ABSTRACT

The gastrointestinal (GI) tract is a favourable ecological niche for a large number of microorganisms, and as in other animal groups, a wide range of microbes colonizes the GI tract of fish. Although GI tract of fish is generally dominated by members of *Bacillota*, *Pseudomonadota* and *Bacteroidota*, bacterial abundance and pattern of dominance, strongly depends upon various biotic and abiotic factors that includes variety of fish, structure and microarchitecture of GI tract, condition of water, temperature, geographical regions, availability of nutrients and oxygen in water.

The loach *Lepidocephalichthys guntea* is a freshwater fish of the family *Cobitidae*. Native to South and Southeast Asia, these loach exhibit market values as well as unique characteristics of intestinal breathing that make it an interesting addition to freshwater aquaculture as well as aquariums. They are bottom-dweller, often seen scavenging for small invertebrates and organic matter in the substrate. During eutrophication or drought these fish come to air water interface and gulp water through mouth that passes to intestine. After consumption of oxygen via posterior intestine, rest air is voided through the rectum.

The first objective of our study was focused on isolation of both aerobic and facultatively anaerobic cultivable intestinal bacteria of *L. guntea* using conventional culture-based techniques. This study was aimed to investigate both the autochthonous and allocthonous gut bacterial population. For this, a set of wild healthy fish were dissected and intestinal bacteria were retrieved using the dilution plate technique on selective media. Gut bacteria of this fish showed considerable species diversity. These isolates were characterized using morphological and biochemical parameters that was further followed by taxonomic identification and construction of evolutionary lineage of each gut isolates. Results showed that *Staphylococcus* spp*., Rhodococcus* sp*., Bacillus* sp., *Solibacillus* sp., *Verticiella* sp., and *Oceanobacillus* sp. were allocthonous gut isolates while *Enterobacter* sp., *Comamonas* sp., *Klebsiella* sp., *Shigella* sp., and *Staphylococcus* spp. reflected the autochthonous gut bacterial population.

In our second objective, these isolates were tested for several environmental stress factors that include temperature, salinity, pH, antibiotic resistance, and heavy metal resistance properties which yield a selection of six different isolates. Selected isolates named as *Shigella* sp. GCP5, *Comamonas* sp. GCA5*, Staphylococcus sp.* GCP4*, Rhodococcus* sp. GG48*, Bacillus* sp. GG161, and *Verticiella* sp. GG226 were subjected to whole genome sequencing and genome mining. Experiments showed that all six gut isolates showed growth when they were subjected to cold and heat stress in laboratory with lowest temperature tolerance of 4 ^{o}C and highest temperature tolerance of 42 ^{o}C. Genome mining revealed that all the isolates carry different sets of cold shock and heat shock response genes which enable them to tolerance the stress. While saline stress was applied on these isolates using NaCl, each of them showed different tolerance range. Genome mining showed that, salinity tolerance was performed majorly by potassium (K^{+}) uptake. Also presence of genes encoding different compatible solutes like glycine betaine, ectoine, and proline were reflected all the gut isolates. Another stress factor was pH, against which these gut isolates showed a wide range of tolerance. The broadest range of pH tolerance was observed in *Shigella* sp. GCP5 that could tolerate a pH range of 3-13. On the contrary, *Rhodococcus* sp. GG48 showed smallest range of pH tolerance from 7-10. Genome mining of GCP5 showed that tolerance of acidic environment was due to presence of a periplasmic protein HDEA in its genome that supports acid resistance in pathogenic enteric bacteria. In the context of high pH, alkali tolerant isolates are expected to manage their metabolism through various strategies, including the proton transport system, enzymes such as proteases, lipases, cellulases, H^{+} coupled ion-transport systems, F0F1 ATPases, and transport systems that coordinate H^{+} transport with other solutes. Genes encoding distinct subunits of Na^{+}/H^{+} antiporters and F0F1 ATPases were disclosed in the genome of all six isolates. Antibiotic resistance profiling of all six gut isolates showed that only *Shigella* sp. GCP5 has a broad spectrum of antibiotic resistance that includes monobactam, penicillin and beta lactam combination agents, ceftriaxone, erythromycin, imipenem and meropenem. A heavy metal resistance profiling was done for these

isolates which showed a broad range of resistance that was distinct for all isolates. Genome mining of the gut isolates showed presence of different sets of heavy metal transportation systems.

Third objective was focused on response of gut bacteria of gill-intesinal breather *L. guntea* during the hypoxic stress and comparison of this response with the changes of gut microbiota of a sole gill-breather fish *Cirrhinus mrigala*. Hypoxia is one of the main risks to fish health in an aquatic environment. High-throughput sequencing was used to examine the anterior and posterior guts of *L. guntea* in both normoxic and hypoxic conditions. According to the community profiling, prolonged exposure to hypoxia increased the diversity and abundance of bacteria in the posterior gut while decreasing both in the anterior gut. Additionally, the anterior and posterior gut's core microbiota showed a significant alteration during hypoxia. Comparative analyses showed that, hypoxia causes more pronounced alterations in the posterior gut bacteria than the anterior gut at various taxonomic levels. As a consequence of hypoxia, several pathogen populations were replaced by potential opportunistic pathogens. A surge in probiotic genera was also seen along with the shift in the pathogenic bacterial population. Similarly, comparison of gut microbiota of *Cirrhinus mrigala* was performed. As dissolved oxygen levels (DO2) decreased from 7±0.5 mg/L to 0.5±0.07 mg/L, a substantial shift was observed in the abundance and diversity of the bacterial population inhabiting the fish gut. The alpha diversity indices showed that the abundance and diversity of gut microflora increased in hypoxia as compared to normoxia. The community profiling indicates in case of hypoxia for both gut regions the number of core microbiota decreases as compared to normoxia. In hypoxic condition, the abundance of Pseudomonadota decreased in both guts while abundance of Firmicutes increased in the anterior gut but decreased in the posterior gut. With this, a rapid rise in probiotic bacteria *Cetobacterium* under hypoxia was observed. With this comparison of gut microbiota of *Lepidocephalichthys guntea* with *Cirrhinus mrigala* to reveal differences in the gut bacterial community during oxygen stressed environmental condition by meta-taxonomic study. The findings have illuminated a significant disparity in the composition of the gut microbiota between these two

species. *L. guntea* exhibited a notably elevated microbial population within its gastrointestinal tract, accompanied by heightened levels of microbial diversity and richness as compared to *C. mrigala*.

The fourth objective was concerned with the immune response provided by the gut immune system of *L. guntea*, when challenged with a pathogenic bacterium. *L. guntea* was experimentally infected with *Aeromonas hydrophila* using intraperitoneal injection followed by bath challenge, and transcriptome data were used to examine the gut immune responses during disease progression and recovery from the diseased state. For the control or uninfected fish (FGC) and the infected fish that were kept for seven days (FGE1) and fifteen days (FGE2), separate water tanks were set up. Coding DNA sequences (CDS) for FGC and FGE1, FGC and FGE2, and FGE1 and FGE2 were analyzed for differential gene expression (DGE). The presence and expression of genes involved in T cell receptor (TCR) signalling pathway, natural killer (NK) cell-mediated cytotoxicity pathway, and complement-mediated pathway, along with a large number of other immune-related proteins, and heat shock protein (HSPs) under various experimental conditions and its relationship to immune modulation of the fish gut was the primary focus of this study. Significant up-and-down regulation of these pathways shows that, in FGE1, the fish's innate immune system was engaged, whereas in FGE2, the majority of innate immune mechanisms were repressed, and adaptive immunity was activated. Expression of genes related to the immune system and heat-shock proteins was induced during this host's immunological response, and this information was then used to build a thorough network relating to immunity and the heat-shock response. This is the first study to examine the relationship between pathogenic bacterial infection, disease reversal, and modification of innate and adaptive immunity as well as heat shock response.