

# **Biotechnology Journal International**

Volume 27, Issue 5, Page 71-81, 2023; Article no.BJI.105556 ISSN: 2456-7051 (Past name: British Biotechnology Journal, Past ISSN: 2231–2927, NLM ID: 101616695)

# The Metagenomic Characterization of Bacterial Community in Aquacultured Shrimp Penaeus monodon

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#### Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

#### Article Information

DOI: 10.9734/BJI/2023/v27i5698

#### **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <u>https://www.sdiarticle5.com/review-history/105556</u>

**Original Research Article** 

Received: 26/06/2023 Accepted: 02/09/2023 Published: 14/09/2023

# ABSTRACT

Aquaculture has grown rapidly during the last few decades due to research and developmental activities but we are still unaware of the various microbial species thriving within the aquaculture systems and their specific roles for knowing that Mmetagenomics study required environmental sample to discover the unexplored microbial community. The aim of this present study is to characterize the gut microbiota of giant tiger shrimp, Penaeus monodon collected gut sample and 16S rRNA gene-based high-throughput sequencing revealed distinct and diverse microbial communities. The results showed a high abundance of Betaproteobacteria, followed by Alphaproteobacteria, Clostridia, Actinobacteria, Gammaproteobacteria and Bacilli found in the gut sample 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. These findings provide valuable information on the microbial community and contribute to controlling the diseases in shrimp farms.

Keywords: Metagenomics; bacterial community; next generation sequencing; 16S rRNA.

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# **1. 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 the expansion and profitability of aquaculture is the lack of disease control [1-6]. 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, bioremediation, probiotics, and as a direct food source for the cultured species has expanded further in the last few decades [7-15]. 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 [16-23]. 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 high throughput sequencing was employed to reveal distinct and diverse microbial communities present in the sample.

# 2. MATERIALS AND METHODS

#### 2.1 Sample Collection and Processing

The present study was carried out in a semiintensive aquaculture system for Penaeus monodon production, located in southern coastal areas of Kerala, India. The aquaculture system operates under semi-intensive management. Approximately 25 harvested 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 guts were isolated with proper care. Gut DNA isolation was done at the CEPCI research centre, in Kollam, Kerala. The isolated gut was immediately transferred to ethanol. Gut DNA extraction was done as per the manufacturer's recommendation. Extracted DNA from the samples was subjected to NanoDrop and GEL Check before being taken for further steps. The NanoDrop readings of 260/280 at a ~ value of 1.8 to 2 are used to determine the DNA's quality.

#### 2.2 Next Generation Sequencing Analysis

Metagenomic nucleic acid extracted from the gut was subjected to 16S rRNA gene-based high throughput sequencing and analysis at Phytocom Pharmaceuticals (P) Ltd, Kalamassery, Kerala, PCR Amplification with V3 India. \_\/4 Primers.40ng of Extracted DNA is used for amplification along with 10 pM of each primer. The amplified 16s PCR Product is purified and subjected to GEL Check and Nanodrop QC. The amplicons from each sample were purified with Ampure beads to remove unused primers and an additional 8 cycles of PCR was performed using Illumina barcoded adapters to prepare the sequencing libraries. Sequencing was performed using Illumina Miseq with a 2x300PE v3 sequencing kit. Raw data identification is done using FASTQC and MULTIQC, followed by trimming of adapters and low-quality reads by TRIMGALORE. The databases used are SILVA / GREENGENES /NCBI. Each read is classified based on percentage coverage and identity. The kraken-build parameter was used to build 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 0.45. The trimmed raw data was used as the input for the Kraken 2 analysis.

# 3. RESULTS

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 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. 16s metagenomic analysis of the sample was performed in Phytocom Pharmaceuticals (P)LTD, Kalamassery, Kerala, India. Analysis of the showed а high abundance results of Betaproteobacteria in the metagenome retrieved from the gut sample followed by Alphaproteobacteria, Clostridia, Actinobacteria. Gammaproteobacteria and Bacilli in the metagenome retrieved from the gut sample. Fig. 1 shows the relative abundance of the most dominant bacterial groups.

Betaproteobacteria was found to be the most abundant phylum in the metagenome retrieved from the gut sample. Betaproteobacteria and Alphaproteobacteria come under the class Proteobacteria. Proteobacteria 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 Iwoffii which is also a proteobacterium. The next abundant phyla of the gut sample was 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 aroups 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 potential bioremediators of the culture systems and play an important role in the degradation of Species of organic matter. the genus Bacteroidetes have been reported to show high antibiotic resistance capacity and have been reported as major vitamin B12 producers in the intestines 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.



Fig. 1. Top 10 genus of Shrimp gut



Fig. 2. Quality control matrices of sequences

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Fig. 3. Analysed classes of Penaeus monodon

	Family												
Prawngut													
0	%	10%	20%	30%	40%	50%	60%	70% 8	0% 90%	6 100%			
Staphy	ococcaceae	Lactobacillaceae	Pseudomonadaceae	Leuconostocaceae	<ul> <li>Micrococcaceae</li> </ul>	Bifidobacteriaceae	Streptococcaceae	Enterobacteriaceae	Prevotellaceae	Bacillaceae			
Flavoba	acteriaceae	Sphingomonadaceae	Moraxellaceae	Campylobacteraceae	Acetobacteraceae	<ul> <li>Hungateiclostridiaceae</li> </ul>	Comamonadaceae	Rhodobacteraceae	<ul> <li>Chitinophagaceae</li> </ul>	Enterococcaceae			
■ Burkho	Ideriaceae	Clostridiaceae	Synechococcaceae	Peptostreptococcaceae	Caulobacteraceae	Lachnospiraceae	Anaerolineaceae	Kosmotogaceae	Corynebacteriaceae	Geobacteraceae			
Bradyri	nizobiaceae	Yersiniaceae	Bacteroidaceae	Xanthomonadaceae	Alcaligenaceae	Vibrionaceae	Zoogloeaceae	Methylobacteriaceae	Rhizobiaceae	Pasteurellaceae			
Methyl	aciliaceae	Actinomycetaceae	Sphingobacteriaceae	Aeromonadaceae	Microbacteriaceae     Spirochaetaceae	Peptoniphilaceae	Brevibacteriaceae	Halomonadaceae	Streptomycetaceae	Muconlasmataceae			
Rhodos	nirillaceae	Chlorobiaceae	Nocardiaceae	<ul> <li>Fusobacteriaceae</li> </ul>	Blattabacteriaceae	Micromonosnoraceae	Desulfomicrobiaceae	Polyangiaceae	Aerococcaceae	Germatimonadaceae			
Oxalob	acteraceae	Neisseriaceae	<ul> <li>Opitutaceae</li> </ul>	<ul> <li>Hyphomicrobiaceae</li> </ul>	<ul> <li>Methylophilaceae</li> </ul>	Verrucomicrobiaceae	<ul> <li>Erythrobacteraceae</li> </ul>	Cvtophagaceae	Leptospiraceae	Atopobiaceae			
Planoco	occaceae	Veillonellaceae	Peptococcaceae	Solibacteraceae	Syntrophomonadaceae	Akkermansiaceae	Dermabacteraceae	<ul> <li>Bdellovibrionaceae</li> </ul>	Candidatus Brocadiaceae	Phyllobacteriaceae			
Rhodoo	cyclaceae	Chloroflexaceae	Rickettsiaceae	Carnobacteriaceae	Dietziaceae	Erwiniaceae	Propionibacteriaceae	<ul> <li>Vicinamibacteraceae</li> </ul>	Anaeromyxobacteraceae	Cellvibrionaceae			
<ul> <li>Hafniad</li> </ul>	:eae	Baekduiaceae	Legionellaceae	Nitrospiraceae	Pseudonocardiaceae	Chroococcaceae	Cyclobacteriaceae	Halieaceae	Idiomarinaceae	<ul> <li>Mycobacteriaceae</li> </ul>			
■ Prolixit	acteraceae	Puniceicoccaceae	Acidobacteriaceae	Desulfobacteraceae	<ul> <li>Hymenobacteraceae</li> </ul>	<ul> <li>Ignavibacteriaceae</li> </ul>	<ul> <li>Myxococcaceae</li> </ul>	Rhodanobacteraceae	Tannerellaceae	Acidimicrobiaceae			
Eggerth	ellaceae	Egicoccaceae	Halobacteriovoraceae	Kofleriaceae	Piscirickettsiaceae	Planctomycetaceae	<ul> <li>Rikenellaceae</li> </ul>	Rubrobacteraceae	Thermaceae	Acidaminococcaceae			
Anaero	halosphaeraceae	Bartonellaceae	Brucellaceae	Christensenellaceae	Deferribacteraceae	Dermacoccaceae	Gemmataceae	Leptotrichiaceae	<ul> <li>Melioribacteraceae</li> </ul>	<ul> <li>Nitrosomonadaceae</li> </ul>			
Simkan	iaceae	Syntrophobacteraceae	Aurantimonadaceae	<ul> <li>Barnesiellaceae</li> </ul>	Cellulomonadaceae	Dermocarpellaceae	Desulfuromonadaceae	Dysgonamonadaceae	Elusimicrobiaceae	Haliscomenobacteraceae			
Methyl	ococcaceae	Nostocaceae	Parachlamydiaceae	rachlamydiaceae 📕 Rhodothermaceae		Streptosporangiaceae	Symbiobacteriaceae	Tepidiformaceae	Xanthobacteraceae	Acholeplasmataceae			
Acidoth	nermaceae	Alteromonadaceae	Chromatiaceae	Chromobacteriaceae	Coriobacteriaceae	Eubacteriaceae	Fimbriimonadaceae	Frankiaceae	Intrasporangiaceae	Isosphaeraceae			
Lacipire	Lacipirellulaceae Pirellulaceae		Porphyromonadaceae	Roseiflexaceae	<ul> <li>Spiroplasmataceae</li> </ul>	Steroidobacteraceae	Thioalkalispiraceae	Thiobacillaceae					

Fig. 4. Analysed families of Penaeus monodon

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![](_page_6_Figure_1.jpeg)

Fig. 5. Analysed phylum of Penaeus monodon

Genus												
Prawngut												
0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%		
<ul> <li>Staphylococcus</li> </ul>	Lactobacillus	■ Pseudomonas	<ul> <li>Leuconostoc</li> </ul>	Pediococcus	<ul> <li>Bifidobacterium</li> </ul>	<ul> <li>Kocuria</li> </ul>	Streptococcus	Prevotella	Bacillus	<ul> <li>Flavobacterium</li> </ul>		
Arcobacter	Acinetobacter	Acetobacter	Enterococcus	Clostridium	Ralstonia	Paracoccus	Sphingomonas	Synechococcus	Mesotoga	Corynebacterium		
Geobacter	Clostridioides	<ul> <li>Weissella</li> </ul>	<ul> <li>Hydrogenophaga</li> </ul>	Lactococcus	Bacteroides	Serratia	Parascardovia	Vibrio	<ul> <li>Rhizobium</li> </ul>	Trueperella		
Aeromonas	Brevibacterium	<ul> <li>Haemophilus</li> </ul>	Pelolinea	Moraxella	Stenotrophomonas	Methylocystis	Brevundimonas	Methylobacterium	<ul> <li>Wolbachia</li> </ul>	Desulfovibrio		
Arthrospira	Lysinibacillus	Deinococcus	Novosphingobium	Treponema	Sphingobium	Ureaplasma	Chlorobium	Fusobacterium	Oscillibacter	<ul> <li>Blattabacterium</li> </ul>		
Halomonas	Sphingobacterium	Curtobacterium	Finegoldia	Rhodococcus	Streptomyces	Brevefilum	Exiguobacterium	Paenibacillus	Anaerococcus	Faecalibacterium		
Desulfomicrobium	Chryseobacterium	Sorangium	Phenylobacterium	Polynucleobacter	Aerococcus	Gemmatimonas	Azospirillum	Cyanobium	Proteus	Neisseria		
Verrucomicrobium	Blautia	Massilia	Ruminococcus	Achromobacter	Leptospira	Providencia	Delftia	Candidatus Solibacter	Aeromicrobium	Akkermansia		
<ul> <li>Asticcacaulis</li> </ul>	Dermabacter	Rummeliibacillus	Alcaligenes	■ Bosea	Azospira	Bdellovibrio	Candidatus Kuenenia	Desulfallas	<ul> <li>Mesorhizobium</li> </ul>	Chloroflexus		
Psychrobacter	<ul> <li>Arachidicoccus</li> </ul>	Dietzia	Kribbella	Luteitalea	Pantoea	Paraburkholderia	Syntrophomonas	Anaeromyxobacter	<ul> <li>Cellvibrio</li> </ul>	Cutibacterium		
Dyadobacter	<ul> <li>Olsenella</li> </ul>	<ul> <li>Campylobacter</li> </ul>	Devosia	Dolosigranulum	<ul> <li>Megasphaera</li> </ul>	Paucibacter	<ul> <li>Veillonella</li> </ul>	Azoarcus	Baekduia	Dysosmobacter		
Lachnoclostridium	Legionella	Nitrospira	Rothia	Burkholderia	Capnocytophaga	Comamonas	Coraliomargarita	Ferrovibrio	Flavisolibacter	Geminocystis		
Glaesserella	Idiomarina	Rickettsia	Chryseolinea	Corallococcus	Ignavibacterium	Plantactinospora	Pontibacter	Tannerella	<ul> <li>Acidimicrobium</li> </ul>	Alistipes		
Anseongella	Brevibacillus	Cyclobacterium	Egicoccus	Haliangium	Halobacteriovorax	Hydrogenovibrio	<ul> <li>Micavibrio</li> </ul>	<ul> <li>Rubrobacter</li> </ul>	Sinomonas	Sphaerotilus		
Actinoplanes	Anaerohalosphaera	Arenimonas	Aurantimicrobium	Bartonella	Castellaniella	Christensenella	Citrobacter	Denitrovibrio	Desulfoglaeba	Gemmata		
<ul> <li>Hungateiclostridium</li> </ul>	Inhella	Jeotgalicoccus	<ul> <li>Kytococcus</li> </ul>	<ul> <li>Mannheimia</li> </ul>	<ul> <li>Melioribacter</li> </ul>	<ul> <li>Micromonospora</li> </ul>	Nocardia	Opitutus	Parvimonas	Phascolarctobacterium		
Simkania	Solitalea	Sphingopyxis	Streptobacillus	Sulfurospirillum	Actinomyces	Atlantibacter	Aureimonas	Barnesiella	Candidatus Protochlamydia	Carnobacterium		
Cellulomonas	Dehalogenimonas	Elusimicrobium	Haliscomenobacter	Komagataeibacter	Luteimonas	Lysobacter	<ul> <li>Methylomonas</li> </ul>	<ul> <li>Morganella</li> </ul>	<ul> <li>Mycobacterium</li> </ul>	Nostoc		
Pedobacter	Porphyrobacter	Proteiniphilum	Rhodoplanes	Saccharomonospora	Scytonema	Slackia	Solibacillus	Spirosoma	Stanieria	<ul> <li>Streptosporangium</li> </ul>		
Symbiobacterium	Tabrizicola	Tepidiforma	Acidothermus	Ahniella	Amycolatopsis	Anoxybacillus	<ul> <li>Blastochloris</li> </ul>	Bythopirellula	Candidatus Koribacter	Candidatus Phytoplasma		
Candidatus Symbiobacter	Chitinophaga	<ul> <li>Collinsella</li> </ul>	Desulfococcus	Desulfosarcina	<ul> <li>Eggerthella</li> </ul>	<ul> <li>Empedobacter</li> </ul>	Escherichia	<ul> <li>Fastidiosipila</li> </ul>	Fimbriimonas	Frankia		
Fuerstia	Gemella	Gordonibacter	<ul> <li>Halobacillus</li> </ul>	Herbaspirillum	Janthinobacterium	Klebsiella	Luteibacter	<ul> <li>Marinobacter</li> </ul>	Methylovorus	Minicystis		
Mycoplasma	Niabella	Oceanobacillus	Ottowia	Paludisphaera	Panacibacter	Pelobacter						

Fig. 6. Analysed genus of Penaeus monodon

							Ord	er									
Prawngut																	
	102	10%	20	<b>1</b> 92	30%	4	0%	50%		-n%	7	192	80%		90%	100	0/
<ul> <li>Bacillales</li> </ul>		Lactobacillales			Pseudomonadales		Micrococcales		070	Enterobacterales		0070	Clostridia	ales	100	/0	
<ul> <li>Bifidobacteriales</li> </ul>		Bacteroidales		<b>=</b> B	Burkholderiales		Flavobacteriales		Rhizobiales			Sphingomonadales					
Rhodospirillales		Fusobacteriales		≡ 0	ampylobacterale	es	Rhodobacterales			Chitinophagales			Corynebacteriales				
Synechococcales		Caulobacterales		Anaerolineales			Kosmotogales			Desulfuromonadales			Cytophagales				
Xanthomonadales		Desulfovibric	Desulfovibrionales		thodocyclales		Coriobacteriales			Nitro:	somonadales		Vibrional	es			
Propionibacteriales		Pasteurellales		■ 0	Oscillatoriales		Actinomycetales			Sphingobacteriales			Aeromonadales				
Tissierellales Rickettsia		Rickettsiales		Streptomycetales		Myxococcales			Spirochaetales			Deinococcales					
Oceanospirillales Mycoplasmatales		tales	Chlorobiales			Nostocales			<ul> <li>Verrucomicrobiales</li> </ul>			<ul> <li>Micromonosporales</li> </ul>					
Neisseriales Gemmatimonadales		nadales	Opitutales		1	Leptospirales		Veillonellales			Bryobact	erales					
Cellvibrionales Rubrobacterales		ales	Bdellovibrionales			Candidatus Brocadiales		Chloroflexales			Alteromo	nadales					
Ignavibacteriales		Desulfobacte	Desulfobacterales		Legionellales		Marinilabiliales		<ul> <li>Nitrospirales</li> </ul>			Pseudono	ocardiales				
Chroococcales		Puniceicoccales		= A	Acidobacteriales		Parachlamydiales		<ul> <li>Acidimicrobiales</li> </ul>			Bacteriov	/oracales				
Chromatiales		Eggerthellales		= E	Egicoccales		Planctomycetales		Streptosporangiales			Thermales					
Thiotrichales		Victivallales		= A	Acidaminococcales		Deferribacterales		Gemmatales			Sedimentisphaerales					
Syntrophobacterales Bacteroidetes Order II. Inc		s Order II. Incertae se	edis Elusimicrobiales			Methylococcales		Pirellulales			Pleurocapsales						
Saprospirales Tepidiformales		es	Acholeplasmatales		25	Acidothermales		Entomoplasmatales			Fimbriimonadales						
Frankiales Isosphaerales		s	■ N	levskiales													

Fig. 7. Analysed order of Penaeus monodon

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, and 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 topmost classes identified by OUT clustering analysis. Bacillales Lactobacillales (85-90%) (90-95%). and Micrococcales. Pseudomonales (80- 85%), 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 top dominant genera were Staphylococcus, Lactobacillus, Pseudomonas, Leuconostoc, Pediococcus, Bifidobacterium, Kocuria, Streptococcus, Prevotella, Bacillus, Flavobacterium. A total of 230 genera were identified from shrimp gut OUT clustering analysis.162 families and 85 Orders were identified.46 classes and 24 phyla were identified. Detailed illustrations of the shrimp gut microbiota communities are explained in the following figures.

# 4. DISCUSSION AND CONCLUSION

Metagenomic analysis of the aquaculture systems will pave the way for elucidating the diversity of microorganisms present in the system and their potential role in the aquaculture system, including the determination of metabolic processes performed by microbes: understanding the biogeochemical cycles of nutrients in the culture systems as well the development/outbreak of diseases [22-26]. 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 concerning the microbial community present in the gut of a semi-intensive shrimp culture system. Microbes are the most dominant group that harbours 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 help to develop pathogen control strategies in the aquaculture systems.

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#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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