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Microbiome analysis of gut bacterial communities of healthy and diseased Malaysian mahseer (*Tor tambroides*) using 16S rRNA metagenomics approach

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ABSTRACT

Aims: The gut microbiota is referred to as an 'extra organ' and is critical in assisting the host in terms of nutrition and immunity. Environmental stressors could alter the gut microbial community and cause gut inflammation. This study aimed to investigate and compare the gut microbiota community between healthy and diseased *Tor tambroides*.
Methodology and results: In this study, such gut microbial alterations were explored using NGS-based 16S rDNA targeted sequencing on the Malaysian mahseer (*T. tambroides*). Three healthy adult and three diseased adult Malaysian mahseers (showing signs of exophthalmia, coelomic distension and petechial haemorrhage) were obtained from LTT Aquaculture Sdn Bhd. Our results revealed significant differences in microbial diversity, composition and function between both populations of *T. tambroides*. Alpha diversity analysis depicts lower diversity of gut microbiota composition in diseased *T. tambroides* as compared to the healthy group. In particular, Enterobacteriaceae, *Aeromonas*, *Bacteroides*, *Vibrio* and *Pseudomonas* were found within gut microbiota of the diseased fishes. In addition, cellulose-degrading bacteria and protease-producing bacteria were identified from the gut of *T. tambroides*.
Conclusion, significance and impact of study: Thus, our findings emphasized on the association between the alteration in gut microbiota composition and infectious abdominal dropsy (IAD) in *T. tambroides*. This finding is important to provide basic information for further diagnosis, prevention and treatment of intestinal diseases in fish.

Keywords: 16S rRNA gene, gut microbiota, infectious abdominal dropsy, Malaysian mahseer, metagenome

INTRODUCTION

Within the past few decades, study of microbiota within gastrointestinal tract (GIT) had been achieving remarkable progress with the discovery of more GIT microbiome communities on a host organism (Li *et al.*, 2016; Liu *et al.*, 2016; Egerton *et al.*, 2018; Tran *et al.*, 2018; Butt and Volkoff, 2019; Tan *et al.*, 2019; Burtseva *et al.*, 2021). As of today, there are two main approaches to discover on gut microbiota community: culture-dependent microbiological methods and culture-independent methods. The classical method involved seeding gut sample directly on either selective or universal media (Hovda *et al.*, 2007; Tarnecki *et al.*, 2017) while the later involved DNA barcoding. For instance, denaturing gel electrophoresis, qPCR and fluorescence *in situ* hybridization (Hovda *et al.*, 2007; Tarnecki *et al.*, 2017; Egerton *et al.*, 2018). As culture-dependent methods are time-consuming and selective, it is unable to provide the entire microbial diversity of complex environments (Hovda *et al.*, 2007). Thus, NGS-based method involving metabarcoding based on 16S rRNA gene is now a popular method among researchers to undercover more uncultured forms of microorganisms and estimate different bacterial groups within the sample as it is able to describe both cultivable and uncultivable bacteria (Tarnecki *et al.*, 2017; Egerton *et al.*, 2018).

Gut microbiota is considered as an 'extra organ' due to its important role in intestinal development, immunological protection, growth and health and homeostasis (O' Hara and Shanahan, 2006). Thus, various studies comparing the gut microbiota composition between healthy and diseased freshwater fish, including largemouth bronze gudgeon (*Coreius guichenoti*) suffering from furunculosis (Li *et al.*, 2016), Crucian carp (*Carassius auratus*) suffering from "red-operculum" disease (Li *et al.*, 2017) and grass carp (*Ctenopharyngodon idellus*) suffering from enteritis had been done to further understand on its role. Myriad diversity of mutualistics, commensal and pathogenic microbes within the intestinal tube would assist the host in terms of protection against infectious agents, nutrients

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