

Identification of host-specific skin-mucus and gut microbiota in snakehead murrel (*Channa striata*) (Bloch, 1793) using metagenomics approach

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ABSTRACT

The host-associated microbiota plays a key role in various biological processes of the host, including immunity, nutrition, and growth. In the present study, we profiled microbiota from the skin-mucus and gut of *Channa striata* (murrel) during feeding and fasting conditions by utilizing the 16S ribosomal RNA gene sequencing approach. Comparison of the microbial profiles showed that the feeding and fasting rhythm can alter the bacterial diversity of skin-mucus and gut-associated bacterial communities. In brief, the skin-mucus and gut microbial communities of murrel consisted of 22 bacterial phyla and were mainly dominated by *Actinobacteria* (66.07–87.78%) and *Proteobacteria* (11.62–31.93%) under both feeding and fasting conditions. *Arthrobacter* and *Rhodococcus*, known for improving fish performance in terms of growth, immunity, and nutrition, were more abundant in both gut and skin-mucus. In addition, *Vogesella*, *Brevundimonas*, *Sphingomonas*, and *Methylobacterium* were present in the skin-mucus and gut samples. PICRUSt analysis revealed several metabolic pathways that were abundant in the fish-fed groups, such as carbohydrate metabolism, amino acid metabolism, membrane transport, energy metabolism, metabolism of cofactors and vitamins, and lipid metabolism. Feeding patterns can alter the diversity of the bacterial community, and the altered microbiota may have a differential role in the metabolic functions of fed and starved fish. This study provides baseline data on the microbial community associated with murrel and their possible contribution to host metabolism. However, these findings need to be further explored at the functional level to determine how dietary shifts regulate gut homeostasis and health.

1. INTRODUCTION

The snakehead murrel *Channa striata* (Bloch, 1793) is a candidate food fish in South Asian countries due to its nutritional and medicinal importance [1]. It has high-quality flesh and enriched taste along with high levels of polyunsaturated fatty acids [2,3]. Evidence suggests that post-operative medicinal application tends to improve wound healing and reduce postoperative pain and discomfort [4,5]. This has also been reported in the treatment of tuberculosis [6], the application of antiulcer [7], the application of antidepressants [8], the treatment of asthma [9], and fertility [10]. Culturing murrel has gained substantial

popularity in India in the last decade due to its wide range of natural habitats, ranging from rivers, swamps, ponds, canals, and lakes to land containing rice fields. Murrels are carnivorous fish species and bottom dwellers and can survive harsh environmental conditions, such as lower oxygen levels and higher amounts of ammonia (NH₃). This air-breathing fish species has typical characteristics, and the epithelial layer of skin is covered with a substantial mucosal layer compared to other fishes, which acts as a physical barrier against infection [11].

Mucosal surfaces of vertebrates, including fishes, are known to colonize a wide range of complex microbial communities and are involved in various biological processes in the host, including the immune system. The gut-associated microbiota, in particular, plays a key role in the immunological and metabolic development of the host [12,13]. Several factors can influence the structure and composition of the host-associated microbiota, such as the

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environment (rearing condition), feeding (nutritional content), and biological conditions [14-16]. Most of the research performed in fish microbiota is focused mainly on gut microbes. Therefore, a change in diet or feeding habits can be considered an important factor that alters host-associated microbiota, which influences the nutritional regulation of host energy balance [17-19]. The feeding and fasting cycle alters the metabolism of the host and microbial load or composition [20]. These studies depict the complex nature of the gut microbial community and imply its dynamics, largely modulated by dietary ingredients and feeding regimes [21-24].

Despite the increasing knowledge of relationships between diet and the gut microbiota, very little is known about microbiota associated with other mucosal surfaces, such as skin. Nevertheless, few studies have been performed to understand the complexity of the skin-mucus-linked microbiota of fish, for example, Atlantic salmon and eel. [25-29] and revealed a shift in their composition due to stress, feeding, and other environmental factors [30,31].

Murrels are emerging as an excellent model due to their carnivorous feeding habits and unique characteristics such as their high level of skin mucosa, which makes them unusual relative to other fish species. Therefore, characterization and understanding of the role of the microbial community-associated skin and gut will serve as baseline information for framing successful farming practices for murrels, including the development of probiotics. At present, advances in next-generation sequencing (NGS) technologies and high-performance computing have brought about paradigm progress in the field of metagenomics, allowing the study of unexplored complex microbiota. In this study, we used the 16S rRNA gene sequencing method to discover and characterize the bacterial composition of the skin-mucus and gut of murrels during feeding and starvation conditions. Furthermore, we performed predictive metabolic pathway analysis (PICRUSt) of the bacterial communities associated with murrels. This metagenomic study will help in the investigation of microbes from the fish skin-mucus and gut linked with metabolism and health/immunity and the detection of novel microbes to aid in the development of probiotics and antibiotics in aquaculture.

2. MATERIALS AND METHODS

2.1. Experiment and DNA Extraction

A total of 100 healthy murrels (5 ± 0.5 g) were collected from the air-breathing catfish rearing facility of the ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, Odisha, India. The collected fish were acclimatized for 2 days in 200 L glass tanks under laboratory conditions at 28.5°C. All fishes were fed twice a day with commercial feed at a rate of 3% of their body weight. The feed was composed of protein (>25%), fat (>2%), carbohydrates (<10%), fiber (<3%), calcium (>0.5%), and phosphorus (>0.5%). After 48 h of acclimatization, these fish were randomly divided into two treatment groups (feeding and fasting) and placed in two 20 L glass tubes with a density of ten fish per tank. The first group of fishes was starved for 48 h, and the other group of fishes designated the control was fed twice a day with commercial feed. After completion of the experiment, fish were collected and immediately transported to the Fish Pathology Laboratory of ICAR-CIFA. The collected fish were anesthetized with 150 mg/L MS222 (tricaine methane sulfonate, Sigma Aldrich) and rinsed with sterile water to prevent cross-contamination. Skin-mucus was collected from three fishes randomly chosen from each treatment group by scraping off the dorsolateral surface of the fish using a sterile scalpel. The mucus samples obtained from fish in each treatment

group were pooled separately and collected in 1.5 ml microcentrifuge tubes. Gut samples from the same fish were collected by aseptically removing the whole intestine from the abdominal cavity and squeezing into a sterile tube. Gut contents from three individuals were pooled together to form a single biological sample. The samples were pooled to eliminate the interindividual variations among fishes receiving the same treatment. All samples were stored at -80°C until further processing.

Genomic DNA from skin-mucus and gut samples was extracted using the QIAamp DNA Mini Kit (Qiagen/Gmbh, Hilden, Germany) according to the manufacturer's protocol. The mucus samples were centrifuged for 5 min before DNA extraction. The quality and quantity of the isolated DNA were assessed using a UV spectrophotometer (Nano Drop™, Thermo Fisher Scientific, Waltham, MA, USA) and stored at -20°C until further analysis.

2.2. Illumina NGS Library Preparation, Sequencing, and Computational Analysis

The V3 to V4 regions of the 16S rRNA gene were amplified by polymerase chain reaction (PCR) using the universal bacterial primer pair (5'-CCTACGGGNGGCWGCAG-3' and 5'-GACTACHVGGGTATCTAATCC-3') following the 16S Metagenomic Sequencing Library Preparation for Illumina MiSeq System (Illumina, San Diego, CA, United States). The V3-V4 primer pairs of 16S rRNA regions are mostly used for bacterial microbiome sequencing using NGS [32]. The 16S rRNA amplicons/fragments were generated using the Platinum® Taq DNA Polymerase High Fidelity Kit (Thermo Fisher Scientific, Italy). The procedure for 16S rRNA gene library preparation and sequencing was adopted from a previous report [33]. In this study, a Nextera XT Index Kit (Illumina, San Diego, CA) was used for fragment ligation with paired-end adapters; subsequently, the quality of libraries was checked using a KAPA library quantification kit as per the procedure. The libraries were then multiplexed, and sequencing was carried out using Illumina MiSeq (San Diego, CA, USA) by paired-end 2×250 bp sequencing chemistry.

The raw reads generated from sequencing were assessed and quality filtered using the following criteria: (i) barcodes/adapters and primers were removed from the sequences; (ii) sequences with an overlapping region below 10 bp were discarded; and (iii) sequences that contained an "N" base >300 bp were trimmed. To reconstruct the original contigs, overlapping paired-end reads were aligned using FLASH v1.2.11 software (<http://sourceforge.net/projects/flashpage>). The resulting reads were subjected to Fast QC for quality checking ($Q>30$), and the adaptor was trimmed using Trimmomatic v0.33. Furthermore, the trimmed paired-end quality read sequences were processed using QIIME v1.9.1 (Quantitative insights into microbial ecology) with default parameters [34]. The filtered reads were clustered using the UCLUST method [35]. Then, the sequence data were assigned to the operational taxonomic units (OTUs) with 97% sequence similarity against the curated chimera-free 16S rRNA database (Greengenes v13.8) through the Ribosomal Database Project (RDP) classifier, and an abundance of OTUs <0.001% of the total sequences was discarded. The taxonomic information of the representative sequence in each OTU was obtained by matching the sequence database using BLAST of QIIME. The bar graph for the samples was generated using QIIME. Alpha diversity metrics were calculated based on a rarefied OTU table (rarefied at the lowest sample size) using "Observed species," "Chao1 index" (species diversity estimator), "Shannon," and "Simpson"

diversity indices. The alpha and beta diversity plots were generated using the phyloseq and microbiomseq packages with ANOVA in R (v4.0.2). A bubble plot was generated using ggplot2 and the reshape2 package in R (v4.0.2). The variation in species composition between/among bacterial communities was measured using an unweighted (presence/absence) UniFrac distance matrix [36,37]. The distance matrices were visualized by principal coordinate analysis (PCoA). A Venn diagram was utilized to recognize and visualize the common core microbiome (<http://bioinformatics.psb.ugent.be/webtools/Venn/>), and statistical analysis was performed using Paleontological Statistics Software Package for Education (PAST) software v3.0 [38]. Differences were considered significant at $P < 0.05$.

To identify the metabolic role/functions of bacterial communities, the PICRUSt algorithm was used with 97% similarity OTUs clustered for each sample [39]. All OTUs were normalized by copy number, and new matrices of predicted functional categories were created using the Cluster of Ortholog Genes (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. The results were summarized at different levels using QIIME. The predicted functional table for PICRUSt analysis is available on the Open Science Framework platform. The heat maps of the hierarchy cluster and metastatic analysis were constructed based on the abundances of each hierarchy.

3. RESULTS

Illumina sequencing generated a total of 1,098,230 raw reads from the gut and skin-mucus samples of murrel. Out of this, 270,003, 294,797, 237,159, and 296,271 total paired-end reads were generated for the four different samples, that is, gut (starved), gut (fed), skin-mucus (starved), and skin-mucus (fed). After quality filtering, we obtained 576,176 high-quality reads that were clustered into 395, 467, 954, and 858 OTUs from each sample type [Table 1]. Due to differences in sequencing depth, the samples were rarefied, and alpha diversity, which represents microbial diversity within samples, was assessed for all skin-mucus and gut samples of murrel. We calculated the diversity using different matrices, such as Shannon, Simpson, Chao1, and observed species [Tables 2 and 3]. The H' value of alpha diversity and the Shannon or Simpson index for skin-mucus of starved fishes were 3.82 and 0.76, respectively, which were significantly higher than those of the other groups ($P < 0.05$). The alpha diversity analysis revealed that the skin-mucus microbiome during fasting has the highest diversity and even distribution of microbes compared to all other groups. Compared to this, the gut microbiome during fasting showed more diversity but less evenness [Figure 1]. A Krona chart that

allows exploration of relative abundances and confidences within the complex hierarchies of metagenome classifications was depicted using quantitative phylogenetic information of skin-mucus and gut samples of starved and fed fishes, respectively [Figure 2]. *Actinobacteria* and *Proteobacteria* were the most abundant phyla among the detected microbes. The taxonomic diversity reveals the contribution of each sample to the overall beta diversity represented by local contribution to beta diversity (LCBD). The starved skin-mucus showed a higher LCBD value than all other samples [Figure 3]. The relative abundances of the complex hierarchies in skin-mucus and gut metagenome classifications of starved and fed fishes were depicted using bubble plots. [Figure 4]. The relative abundances of *Arthobacter* and *Rhodococcus* were highest in all samples, with *Vogesella*, *Micrococcus* and *Acinetobacter* being more abundant in skin-mucus under starved conditions than in the rest of the samples. The metagenome data were deposited in the GenBank database of NCBI as a Short Read Archive (Accession numbers: SRR10662778; SRR10662777; SRR10662776; SRR10662775). The skin-mucus microbiome at the phylum level was more abundant in fed fishes than in starved fishes. Alterations in the abundance and diversity of microbes were also observed at different levels of taxa and OTUs in the skin-mucus and gut of murrel. The Shannon diversity index was calculated for each treated and control fish based on the 95% most abundant taxa in these samples.

3.1. Gut Microbial Communities of Murrel

The bacterial diversity linked with the murrel gut consisted of a total of 14 and 12 different bacterial phyla in starved and fed fishes, respectively [Figure 5]. *Actinobacteria* and *Proteobacteria* represented the most abundant phyla, which together accounted for 99% of the total population in the gut of both samples. *Actinobacteria* was the most abundant phylum in the gut, with a slightly higher abundance (87.78%) in the gut fed than in the gut starved (86.15%). *Proteobacteria* was found to be the second-highest dominant phylum, representing 11.62% and 13.11% in the gut of fed and starved fishes, respectively. Overall, 12 phyla were present in gut samples of both starved and fed fishes. Other phyla, such as *Firmicutes* (0.30%), *Verrucomicrobia* (0.12%), *Planctomycetes* (0.0908%), *Cyanobacteria* (0.0281%), *Bacteroidetes* (0.0275%), and *Fusobacteria* (0.0013%), were identified in the gut of fed fishes. In starved fishes, the abundances of these phyla were represented as *Firmicutes* (0.26%), *Planctomycetes* (0.13%), *Verrucomicrobia* (0.13%), *Fusobacteria* (0.1038%), *Cyanobacteria* (0.0458%), and *Bacteroidetes* (0.0365%). Interestingly, this study identified the presence of two phyla, TM7 (0.0021%) and *Chlamydiae*

Table 1: Metagenomics read statistics showing total number of paired end and processed reads along with the number of OTUs in each taxonomic rank in the skin-mucus and gut microbiome of starved and fed fish.

Category	Skin-mucus (Starved)	Skin-mucus (Fed)	Gut (Starved)	Gut (Fed)
Total paired end reads	237159	296271	270003	294797
Processed reads	232637	290887	264995	289224
Total identified rRNA sequences	119887	160201	139681	156407
Total OTUs picked	954	858	395	467
Phylum	16	20	14	12
Class	34	40	25	25
Family	118	131	93	98
Order	48	46	54	67
Genus	255	225	142	165
Species	319	267	160	199

Table 2: Alpha diversity index across various matrices. The alpha diversities in the skin-mucus of starved fishes were highest for all the matrices.

Samples	Shannon	Simpson	Chao1	Observed species
Skin-mucus (Starved)	3.8253329	0.76399317	1359.06410	954.0
Skin-mucus (Fed)	2.2693090	0.52400006	1261.277027	858.0
Gut (Starved)	2.16310180	0.555559836	512.424242	395.0
Gut (Fed)	1.99158492	0.498923358	649.397058	467.0

Table 3: Functional diversity index calculating using QIIME: The functional diversity was classified based on KEGG and COG classification system and the indices were calculated for Shannon, Simpson, and Chao1.

Samples	Shannon	Simpson	Chao1
KEGG			
Skin-mucus (Starved)	10.9228537996	0.9992140606	5812.38709677
Skin-mucus (Fed)	10.6795682317	0.9990719864	5753.72727273
Gut (Starved)	10.6957903364	0.9990861413	5569.36842105
Gut (Fed)	10.6523593585	0.9990617187	5502.95854922
COG			
Skin-mucus (Starved)	10.5522169053	0.9985284645	4360.15
Skin-mucus (Fed)	10.3474153491	0.9983079074	4336.5
Gut (Starved)	10.3598447277	0.9983065096	4274.875
Gut (Fed)	10.3336271975	0.9983042549	4265.58823529

KEGG: Encyclopedia of Genes and Genomes, COG: Cluster of Ortholog Genes

(0.0007%), found exclusively in starved fishes. The taxonomic distribution at the order level showed the composition of different groups of *Actinobacteria*, such as *Actinomycetales*, *Acidimicrobiales*, *Solirubrobacterales*, and *Gaiellales*, which were found to be abundant in both fishes. In the *Proteobacteria* phylum, out of 22 orders, *Pseudomonadales* was highly abundant.

At the genus level, the taxonomic composition of microbes detected a total of 142 and 165 genera in the gut of both starved and fed fish. We detected approximately 64–91 unclassified genera in both samples. *Arthrobacter* (64.97–69.54%) was the most abundant genus, followed by *Rhodococcus* (13.12–16.82%), *Sphingomonas* (1.67–2.62%), *Brevundimonas* (2.50–2.55%), and *Methylobacterium* (1.17–1.46%), in both gut samples. Interestingly, the gut of starved fish was enriched with a higher percentage of *Rhodococcus* than that of fed fish. In contrast, we detected a high level of *Arthrobacter* in the gut samples of fish fed to the counterpart of starved fish. At the family level, *Micrococcaceae* (68.19–74.26%) was most diverse in the gut, followed by *Nocardiaceae* (13.12–16.82%), *Moraxellaceae* (3.94–3.99%), *Caulobacteraceae* (2.55–2.6%), *Sphingomionadaceae* (1.71–2.69%), etc. In the *Arthrobacter* genera, various species, such as *Arthrobacter nitroguajacolicus*, *Arthrobacter oxydans*, *Arthrobacter psychrolactophilus*, *Arthrobacter woluwensis*, and *Arthrobacter nicotinovorans*, were detected, among which *A. nitroguajacolicus* was the most abundant species in both samples (69.40% and 64.35% in gut samples of fed and starved fish, respectively). Among the *Rhodococcus* genera, species such as *Rhodococcus fascians* and *Rhodococcus rubur* were reported, and *R. fascians* was most abundant, at 16.45% and 12.87% in gut samples of both starved and fed fish, respectively.

3.2. Skin-mucus Microbiome of Murrel

A total of 954 and 858 OTUs of bacterial communities associated with skin-mucus at taxonomic resolution were identified in starved and fed fishes, respectively. As per the analyzed dataset, 16 and 20 phyla were present in the skin-mucus of both starved and fed fish, respectively. *Actinobacteria* was predominant, with a relative abundance of 66.07% and 86.48%, followed by other phyla, such as *Proteobacteria* (31.93% and 11.99%), *Firmicutes* (0.92% and 0.93%), *Fusobacteria* (0.32% and 0.001%), *Bacteroidetes* (0.22% and 0.18%), *Thermi* (0.26% and 1.35%), and *Verrucomicrobia* (0.092% and 0.094%), enriched in the skin-mucus of starved and fed fish, respectively [Figure 5]. At the order level, a total of 54 (skin-mucus starved) and 67 (skin-mucus fed) OTUs were found, and 4–6 unclassified orders were detected. The *Actinomycetales* order (*Actinobacteria* phyla) was abundant (66.08%, 86.45%) in the skin-mucus of starved and fed fish, followed by *Pseudomonadales* from *Proteobacteria* (8.88%, 3.02%), *Neisseriales* (8.65%, 0.88%), *Burkholderiales* (3.29%, 0.45%), *Caulobacterales* (3.24%, 2.62%), *Sphingomonadales* (2.95%, 1.71%), and *Bacillales* from *Firmicutes* (0.68%, 0.64%).

The taxonomic resolution at the genus level detected 255 and 224 genera in skin-mucus samples of starved and fed fish. A total of 124 unclassified genera were recognized in both murrel skin-mucus samples. *Arthrobacter* (order; *Actinomycetales*) was predominant, with relative abundances of 46.33% and 67.62% in the skin-mucus of starved and fed fish, respectively. *Rhodococcus* (order: *Actinomycetales*) showed the highest abundance (13.75%) in the skin-mucus of fed fish and lower abundance (10.73%) in the skin-mucus of starved fish. *Brevundimonas* (2.52% and 1.87%) and *Methylobacterium* (0.92% and 0.80%) were found in the skin-mucus of both fishes but showed the highest abundance in the skin-mucus of fed fish. Bacterial genera such as *Micrococcus* (4.18% and 0.04), *Acinetobacter* (3.99% and 0.24%), *Enhydrobacter* (1.98% and 0.29%), *Sphingomonas* (1.75% and 1.61%), and *Pseudomonas* (1.18% and 0.27%) showed varying levels of abundance in the skin-mucus of starved and fed fishes. In the *Arthrobacter* genera, various species, such as *A. nitroguajacolicus*, *A. oxydans*, *A. psychrolactophilus*, *A. woluwensis*, and *A. nicotinovorans*, were detected, and among them, *A. nitroguajacolicus* was the most abundant species in both samples (69.40% and 64.35% in the skin-mucus). *A. nitroguajacolicus* was identified to be most abundant, consisting of 46.22% and 67.49%, followed by *R. fascians* (genus; *Rhodococcus*), attaining 10.49% and 13.54% in the skin-mucus of starved and fed fishes, respectively.

3.3. Comparative Studies of the Microbiome in the Skin-Mucus and Gut of Murrel

A total of 11 phyla were commonly present among all samples, namely, *Actinobacteria* (87.78–66.07%), *Proteobacteria* (31.93–11.62%), *Firmicutes* (0.93–0.26%), *Planctomycetes* (0.13–0.05%), *Verrucomicrobia* (0.13–0.092%), *Fusobacteria* (0.32–0.001%), *Cyanobacteria* (0.05–0.02%), *Bacteroidetes* (0.22–0.02%), *Chloroflexi* (0.01–0.0006%), and *Acidobacteria* (0.0083–0.0025%) [Figure 5]. A higher percentage of *Firmicutes* and *Cyanobacteria* was identified in the skin-mucus of fed fishes. Similarly, the highest percentages of *Proteobacteria*, *Fusobacteria*, and *Bacteroidetes* were observed in the skin-mucus of starved fishes. At the genus level, 142–255 different bacterial genera were identified in all samples. A total of 184 genera were reported in the starved fishes, and 142 different genera were found in the skin-mucus of fed fishes. *Arthrobacter*, which was observed at the highest percentage in the gut sample of fed fishes, was, however, found to have the lowest percentage in

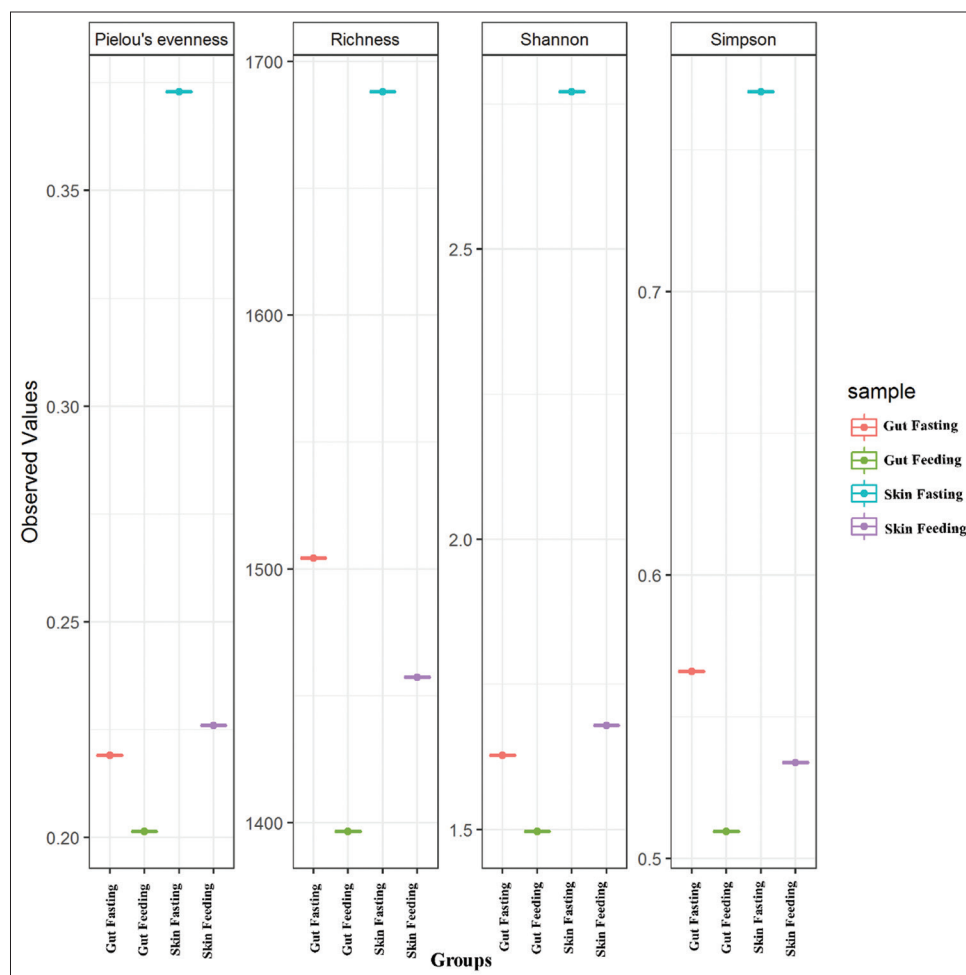


Figure 1: Alpha diversity indices (Pielou's evenness, Richness, Shannon and Simpson) for 4 groups of samples (gut fasting, gut feeding, skin-mucus fasting, and skin-mucus feeding).

the skin-mucus of starved fishes. Unclassified bacteria in the four samples accounted for 13.23%, 9.26%, 10.37%, and 10.40% of the total reads for the skin-mucus and gut of starved and fed fishes, respectively. *Vogesella* was abundant in the skin-mucus of starved fish (8.61%) compared to the skin-mucus of fed fish (0.8839), gut of starved fish (0), and gut of fed fish (0.0019). The abundances of *Arthrobacter*, *Rhodococcus*, *Brevundimonas*, and *Sphingomonas* increased in fed fish compared to starved fish. The principal coordinate analysis plot in [Figure 6](#) shows principal coordinates PC1 versus PC2. In these analyses, a large shift in microbiota was observed in the skin-mucus of fishes after starvation. Skin-mucus (starved) had the highest OTU and clustered uniquely among the datasets in the PCoA plot. The gut-fed and gut-starved samples did not show a large shift in microbiota. A Venn diagram depicted the distribution of the common bacterial populations as well as the unique microbiota among the skin-mucus and gut of starved and fed fishes [[Figure 7](#)].

3.4. Functional Classification

All 16S rRNA sequences were taken for the functional analysis study and predicted by using PICRUSt tools. The pathway was categorized up to three functional levels, that is, level 1, level 2, and level 3. The metabolism was reported as the most abundant function at level 1, for which skin-mucus and gut (in feeding condition) showed similar highest values, that is, 28.08% and 27.63%, respectively, compared to skin-mucus and gut (in starved condition), that is, 18.62% and 25.65%.

The most differentially abundant KEGG pathway in level 2 was carbohydrate metabolism, amino acid metabolism, membrane transport, energy metabolism, lipid metabolism, and cofactors and vitamins; however, all these pathways were significantly higher under feeding conditions than under starvation conditions [[Supplementary Figure S1](#)]. Furthermore, 329 KEGG pathways were identified at level 3, out of which 12 pathways detected were significantly different between groups through metastatic analysis.

A total of 62.95% of murrel metagenomic sequences were observed to cluster to the COG functional groups through COG cluster analysis. At level 1, metabolism was the abundant COG detected in skin-mucus starved, skin-mucus fed, gut starved, and gut fed fishes at 44.26%, 45.82%, 45.91%, and 45.88%, respectively, followed by information storage and cellular processing and signaling. The top differentially abundant pathway (level 2) functions are depicted [[Supplementary Figure S2](#)], and the COG abundance of the skin-mucus of fed murrel was higher than that of the skin-mucus of starved fish. A heatmap was built to characterize and identify the KEGG categories (level 2) in different tissues [[Supplementary Figure S3](#)]. The Spearman correlation between selected pathways is shown with *P*-value; red indicates the highest *P*-value, and blue represents the lowest *P*-value. Larger (absolute) values were found for lipid metabolism, carbohydrate metabolism, amino acid metabolism, environmental metabolism, and metabolism of vitamins and cofactors.

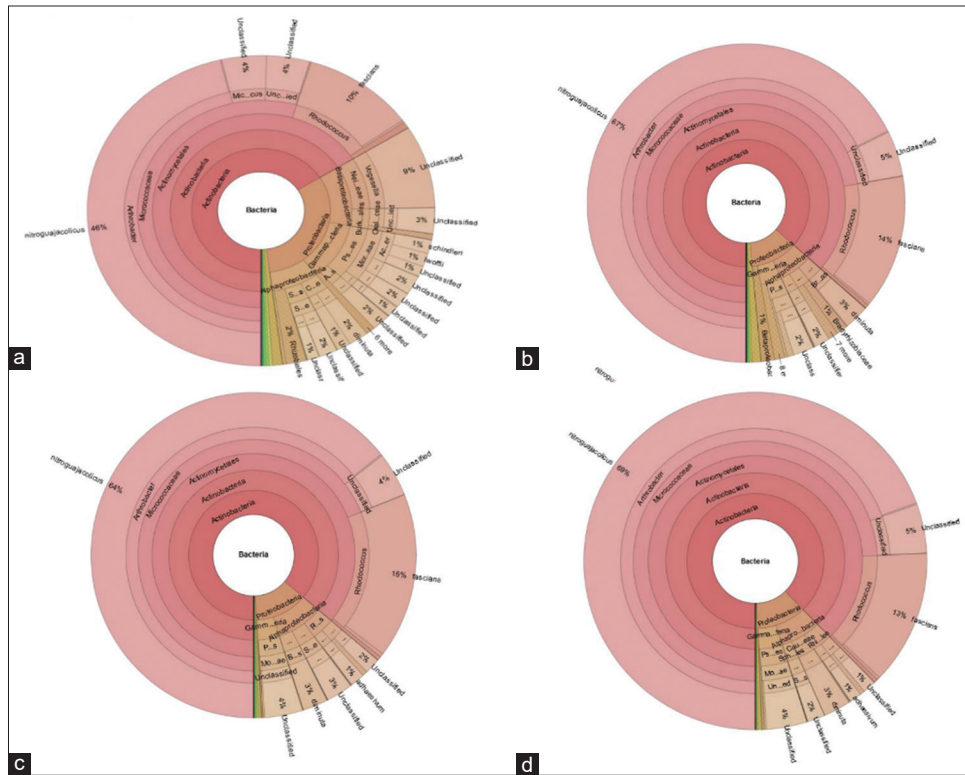


Figure 2: Krona chart for the taxonomic classification of the bacterial metagenome from *Channa striata* (a) skin-mucus (starved), (b) Skin-mucus (fed), (c) Gut (starved), and (d) Gut (fed). The inner circle represents higher taxonomic ranks, while more detailed taxonomic ranks (up to genus level) are presented in the outer circles.

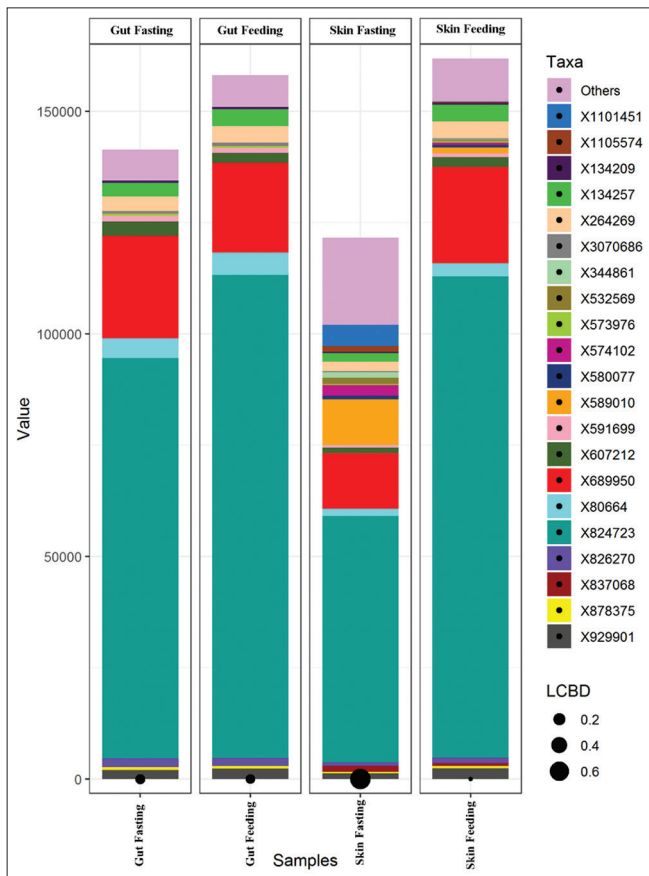


Figure 3: Taxonomic diversity of gut and skin-mucus microbiome in feeding and fasting conditions.

4. DISCUSSION

The bacterial composition plays an important role in nutrition, immunity, and the development of vertebrates, including fishes [40]. Various studies have depicted an association of the bacterial community with host health [41-43]. NGS technologies have been utilized to identify and decipher the functional role of microbiota in several fish species [44-47]. Earlier studies have reported that *Proteobacteria*, *Fusobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Verrucomicrobia* are the predominant phyla in the gut of fish species. The microbial composition of the gut has experienced increased attention due to its crucial role in fish health and nutrition [48]. In this study, OTUs of the other groups were clustered based on the threshold level (approx. 97%), which showed the wealth of microbial content [49]. An earlier report suggested that *Proteobacteria* and *Firmicutes* are dominant and ubiquitous phyla in fishes, and a few reports have indicated that *Fusobacteria* and *Proteobacteria* are abundant phyla in freshwater fishes [45,50,51].

In contrast, our study indicated that *Actinobacteria* and *Proteobacteria* represented the predominant phyla, accounting for 95–99% of the total microbial composition in the gut and skin-mucus of murrel. The feeding regime mainly plays a key role in establishing bacterial diversity in the gut [23,52,53]. In this study, we revealed a smaller difference in the microbial diversity in both samples of starved and fed fish. The gut microbiota of rainbow trout (*Oncorhynchus mykiss*) increased in diversity (dominant phyla: *Firmicutes* and *Proteobacteria*) after the onset of first feeding [54,55]. These studies indicated that feeding habit or regime affects the gut microbiome in fishes [56-59]. Recently, Ramirez and Romero reported that bacterial communities were altered in gut samples of farmed and wild yellowtail kingfish (*Seriola lalandi*) due to feeding habits [60].

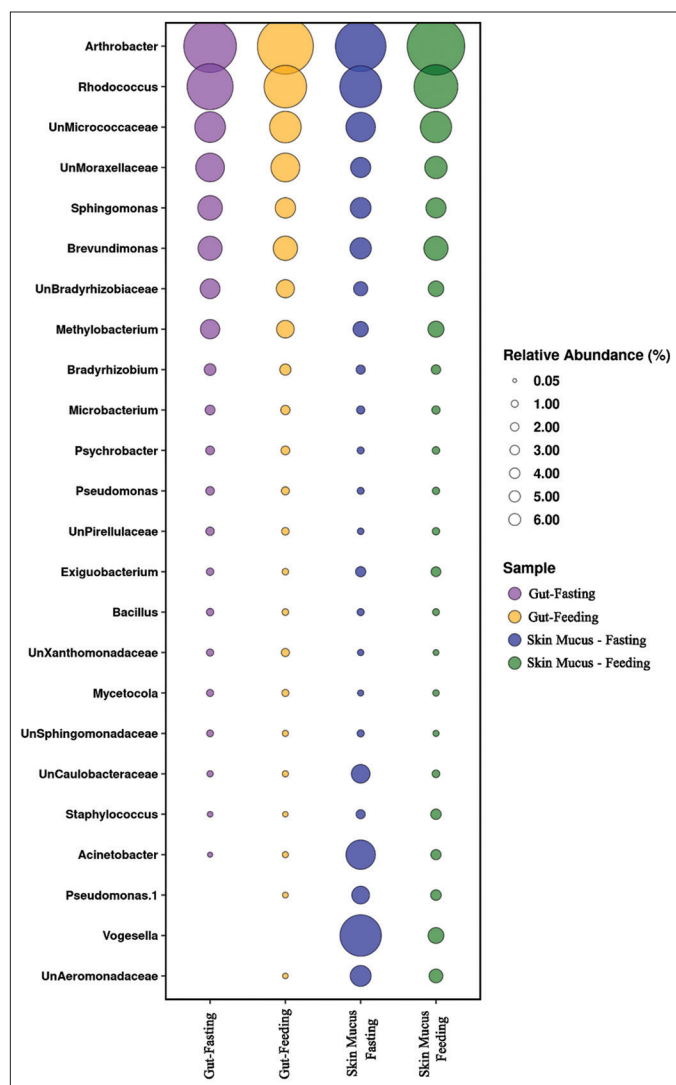


Figure 4: Bubble plot for four groups of samples (skin-mucus fasting, skin-mucus feeding, gut fasting, and gut feeding) showing the relative abundance of top genera.

In this study, *Actinobacteria* was the predominant phylum observed in both starved and fed fish and was reported to be widely distributed in aquatic ecosystems. Interestingly, *Actinobacteria* groups are mainly helpful in the production of secondary metabolites and antibiotics to prevent the growth of pathogenic bacteria [61]. Earlier studies indicated that *Actinobacteria* play a key role in metabolism and immunity in gibel carp (*Carassius auratus gibelio*) fed traditional feed [62]. An increase in the abundance of *Actinobacteria* during the fasting phase may maintain/protect fish health by producing secondary metabolites. In *Schizothorax zarudnyi* and *S. altidorsalis*, the diversity of *Actinobacteria* in the gut indicated antibacterial (antimicrobial resistance, AMR) activity against the pathogenic bacteria of humans and fish and has the potential to generate biologically active compounds [63]. The key role of these phyla is suggested to be involvement in energy and nutrient cycling [64]. At the genus level, we detected *Arthrobacter* as an abundant genus, which showed a resolution of approximately 46.33% in the skin-mucus of starved fish and 67.62% in fed fish. In contrast, these genera were abundant in gut samples of fed fishes compared to those of starved fishes. The *Arthrobacter* genus is known to colonize bacterial growth

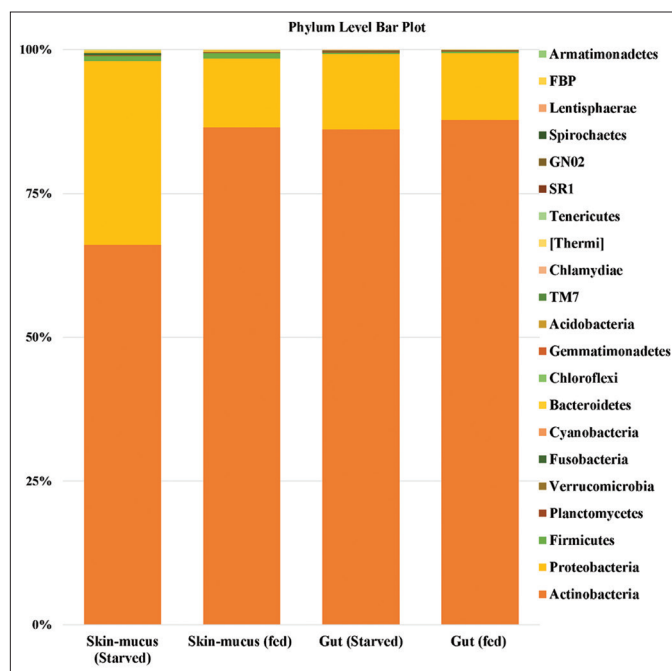


Figure 5: Stacked column bar graph showing the distribution and abundances of bacteria at the phylum level in *Channa striata* fed with control formulated diet and starved. Y-axis represented the percentage of abundance and X-axis represents the different sample.

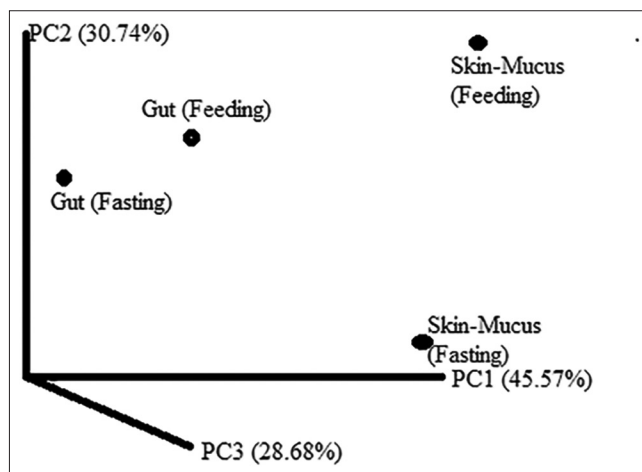


Figure 6: Principal coordinate analysis plot (PC1 vs PC2) based on unweighted UniFrac distance matrix of four samples (Skin-mucus Fasting, Skin-mucus Feeding, Gut Fasting, and Gut Feeding).

in the gut of freshwater fishes [65]. Interestingly, at the species level, the abundance of *Arthrobacter nitroguajacolicus* indicated a unique role in murrel. This species has a vital role as a biocatalyst (nitrile-converting enzyme) for the production of acrylic acid from acrylonitrile through hydrolysis [66]. This group of bacteria is found in diverse ecologically enriched environments and is observed to thrive in stressful situations [67]. *Arthrobacter nitroguajacolicus* R61a is able to utilize quinaldine as a source of carbon and energy for degradation, as reported by Stephan Kolkenbrock [68]. This work reported the presence of essential bacterial groups responsible for producing biologically active compounds with antibacterial activity and their synthesis. Diversity indices such as Shannon and Simpson

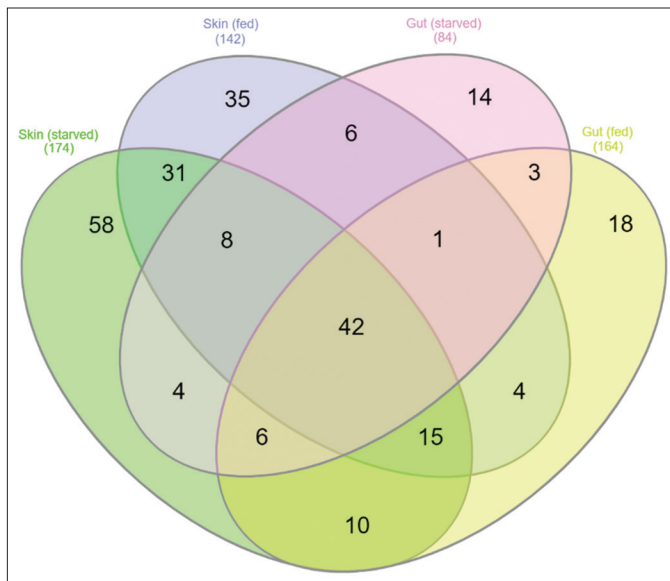


Figure 7: Venn diagram of the unique and shared bacteria in genera level identified in four different types of samples.

indices were measured based on the richness and evenness of bacterial species [69].

Rhodococcus (order: *Actinomycetales*) was the second-highest and most abundant genus in the murrel gut and skin-mucus. Earlier studies indicated that this genus has the ability to produce phytase in *Catla catla* [70]. *Rhodococcus* spp. has applications in bioprocess technology, bioremediation, pigment synthesis, and probiotics in aquaculture [71,72]. The gene encoding virulence factors have been identified from *Rhodococcus fascians* [73] and used as a catalyst for triacylglycerol synthesis [74].

The other phylum, *Proteobacteria*, was dominant in the gut and skin-mucus samples of murrel, which was also reported in *Plectropomus leopardus* during fasting conditions [75]. In this study, *Vogesella* (*Proteobacteria*) dominated the gut of starved fishes compared to fed fishes. *Gammaproteobacteria* were reported to be dominant in the gut of fishes during fasting, but in our study, these bacterial groups were abundant in the skin-mucus of murrel. *Gammaproteobacteria* were dominant in the gut and skin-mucus of fish from both the sea and rearing tanks [76-78]. The alteration in microbiome during starvation and feeding also affects the nutrition of the host and shows the linkage between host-microbe interactions [15,16,79]. It was reported that *Firmicutes* levels were altered during high intake of feed and indicated an inverse correlation with *Bacteroidetes* [80,81]. Our results showed that *Alphaproteobacteria* was dominant in the gut and skin-mucus of fishes. *Brevundimonas* (*Caulobacteraceae*), *Sphingomonas* (*Sphingomonadaceae*), *Methylobacterium* (*Methylobacteriaceae*), and an unclassified genus of the *Moraxellaceae* family of the dominant genus were identified in both the gut and skin-mucus. Earlier studies implied that *Brevundimonas diminuta* can be used as an important strain in water filtration systems as a quality controller for bacterial assays, given its antibiotic susceptibility [82]. *Sphingomonas* spp. has been utilized as a biocatalyst in bioremediation [83] and for generating exopolysaccharides as a gelling agent for the pharmaceutical and food industries [84]. It also has the ability to produce carotenoids, for example, β -carotene and nostoxanthin [85]. *Alphaproteobacteria* (59% of the total *Proteobacteria*) were abundant in the butterfly fish *Chaetodon vagabundus* [86]. In our study, we could not detect

the abundance of *Firmicutes* and *Bacteroidetes*, possibly due to the carnivorous feeding habit of murrel, as earlier studies indicated that these groups of bacteria are abundant in fishes, where microbes help to degrade polysaccharides during metabolism [87]. Similarly, in *T. tambroides*, the gut microbiota is represented by high levels of *Firmicutes* and *Bacteroidetes* due to their habitat in the lake, which was enriched with periphyton algae [88]. Our results showed that *Actinobacteria* was the predominant phylum among the total microbiota of the skin-mucus and gut of murrel. Interestingly, the present study revealed that the microbiota composition of murrel was not affected by altered feeding conditions at a significant level, while earlier reports implied that microbiota was affected by host factors (genetics) as well as the environment [52,89,90].

5. CONCLUSION

This study provides baseline data on the microbial community associated with murrel and their possible contribution to host metabolism. This study revealed skin-mucus and gut microbial communities of murrel consisted of 22 bacterial phyla and were mainly dominated by *Actinobacteria* (66.07–87.78%) and *Proteobacteria* (11.62–31.93%) under both feeding and fasting conditions. *Arthrobacter* and *Rhodococcus*, known for improving fish performance in terms of growth, immunity, and nutrition, were more abundant in both gut and skin-mucus. Further, work is required to understand shifts in skin-mucus and gut microbiota after a prolonged period of starvation. This might reveal a significant alteration in the microbial profile and facilitate the discovery of novel microbes involved in starvation.

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7. AUTHORS' CONTRIBUTIONS

Jitendra Kumar Sundaray and Kiran D Rasal participated in conceptualization and designing of experiment. Sangita Dixit and Manohar Vasam acquired the data. Sangita Dixit, Pragyana Paramita Swain, Mir Asif Iquebal, Sarika Jaiswal and UB Angadi analyzed/interpreted the data. Kiran D Rasal, Pragyana Paramita Swain and Jitendra Kumar Sundaray drafted the manuscript. Prabhugouda Siryappagoudar and Dinesh Kumar did critical revision of manuscript. Mir Asif Iquebal, Sarika Jaiswal and UB Angadi carried out statistical analysis of the data. Rajesh Kumar and Jackson Debbarma participated in technical or material support, Jitendra Kumar Sundaray supervised the experiment. Jitendra Kumar Sundaray and Anil Rai approved the final manuscript.

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9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The research undertaken complies with the current animal welfare laws in India. The care and treatment of animals used in this study were in accordance with the ethical guidelines of ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, India.

11. DATA AVAILABILITY

The sequences generated in this study are deposited into the Genbank database of NCBI as Short Read Archive with Accession number: SRR10662778; SRR10662777; SRR10662776; SRR10662775.

12. PUBLISHER'S NOTE

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