Analysis of Taxonomic Compositions, Metabolic Pathways and Antibiotic Resistance Genes in Fish Gut Microbiome by Shotgun Metagenomics

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Abstract : Characterization of diverse microbial communities in specific environment plays a crucial role in the better understanding of their functional relationship with the ecosystem. It is now well established that gut microbiome of fish is not the simple replication of microbiota of surrounding local habitat, and extensive species, dietary, physiological and metabolic variations in fishes may have a significant impact on its composition. Moreover, overuse of antibiotics in human, veterinary and aquaculture medicine has led to rapid emergence and propagation of antibiotic resistance genes (ARGs) in the aquatic environment. Microbial communities harboring specific ARGs not only get a preferential edge during selective antibiotic exposure but also possess the significant risk of ARGs transfer to other non-resistance bacteria within the confined environments. This phenomenon may lead to the emergence of habitat-specific microbial resistomes and subsequent emergence of virulent antibiotic-resistant pathogens with severe fish and consumer health consequences. In this study, gut microbiota of freshwater carp (Labeo rohita) was investigated by shotgun metagenomics to understand its taxonomic composition and functional capabilities. Metagenomic DNA, extracted from the fish gut, was subjected to sequencing on Illumina NextSeq to generate paired-end (PE) 2 x 150 bp sequencing reads. After the QC of raw sequencing data by Trimmomatic, taxonomic analysis by Kraken2 taxonomic sequence classification system revealed the presence of 36 phyla, 326 families and 985 genera in the fish gut microbiome. At phylum level, Proteobacteria accounted for more than three-fourths of total bacterial populations followed by Actinobacteria (14%) and Cyanobacteria (3%). Commonly used probiotic bacteria (Bacillus, Lactobacillus, Streptococcus, and Lactococcus) were found to be very less prevalent in fish gut. After sequencing data assembly by MEGAHIT v1.1.2 assembler and PROKKA automated analysis pipeline, pathway analysis revealed the presence of 1,608 Metacyc pathways in the fish gut microbiome. Biosynthesis pathways were found to be the most dominant (51%) followed by degradation (39%), energy-metabolism (4%) and fermentation (2%). Almost one-third (33%) of biosynthesis pathways were involved in the synthesis of secondary metabolites. Metabolic pathways for the biosynthesis of 35 antibiotic types were also present, and these accounted for 5% of overall metabolic pathways in the fish gut microbiome. Fifty-one different types of antibiotic resistance genes (ARGs) belonging to 15 antimicrobial resistance (AMR) gene families and conferring resistance against 24 antibiotic types were detected in fish gut. More than 90% ARGs in fish gut microbiome were against beta-lactams (penicillins, cephalosporins, penems, and monobactams). Resistance against tetracycline, macrolides, fluoroquinolones, and phenicols ranged from 0.7% to 1.3%. Some of the ARGs for multi-drug resistance were also found to be located on sequences of plasmid origin. The presence of pathogenic bacteria and ARGs on plasmid sequences suggested the potential risk due to horizontal gene transfer in the confined gut environment.

Keywords : antibiotic resistance, fish gut, metabolic pathways, microbial diversity

Conference Title : ICFAM 2018 : International Conference on Fisheries and Aquaculture Management
Conference Location : Tokyo, Japan
Conference Dates : November 12-13, 2018