Unveiling the Gut and Flesh Microbiome Signature and Diversity of the Bangladesh National Fish Hilsa (Tenualosa ilisha)

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Article

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Abstract

Fish microbiome science is progressing fast, but it is mostly restricted to farmed or laboratory fish species compared to natural or marine fish populations. The aim of this study was to unveil the gut and flesh microbiome signature and diversity of the anadromous fish, *Tenualosa ilisha* (hilsa), the national fish of Bangladesh. We analysed 18 samples including 15 gut (intestinal contents) and three flesh samples from 18 individual hilsa fishes collected from three major habitats (e.g., freshwater, brackish water and marine water) of hilsa in Bangladesh through 16S rRNA (V3 – V4 region) gene sequencing-based metagenomics. A total of 325 operational taxonomic units (OTUs) including 67 and 258 in flesh and gut samples, respectively were identified. The observed OTUs were represented by six phyla, nine classes, 19 orders, 26 families and 40 genera of bacteria. Our findings revealed substantial taxonomic variability between sample categories (i.e., gut and flesh; $p = 0.0127$; Kruskal Wallis test), and habitats (freshwater, brackish water, and marine water; ($p = 0.007$; Kruskal Wallis test) of the hilsa fishes, indicated by their higher degree of shared microbiota. Of the identified genera, *Vagococcus*, *Morganella*, *Enterobacter*, *Plesiomonas*, *Shigella*, *Clostridium*, *Klebsiella*, *Serratia*, *Aeromonas*, *Macrococcus*, *Staphylococcus*, *Proteus*, *Hafnia* etc. were the major bacterial genera detected in hilsa fish. Importantly, we detected six genera such as *Sinobaca*, *Synechococcus*, *Gemmata*, *Serinicoccus*, *Saccharopolyspora*, and *Paulinella* seem to be specific to the hilsa fishes. Our data provided evidence for the existence of both unique and shared bacteriomes with probiotics potentials in the gut and flesh of hilsa fishes, which might be taken into consideration for undertaking future microbiome study in this economically important fish species.

Introduction

Hilsa (*Tenualosa ilisha*) is the national fish and geographical indication (GI) product and also known as iconic flagship species of Bangladesh. It is also known as the ilish, hilsa, hilsa herring or hilsa shad, which is a species of fish related to the herring, in the family *Clupeidae* [1] . Because of its special flavour and taste, the economic value and demand for this fish in worldwide is very high. Globally, hilsa is the most important commercial transboundary species of the Bay of Bengal, but Bangladesh enjoys the major share (86%), followed by India (8%), Myanmar (4%) and the rest by other countries. Hilsa has also sociocultural and religious values and its non-consumptive value estimated approximately US$0.36 billion per annum [2] . The fishery directly employs 0.5 million fishers and another 2.5 million actors are associated in its value chain [3] . This highly popular and expensive fish contributes about 12% of the total fish production and about 1.15% of the GDP (gross domestic product) in Bangladesh [4] . It is widely distributed in Southeast and South Asia [5,6] , ranging from China Sea, Bay of Bengal, Arabian Sea, Red Sea to Persian Gulf, and is also found in coastal areas, estuaries, brackish and freshwater rivers [6,7] . Hilsa is a marine fish but it comes to freshwater during spawning. Though hilsa is generally considered an anadromous species, it is found in all the principal rivers more or less round the year. For a migratory fish like hilsa, a homogeneous stock thus is not normally expected [3] .

Microbial communities in hilsa fish guts can enhance host metabolic capacity through beneficial effects on nutrient digestion and assimilation, and can protect the host from invasive pathogens [8]. Gut microbiota, which facilitate host homeostasis [9,10], have been analysed in many fish species [11,12] but rarely in the hilsa fish [4]. The gut microbiota of fish is known to play crucial roles in digestion, nutrition, immunity, reproduction and the overall health of fish [13]. Besides resident microbes, the fish gut is also considered the principal reservoir for the colonization of pathogenic bacteria [14]. The gut microbiome is influenced by a myriad of factors and it is difficult to ascertain the individual effects of each of these factors. The colonization of fish gut starts early in the larval stage and is continuously driven towards achievement of a complex assemblage of gut associated microbes [15]. Moreover, unhygienic handling, poor preservation and transportation methods could make this fish as a potential carrier for the transmission of pathogenic bacteria. In a previous study, Foysal et al. characterized several pathogenic bacteria in marketed hilsa fish in

Bangladesh [4]. Most research conducted in fish microbiota has been focused on gut-associated microbiota and its role regulating physiology and health in host, particularly in aquaculture fish species [16]. So far, no report has been published on any disease of hilsa fish. Nothing is known about the gut microbiome of this unique national fish. We employed the high-throughput 16S rRNA gene amplicon sequencing technique to gain insights into the microbiome composition and diversity of gut and flesh microbiomes of the hilsa fishes in three major habitats of Bangladesh. Culture-independent 16S rRNA gene amplicon sequencing-based investigation of the hilsa fish microbiome suggested that both microbiome signature and diversity could vary according to sample category and habitat of the hosts.

Results

In order to unveil the microbiome signature and diversity in the gut and flesh samples of hilsa fish collected from three habitats (e.g., FW, BW and MW) of Bangladesh, we analysed 15 gut samples (e.g., intestinal contents) and three flesh samples through 16S rRNA amplicon sequencing. The study sampling information, demographics, amplicon sequence related data, assigned operational taxonomic units (OTUs) per sample and SRA (sequence read archives) accession numbers of the study subjects are summarized in Table 1. The mean length, girth and weight of the hilsa fishes were 38.83 cm, 24.31 cm and 786.17 gm (**Table S1**). Among these hilsa fishes, 15 samples were from gut (83.33%) and three were flesh (16.67%). The 16S rRNA gene amplicon sequencing of the 18 hilsa fish samples generated 3,696,608 raw reads (average: 205,367 reads/sample), of which 390,289 quality reads (10.56%) mapped to 325 OTUs of bacteria. Among the observed OTUs, 67 and 258 OTUs were identified in flesh and gut samples, respectively (Table 1).

To elucidate whether diversity of the hilsa fish varies according to sample categories (e.g., gut and flesh) and habitats (e.g., FW, BW and MW), we examined both within sample (alpha) and across the samples (beta) diversities of the detected bacterial communities (Fig. 1). The alpha diversity measured using Observed species, Chao1, Shannon, Simpson, InvSimpson and Fisher indices showed significant differences in bacterial community richness, keeping substantially higher diversity in gut samples associated bacteriomes ($p = 0.05$; Wilcoxon test) than the flesh samples (Fig. 1A). The within sample diversities were more distinct according to the habitats of the hilsa fishes where higher diversity was estimated in FW followed by BW and MW ($p = 0.05$; Wilcoxon test) (Fig. 1B). The Bray–Curtis dissimilarity distance estimated principal coordinate analysis (PCoA) plot showed that bacteriome composition in hilsa fishes differed significantly according to sample categories (gut versus flesh, $p = 0.03$, $R^2 = 0.076$, PERMANOVA test) (Fig. 1C). Moreover, the beta diversity of the bacteriomes also varied significantly according to the host habitats ($p = 0.05$, $R^2 = 0.246$, PERMANOVA test) (Fig. 1D). The observed OTUs were represented by six phyla, nine classes, 19 orders, 26 families and 40 genera of bacteria (**Table S2**). At phylum level, the hilsa fish bacteriome was dominated by Firmicutes, Proteobacteria, and Planctomycetes, comprising > 97.5% of the total abundances (**Data S1**). Among these phyla, Firmicutes was the most abundant phylum with a relative abundance of 62.71% and 88.0% in gut and flesh samples, respectively. Proteobacteria was found as the second top abundant bacterial phylum with 23.11% and 7.66% relative abundances in gut and flesh, respectively (**Data S1**). By comparing the relative abundances of the detected phyla across the habitats of the hilsa fish, we found that Firmicutes was predominant bacterial phylum in FW, BW and MW (> 65.0% relative abundances). Likewise, Proteobacteria was the second most predominating phylum in FW (16.97%) and BW (24.52%) while Planctomycetes remained as the second most predominant phylum (15.39%) in MW. The rest of phyla also differed significantly between gut and flesh samples and across the habitats (Fig. 2, **Data S1**). By comparing the bacterial taxa at order level, we found that *Lactobacillales* was the top most abundant (76.98%) bacterial order in gut whereas *Enterobacteriales* was the predominant order in flesh samples with a relative abundance of 86.83% (Fig. 3, **Data S1**). The relative abundance of these two bacterial orders also varied across the habitats of the hilsa fish. For instance, *Lactobacillales* and *Enterobacteriales* were detected with relative abundances of 82.63% and 15.36%, respectively in FW, while *Enterobacteriales* and *Lactobacillales* had 92.45% and 2.20% abundances, respectively in BW, and 79.67% and 13.39% abundances, respectively in MW (Fig. 3). *Enterobacteriaceae* (89.64%), *Aeromonadaceae*

(4.84%) and *Moraxellaceae* (1.31%) were the top abundant families in the gut of hilsa fishes whereas *Enterococcaceae* (82.91%) and *Enterobacteriaceae* (15.44%) were the most predominating bacterial families in the flesh (**Fig. S1, Data S1**). Despite, having had relatively lower abundances (< 2.0%), rest of the bacterial orders and families also showed discriminations according to sample groups and habitats (**Data S1**).

We also demonstrated noteworthy differences in both composition and the relative abundances of bacterial taxa at genus-level according to sample categories (gut and flesh; $p = 0.0127$; Kruskal Wallis test) and habitats (FW, BW and MW; $p = 0.007$; Kruskal Wallis test) of the hilsa fishes. In this study, we detected 40 bacterial genera in gut and 30 genera in flesh samples of the hilsa fishes, of which 30 (75%) were identified as the shared genera between the gut and flesh (**Fig. S2a**). Likewise, 40, 35 and 22 bacterial genera were detected in FW, BW and MW, and of them, 18 (45%) genera were found to be shared in all of the three habitats (**Fig. S2b, Table S2**). By studying the phylogenetic relationship of the identified bacterial genera, we found that majority of these genera (n = 18) belonged to *Gammaproteobacteria* followed by *Bacilli* (n = 15), *Actinobacteria* (n = 2), *Planctomycetia* (n = 2) and others (n = 3) (**Fig. S3**).

To examine whether genus level composition and relative abundance of the bacteria vary between the sample categories and across the host habitats, we performed pairwise Kruskal–Wallis test of the relative abundances of all genera identified (Figs. 4 and 5). Although, 40 bacterial genera were detected, twenty-five genera had differentially abundant OTUs in the study samples and host habitats ($p < 0.05$, Kruskal–Wallis test) (Fig. 5). Of the identified genera, *Vagococcus* (68.67%), *Morganella* (13.57%), *Enterobacter* (5.37%), *Plesiomonas* (3.56%), *Shigella* (1.75%), *Clostridium* (1.58%) were the top abundant genera in the gut of hilsa fishes. Conversely, *Enterobacter* (19.87%), *Serratia* (19.85%), *Aeromonas* (18.56), *Klebsiella* (14.33%), *Acinetobacter* (4.85%), *Vagococcus* (4.21%), *Macrococcus* (2.84%), *Clostridium* (2.55%), *Pseudomonas* (1.97%), *Shigella* (1.69%), *Hafnia* (1.59%), *Plesiomonas* (1.51%), *Weissella* (1.1%), and *Morganella* (1.0%) were the predominating genera in flesh of hilsa fishes (Fig. 4A). Despite having lower relative abundances, the gut samples had sole association of ten genera such as *Proteus*, *Cronobacter*, *Synechococcus*, *Streptococcus*, *Photobacterium*, *Lactobacillus*, *Peptoniphilus*, *Gemmata*, *Serinicoccus*, and *Saccharopolyspora* (**Data S1**).

The FW samples were dominated by *Vagococcus* (69.54%), *Morganella* (13.81%), *Enterobacter* (4.98%), *Plesiomonas* (3.59%), *Shigella* (1.68%) and *Clostridium* (1.45%). In addition, BW samples were mostly dominated by *Enterobacter* (23.13%), *Proteus* (14.59%), *Vagococcus* (12.68%), *Aeromonas* (11.14%), *Serratia* (7.55%), *Cronobacter* (6.98%), *Klebsiella* (6.31%), *Clostridium* (5.18%), *Shigella* (3.36%), *Acinetobacter* (3.30%), and *Plesiomonas* (1.79%). On the other hand, *Serratia* (35.11%), *Morganella* (20.68%), *Vagococcus* (17.69%), *Erwinia* (7.02%), *Klebsiella* (4.81%), *Shigella* (3.90%), *Staphylococcus* (3.77%), *Sinobaca* (2.60%), and *Clostridium* (1.04%) were the predominating bacterial genera detected in the MW samples of the hilsa fishes (Fig. 4B). Besides, *Vagococcus*, *Shigella* and *Morganella* had significant association with gut ($p < 0.05$, Kruskal Wallis test) while *Acinetobacter*, *Aeromonas*, *Hafnia*, *Klebsiella*, *Pseudomonas*, *Enterobacter*, *Weissella*, *Salmonella* and *Serratia* had substantial association with flesh samples ($p < 0.05$, Kruskal Wallis test) of the hilsa fishes (Fig. 5A). Likewise, *Acinetobacter*, *Klebsiella*, *Pseudomonas* and *Planctomyces* in FW, *Enterobacter*, *Morganella*, *Klebsiella* and *Shigella* in BW, and *Proteus* in MW had significant correlations ($p < 0.05$, Kruskal Wallis test). Remarkably, *Lactococcus*, *Macrococcus* and *Vagococcus* had stronger correlation ($p < 0.05$, Kruskal Wallis test) with all of the samples from three habitats (i.e., FW, BW and MW) (Fig. 5B). Interestingly, of the identified 40 bacterial genera, six genera (e.g., *Sinobaca*, *Synechococcus*, *Gemmata*, *Serinicoccus*, *Saccharopolyspora*, and *Paulinella*) have not been identified in any aquatic and marine fish species. Though, rest of the genera had relatively lower abundances (< 1.0%), but their relative abundances differed in two sample groups (gut vs flesh), and across three habitats (e.g., FW, BW and MW) of the hilsa fishes (Fig. 4, **Data S1**).

Discussion

Unveiling the signature and diversity of microbial communities in the national fish of Bangladesh i.e., *Tenualosa ilisha* or hilsa in their different habitats through next generation sequencing (NGS) is one of the basic foci in aquatic and marine research. Targeted amplicon sequencing based 16S rRNA metagenomics approach used in this study successful identified a number of bacterial taxa in the gut and flesh of hilsa fishes collected from three distinct habitats (e.g., FW, BW and MW) of Bangladesh. To our knowledge, this is the first of such study aimed at unveiling the microbiota of wild hilsa fish using 16S rRNA gene (V3-V4 regions) sequencing. Using this high throughput NGS approach, we unveiled the composition and relative abundances of the bacteriomes of hilsa fish and its major habitats in Bangladesh with high precision of taxonomic classification using thousands of assigned reads. The three most common and abundant phyla, in this study (Firmicutes, Proteobacteria, and Planctomycetes) had earlier been reported in carriage water of ornamental fish and commercially important fishes from different continents [17,18]. Moreover, majority of the bacterial phyla detected in this study, irrespective of sample categories and habitats of the hilsa fishes, have been reported to comprise a large proportion of the gut microbiota in many fish species [13,19,20]. One of the hallmark findings of this study was the identification of many beneficial and/or probiotics bacterial genera in both gut and flesh samples of the hilsa fishes across the tree habitats. Out of the 40 genera detected in this study, *Vagococcus*, *Morganella*, *Serratia*, *Enterobacter*, *Aeromonas*, and *Klebsiella* were the dominant genera of the as observed in both gut and flesh samples. The genus *Vagococcus* consists of 13 species, and majority of these species have recently been used in aquaculture as promising probiotics candidates for marine fish species [21]. However, an emerging concern regarding the safety of *Vagococcus* spp. as a probiotic agent in aquaculture does not guarantee zero risks. *Morganella* is a Gram-negative, rod-shaped, aerobic and facultatively anaerobic bacterium commonly found in freshwater, soil, and normal flora of intestinal tracts in animals [22] and fishes [23] and fish products [24]. *Enterobacter* spp. has already been proved as a prospective probiotic for aquaculture applications, and showed extensive resistance to bacterial infection, no pathogenicity to the host, and stronger environmental tolerance [25]. In a recent study, Tang et al. isolated *E. asburiae* C28 from the intestine of *Carassius auratus*, which reduced the load of potential pathogens, increased the number of potential probiotics in the host gut, and decreased the death rate of *C. auratus* challenged by *A. hydrophila*[26]. Besides these genera, diverse probiotic bacterial genera, including *Plesiomonas*, *Pseudomonas*, *Acinetobacter*, *Streptococcus* and *Staphylococcus*, were identified from the both gut and flesh samples of hilsa fishes from three important habitats in Bangladesh. Member of the *Plesiomonas* genus naturally inhabits freshwater and marine environments, and has already been isolated from different aquatic and marine species [27,28]. *Aeromonas* species can be found in a variety of aquatic and environmental habitats including freshwater, sediment, estuaries, seaweed, sea grass, used water, drinking water and food [29]. *Aeromonas* sp. and *Pseudomonas* sp. are the most prevalent bacteria isolated from carp culture systems [30]. Different microbial species have been reported and introduced as probiotics in the aquaculture industry, including Gram-negative bacteria such as those of the genera *Aeromonas*, *Enterobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Acinetobacter*, *Rhodopseudomonas*, and *Vibrio*, and Gram-positive bacteria such as those of the genera *Bacillus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Microbacterium*, *Micrococcus*, *Streptococcus*, and *Streptomyces*[31,32]. However, the isolation and efficacy of probiotic microorganisms that originated from hilsa fish have not been reported thus far. Another important finding of this study was the identification of some bacterial genera (e.g., *Sinobaca*, *Synechococcus*, *Gemmata*, *Serinicoccus*, *Saccharopolyspora*, and *Paulinella*) that have not been reported in any aquatic and marine fish species. However, some of these genera (e.g., *Synechococcus*, *Gemmata*, *Saccharopolyspora*, and *Paulinella*) have been identified as a part of photosynthetic ocean microbiomes of diverse salinity and seasons [33,34]. Furthermore, *Lactococcus*, *Morganella*, *Enterococcus*, *Aeromonas*, *Shewanella*, *Pediococcus*, *Leuconostoc*, *Saccharopolyspora*, and *Lactobacillus*, identified in this study are common gut probiotic bacteria used in the aquaculture industries [17,33,35,36]. The involvement of probiotics in nutrition, disease resistance and other beneficial activities in different fish species has proven beyond any doubt. These probiotics microbes of the hilsa fishes may

enhance the nutritional value, flavour and texture, produce antioxidant and antimicrobial compounds, and stimulate the immune functions.

Conclusion

The 16S rRNA gene-based metagenomics data and extracted taxonomic information of gut and flesh bacteriome of hilsa fish in three habitats (e.g., freshwater, brackish water and marine water) has laid a foundation for shedding light in the microbiome of this economically important anadromous and important trans-boundary fish that lives in the Bay of Bengal and moves to migrates to the upstream rivers of Bangladesh and some south Asian countries for feeding, breeding, and the nursing of offspring. The results obtained indicate a strong influence of hilsa fish habitats on the composition of its microbiota. Our results provide microbiota composition and structure, comprising beneficial probiotics (e.g., *Vagococcus*, *Morganella*, *Enterobacter*, *Aeromonas*, *Pseudomonas*, *Plesiomonas*, *Acinetobacter*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, etc.) bacteria which might be taken into considerations for prospective aquaculture applications. Taken together, the bacteriome data and taxonomic observations reported herein this study pave the way for further comprehensive investigations on the co-evolution of hilsa fish microbiomes specially to understand the role of gut and flesh microbiota in host metabolism and immunity.

Materials and Methods

Ethics statement

The Animal Research Ethics Committee (AREC) of the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh, reviewed and approved the experimental procedures of this study (Reference number: FVMAS/AREC/2023/6679). The fish sampling protocol complied with the ARRIVE 2.0 guidelines [37], and followed the guidelines of Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The study location was not privately owned or protected in any way, and the study did not utilize endangered or protected species.

Sample collection, processing and genomic DNA extraction

Hilsa fishes (N = 18) were collected from three major habitats in Bangladesh between September and October 2021 (Table 1). The fishes were caught using a gillnet by local fisherman, immediately placed on ice (at 4°C) and transported to laboratory at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) for further processing and sampling (Figs. 4a **– c**). Sampling of their intestinal contents (n = 15) and flesh (n = 3) (**Figs. S4d – f**) were performed within 48 h after collection and storage at 4°C. Fish scales were removed by sterile forceps under aseptic conditions, and fleshes (100 mg) from each fish were collected in sterile 15 mL centrifuge tubes. Fish guts were excised to collect the lower stomach contents (100 mg) of each fish in sterile 15 mL centrifuge tubes, and stored at -80°C until DNA extraction. Total genomic DNA was extracted from each specimen using DNeasy Blood and Tissue Kit (Qiagen, Crawley, UK) following the manufacturer's instructions and previously published protocols [10,38]. The purity and concentration of the extracted DNA were measured using a NanoDrop 2000c spectrophotometer (Thermo-Fisher Scientific, Waltham, MA).

Library preparation, sequencing and bioinformatics analysis

We amplified the genomic DNA by targeting the V3 – V4 regions of 16S rRNA gene with 30 µL final volume containing 3 µL template DNA, 15 µL master mix (BioLabs, USA), 1.5 µL of each V3 – V4 forward (341f: 5'-CCTACGGGNGGCWGCAG-3') and reverse (785r: 5'-GACTACHVGGGTATCTA ATCC-3') primers [39], and 9 µL ddH2O. A 25 cycle of amplicon PCR

including initial denaturation at 95°C for 3 min, denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s and elongation at 72°C for 30 s was performed for quantified DNA with the final extension of 5 min at 72°C in a thermal cycler (Analytik Jena, Germany) [40]. The PCR amplicons were visualized in 1.5% agarose gel. We purified the amplified PCR products using the Agencourt Ampure XP beads (Beckman Coulter, Brea, USA). The Nextera XT index kit (Illumina, San Diego, USA) was used for paired-end library preparation according to Illumina standard protocol (Part# 15044223 Rev. B). Paired-end (2 × 150 bp) sequencing of the prepared library pools was done using Illumina NextSeq 550 platform (Illumina, USA) at the Illumina Genome Sequencing laboratory of the IBGE, BSMRAU. FastQC v0.11.9 [41] and Trimmomatic v0.39 [42] (set parameters- leading:20, slidingwindow:4:20:20, trailing:20, minlen = 36) [43] were used to check and remove Illumina adapters, known Illumina artifacts, and phiX reads, respectively. We used QIIME 2 (2023.2.0) and associated plugins [44] to process the demultiplexed sequences., and the SILVA database v.138 [45] assigned these processed sequences (with ≥ 98% identity) into operational taxonomic units (OTUs). Default parameters were used for bioinformatic analyses except where otherwise stated.

Statistical analysis

R programming language (v4.1.1) was used for the downstream analysis including alpha-beta diversity, microbial composition and statistical comparison. To estimate the within sample diversity (α-diversity), the observed OTUs, Shannon and Simpson diversity indices were calculated in microbiomeSeq (http://www.github.com/umerijaz/microbiomeSeq) and visualized using phyloseq R package (v1.34.0) [46]. Non-parametric Kruskal Wallis test was used to estimate the differences in bacterial diversity between the sample categories and host habitats [47].

Table 1
Study sample information, SRA accession numbers of the 16S rRNA amplicon sequences and OTUs (operational taxonomic units) mapped against bacterial taxa.

Sample ID	Collection site	Coordinate	Habitat	Source	No. of raw reads	No. of mapped reads	No. of observed OTUs	SRA accessions
CG1	Confluence of Meghna and Padma River, Chandpur	23.2321° N, 90.6631° E	Freshwater	Gut	325,812	33,295	22	SRR24402593
CG2	Confluence of Meghna and Padma River, Chandpur	23.2321° N, 90.6631° E	Freshwater	Gut	140,124	12,418	22	SRR24402592
CG3	Confluence of Meghna and Padma River, Chandpur	23.2321° N, 90.6631° E	Freshwater	Gut	119,676	4,738	10	SRR24402608
CG4	Confluence of Meghna and Padma River, Chandpur	23.2321° N, 90.6631° E	Freshwater	Gut	697,544	291,731	23	SRR24402607
CG5	Confluence of Meghna and Padma River, Chandpur	23.2321° N, 90.6631° E	Freshwater	Gut	131,276	9,514	20	SRR24402606
RG3	Padma River, Rajshahi	24.3745° N, 88.6042° E	Freshwater	Gut	194,464	2,256	18	SRR24402605
MG1	Meghna River, Munshiganj	23.5422° N, 90.5305° E	Freshwater	Gut	165,096	1,333	15	SRR24402604
MG2	Meghna River, Munshiganj	23.5422° N, 90.5305° E	Freshwater	Gut	126,908	7,353	15	SRR24402602
MG4	Meghna River, Munshiganj	23.5422° N, 90.5305° E	Freshwater	Gut	260,420	3,077	11	SRR24402601
PG1	Payra River, Patuakhali	22.3586° N, 90.3317° E	Brackish water	Gut	149,988	611	23	SRR24402600
PG2	Payra River, Patuakhali	22.3586° N, 90.3317° E	Brackish water	Gut	119,052	1,142	11	SRR24402598
PG3	Payra River, Patuakhali	22.3586° N, 90.3317° E	Brackish water	Gut	361,312	8,111	20	SRR24402599

Sample ID	Collection site	Coordinate	Habitat	Source	No. of raw reads	No. of mapped reads	No. of observed OTUs	SRA accessions
PG5	Payra River, Patuakhali	22.3586° N, 90.3317° E	Brackish water	Gut	178,372	3,982	18	SRR24402597
XG1	Bay of Bengal, Cox's Bazar	21.4272° N, 92.0058° E	Marine water	Gut	112,684	509	16	SRR24402596
XG3	Bay of Bengal, Cox's Bazar	21.4272° N, 92.0058° E	Marine water	Gut	151,480	260	14	SRR24402595
CF4	Confluence of Meghna and Padma River, Chandpur	23.2321° N, 90.6631° E	Freshwater	Flesh	180,944	4,994	16	SRR24402609
CF5	Confluence of Meghna and Padma River, Chandpur	23.2321° N, 90.6631° E	Freshwater	Flesh	106,604	2,226	14	SRR24402603
PF4	Payra River, Patuakhali	22.3586° N, 90.3317° E	Brackish water	Flesh	174,852	2,739	17	SRR24402594

Declarations

Data availability

The 16S rRNA gene amplicon sequencing data are available at the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA964437. The accession numbers for all 18 SRA experiments are listed in **Table 1**.

Authors' contributions

The study was planned and designed by TI. MNH, AQMRI and MSR contributed to sample collection, processing, data curation, bioinformatics analysis and writing original manuscript. TIS and TI critically reviewed and edited the manuscript. The final manuscript was read and approved by all authors.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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Figures

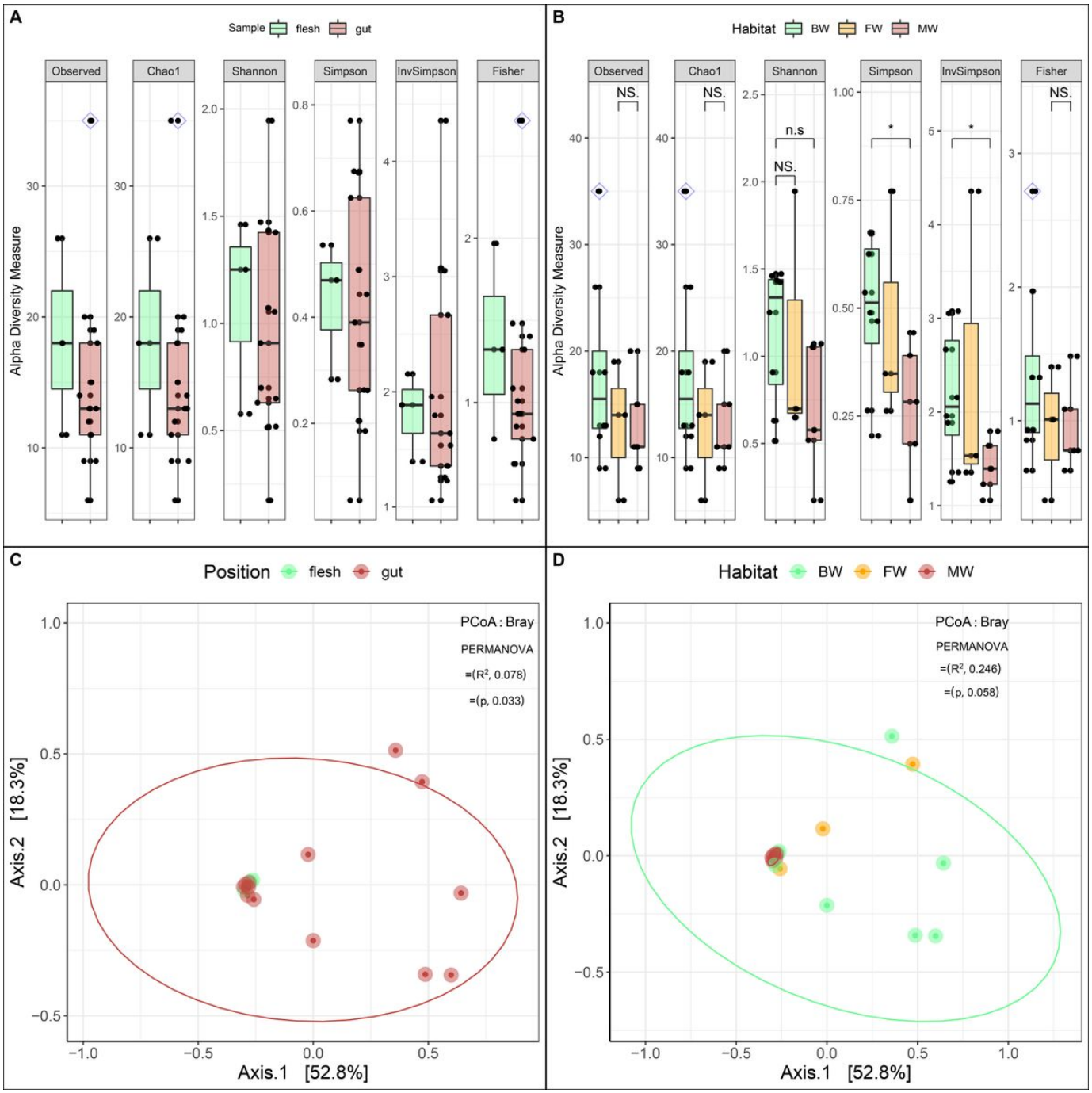

Figure 1

Bacteriome diversity in hilsa fish. (A) Within subject (Alpha) diversity measure. Observed, Chao1, Shannon, Simpson, InvSimpson and Fisher indices estimated within sample bacterial diversity in gut and flesh samples are plotted on boxplots and comparisons are made with pairwise Wilcoxon rank sum tests. Significance level (p-value) 0.01 and 0.05 are represented by the symbols "**", and "*", respectively. (B) Shannon and Simpson indices estimated within sample bacterial diversity in according to host habitat i.e., freshwater (FW), brackish water (BW) and marine water (MW). Between subject (Beta) diversity measure according to (C) sample categories. i.e., flesh versus gut, and (D) host habitat i.e., FW, BW and MW. Bacterial beta diversity was calculated using Bray-Curtis dissimilarity distance method, and visualized on principal coordinate analysis (PCoA) plots. The samples are coloured according to categories (e.g., gut: cherry red and flesh: dragon green) and host habitat (e.g., FW: cheese orange, BW: dragon green and MW: cherry red) and joined with the respective ellipses. Pairwise comparisons on a distance matrix using PERMANOVA test under

reduced model shows significant bacterial community differences between sample categories (p = 0.03, R^2 = 0.076) and across the habitats (p = 0.05, R^2 = 0.246). NS refers to non-significant.

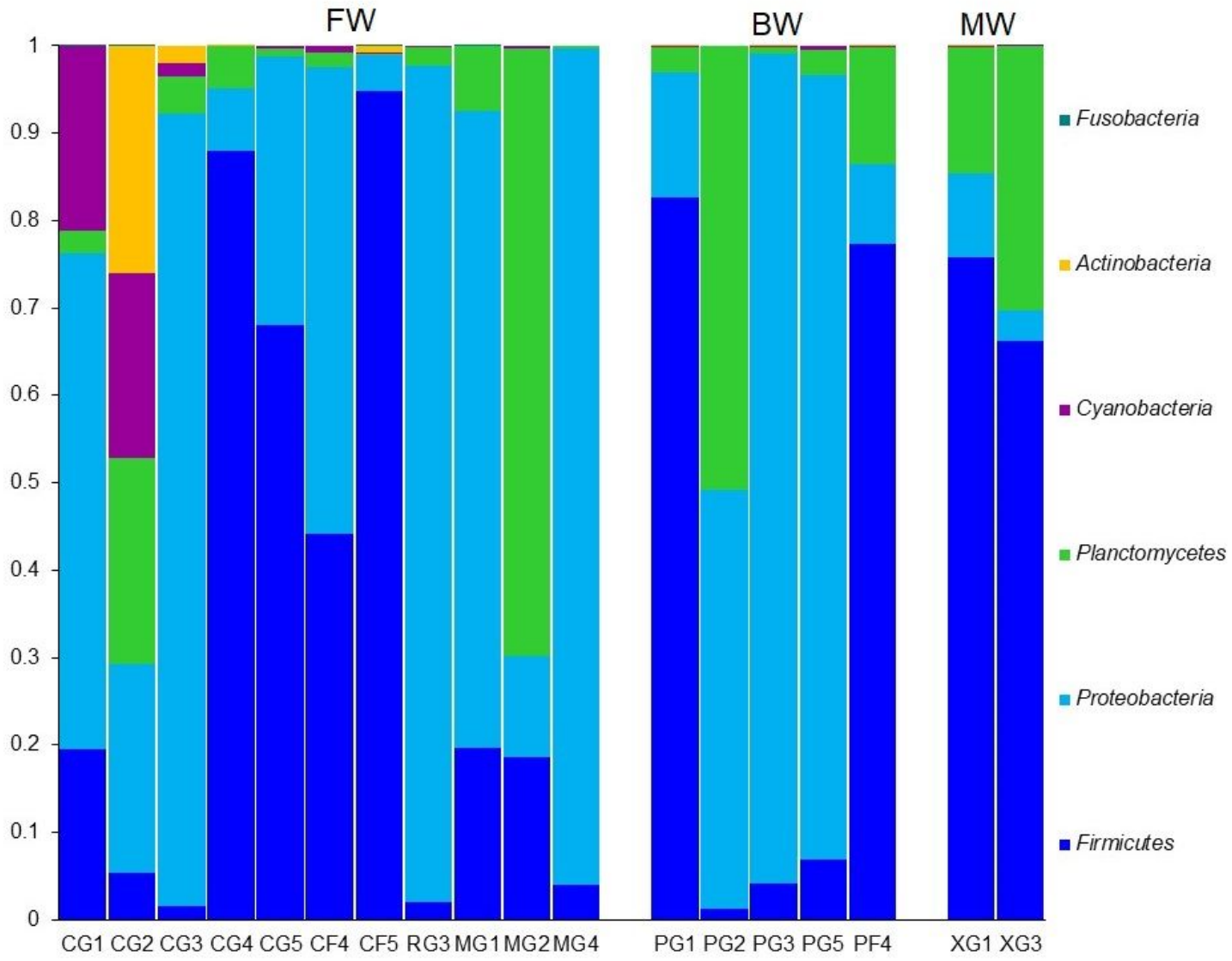

Figure 2

The phylum-level taxonomic abundance of bacteria in freshwater (FW), brackish water (BW) and marine water (MW) samples. Each stacked bar plot represents the abundance of bacterial phyla in each sample of the corresponding category.

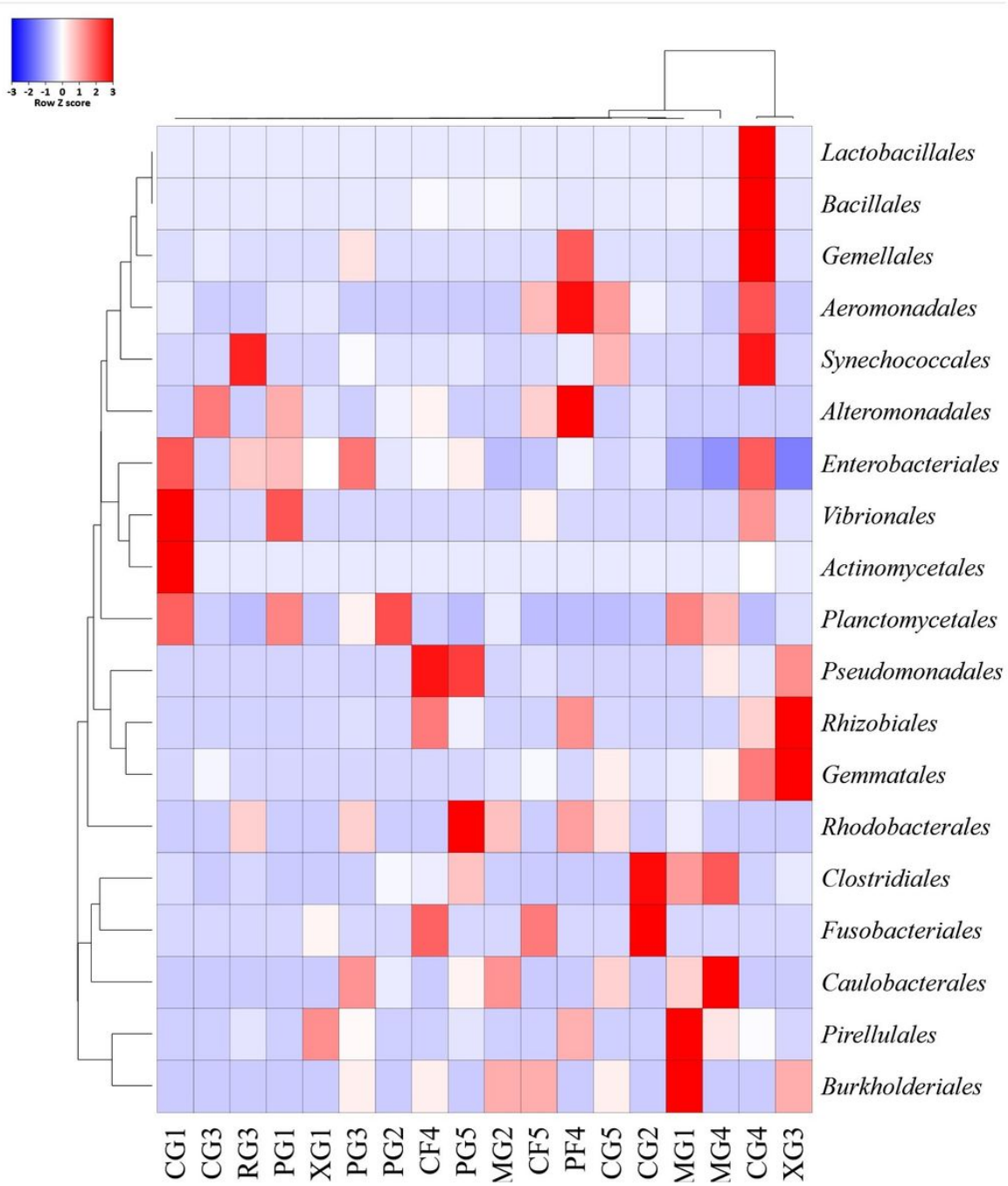

Figure 3

Taxonomic profile of bacteriomes in hilsa fish at the order level. Heatmap showing the average relative abundances and hierarchical clustering of the orders of bacteria in the study samples. The colour bar (row Z score) at the top represents the relative abundance of the bacterial orders in the corresponding samples. The colour codes indicate the presence and completeness of each bacterial taxa, expressed as a value between =3 (lowest abundance) and 3 (highest abundance). The red colour indicates the more abundant patterns, while blue cells account for less abundant orders in that particular sample.

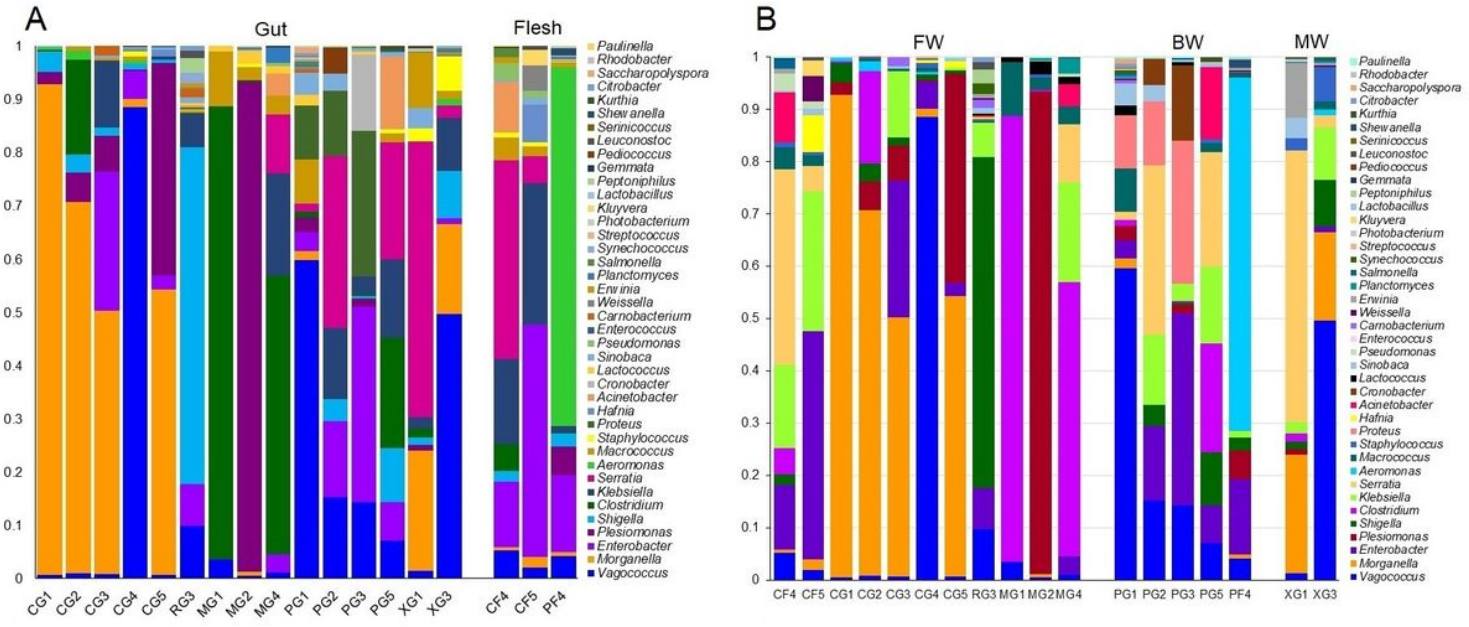

Figure 4

The genus-level taxonomic profile of bacteriomes. (A) The bar plots representing the relative abundance of 40 bacterial genera in gut (CG1-XG3) and flesh (CF4, CF5 and PF4) samples of the hilsa fish. (B) The bar plots representing the relative abundance of 40 bacterial genera in freshwater (FW), brackish water (BW) and marine water (MW) samples. Each stacked bar plot represents the abundance of bacterial genera in each sample of the corresponding category. Notable differences in bacterial genera are those where the taxon is abundant in gut samples, and effectively not detected in the flesh samples. The distribution and relative abundance of the bacterial genera in the study metagenomes are also available in Data S1.

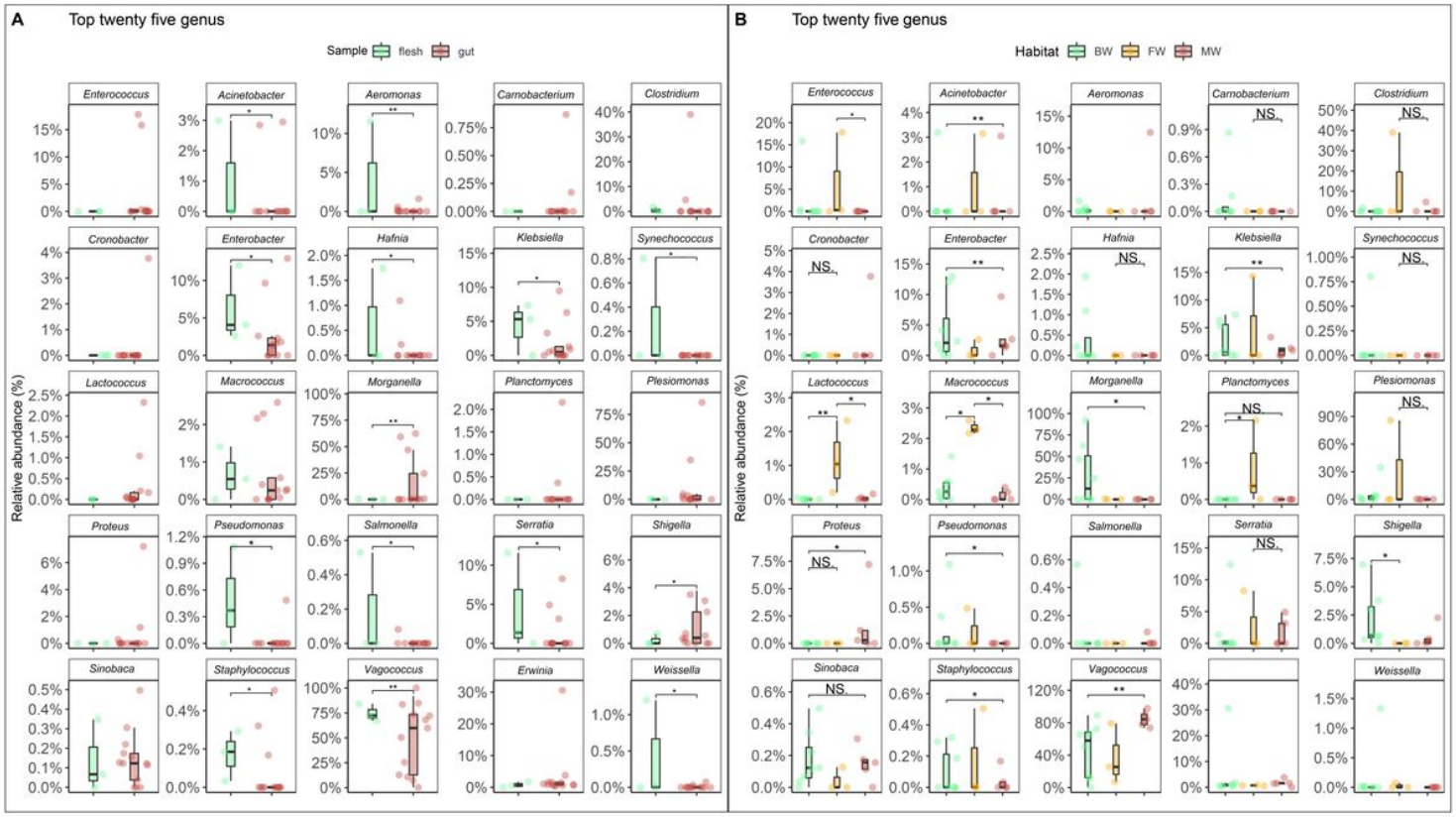

Figure 5

Mean relative abundance and within-group divergence of top abundant 25 bacterial genera. Relative abundances of top 25 abundant genera and within-group (i.e., sample: gut and flesh, and habitat: FW, BW and MW) divergence by use of Bray–Curtis dissimilarity. In the boxplots, the middle line represents the median, the lower hinge corresponds to the first quartile (25^{th} percentile), the upper hinge corresponds to the third quartile (75^{th} percentile), the whiskers extend to the largest and smallest values. The samples are coloured according to (A) sample categories (e.g., gut: cherry red and flesh: dragon green) and (B) host habitats (e.g., FW: cheese orange, BW: dragon green and MW: cherry red). Pairwise comparisons were done by use of the Kruskal–Wallis test. All *p* values are adjusted. We considered *p* values less than 0.05 and adjusted *p* values less than 0.1 to be significant. NS refers to non-significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- DataS1.xlsx
- SupplementaryFiguresandTables.pdf