Comparative Study of Bacterial Flora in Intestinal Contents of Wild and Farmed *Hypophthalamichthys molitrix* Through High-Throughput Sequencing

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INTRODUCTION

The gastrointestinal tracts of vertebrates are inhabited by very complex assemblage of micro-organisms known as gut micro biota and it has very important role in vertebrate health (Sekirov *et al*., 2010). Although all vertebrates resides a gut micro biota, but at world level most of the research has been done on Mammals and it has disclosed that there is intriguing associations between gut micro biota and diet, anatomy, health and phylogeny of the host (Weldon *et al*., 2015). Up to now 28000 identified fish species represent a much diversified range of ecologies, physiologies and natural histories. Despite of the fact that fish constitutes nearly half of total vertebrate diversity a very little is known about fish gut micro biota (Levêque *et al*., 2007). Fish also harbors various kinds of micro-organisms in its gastrointestinal tracts and it is also of very importance for the health of the specific piscine host (Ringø *et al*., 2016). In world people depends for at least 20% of their protein on fish and consume approximately 20 kg of fish per capita per annum, out of it aquaculture contributes 43.1% of fish production at global level and it is growing with rate of more than 6% per year. Despite of it, fish farming still faces problems like inexplicable mortalities, suboptimal growth, infliction if different diseases and variable low quality of juveniles (Attramadal *et al*., 2012). For all these kind of problems Fish-microbe interactions have been suggested to be a key factor and regarded to be the key factor for this kind of lack of reproducibility issues (Vadstein *et a*l., 2013).

Silver Carp (Family: Cyprinidae) is a very common fish at global level and considered to be the best for stocking and aquaculture. It is regarded to be the most important and appropriate specie of cultured fish globally (Kamilov *et al*., 2014). In Pakistan the Silver Carp is the most dominant fish in freshwater biomes of the Punjab and other provinces because of their appropriate reproductive potential and easy feeding (Khan *et al*., 2011). Because of high fish consumption at global level, research in gut micro biota has been very fascinating and happening since long time but as aquaculture industry is expanding the interest in this field is also increasing rapidly (Egerton *et al*., 2018). In the start, conventional culture-dependent methods were used and it has been revealed that by using these kind of teqniques not more than 10% of the total microbiota can be isolated (Hiergeist *et al*., 2015). High through-put next-generation sequencing is the latest method of analysis at molecular level. These are fast, swift and cost-effective and also deliver in-depth and highly accurate sequence data that gives much greater information on even low abundance micro biota (Rimoldi *et al*., 2018).

OBJECTIVES

1. To determine the gut bacterial community of wild and farmed *H. molitrix*, using data from high- throughput sequencing of a single “universal” bacterial marker 16s rRNA.

2. To compare the bacterial communities in Gut of wild and farmed *H. molitrix* through next generation sequencing.

MATERIALS AND METHODS

Wild type specimen was caught at natural unconfined location (River Indus, Khushal Garh Kohat) and farmed specimens were taken from aquaculture, a confined location (Tanda Fish Hatchery Kohat). After dissection, the intestinal contents were carefully collected and genomic DNA was separately extracted and stored (Ghanbari *et al*., 2015). The integrity of the DNA samples was checked visually using agarose gel (containing ethidium bromide) electrophoresis and quantified using fluorometer. The DNA concentration was determined by using a fluorescence spectrophotometer (Romero and Navarrete., 2006). PCRs were performed and in the process universal forward and reverse primer were used. The products amplified by PCR were then examined by gel electrophoresis and then purified by using the Gel Extraction Kit so that any unspecific DNA fragments can be removed and quantitated by using Bio analyzer (Navarrete *et al*., 2012). Two samples with excellent DNA bands were selected and were sequenced. DNA samples were submitted to the Biotechnology Center for preparation and next generation sequencing illuminia of 16S gene amplicons. Cloned 16S rRNA gene sequences obtained in this study were then deposited in the GenBank database and MG RAST (Van Kessel *et al*., 2011).

RESULTS & DISCUSSIONS

Total of 86871 sequences were detected in domestic and 133194 in wild fish. These were then delineated into maximum of 1038 OTUs with 99% sequence similarity ratio. Average read length was 300 base pairs. Total of 15 various phyla were detected and identified, dominated by Proteobacteri, Bacteroidetes and Firmicutes in both samples, with very different relative abundance. Total of 21 different classes were clearly detected and identified. Most prevalent communities at class level were Gammaproteo bacteria, Bacteroidia, Clostridia and Bacilli with very different relative abundance. Total 26 different orders were identified, dominated by Cardiobacteriales, Clostridiales, Flavobacteriales, Pseudomonadales, Bacteroidales, Lactobacillales and Enterobacteriales with very different relative abundance. Total of 61 families were identified. Most prevalent families were Bifidobacteriaceae, Corynebacteriaceae, Microbacteriaceae, Eggerthellaceae, Peptococcaceae with very high relative abundance. Total of 140 different Genus were identified distinctively. Most prominent Genuses were Koukoulia, Myroides, Ignatzschineria, Pseudomonas, Bacteroides with considerable variation in relative abundance in different samples. The most considerable diversity was detected at species level. Total of 125 species were identified and high differences were present in their relative abundance. The most abundant speciespresent were *Pseudomonas; Poultry manure MUC7, Bacteroides SB5, bacterium 28W232, Bacteroides salanitronis* and *Bacteroidaceae SV452*. Considerable differences were present in various taxa especially at Genus and species level. The micro biota of domestic fish was greatly diversified than the wild fish.

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