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Comparative profiling of microbial community of three economically important fishes reared in sea cages under tropical offshore environment

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ABSTRACT

The present study was undertaken to evaluate the microbial composition of farmed cobia pompano and milkfish, reared in sea-cages by culture-independent methods. This study would serve as a basis for assessing the general health of fish, identifying the dominant bacterial species present in the gut for future probiotic work and in early detection of potential pathogens. High-throughput sequencing of V3-V4 hyper variable regions of 16S rDNA on Illumina MiSeq platform facilitated unravelling of composite bacterial population. Analysis of 1.3 million quality-filtered sequences revealed high microbial diversity. Characteristic marine fish gut microbes: *Vibrio* and *Photobacterium* spp. showed prevalence in cobia and pompano whereas *Pelomonas* and *Fusobacterium* spp. dominated the gut of milkfish. Pompano hindgut with 10,537 operational taxonomy units (OTUs) exhibited the highest alpha-diversity index followed by cobia (10,435) and milkfish (2799). Additionally unique and shared OTUs in each gut type were identified. Gammaproteobacteria dominated in cobia and pompano while Betaproteobacteria showed prevalence in milkfish. We obtained 96 shared OTUs among the three species though the numbers of reads were highly variable. These differences in microbiota of farmed fish reared in same environment were presumably due to differences in the gut morphology, physiological behavior and host specificity.

1. Introduction

Rising demand for fin fishes in the national and international market calls for promoting intensive sea-cage farming in India. Mariculture provides an option for supplementing marine capture fisheries and enhances employment opportunities (Ayyappan, 2014) Bestowed with a long coast line of 8129 km, an Exclusive Economic Zone (EEZ) of 2.02 million sq. km and a continental shelf area of 0.53 million sq. km, India possesses an ideal geographical resource for sea farming. Further based on Food and Agriculture Organization report (2014) India is positioned among the top 10 potent nations in case of mariculture activities (FAO, 2014). In spite of the availability of resources, mariculture in India is still in its early stages of development with few sea farming demonstrations taken up by Government Organizations since 2003 (Philipose et al., 2013a; Philipose et al., 2013b; Remani et al., 2004; Vijayakumaran et al., 2009). Among the hatchery bred species available for commercial farming, cobia (*Rachycentron canadum*), seabass (*Lates calcarifer*) and pompano (*Trachinotus blochii*) are the three important mariculture species for which the hatchery technology is standardized and commercial seed production is initiated (Gopakumar et al., 2011; Thirunavukkarasu et al., 2009; Nazar et al., 2014). Besides we also attempted to tap the wild resource of milkfish (*Chanos chanos*) seeds available in bounty in the coastal water of Gulf of Mannar for promoting capture based mariculture. Since the inception of sea farming in India, no comprehensive report exists on microbial diversity and potent pathogens associated with disease outbreak and health management during the culture practices of these farmed fishes.

The bacterial microbiota associated with aquatic organisms is diverse and profoundly influence their immunity, nutrient absorption and digestion (Nayak, 2010; Perez et al., 2010; Llewellyn et al., 2014). Because of the significant role of microbiota in the growth and health management, many investigations have been carried out to examine the microbial diversity in finfishes based on culture-dependent or Denaturing Gradient Gel Electrophoresis (DGGE)/Temperature Gradient Gel Electrophoresis (TGGE) combined 16S rDNA sequencing methods (Kim et al. 2007; Hovda et al. 2007). The scope of these methodologies are limited and biased with low output compared to the high throughput next-generation sequencing which has fetched several folds more sequences for a targeted DNA biomarker both in farmed (Li et al. 2015; Zarkasi et al. 2014; Wu et al. 2013; Wong et al. 2013) as well as in wild collected fishes (Givens et al. 2015; Larsen et al. 2014). Earlier analysis of microbiota associated with fish gut based on pyrosequencing

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had projected equivocal role of feed in determining the host microbiota but a consensus was deduced in terms of host specificity as the vital factor in shaping the gut microbiota (Desai et al. 2012; Roeselers et al. 2011; Wu et al. 2012; Kormas et al. 2014). Therefore, the objective of the current study was to determine the bacterial diversity in farmed fish for better health management under culture conditions. To accomplish this, sequencing of gut samples on Illumina MiSeq platform was undertaken to detect biodiversity with emphasis on development of probiotics. The bacterial species abundance obtained by next generation sequencing will also aid in designing specific probes for early detection of pathogens in symptomatic fishes.

2. Materials and methods

Cobia (1000 Nos.) and pompano (5000 Nos.) fingerlings weighing 15–20 g were procured from the Regional Center of Central Marine Fisheries Research Institute, Mandapam whereas milkfish fingerlings (20,000 Nos.) weighing 10–15 g were collected from wild. These fingerlings were stocked in 2 m dia × 2 m depth nursery cages deployed inside the 9 m dia × 7 m depth HDPE grow out cages moored off at Olaikuda (N 9°20′07.34″ E 79°19′47.91″), off Rameshwaram, Tamil Nadu, India (Fig. 1). The stocking density during the nursery phase was approximately 2.5 kg biomass per cubic metre. They were reared in these cages for one month and were fed with slow sinking pellet feed (4 mm size, 6% of their body weight), twice a day. After 40 days, the fishes (weighing on an average 30 g) were transferred to 9 m dia HDPE cages. During the grow out phase, cobia were fed with low priced sardines whereas milkfish and pompano were provided with 9–13 mm commercial pellet comprising 40% crude protein 15% crude fat, 9% ash, 2.4% crude fibre (Rudra feed, India). The feeding strategy, stocking density and digestive physiology of the three candidate species are provided in Table 1.

Fishes were randomly selected and caught before feeding using a fish net and immediately lifted on board a boat for further examination.

2.1. Sample preparation for culture independent microbial analysis

After eight months of culture, five individuals of each fish species (cobia, milkfish and pompano) were collected and kept alive in aerated FRP tanks holding sea water until processing (approximately 3 h) for microbiota analysis. Average size of individuals sampled were, 804 ± 34.0, 480 ± 28.3, 310 ± 11.0 mm for cobia, milkfish and pompano respectively. Prior to gut sampling, fishes were euthanized with 300 mg/l tricaine methanesulfonate. The lower third of the intestine was aseptically removed from each individual and the gut content was squeezed out from the intestine. The respective gut samples were pooled for all 5 individuals of the same fish species and placed in RNA*later* overnight at 4 °C and then stored at −80 °C until further use. Prior to homogenization, samples stored in RNA*later* were thawed and centrifuged at 5000*g* for 10 min. Supernatant was discarded. The samples were washed with sterile phosphate buffer solution three times to remove excess salts present in RNA*later*. Intestinal samples were homogenized using liquid nitrogen. DNA was extracted from 25 mg of intestinal tissue using DNeasy blood and tissue kit (Qiagen) following manufacturer's instructions including pretreatment with lysozyme for lysis of Gram positive bacteria. Integrity and purity of the DNA were checked using Qubit and Agilent 2100 Bioanalyzer. Prior to 16S amplicon sequencing, PCR was performed, using 27F and 1492R universal primers. The samples were then amplified for the V3-V4 hyper variable regions. The samples that gave positive amplification were subjected to next generation sequencing on Illumina MiSeq platform (Genotypic technologies Pvt. Ltd., Bengaluru, India.)

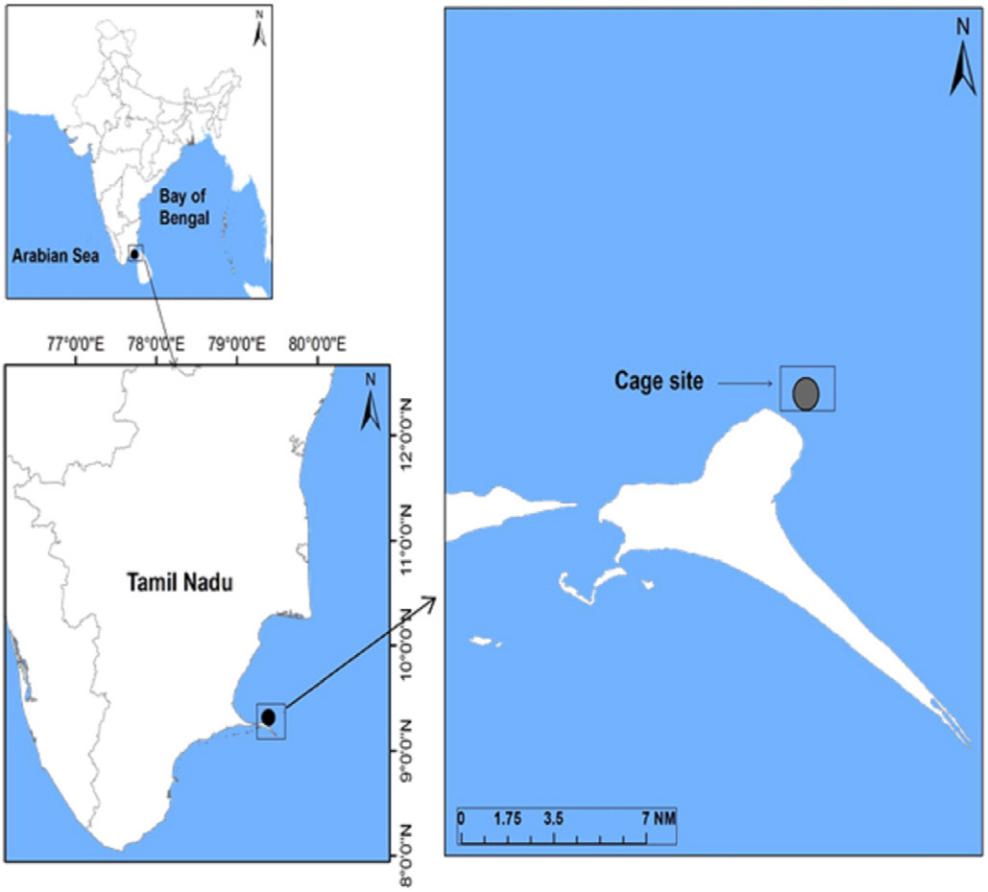

Fig. 1. Map showing the cage culture site at Olaikuda, Rameshwaram Island, Southeast coast of India.