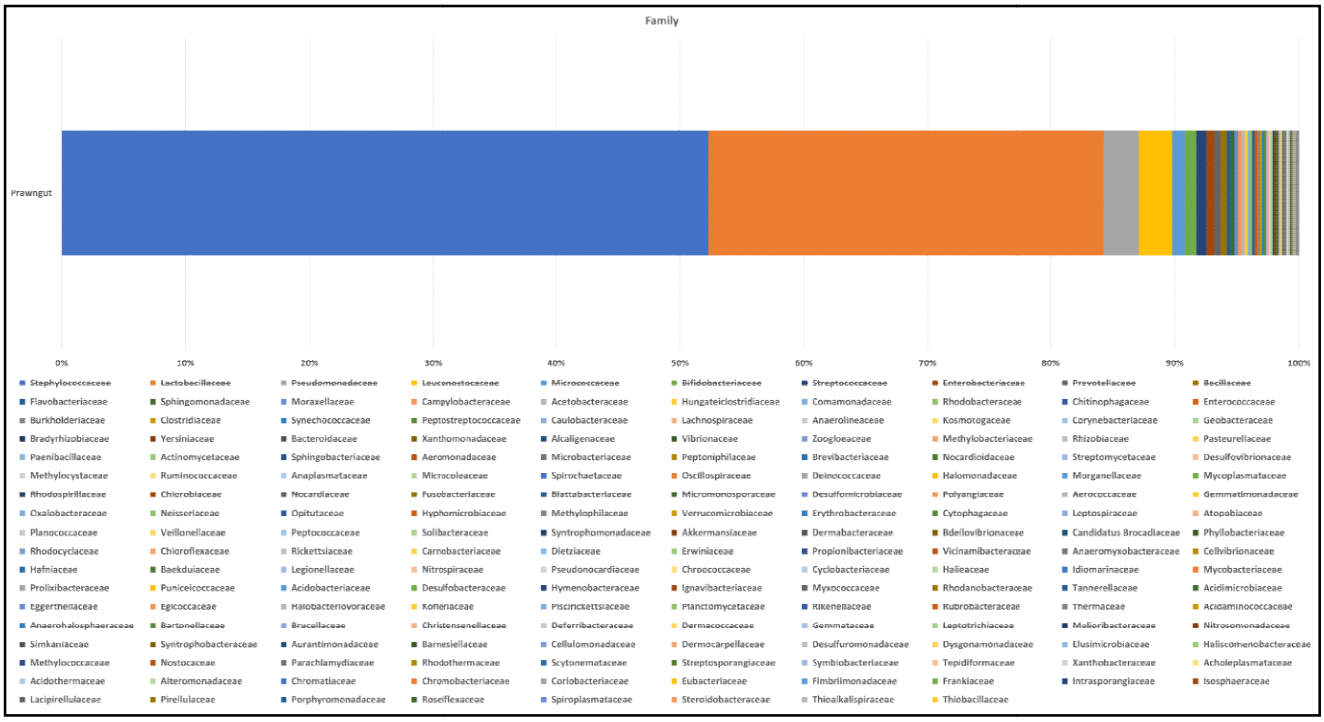
RESULTS AND DISCUSSION

The microbial diversity is assumed to be greater in aquaculture systems due to the presence of nitrogenous and phosphorous metabolites as well as organic matter. Most of the microbial species flourishing within the aquaculture systems and their specific roles still remain mystifying. In this regard, metagenomics can provide additional information regarding the understanding of the microbial diversity that thrives within the aquaculture systems. The present study is a preliminary attempt to explore the microbial diversity present in the gut of an aquaculture pond employing metagenomics. Next-generation sequencing of the gut sample revealed distinct and diverse microbial communities present in the sample. Analysis of the results showed a high abundance of Betaproteobacteria in the metagenome retrieved from gut sample followed by Alphaproteobacteria, Clostridia, Actinobacteria, Gammaproteobacteria and Bacilli in the metagenome retrieved from the gut sample. Figure 1 shows the relative abundance of the most dominant bacterial groups (ten most abundant phylum, ten most abundant genus, and ten most abundant species). Betaproteobacteria was found to be the most abundant phylum in the metagenome retrieved from the gut sample. Betaproteobacteria and Alphaproteobacteria comes under the class Proteobacteria.



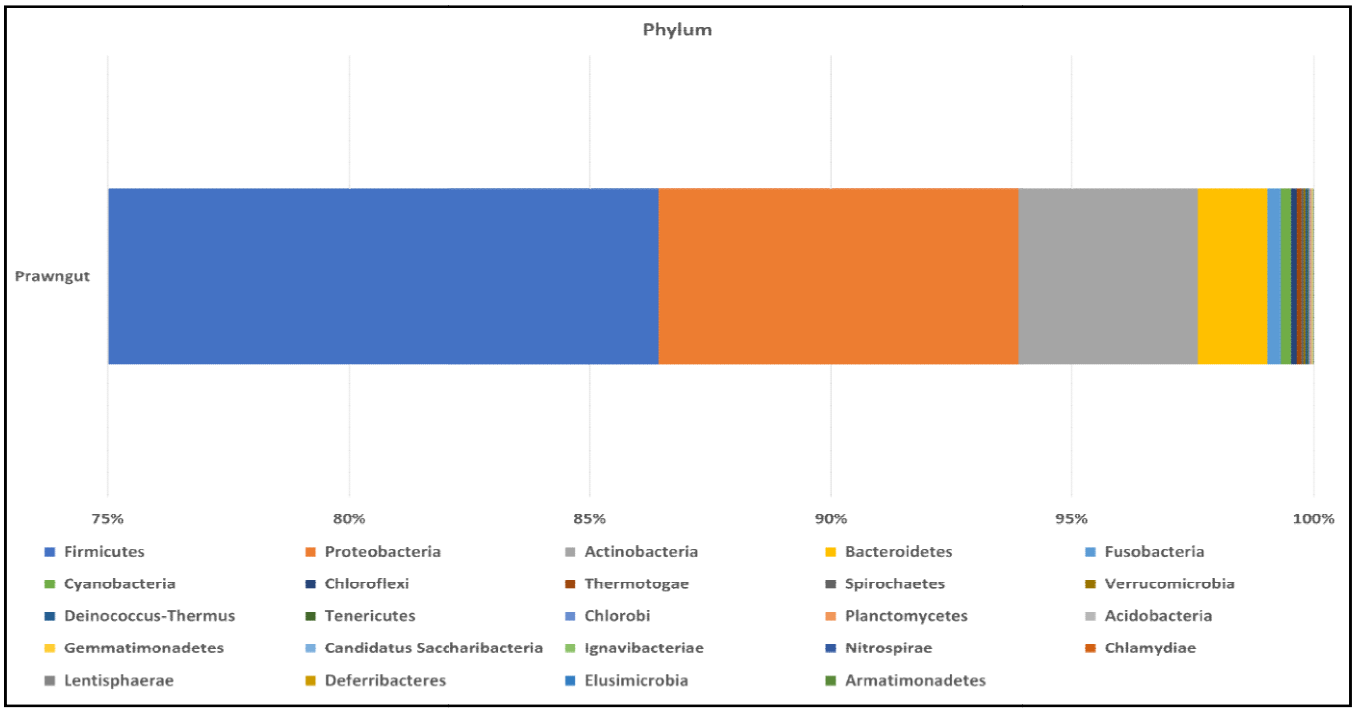


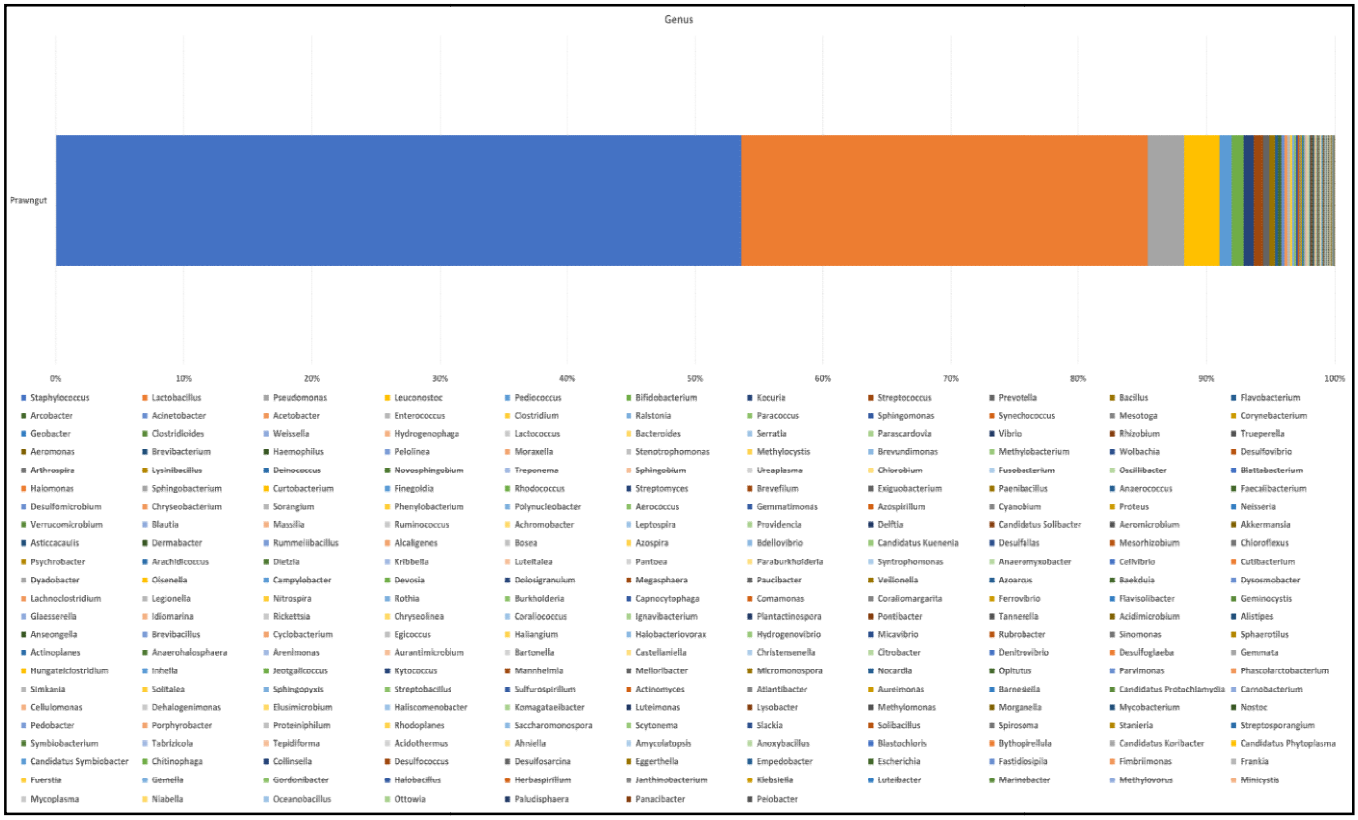




Proteobacterias play essential roles in nutrient cycling and mineralization of organic compounds and are found to be widely distributed in the marine environment. Previous studies have reported that proteobacteria dominate the gut microbiome of penaeid shrimps. Proteobacteria have been reported to be more abundant in the shrimp intestines and are associated with slow growth performance and potential risk of disease. Some of the bacteria from this phylum are responsible for nitrogen fixation also. Most of the OTUs assigned to this phylum were assigned to be Vibrio harveyi, the potential shrimp pathogen that usually results in mass mortality. The high abundance of V. harveyi in the gut shows the risk associated with the pond. Another potential pathogen that could detected in the gut samples was Acinetobacter lwoffii which is also a proteobacterium. The next abundant phyla of the gut sample were Verrucomicrobia, capable of oxidizing a range of complex polymeric carbon compounds, enhancing the capacity of organic matter degradation in toxic sediments. Bacteroidetes are a group of the intestinal microbiome that are beneficial to the host organism and abundant in the gut samples.

This phylum includes some of the most abundant groups in the marine systems after proteobacteria. Most of the OTUs assigned to this phylum were further classified as belonging to the class Flavobacteria and the order Flavobacteriales. Flavobacteria are considered as potential bioremediators of the culture systems and play an important role in the degradation of organic matter. Species of the genus Bacteroidetes have been reported to show high antibiotic resistance capacity and have been reported as a major vitamin B12 producer in the intestine of shrimps and finfishes. Other abundant phyla identified in the gut samples were beneficial bacteria belonging to Clostridia. Clostridia are strictly anaerobic to aerotolerant sporeforming bacilli found in soil as well as in normal intestinal flora of man and animals.Operational taxonomic units (OTUs) cluster analysis showed that the two samples tested were divided into 24 phyla and 230 genera. Percentile calculation of phylum, class, order, family, genus, species of shrimp gut observed from metagenomic analysis were obtained as follows. Firmicutes (90-95%), Proteobacteria (85-90%), Actinobacteria (80-85%) Bacteriodetes and

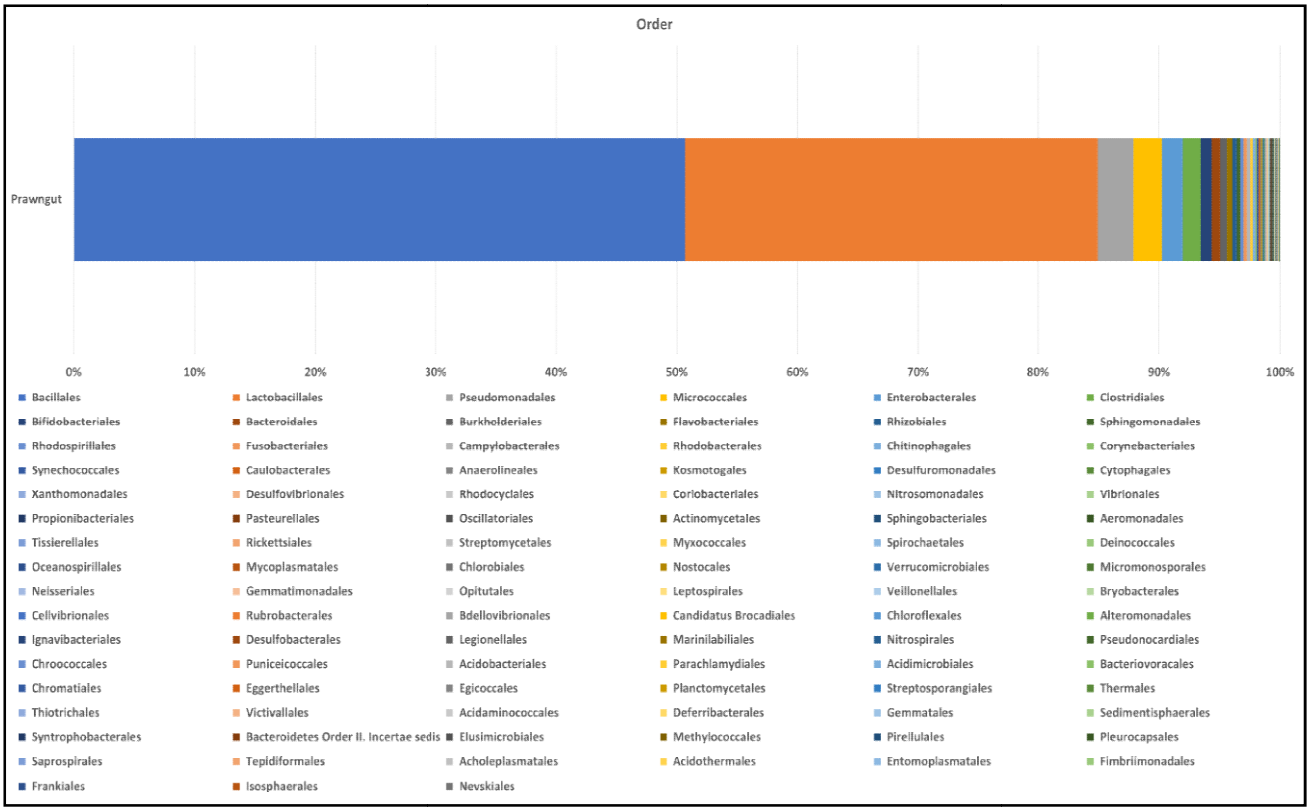




Fusobacteria (75-80%) were the most abundant phyla. At the class level, Bacilli (90-95%), was the most abundant class. Gammaproteobacteria (85-90%), was the second most abundant class. Actinobacteria (80-85%), Clostridia (75-80%)), Alphaproteobacteria and Betaproteobacteria were the top most classes identified by OUT clustering analysis. Bacillales (90-95%), Lactobacillales (85-90%), and Pseudomonales (80- 85%), Micrococcales, Enterobacterales, Clostridiales (75-80) were the top most abundant orders. When the OTUs were considered at the genus level, a high diversity of microbes was identified. A total of 230 genera were detected in all the samples.

The genus level accounting for the largest proportion was Staphylococcus (90-95%). The top10 dominant genera were Staphylococcus, Lactobacillus, Pseudomonas, Leuconostoc, Pediococcus, Bifidobacterium, Kocuria, Streptococcus, Prevotella, Bacillus, Flavobacterium.

Total of 230 genera were identified from shrimp gut OUT clustering analysis.162 family and 85 Order were identified.46 classes and 24 phyla were identified. Detailed illustration of the shrimp gut microbiota communities were explained in the following Figures.



DISCUSSION

Metagenomic analysis of the aquaculture systems will definitely pave way for elucidating the diversity of microorganisms present in the system and its potential role in the aquaculture system, including determination of metabolic processes performed by microbes; understanding the biogeochemical cycles of nutrients in the culture systems as well the development/outbreak of diseases. In conclusion, taxonomic profiles of microbiotas in the sediment of shrimp farming environments were investigated in this study employing metagenomics. The present study provides preliminary data with respect to the microbial community present in the gut of a semi-intensive shrimp culture system. Microbes are the most dominant group that harbors much in the sediments of shrimp ponds. The metagenomic analysis provides a better idea about the microbial communities present in an aquaculture system, especially the uncultivable ones. The present study emphasizes the application of metagenomics in exploring the microbial diversity of aquaculture systems, which might help detect pathogens within the system and helps to develop pathogen control strategies in the aquaculture systems.

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