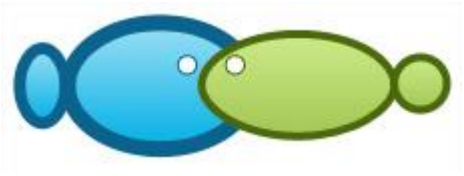

# Gut microbiome associated with cultured Malaysian mahseer *Tor tambroides*

[1]Shumpei Iehata, [1]Mohamad Z. M. Nosi, [2]Muhd Danish-Daniel, [1]Noor E. Sharifah

[1] Faculty of Fisheries and Food Science, University of Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia; [2] Institute of Marine Biotechnology, University of Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia. Corresponding author: S. Iehata, shumpei@umt.edu.my

**Abstract**. In this study, 16S rRNA gene amplicon sequencing and PICRUSt analysis were carried out to better understand the cultured Malaysian mahseer (*Tor tambroides*) associated gut microbiome profile from three different culture farms. Principal Coordinates Analysis demonstrated that gut microbiome diversity had similar trend within same culture location and divergent among different location. 16S rRNA gene amplicon sequencing result showed that the dominant phylum such as Actinobacteria, Bacteroidetes, Firmicutes, and Fusobacteria was distinctly depending on their location, although phylum Proteobacteria was one of the core-gut bacterial community regardless of the location. Interestingly, some *Propionibacteriaceae*, which produce propionic acid by fermented plant materials, were detected only in Terengganu sample that fed only vegetables. Similarly, PICRUSt result showed the highest propionate metabolism in Terengganu. These results indicate that gut microbiome composition of *T. tambroides* seems to vary significantly among different culture locations and were highly dependent on their culture location, especially on their diet type. Furthermore, we found some beneficial bacterial DNA which have high possibility to be utilized as potential probiotics for *T. tambroides* culture. The present study provides new insights into the understanding on the gut microbiome structure of *T. tambroides* from different culture locations, and provides the relevant management information of combination of diet type and bacterial manipulation for development of sustainable aquaculture practices for this species, although we need further research on comparative analysis of the microbiome among gut and environmental sample.

**Key Words**: 16S rRNA gene amplicon sequencing, linear discriminant analysis (LDA) effect size (LEfSe), Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), *Propionibacteriaceae.*

**Introduction**. It is known that gut microbiota plays crucial role in host metabolism, nutrition, and health maintenance (Nicholson et al 2005; Nayak 2010). Moreover, Ramírez et al (2018) reported that understanding the gut bacterial community composition could give the relevant information on the fish diet requirements for the development of sustainable aquaculture. On the other hand, fish gut microbiota composition has been affected by several factors including diet composition, gut shape, host habitat and surrounding environment (McIntosh et al 2008; Sullam et al 2012; Wong & Rawls 2012; Eichmiller et al 2016; Egerton et al 2018). In particular, Ringø et al (2016) suggested that gut microbiota composition of aquatic animals are more sensitive to dietary changes than that of terrestrial animals. Similarly, Miyake et al (2015) indicated that diet type is strongly influenced on fish gut microbiota composition. Until now, it has been gradually revealed the fish bacterial community composition by means of NGS approach (Star et al 2013; Xia et al 2014; Ghanbari et al 2015; Li et al 2015; Li et al 2018b). In the recent years, the cyprinid gut microbiota composition and their diversity have been well documented in the grass carp, *Ctenopharyngodon idella* (Han et al 2010; Wu et al 2012). In addition, there are increasing amount of studies concerning to the gut microbiota in cyprinids using NGS approach as well in other fish species (Wu et al 2013; Ni et al 2014; Ye et al 2014; Kashinskaya et al 2015; Borsodi et al 2017; Li et al 2017a; Tran et al 2018a). However, there are only a few reports on Malaysian mahseer

(*Tor tambroides*) associated gut microbiome composition unlike other cyprinid. Our previous research by combination of traditional culture-depend method and PCR-DGGE revealed that gut bacterial community composition of cultured *T. tambroides* was continuously shifted in different developmental stages (Mohd Nosi et al 2018). Moreover, Tan et al (2019) showed different gut bacterial community composition between wild and captive *T. tambroides* and more diverse in wild *T. tambroides* gut bacterial community than captive one using NGS. Since *T. tambroides* is one of the most valuable freshwater fish with high demand in Malaysia and known as slow-growing species in the cyprinid family (Ng et al 2008), there are several researches that focused on their feed development especially dietary lipid composition (Ng et al 2011; Kamarudin et al 2018). To our concern, the information on gut microbiome of *T. tambroides* is still limited unlike other research like nutritional aspects and breeding. The aim of present study is to reveal comprehensive gut microbiome composition associated with cultured *T. tambroides* with different diets at different culture locations by 16S rRNA gene amplicon sequencing. Comparative analysis of different culture locations sample fed different diets might show core bacteria that could have high possibility to be utilized as probiotics to improve the growth of this slow growing carp.

## Material and Method

***Samples preparation***. *T. tambroides* were collected from three different local farmer at Kota Bharu (6°4'30.245" N, 102°17'20.183" E) for Kelantan sample, Kuantan (3°47'39.811" N, 103°16'0.174" E) for Pahang sample, and Kuala Berang (4°53'15.173" N, 103°2'24.529" E) for Terengganu sample. Each *T. tambroides* was fed with combination of commercial pellet (Akafuji, JPD, Japan) and fruits (banana, papaya) for Kelantan sample, fed with combination of commercial pellet (AquaDine®, USA) and fruits (banana, grape) for Pahang sample, and fed with only vegetables such as cabbage and yardlong bean for Terengganu sample. Three healthy samples from each location; Kelantan (752.68±91.90 g), Pahang (1,158.34±201.27 g), and Terengganu (412.28±144.66 g) were randomly collected and anesthetized using a dose (100 mg $L^{-1}$) of tricaine methanesulfonate (Finquel MS-222, Sigma-Aldrich, Missouri, USA). After removing any debris and external bacteria on fish body surface using sterile 0.01 M phosphate buffer saline (PBS; Sigma-Aldrich Cheme GmbH, Munich, Germany) and 70 % ethanol, whole gut was aseptically taken, washed three times with sterile 0.01 M PBS, transferred into sterile tubes, and stored at -80°C for 16S rRNA gene amplicon sequencing analysis. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. And the studies in this manuscript were approved by UMT Biosecurity and Ethics Committee (UMT/JKEPHMK/2020/45).

***DNA extraction, PCR amplification, Library preparation, and Bioinformatic analysis for 16S rRNA gene amplicon sequencing analysis using Illumina Miseq Platform***. Promega DNA purification system (Promega, Madison, WI, USA) was used for bacterial genomic DNA from whole gut sample from each location. Then, DNA concentration and quality was evaluated by 1% w $v^{-1}$ agarose gel electrophoresis, Nanodrop™ 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and Qubit® 2.0 Fluorometer (Invitrogen, Merelbeke, Belgium). Thereafter, each genomic DNA sample was amplified for V4 region of 16S rRNA gene with unique 8bp barcord containing universal primers (515F/806R), purified using Qiagen Gel Extraction kit (Qiagen, Germany), and then sequencing was conducted using the Illumina MiSeq platform (Illumina, Sandiego, USA) at Shanghai Megiddo Biological Pharmaceutical Co., Ltd. (Shanghai, China).

Bioinformatics analyses were conducted using QIIME (Quantitative Insights Into Microbial Ecology) ver 1.9.1 (Caporaso et al 2010). Pairs of reads from the original DNA fragments were merged by PEAR. The fastq-formatted sequences were quality checked, and ambiguous sequences were filtered with quality score of 30 and 80 % coverage using FASTX-toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and usearch (https://www.drive5.com/usearch/), respectively. After pre-processing, reference-based and chimera sequences were checked and removed from the cleaned sequences using

usearch61, and operational taxonomic unit (OTU) clustering was performed with a 0.97 threshold using uclust (Edgar 2010) and representative sequence were assigned to reference sequence in the SILVA database. Thereafter, five alpha diversity metrics (Observed OTUs, rarefaction curve, invsimpson, Chao1, and Shannon index) for each sample were estimated using QIIME. To evaluate the community divergence (beta diversity), we used Principal Coordinates Analysis (PCOA) based on weighted UniFrac distance to visualize the community composition differences among different culture locations using QIIME, and Venn diagrams to illustrate shared bacterial OTUs within same location sample and among different culture locations using MOTHUR (Schloss et al 2009). We also performed linear discriminant analysis effect size (LEfSe) (Segata et al 2011) to identify the differences of relative abundance of bacterial taxa among different culture location sample using the Huttenhower laboratory Galaxy web application (http://huttenhower.sph.harvard.edu/galaxy/). The Kruskal–Wallis test was conducted to identify bacterial taxa that are significantly different ($P<0.05$) in relative abundance among different samples. The linear discriminant analysis (LDA) identifies the effect size with which these taxa differentiate the samples with thresholds of a log-transformed LDA score of 4.0. All sequencing data (three replicate of *T. tambroides* gut microbiome from three different culture locations) were submitted to NCBI Sequence Read Archive (SRA) under accession number of PRJNA575603.

***Predicted gut microbiome function using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States)***. PICRUSt was used to predict the metabolic functions of the microbial communities in each sample using Galaxy web application (Langille et al 2013). After pick OTU at 0.97 threshold with GreenGene database (version 13.8), normalization of 16SrRNA copy numbers and the microbiome functions categorized at levels 2 and 3 were predicted with referenced to Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology (KO) database (Kanehisa et al 2012). Triplicate samples were used in all experiments. The assumptions of normal distributions and homogeneity of variances were checked before analysis. Data from each location sample was subjected to one-way analysis of variance (ANOVA) using SPSS (version 22; IBM, Chicago, IL, USA) followed by Tukey multiple range test to compare the differences between treatments, where significant differences ($P<0.05$) were observed.

## Results

***16S rRNA gene metagenomic sequencing analysis***. After demultiplexing, filtering out poor quality sequences and removing Chimeric sequences, 16S rRNA gene metagenomic sequencing, we obtained a total of 327,203 bacterial sequences and the average number of sequences per each location samples ranged from 35,101±3,536 to 37,375 ± 5,297, and the average number of OTUs per each location was from 924±107 to 1,912±481 (Table 1). Alpha diversity index (Invsimpson, Shannon index, and Chao1) showed that Terengganu sample was lower than the other locations (Kelantan and Pahang) (Table 1).

Table 1

Summary of alpha diversity index of captive *Tor tambroides* gut microbiome associated with different locations in Malaysia

| *Location* | *No of sequences* | *No of OTU* | *Invsimpson* | *Shannon index* | *Chao1* |
|---|---|---|---|---|---|
| Kelantan | 37375±5297 | 1912±481 | 6.12±3.37 | 4.53±0.71 | 7019.3±2230.4 |
| Pahang | 36591±4497 | 1736±623 | 6.64±0.54 | 4.32±0.44 | 8422.6±4778.0 |
| Terengganu | 35101±3536 | 924±107 | 4.70±2.70 | 3.20±0.69 | 3480.3±425.1 |

To investigate the gut microbiome in different culture locations, the shared and unique OTUs were analyzed using a pairwise comparison and Venn diagram (Figure 1). Figure 1a

shows that less than 20% of OTUs was shared between each location samples, and the ration of core OTUs was only 3.55% among different culture locations. The number of shared OTUs varied from 37 to 143 and that of unique OTUs varied from 136 to 432, and core OTUs among these samples was 31 (Figure 1b). PCOA analysis based on weighted UniFrac distance revealed a different gut bacterial composition among different sample locations (Figure 2).

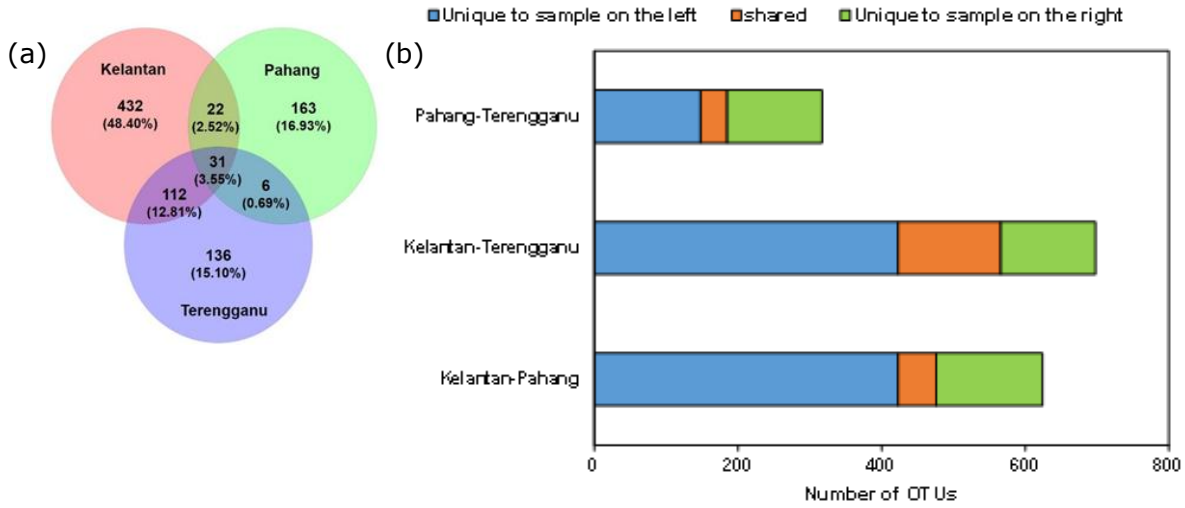


Figure 1. Unique and shared OTUs in different culture location of *Tor tambroides* gut samples. (a) The amount of shared and unique OTUs between different culture locations gut samples at 30% cut off level. Venn diagram of shared and unique OTUs (b) between different culture location gut samples. The percentage in the Venn diagram shows the ratio of the sequences associated to the OTUs in total sequences.

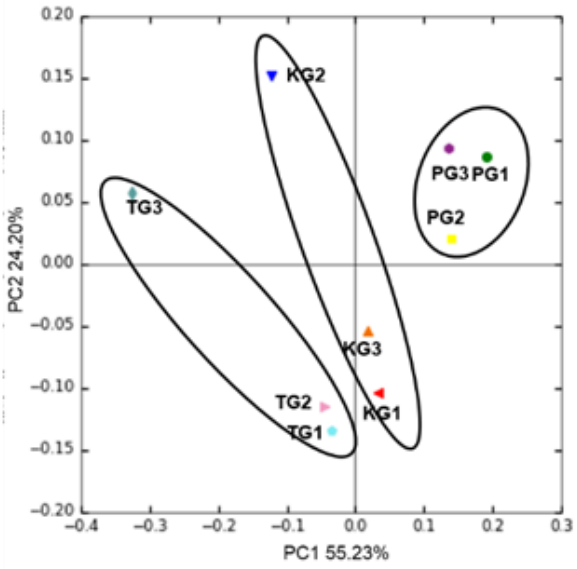


Figure 2. Beta diversity estimation of *Tor tambroides* gut microbiome in the different culture locations using Principal Coordinates Analysis (PCOA) based on weighted UniFrac distances.

***Gut microbiome composition of different culture locations***. The abundance of 6 major phyla was observed from the gut microbiome composition related with *T. tambroides* across three different culture locations (Figure 3a). Phylum Proteobacteria was the prominent phyla in Kelantan and Terengganu samples as representing 57.7 and 57.8% respectively, whereas that phyla was one of prominent phyla in Pahang sample with 37.4%. The phylogenetic affiliation of those OTUs with marked different abundance was *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Fusobacteia*. *Actinobacteia* accounted

for 1.41, 0.028, and 15.92 % of total sequences in Kelantan, Pahang, and Terengganu respectively, whereas *Bacteroidetes* was 2.29, 18.36, and 2.35 % of total sequences in Kelantan, Pahang, and Terengganu respectively. The ration of *Fusobacter* in Pahang was one of prominent phyla in Pahang sample with 35.84%, while 13.34% in Kelantan and only 0.98% in Terengganu. The abundance of phylum Firmicutes was 15.56 and 10.80% in Kelantan and Terengganu, but dropped to 2.66% in Pahang sample. On the other hand, the abundance of phylum Tenericutes ranged from 3.37 to 11.56% across three different culture locations. There was substantially difference between 18 bacterial taxa (Figure 3b). Of these 18 bacterial taxa, 4 bacterial taxa (*Blautia*, *Acinetobacter*, *Pseudomonas*, and *Pseudoalteromonas*) showed much higher relative abundance in Kelantan sample, 4 bacterial taxa (*Bacteroides*, *Cetobacter*, and *Shewanella*) in Pahang sample, and 5 bacterial taxa (Actinomycetales, *Propionicimonas*, Propionibacteriaceae, *Proteiniclasticum*, and Aeromonadaceae) in Terengganu sample. On the other hand, the relative abundance of Genus *Citrobacter* in Kelantan sample and Genus *Plesiomonas* in Terengganu sample was much lower compare to other sample locations (Figure 3b).

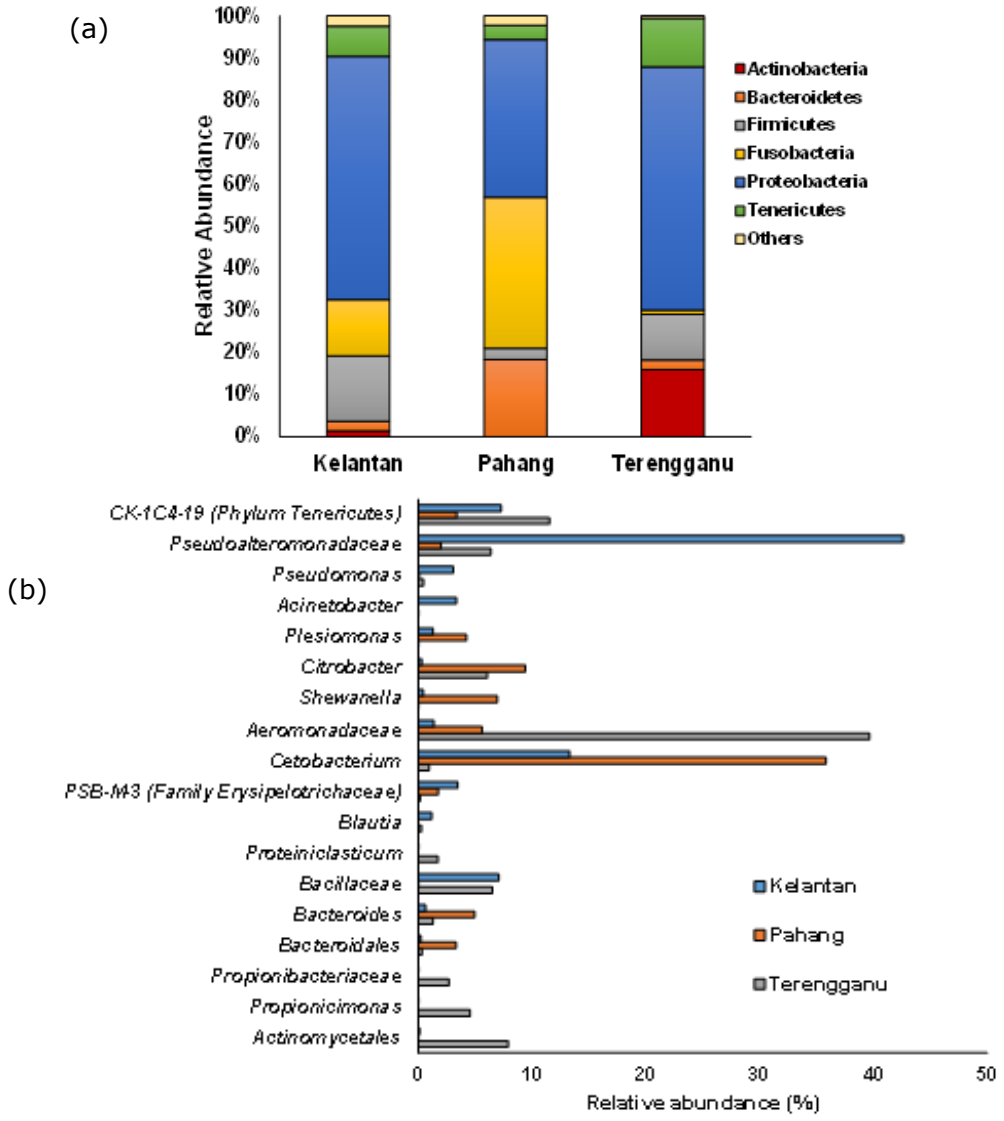


Figure 3. Difference of gut microbiome across different culture location sample of *Tor tambroides* gut. (a) Microbial community-relative abundance at phylum level with rare taxa cut off at < 3 % relative abundance on the gut of *T. tambroides* associated with different culture locations. (b) The 18 bacterial taxonomic rank showing substantially different in abundance across different culture location sample of *T. tambroides* gut.

LEfSe (LDA>4.0, P<0.05) revealed that a total of 20 bacterial biomarkers at different bacterial taxonomic level were differentially abundant among three different culture locations (Figure 4). Shewanellaceae, Alteromonadales, and Fusobacteria were the superior biomarkers in Pahang sample, and the superior bacterial biomarker in Kelantan was Prevotellaceae and Pseudomonadales. On the other hand, Actinomycetales including Propionibacteriaceae was the top order level biomarker in Terengganu sample (Figure 4).

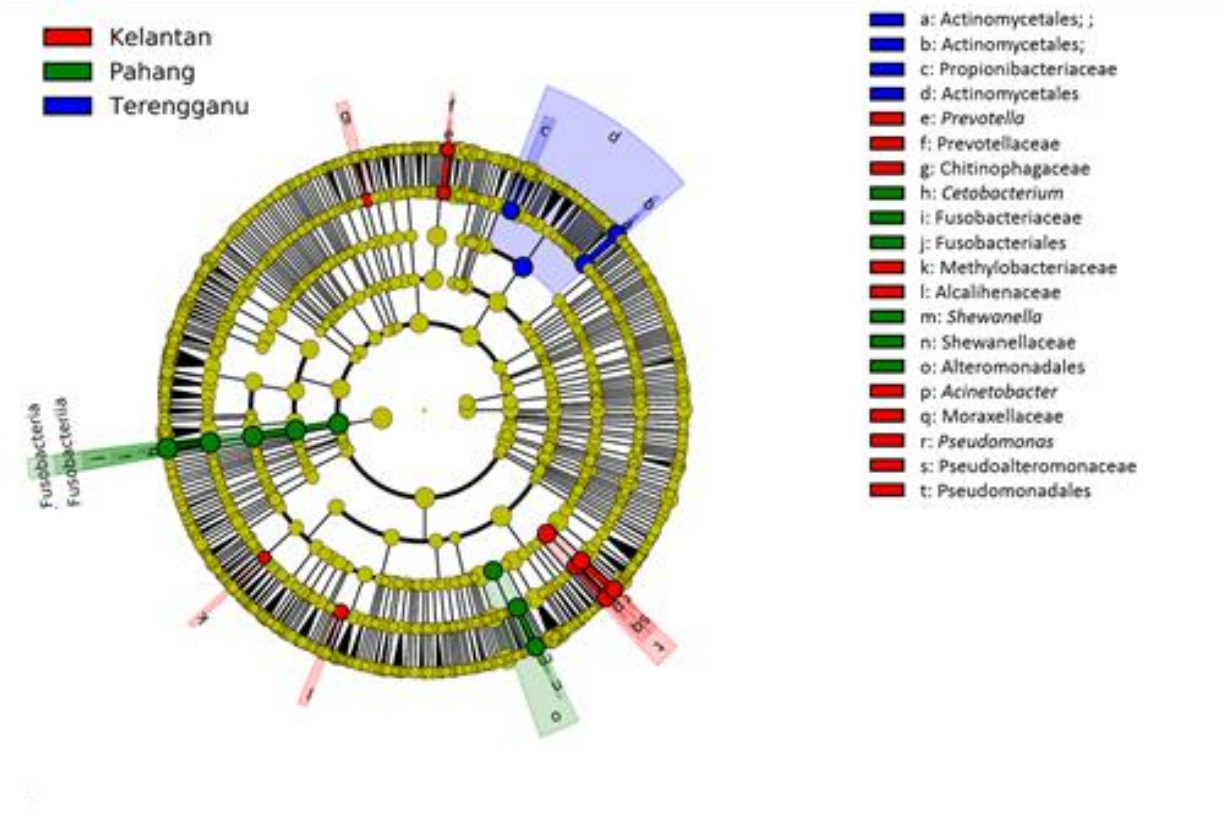


Figure 4. Unique community composition of biomarkers by the linear discriminant analysis (LDA) effect size (LEfSe) (LDA score >4.0, Kruskal–Wallis test, P<0.05) analysis shows the discriminating bacterial taxa among three different culture location of *T. tambroides* gut.

***Predicted gut microbiome function using PICRUSt***. PICRUSt result carried on to predict the *T. tambroides* gut microbiome function in three different location with different diet showed the divergence of the nine gene function at level 3 with statistically significant differences at P<0.05 (Figure 5). Of these 9 gene functions, the abundance of seven metabolism including (Fructose and mannose, Galactose, Glycoxylate and dicarboxylate, Pentose and glucuronate, and Starch and sucrose metabolism) in Pahang sample showed highest function than other two locational samples, whereas fatty acid metabolism was highest and Propionate metabolism exhibited significantly higher function in fish gut sample from Terengganu (Figure 5).

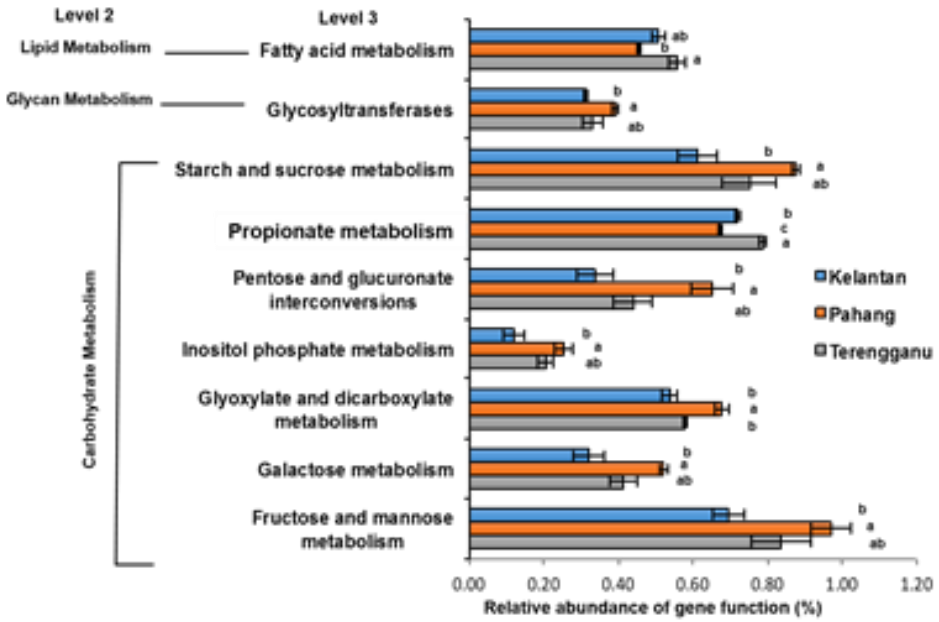


Figure 5. Comparison in the relative abundance of PICRUSt generated gene functional profile of gut microbiome among different location sample *Tor tambroides*. Values are means (±SE) of triplicates of each location sample and the mean values followed by the different superscript letter in gene categories at level 3 indicate significant difference ($P<0.05$) among different location sample. If the effects were significant, ANOVA was followed by Tukey test.

**Discussion**. In the present study, 16S rRNA gene amplicon sequence was performed to characterize of *T. tambroides* related gut microbiome composition and their predicted metabolic gene function. Michl et al (2017) indicated that alpha diversity index was decreased with increasing of plant based protein level in the diet. This supported our alpha diversity result, in which Terengganu sample fed plant based diet showed the lowest alpha diversity index. Kashinskaya et al (2018) showed higher similarity of gut microbiota in the Prussian carp (*Carassius gibelio*) with the food source compared to the external environments. Ni et al (2012) reported that there is no significant difference of *C. idella* gut microbiota composition within cultured or natural captive at different sampling time, suggesting that the carp gut microbiota composition is less influenced by their habitat such as aquaculture water. These information support our findings from PCOA result of 16S rRNA gene amplicon sequencing analysis.

16S rRNA gene amplicon sequencing result in the present study showed that Proteobacteria was one of predominant phylum regardless of location. Interestingly, Firmicutes and Fusobacteria constituted core gut microbiome in Kelantan sample, and Bacteroidetes and Fusobacteria were secondary dominated in Pahang sample. On the other hand, Actinobacteria and Firmicutes were the predominant phyla in Terengganu sample. These results indicate that although phylum Proteobacteria was mainly composed of indigenous bacteria in *T. tambroides* gut, other core gut microbiome composition might be attributed to their environmental factors such as diets. In particular, the abundance of genus *Cetobacterium* of Fusobacteria from Terengganu sample was substantially lower compared to the other two locations, while some Actinobacteria such as *Propionibacteriaceae* detected from only Terengganu sample that fed only vegetables. Similarly, LEfSe results showed that relative abundance of Actinobacteria was significantly higher in Terengganu sample. This is consistent with our PICRUSt result in which the abundance of Propionate metabolism exhibited highest activity in Terengganu, whilst that in Pahang showed lowest metabolic activity. These results might be explained due to their diet types, that Kelantan and Pahang were fed combination of artificial diet and fruits, whereas Terengganu fed only vegetable. This is supported by several previous reports in which gut microbiota was influenced by their feeding habits (Kashinskaya et al 2018; Ingerslev et al 2014; Li et al 2017b; Li et al

2018a; Tran et al 2018b). Moreover, Michl et al (2017) suggested that both gut microbiome community and their diversity are strongly affected by plant base protein level in diet. Liu et al (2016) indicated the abundance of *Cetobacterium* was significantly higher in carnivore fish gut than herbivore fish gut. In addition, there are some reports that plant base diet could significantly reduce the abundance of *Cetobacterium* in *C. idella* gut bacterial community (Hao et al 2017; Feng et al 2019). Similarly, there was no detection from plant based diet fed rainbow trout (*Oncorhynchus mykiss*) gut bacterial community (Gatesoup et al 2018). Tsuchiya et al (2008) reported acetic and propionic acid production as well as Vitamin B12 (cobalamin) via fermentative metabolism by *Cetobacterium*. On the other hand, Actinobacteria was one of gut bacterial member in both freshwater and marine fish (Ghanbari et al 2015; Warnecke et al 2004; Jami et al 2015; Song et al 2016; Tran et al 2017). Some *Propionibacteriaceae* of Actinobacteria produced propionic acid by plant and glucose fermentation, and considered as one of possible probiotics for human (Falentin et al 2010; Nielsen et al 2012; Pophaly et al 2012; Zárate et al 2017). Moreover, Chamlagain et al (2018) reported that *Propionibacterium* produced Vitamin B12 by cereal material fermentation. Thus, these might be the explanation about the lowest abundance of *Cetobacterium* and highest abundance of Actinomycetes like *Propionibacteriaceae* found in Terengganu sample compare to another two locations, since *Propionibacteriaceae* detected from Terengganu sample might play a role to host nutrition such as providing Vitamin B12 and propionic acid via vegetable fermentation in place of *Cetobacterium*.

In addition, we found some beneficial bacteria such as *Aeromonas*, *Citrobacter*, *Pseudoalteromonas* and *Shewanella*, since these bacteria contribute to host nutrition by producing some digestive enzyme (Ray et al 2012). The genus *Aeromonas* was the main component of the gut at all the growth stage, and both wild and cultured *T. tambroides* gut, whereas *Citrobacter* and *Shewanella* were one of gut bacteria community member of *T. tambroides* (Mohd Nosi et al 2018; Tan et al 2019). It is well-known that certain *Aeromonas* species play significant role in the production of enzyme and help in digestion process (Banerjee & Ray 2017). *Citrobacter* has been known as the bacteria that possess some digestive enzyme such as amylase, protease, and cellulose activity (Wu et al 2012; Ye et al 2014; Ray et al 2010). *Shewanella* was known as main PUFA (poly unsaturated fatty acid) producing bacteria from fish and invertebrate gut (Monroig et al 2013). Furthermore, there are some reports on utilization of *Shewanella* as probiotics to improve fish gut environment and their growth (Asaduzzaman et al 2018; Lobo et al 2014). Similarly, genus *Pseudoalteromonas* has been reported as antimicrobial metabolites producing bacteria and probiotics utilization (Offret et al 2016; Wang et al 2018). These suggest the high possibility that these bacteria could be the indigenous bacteria in *T. tambroides* gut microbiome that contribute to host digestion.

**Conclusions**. The present study is the first study comprehensively investigated on the gut microbime composition and their predicted functional gene associated with economically important *T. tambroides* under different culture locations using 16S rRNA gene amplicon sequencing. *T. tambroides* gut samples from different locations were composed of unique and various microbiome composition, and impacted by their culture condition. The core bacteria in all location sample comprised phylum Proteobacteria, whereas the abundance of Actinobacteria, Bacteroidetes, Firmicutes, and Fusobacteria were mainly depending on the culture condition and their diet type. In particular, the abundance of *Cetobacterium* and *Propionibacteriaceae* seems to be strongly affected by the diet type and these bacteria might be key bacteria in *T. tambroides* gut microbiome to have an important role in host digestion. Moreover, some beneficial bacteria for potential probiotics were found. However, further investigations should be carried out in combination with specific factors such as type of food sources, and abiotic factor including water quality as well as comparative analysis of the microbiome composition among gut and environmental sample to obtain more comprehensive information regarding to gut microbiome composition in *T. tambroides*.

**Acknowledgements**. This research was kindly funded by Ministry of Higher Education (MOHE) of Malaysia under grant of Fundamental Research Grant Scheme (FRGS) (Vot 59370).

**References**

Asaduzzaman M., Iehata S., Akter S., Kader M. A., Ghosh S. K., Khan M. N. A., Abol-Munafi A. B., 2018 Effects of host gut-derived probiotic bacteria on gut morphology, microbiota composition and volatile short chain fatty acids production of Malaysian Mahseer *Tor tambroides*. Aquaculture Reports 9:53-61.

Banerjee G., Ray A. K., 2017 Bacterial symbiosis in the fish gut and its role in health and metabolism. Symbiosis 72:1-11.

Borsodi A. K., Szabó A., Krett G., Felföldi T., Specziár A., Boros G., 2017 Gut content microbiota of introduced bigheaded carps (*Hypophthalmichthys* spp.) inhabiting the largest shallow lake in Central Europe. Microbiological Research 195:40-50.

Caporaso J. G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F. D., Costello E. K., Fierer N., Peña A. G., Goodrich J. K., Gordon J. I., Huttley G. A., Kelley S. T., Knights D., Koenig J. E., Ley R. E., Lozupone C. A., Mcdonald D., Muegge B. D., Pirrung M., Reeder J., Sevinsky J. R., Turnbaugh P. J., Walters W. A., Widmann J., Yatsunenko T., Zaneveld J., Knight R., 2010 QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335-336.

Chamlagain B., Sugito T. A., Deptula P., Edelmann M., Kariluoto S., Varmanen P., Piironen V., 2018 *In situ* production of active vitamin B12 in cereal matrices using *Propionibacterium freudenreichii*. Food Science & Nutrition 6:67-76.

Edgar R. C., 2010 Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460-2461.

Egerton S., Culloty S., Whooley J., Stanton C., Ross R. P., 2018 The gut microbiota of marine fish. Frontiers in Microbiology 9 doi.org/10.3389/fmicb.2018.00873

Eichmiller J. J., Hamilton M. J., Staley C., Sadowsky M. J., Sorensen P. W., 2016 Environment shapes the fecal microbiome of invasive carp species. Microbiome 4:44. doi.org/10.1186/s40168-016-0190-1

Falentin H., Deutsch S. M., Jan G., Loux V., Thierry A., Parayre S., Maillard M. B., Dherbécourt J., Cousin F. J., Jardin J., Siguier P., Couloux A., Barbe V., Vacherie B., Wincker P., Gibrat J. C., Gaillardin C., Lortal S., 2010 The complete genome of *Propionibacterium freudenreichii* CIRM-BIA1T, a hardy Actinobacterium with food and probiotic applications. PloS one 5:e11748. doi.org/10.1371/journal.pone.0011748

Feng W., Zhang J., Jakovlić I., Xiong F., Wu S., Zou H., Li W., Li M., Wang G., 2019 Gut segments outweigh the diet in shaping the intestinal microbiota composition in grass carp *Ctenopharyngodon idellus*. AMB Express 9:44. doi.org/10.1186/s13568-019-0770-0

Gatesoupe F. J., Fauconneau B., Deborde C., Madji Hounoum B., Jacob D., Moing A., Corraze G., Médale F., 2018 Intestinal microbiota in rainbow trout, *Oncorhynchus mykiss*, fed diets with different levels of fish-based and plant ingredients: A correlative approach with some plasma metabolites. Aquaculture Nutrition 24:1563-1576.

Ghanbari M., Kneifel W., Domig K. J., 2015 A new view of the fish gut microbiome: advances from next-generation sequencing. Aquaculture 448:464-475.

Han S., Liu Y., Zhou Z., He S., Cao Y., Shi P., Yao B., Ringø E., 2010 Analysis of bacterial diversity in the intestine of grass carp (*Ctenopharyngodon idellus*) based on 16S rDNA gene sequences. Aquaculture Research 42:47-56.

Hao Y. T., Wu S. G., Xiong F., Tran N. T., Jakovlić I., Zou H., Li W. X., Wang G. T., 2017 Succession and fermentation products of grass carp (*Ctenopharyngodon idellus*) hindgut microbiota in response to an extreme dietary shift. Frontiers in Microbiology 8:1585. doi.org/10.3389/fmicb.2017.01585

Ingerslev H. C., von Gersdorff Jørgensen L., Strube M. L., Larsen N., Dalsgaard I., Boye M., Madsen L., 2014 The development of the gut microbiota in rainbow trout

(*Oncorhynchus mykiss*) is affected by first feeding and diet type. Aquaculture 424:24-34.
Jami M., Ghanbari M., Kneifel W., Domig K. J., 2015 Phylogenetic diversity and biological activity of culturable Actinobacteria isolated from freshwater fish gut microbiota. Microbiological Research 175:6-15.
Kamarudin M. S., Bami M. L., Arshad A., Saad C. R., Ebrahimi M., 2018 Preliminary study of the performance of crude illipe oil (*Shorea macrophylla*) as a dietary lipid source for riverine cyprinid *Tor tambroides*. Fish Science 84:385-397.
Kanehisa M., Goto S., Sato Y., Furumichi M., Tanabe M., 2012 KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Research 40*:*D109-D114.
Kashinskaya E. N., Belkova N. L., Izvekova G. I., Simonov E. P., Andree K. B., Glupov V. V., Baturina O. A., Kabilov M. R., Solovyev M. M., 2015 A comparative study on microbiota from the intestine of Prussian carp (*Carassius gibelio*) and their aquatic environmental compartments, using different molecular methods. Journal of Applied Microbiology 119:948-961.
Kashinskaya E. N., Simonov E. P., Kabilov M. R., Izvekova G. I., Andree K. B., Solovyev M. M., 2018 Diet and other environmental factors shape the bacterial communities of fish gut in an eutrophic lake. Journal of Applied Microbiology 125:1626-1641.
Langille M., Zaneveld J., Caporaso J., McDonald D., Knights D., Reyes J. A., Clemente J. C., Burkepile D. E., Thurber R. L. V., Knight R., Beiko R. G., Huttenhower C., 2013 Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology 31:814–821.
Li T., Long M., Gatesoupe F. J., Zhang Q., Li A., Gong X., 2015 Comparative analysis of the intestinal bacterial communities in different species of carp by pyrosequencing. Microbial Ecology 69:25-36.
Li X., Yu Y., Li C., Yan Q., 2018a Comparative study on the gut microbiotas of four economically important Asian carp species. Science China Life Sciences 61:696-705.
Li X., Zhou L., Yu Y., Ni J., Xu W., Yan Q., 2017a Composition of gut microbiota in the gibel carp (*Carassius auratus gibelio*) varies with host development. Microbial Ecology 7(74):239-249.
Li H., Zhu Y., Zhao F., Song S., Li Y., Xu X., Zhou G., Li C., 2017b Fish oil, lard and soybean oil differentially shape gut microbiota of middle-aged rats. Scientific Reports 7:8256. doi:10.1038/s41598-017-00969-0
Li X., Zhu Y., Ringø E., Wang X., Gong J., Yang D., 2018b Intestinal microbiome and its potential functions in bighead carp (*Aristichthys nobilis*) under different feeding strategies. PeerJ 6:e6000. doi.org/10.7717/peerj.6000
Liu H., Guo X., Gooneratne R., Lai R., Zeng C., Zhan F., Wang W., 2016 The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. Scientific Reports 6:24340. doi:10.1038/srep24340
Lobo C., Moreno-Ventas X., Tapia-Paniagua S., Rodríguez C., Morinigo M. A., de La Banda I. G., 2014 Dietary probiotic supplementation (*Shewanella putrefaciens* Pdp11) modulates gut microbiota and promotes growth and condition in *Senegalese sole* larviculture. Fish Physiology and Biochemistry 40:295-309.
McIntosh D., Ji B., Forward B. S., Puvanendran V., Boyce D., Ritchie R., 2008 Culture-independent characterization of the bacterial populations associated with cod (*Gadus morhua L.*) and live feed at an experimental hatchery facility using denaturing gradient gel electrophoresis. Aquaculture 275:42-50.
Michl S. C., Ratten J. M., Beyer M., Hasler M., LaRoche J., Schulz C., 2017 The malleable gut microbiome of juvenile rainbow trout (*Oncorhynchus mykiss*): Diet-dependent shifts of bacterial community structures. PloS one 12:e0177735. doi.org/10.1371/journal.pone.0177735
Miyake S., Ngugi D. K., Stingl U., 2015 Diet strongly influences the gut microbiota of surgeonfishes. Molecular Ecology 24(3):656-672.
Mohd Nosi M. Z., Syed Jamil Fadaak S. N. E., Muhammad M. D. D., Iehata S., 2018 Assessment of gut microbiota in different developmental stages of Malaysian Mahseer (*Tor tambroides*). Aquaculture Research 49:2977–2987.

Monroig Ó., Tocher D., Navarro J., 2013 Biosynthesis of polyunsaturated fatty acids in marine invertebrates: recent advances in molecular mechanisms. Marine Drugs 11:3998-4018.
Nayak S. K., 2010 Role of gastrointestinal microbiota in fish. Aquaculture Research 41:1553-1573.
Ng W. K., Abdullah N., De Silva S. S., 2008 The dietary protein requirement of the Malaysian mahseer, *Tor tambroides* (Bleeker), and the lack of protein-sparing action by dietary lipid. Aquaculture 284:201-206.
Ng W. K., Andin V. C., 2011 The Malaysian mahseer, *Tor tambroides* (Bleeker), requires low dietary lipid levels with a preference for lipid sources with high omega-6 and low omega-3 polyunsaturated fatty acids. Aquaculture 322-323:82-89.
Ni J., Yan Q., Yu Y., Zhang T., 2014 Factors influencing the grass carp gut microbiome and its effect on metabolism. FEMS Microbiology Ecology 87:704-714.
Ni J., Yu Y., Zhang T., Gao L., 2012 Comparison of intestinal bacterial communities in grass carp, *Ctenopharyngodon idellus*, from two different habitats. Chinese Journal of Oceanology Limnology 30:757-765.
Nicholson J. K., Holmes E., Wilson I. D., 2005 Gut microorganisms, mammalian metabolism and personalized health care. Nature Reviews Microbiology 3:431–438.
Nielsen J. L., Nguyen H., Meyer R. L., Nielsen P. H., 2012 Identification of glucose-fermenting bacteria in a full-scale enhanced biological phosphorus removal plant by stable isotope probing. Microbiology 158:1818-1825.
Offret C., Desriac F., Le Chevalier P., Mounier J., Jégou C., Fleury Y., 2016 Spotlight on antimicrobial metabolites from the marine bacteria *Pseudoalteromonas*: chemodiversity and ecological significance. Marine Drugs 14:129. doi.org/10.3390/md14070129
Pophaly S. D., Tomar S. K., De S., Singh R., 2012 Multifaceted attributes of dairy propionibacteria: a review. World Journal of Microbiology and Biotechnology 28:3081-3095.
Ramírez C., Coronado J., Silva A., Romero J., 2018 Cetobacterium is a major component of the microbiome of giant amazonian fish (*Arapaima gigas*) in Ecuador. Animals 8:189. doi.org/10.3390/ani8110189
Ray A. K., Roy T., Mondal S., Ringø E., 2010 Identification of gut-associated amylase, cellulose and protease-producing bacteria in three species of Indian major carps. Aquaculture Research 41:1462-1469.
Ray A. K., Ghosh K., Ringø E., 2012 Enzyme-producing bacteria isolated from fish gut: a review. Aquaculture Nutrition 18:465-492.
Ringø E., Zhou Z., Vecino J. L. G., Wadsworth S., Romero J., Krogdahl Å., Olsen R. E., Dimitroglou A., Foey A., Davies S., Owen M., Lauzon H. L., De Schryver P., Bossier P., Sperstad S., Merrifield D. L., 2016 Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? Aquaculture Nutrition 22:219-282.
Schloss P. D., Westcott S. L., Ryabin T., Hall J. R., Hartmann M., Hollister E. B., Lesniewski R. A., Oakley B. B., Parks D. H., Robinson C. J., Sahl J. W., Stres B., Thalinger G. G., Van Horn D. J., Weber C. F., 2009 Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied Environmental Microbiology 75:7537-7541.
Segata N., Izard J., Waldron L., Gevers D., Miropolsky L., Garrett W. S., Huttenhower C., 2011 Metagenomic biomarker discovery and explanation. Genome Biology 12:R60. doi:10.1186/gb-2011-12-6-r60
Song W., Li L., Huang H., Jiang K., Zhang F., Chen X., Zhao M., Ma L., 2016 The gut microbial community of antarctic fish detected by 16S rRNA gene sequence analysis. BioMed Research International doi.org/10.1155/2016/3241529
Star B., Haverkamp T. H., Jentoft S., Jakobsen K. S., 2013 Next generation sequencing shows high variation of the intestinal microbial species composition in Atlantic cod caught at a single location. BMC Microbiology 13: 248. doi.org/10.1186/1471-2180-13-248

Sullam K. E., Essinger S. D., Lozupone C. A., O'connor M. P., Rosen G. L., Knight R., Kilham S. S., Russell J. A., 2012 Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. Molecular Ecology 21:3363-3378.
Tan C. K., Natrah I., Suyub I. B., Edward M. J., Kaman N., Samsudin A. A., 2019 Comparative study of gut microbiota in wild and captive Malaysian Mahseer (*Tor tambroides*). MicrobiologyOpen e00734. doi.org/10.1002/mbo3.734
Tran N. T., Wang G. T., Wu S. G., 2017 A review of intestinal microbes in grass carp *Ctenopharyngodon idellus* (Valenciennes). Aquaculture Research 48:3287-3297.
Tran N. T., Xiong F., Hao Y. T., Zhang J., Wu S. G., Wang G. T., 2018a Starvation influences the microbiota assembly and expression of immunity-related genes in the intestine of grass carp (*Ctenopharyngodon idellus*). Aquaculture 489:121-129.
Tran N. T., Zhang J., Xiong F., Wang G. T., Li W. X., Wu S. G., 2018b Altered gut microbiota associated with intestinal disease in grass carp (*Ctenopharyngodon idellus*). World Journal of Microbiology and Biotechnology 34:71. doi.org/10.1007/s11274-018-2447-2
Tsuchiya C., Sakata T., Sugita H., 2008 Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. Letters in Applied Microbiology 46:43-48.
Wang H., Wang C., Tang Y., Sun B., Huang J., Song X., 2018 *Pseudoalteromonas* probiotics as potential biocontrol agents improve the survival of *Penaeus vannamei* challenged with acute hepatopancreatic necrosis disease (AHPND)-causing *Vibrio parahaemolyticus*. Aquaculture 494:30-36.
Warnecke F., Amann R., Pernthaler J., 2004 Actinobacterial 16S rRNA genes from freshwater habitats cluster in four distinct lineages. Environmental Microbiology 6:242-253.
Wong S., Rawls J. F., 2012 Intestinal microbiota composition in fishes is influenced by host ecology and environment. Molecular Ecology 21:3100-3102.
Wu S., Wang G., Angert E. R., Wang W., Li W., Zou H., 2012 Composition, diversity, and origin of the bacterial community in grass carp intestine. Plos One 7:e30440. doi:10.1371/journal.pone.0030440
Wu S. G., Tian J. Y., Gatesoupe F. J., Li W. X., Zou H., Yang B. J., Wang G. T., 2013 Intestinal microbiota of gibel carp (*Carassius auratus gibelio*) and its origin as revealed by 454 pyrosequencing. World Journal of Microbiology and Biotechnology 29:1585-1595.
Xia J. H., Lin G., Fu G. H., Wan Z. Y., Lee M., Wang L., Liu X. J., Yue G. H., 2014 The intestinal microbiome of fish under starvation. BMC Genomics 15:266. doi.org/10.1186/1471-2164-15-266
Ye L., Amberg J., Chapman D., Gaikowski M., Liu W. T., 2014 Fish gut microbiota analysis differentiates physiology and behavior of invasive Asian carp and indigenous American fish. The ISME Journal 8:541. doi.org/10.1038/ismej.2013.181
Zárate G., Sáez G. D., Chaia A. P., 2017 Dairy propionibacteria prevent the proliferative effect of plant lectins on SW480 cells and protect the metabolic activity of the intestinal microbiota *in vitro*. Anaerobe 44:58-65.
*** https://www.drive5.com/usearch/
*** http://hannonlab.cshl.edu/fastx_toolkit/
*** http://huttenhower.sph.harvard.edu/galaxy/

Received: 16 October 2020. Accepted: 20 November 2020. Published online: 24 January 2021.
Authors:
Shumpei Iehata, University of Malaysia Terengganu, Faculty of Fisheries and Food Science, Malaysia, Terengganu, 21030 Kuala Terengganu, e-mail: shumpei@umt.edu.my
Mohamad Zaimin Mohd Nosi, University of Malaysia Terengganu, Faculty of Fisheries and Food Science, Malaysia, Terengganu, 21030 Kuala Terengganu, e-mail: aminichigo92@gmail.com
Muhd Danish-Daniel, University of Malaysia Terengganu, Institute of Marine Biotechnology, Malaysia, Terengganu, 21030 Kuala Terengganu, e-mail: mdda@umt.edu.my
Noor Emilia Sharifah, University of Malaysia Terengganu, Faculty of Fisheries and Food Science, Malaysia, Terengganu, 21030 Kuala Terengganu, e-mail: emilia@umt.edu.my

How to cite this article:
Iehata S., Nosi M. Z. M., Danish-Daniel M., Sharifah N. E., 2021 Gut microbiome associated with cultured Malaysian mahseer *Tor tambroides*. AACL Bioflux 14(1):46-58.