Association of Aquaculture Environment Microbiota with Metabolism in American Shad (*Alosa Sapidissima*)

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Abstract

The environment microbiome affects the growth and development of fish species. Information of the environment microbiome is beneficial to increase the production of fish in different aquaculture systems. In this study we analyzed differences in environmental microbial composition, intestinal metabolites and differentially expressed genes (DEGs) in American shad living in tank aquaculture and pond aquaculture environment. The results demonstrated that the dominant phyla were Bacteroidetes, Actinobacteria, Fusobacteria, Gemmatimonadetes, Firmicutes, Acidobacteria, Nitrospirae and Epsilonbacteraeota in two different environments. There were significantly changed of metabolites in different aquaculture environment, including DP-ethanolamine, L-proline, sulfuric acid, L-valine, L-tryptophan, creatinine, uric acid and L-isoleucine. The transcriptome data revealed eight genes (As23G026314, As04G005148, As21G024434, As04G005193, As23G026314, As13G016035, As02G001872 and As07G009244) related to metabolisms significantly changed in pond aquaculture group compared to tank aquaculture group. In addition, the body weight, amino acid metabolism, and glycerophospholipid metabolism of American shad also significantly changed in the pond aquaculture environment. Therefore, identifying the predominant microbiome in the aquaculture environment may be prevent the disease from occurring and maintain healthy fish reared in the aquaculture environment.

Introduction

Microorganisms play the critical role in the sediment and water of various aquaculture environments due to participating in biogeochemical cycles and food network interactions (Wörmer et al., 2019). Bacteria and some kinds of eukaryotic microorganisms involve in maintain a balance of elements (such as nitrogen, phosphorus, sulfur) and participate in a range of physiological functions in the aquatic environment and sediment (Grujcic et al., 2018). The nutrients of aquatic organisms usually sink into the sediment of aquaculture water and eventually become a source of nutrients for bacterial and eukaryotic (Shaughnessy et al., 2019). In addition, microorganisms affect the quality of water, such as involved in aerobic chemoheterotrophy and phototrophy, organic matter decomposition, and sulfur compound transformation, which indirectly affected the growth of aquatic organisms (Kumar et al., 2019; Mahmoudi et al., 2017; Ul-Hasan et al., 2019). Transcriptomics and metabolomics are often used to analyze the specific signaling pathways associated with environment, physiology and health (Beale et al., 2016). The transcriptomics and metabolomics could reveal the critical signaling pathways and metabolism regulation networks, which affecting the aquatic organism's carbohydrate metabolism and growth (Su et al. 2021; Qiu et al., 2020; Zhou et al., 2017). To understand the mechanism of environmental influence on the growth of aquatic organisms, the related transcriptomics and metabolomics have integrated into the same study.

American shad (*Alosa sapidissima*) is an anadromous species and mainly originates in the western Atlantic Ocean and the east coast of Canada and the United States (Nack et al., 2019). To alternative the Reeves shad (*Tenualosa reevesii*), artificial farming techniques of American shad are becoming mature in China (Liu et al., 2021). However, American shad is vulnerable and overreacting to the environment, and any improper handling will result in the death of this species. Some researchers have focused on enhancing the reproductive capacity and improving the breeding conditions of American shad, including artificially induced spawning, environmental condition regulation, and breeding improved variety (St. Pierre, 1992; Liu et al., 2006). The breeding conditions, such as water quality, temperature, illumination intensity, and oxygen content, can induce oxidative stress reaction, enhance mortality, and affect the growth and development of American shad. It has suggested that the environmental requirements of its ontogenetic development are vital for the growth performance of American shad broodstocks (Liu et al., 2021).

Pond aquaculture plays an important role in the freshwater aquaculture of China, and the application area nearly accounts for 50% of the country's aquaculture (National Bureau of Fisheries and China Society of Fisheries, 2019). However, commercial aquaculture models have also emerged in China, especially in American shad aquaculture (Liu et al., 2021). The microbial species in the water and sediment are closely relevant to the fish culture environment, because of the activities of the aquaculture species and the characteristics of environmental requirements in aquaculture, including dissolved oxygen, lighting, and the frequency of mechanical stirring. Microorganisms in commercial aquaculture and pond aquaculture are also closely related to the culture environment of fish (Zhao et al., 2015). The growth and development of American shad were different in tank aquaculture and pond aquaculture. The correlation between the development of American shad and microorganisms has seldom been analyzed in the aquaculture environment. In addition, the characteristics and differences of microorganisms' distributions in tank and pond aquaculture are still unclear. Thus, in the survey, we compared microorganisms in two different aquaculture tankage in Hongze of

China and analyzed their influence on the growth and development, intestinal metabolites, and differentially expressed genes (DEGs) in the gut of American shad. The purpose is to reveal the differences in the distribution patterns of microorganism species between tank aquaculture and pond aquaculture and show the influence on growth, metabolites, and transcription of crucial genes in different aquaculture environments. This study provides the information to evaluate growth parameters in the different aquatic pond, which is helpful to improve the efficiency of pond aquaculture.

Materials And Methods

Chemicals and reagents

Water, methanol, acetonitrile, and formic acid were purchased from CNW Technologies GmbH (Düsseldorf, Germany). L-2-chlorophenylalanine was purchased from Shanghai Hengchuang Biotechnology Co., Ltd. (Shanghai, China). DNeasy PowerSoil Kit (Cat. No. 12888) and QIAamp 96 PowerFecal QIAcube HT Kit (Cat. No. 51531) were purchased from QIAGEN (Germany). Qubit dsDNA Assay Kit (Cat. No. Q32854) was purchased from Life Technologies (America). Tks Gflex DNA Polymerase (Cat. No. R060B) was purchased from Takara (Japan).

Fish culture conditions, treatments and sample collection

Two-month-old fish were raised in a greenhouse tank recirculating aquaculture system (RAS) and underwent sixth-generation selection in indoor concrete tanks at Hongze Fishseeds Biotech. Inc., Jiangsu, China. RAS can ensure the excellence of the water quality due to the physical filtration, oxygenation and bio-filtration (Liu et al., 2021). The system was a specially designed dual-drain RAS equipped with an online infrared dome camera to monitor the growth, maturation and natural spawning behavior of shad broodstocks. Other two-month-old fish were also raised in a greenhouse pond aquaculture system at Hongze Fishseeds Biotech. Inc., Jiangsu, China. Temperature and dissolved oxygen were measured by a YSI 550A (Y.S.I. Environmental Inc.) every morning in both locations. Water quality was monitored by testing ammonia and nitrite levels every four days in both locations. Both recirculating aquaculture system and the pond were maintained under a natural photoperiod in the greenhouse, with the sunlight intensity attenuated by a roof cover with 40% light penetration. The daily average light intensity ranged from 800 to 1600 lux. Fish were fed commercial pellets three times a day for 20 min each time during the entire culture period.

Sixty fish (two months postfertilization) were randomly selected and divided into three tanks or three ponds (20 fish per tank or pond). After thirteen month cultured, the ten-individual fish from each tank and pond ($n = 3$) sampled to measure the body weight. Then these fish were washed with purified water and euthanized. The intestinal parts were dissected and gathered for metabolites and RNA. Four biological replicates per group were used for LC-MS analysis and three biological replicates per group were used for RNA-Seq analysis. The sediment at the bottom of the tank aquaculture and pond aquaculture was also collected. Four tubes ($>2\text{mg}/\text{tube}$) of sediment were gathered for each group to ensure a sufficient number of DNA.

Sample preparation and analysis

Metabolite extraction

The process of metabolite extraction referenced Lu et al., (2016). The ACQUITY UHPLC system (Waters Corporation, Milford, USA) coupled to an AB SCIEX Triple TOF 5600 System (AB SCIEX, Framingham, MA) was used to reveal the metabolic profiles with ESI. The modes of positive and negative ion executed by using the C18 column. Water/formic acid and acetonitrile/formic acid were used to elute, and the separation parameter followed the instructions.

RNA extraction, library construction and sequencing

RNA was extracted from 3 mg of intestinal tissue using a mirVana miRNA Isolation Kit (Ambion). The RNA-Seq process was performed as described in Zhang et al. (2021). RNA integrity was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Samples with an RNA integrity number (RIN) ≥ 7 were used for subsequent analysis. Libraries were constructed using a TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Then, these libraries were sequenced on an Illumina sequencing platform (Illumina HiSeq X Ten), and 125 bp/150 bp paired-end reads were generated.

DNA extraction and library construction

DNA was extracted from gut samples using TruSeq Nano DNA LT Sample Preparation Kit (Illumina). The V4 region of bacterial 16S rRNA was amplified using DNA samples. Agencourt AMPure XP (BECKMAN COULTER, USA) was used for purifying PCR products. Sequencing libraries were constructed using KAPA Library Quantification Kits (Illumina, USA). The Illumina MiSeq platform was used for paired-end sequencing (Kapa Biosystems, USA).

Data preprocessing and statistical analysis

LC-MS metabolomics data analysis

LC-MS metabolomic analysis was done by OE Biotech Co. Ltd. (Shanghai, China). Progenesis QI software was used for acquired LC-MS raw data analysis (Waters Corporation, Milford, USA). Progenesis QI Data Processing Software was used to analyze the metabolites (Waters Corporation, Milford, USA). The public databases were referenced to explore, including <http://www.hmdb.ca/>; <http://www.lipidmaps.org/> and in-house-developed databases. The changes of metabolite were analyzed by Principal component analysis (PCA) and orthogonal partial least-squares-discriminant analysis (OPLS-DA). The Hotelling's T² region shows the 95% confidence interval in the model. Variable importance in the projection (VIP) shows the contribution rate of each variable in the OPLS-DA model. The differential metabolites were obtained based on the combination of a statistically significant threshold of variable influence on projection (VIP) values selected from the OPLS-DA model and p values from a two-tailed Student's t-test on the normalized peak areas. The differential metabolites were with VIP>1 and p values<0.05.

RNA sequencing data analysis

Transcriptome sequencing and analysis were also done by OE Biotech Co., Ltd. Trimmomatic was used to process raw data (raw reads) (Bolger et al., 2014). Clean reads were obtained by removing poly-N sequences and low-quality reads. Hisat2 was used to map the clean reads and genome (Kim et al., 2015). For transcript-level quantification, bowtie2 was used to analyze the values of the transcript. DESeq (2012) functions were used to identify the DEGs based on SizeFactors and nbinom Test. The significantly differential expression was showed with the thresholds of $P < 0.05$ and fold change >2 or fold change < 0.5 . The transcript expression patterns were executed by hierarchical cluster analysis of DEGs. DEGs were analyzed by GO term enrichment and KEGG pathway enrichment following the hypergeometric distribution (Kanehisa et al., 2008).

16S rRNA metagenetics and bioinformatics analysis

FASTQ format was used for the raw sequence data. Trimmomatic software (Bolger et al., 2014) was used to preprocess the paired-end reads and eliminated ambiguous bases (N). FLASH software was used to assemble the left paired-end reads (Reyon et al., 2012) as follows: overlapping was from 10bp to 200bp, and the mismatch rate was from 0% to 20%. QIIME software was used to denoise and clean the reads (Caporaso et al., 2010) (version 1.8.0). The primer sequences were removed from the clean reads, and Vsearch software was used to execute the OTUs (Rognes et al., 2016). QIIME package was used to select the representative read in every OTU. RDP classifier was used to annotate and blast the usual reads (Wang et al., 2007). All representative reads were annotated and blasted against Unite database (ITSs rDNA) using blast (Lobo et al., 2008).

Functional analysis of integrated metabolomic and transcriptomic data

KEGG database (<https://www.kegg.jp/>) was used to define the metabolic pathways and integrated metabolomic and transcriptomic datasets. The markers list was introduced into the KEGG database to find out metabolic pathways induced by ENR treatment. The specific metabolite is related to a particular gene if they shared one common KEGG pathway.

Results

Bodyweight

As shown in Fig. 1, the body weight (577.11 ± 21.56 g) was significantly increased in the pond aquaculture groups compared to that in the tank aquaculture group (559.63 ± 20.19 g).

Metabolomic Alterations Induced By Different Aquaculture Environments

Metabolomic analysis was done to analyze the alterations in metabolic profiles of fish gut cultured in tank aquaculture and pond aquaculture. Ninety-four metabolites significantly changed ($p < 0.05$) after culture in the pond aquaculture (72 metabolites upregulated and 22 metabolites downregulated) (Supplementary Fig. 1). Several of the metabolites were associated with the tank aquaculture and the pond aquaculture (Supplementary Fig. 1). The heatmap shows the relative changes in the metabolite concentrations of each group with colors (Fig. 2A). Red and blue represent the upregulation and downregulation of metabolites, respectively. The OPLS-DA score plot shows a clear separation of metabolic profiles between the tank aquaculture group and the pond aquaculture group (Fig. 2B). In the OPLS-DA model, $R_2X = 0.787$, $R_2Y = 0.93$, $Q^2(\text{cum}) = 0.441$ and $R^2 = 0.943$, indicating the usability of the model. The VIP values and coefficients of the OPLS-DA model were analyzed to find out the effect of metabolites on metabolic alterations in different aquaculture environments (Supplementary material 1). The most metabolites affected in pond aquaculture were related to aminoacyl-tRNA biosynthesis, phenylalanine, tyrosine and tryptophan biosynthesis, ABC transporters and valine, leucine and isoleucine biosynthesis metabolic pathways. Metabolic pathways of top-20 were shown in Fig. 2C. Four metabolic pathways were significantly different at $P > 0.01$, while seven metabolic pathways were significantly different at $P > 0.05$. The coefficients and p-value of the metabolic pathways were analyzed to show the correlation of metabolites on metabolomic alterations in the different aquaculture environments (Supplementary material 2). The significantly enriched pathway was selected to construct a bubble diagram. Figure 2D showed that among the altered metabolic pathways, the rich factor was the largest for the aminoacyl – tRNA biosynthesis and ABC transporters pathways. And these two pathways were related to three common metabolites such as L-Histidine, L-Lysine and L-Valine.

Transcriptomics Of Different Cultured Environments

276 DEGs were found in the tank aquaculture and the pond aquaculture treatment groups ($P < 0.05$), precisely, 169 upregulated and 107 downregulated. Two parts of hierarchical clusters were shown in different cultured environments according to DEGs expression levels (Fig. 3A, cluster A and cluster B). Cluster A represented upregulated genes, while cluster B represented downregulated genes. As shown in Table 1, the top-twenty of DEGs involved in different molecular functions, such as protein binding, succinate dehydrogenase activity, iron ion binding, inorganic anion exchanger activity and motor activity. The volcano plot revealed the overall distribution of the DEGs (Supplementary Fig. 2). The distribution comparison diagram of DEGs and all genes by GO enrichment analysis at level 2 was shown in Fig. 3B. The three major categories, such as biological processes, cellular components and molecular functions, were revealed in Fig. 3B. The distribution comparison diagram of upregulated and downregulated DEGs were shown in GO level 2 analysis (Supplementary Fig. 3). Among the identified pathways, the thirty most important pathways involved in lipid metabolism and energy metabolism were showed in Fig. 3C, and some DEGs involved in growth and development. KEGG database showed the diverse ranges of functionally characterize DEGs, with the 276 DEGs between the tank aquaculture and the pond aquaculture groups assigned to 168 pathways. The top-twenty pathways identified by KEGG enrichment analysis were the focus. A bubble diagram was constructed using the $-\log_{10}$ (p-value) to visualize the expression patterns of DEGs. The pathways of DEGs were mainly included viral myocarditis, immunodeficiency, allograft rejection, and autoimmune thyroid disease pathways (Fig. 3D).

Association between the altered pathways with metabolites and transcriptomic pathway

The KEGG database were used to analyze the effects of two environment on transcriptomics pathway and metabolomics pathway. The pond aquaculture groups induced significant alterations in the metabolites and genes in the gut of American shad compared to the tank aquaculture groups. To investigate the potential associations, Venn diagrams (Fig. 4A) were generated by identifying the common KEGG pathways. Five of the common metabolite pathways (glycerophospholipid metabolism, arginine and proline metabolism, purine metabolism, valine, leucine and isoleucine degradation, and glycine, serine and threonine metabolism) shared nine genes (As13G016035, As21G024434, As04G005193, As04G005148, As02G001872, As07G009244, As15G018635, As13G016748, and As23G026314) and eight metabolites (CDP-ethanolamine, L-proline, sulfuric acid, L-valine, L-tryptophan, creatinine, uric acid and L-isoleucine). The total metabolites levels increased upon pond aquaculture, and the nine genes expression changed (Table 2 in detail). The association network map was constructed based on the results of the association analysis of differential genes and differential metabolites (Fig. 4B). The total differential metabolites levels increased upon pond aquaculture, whereas up to 5 genes (As13G016035, As02G001872, As07G009244, As15G018635 and As23G026314) related to the glycine, serine and threonine metabolism, purine metabolism, arginine and proline metabolism, glycerophospholipid metabolism appeared to downregulate (Table 2 in detail, in Fig. 5A-D). In addition, the total differential metabolites levels increased upon pond

aquaculture, whereas up to 4 genes (As13G016748, As21G024434, As04G005193 and As04G005148) related to valine, leucine and isoleucine degradation and glycerophospholipid metabolism appeared to be upregulated (Table 2 and, in detail, in Fig. 5E). The effect of metabolites in different aquaculture environmental remains to be investigated.

Microbial Community Analysis

Microbial community analysis according to high-throughput sequencing was done to reveal the changes of microbiome community in the tank and pond aquaculture environment. The two environment samples were all dominated by the phyla of Proteobacteria and Bacteroidetes (Fig. 6A). The compositions of the microbial communities in all the samples were similar at the rank of the phylum. Proteobacteria was the most abundant bacterial phylum in all eight samples, accounting for approximately 60% of the total species. The number of proteobacteria was more significant in the pond environment compared to the tank environment. The relative changes in the microbial abundance showed with colors in the heatmap (Fig. 6B). We use alpha diversity indices of the Chao index (Fig. 6C), and the Shannon index (Fig. 6D) to confirm the results. This is a significantly higher diversity of the microbiome in the pond environment than the tank environment group. We performed beta diversity index, PCA analysis, PCoA analysis, and UPGMA sample hierarchical cluster analysis to determine the classified bacterial taxa between different groups (Fig. 7). In addition, a biomarker analysis using the linear discriminant analysis (LDA) effect size (LEfSe) method was executed to determine the classified bacterial taxa with significant differences in abundance. As shown in Fig. 8A and B, twenty-eight bacterial clades showed statistically significant changes with an LDA threshold of 4.1. Nine bacteria were significantly enriched in pond aquaculture environment group, while nineteen clades showed an abundance advantage in the tank aquaculture environment group. The relative abundance of phylum was shown in Fig. 8C, bacteroidia, alphaproteobacteria, fusobacteria, firmicutes, gemmatimonadetes, acidobacteria, nitrospirae and epsilonbacteraeota were enriched in pond aquaculture environment samples. The species correlation at various taxonomic levels reflected in the species correlation network map (Fig. 8D).

Discussion

Our research reveals the differences in the sediment microbial communities in tank aquaculture and pond aquaculture of American shad (*Alosa sapidissima*). We analyzed the microbiome in the system and provided information on the effect of the environment factor on the American shad growth. The result showed that the microbiome was dominated by the phyla of Proteobacteria, Fusobacteria, Bacteroidetes and Actinobacteria in the pond aquaculture environment. This result is consistent with other findings in the pond environments. For example, Dai et al., (2021) found bacteroidetes, proteobacteria and actinobacteria were more abundant in the pond environment. The pond aquaculture induced significant alterations in the metabolomes and transcriptomic pathways of the gut compared to tank aquaculture. For example, the total amino acid metabolites level increased upon pond aquaculture, whereas up to 5 genes related to the pathway appeared to be downregulated. DP-ethanolamine, L-proline, sulfuric acid, L-valine, L-tryptophan, creatinine, uric acid, and L-isoleucine increased in the pond aquaculture group and positively correlated with the family of B8 (Fusobacteria;f_Fusobacteriaceae) and B9 (Planctomycetes; f_Gemmataceae). Similarly, lysine increased in the pond aquaculture group and positively correlated with the families of B5 (Chlamydiae;f_unassigned), B14 (Proteobacteria;f_unassigned) and B16 (Proteobacteria; f_Neisseriaceae). Fusobacteriaceae, aeromonadaceae and moraxellaceae were enriched in the pond environment compared to the tank environment. Microorganisms in sediment were mainly related to the enzymes of organic matter decomposition (Dai et al., 2021), which served as a natural nutritional supplement to enhance aquatic organisms' growth (Addo et al., 2021). The nutritional supplement is closely related to the growth development of aquatic animals. The results indicated that greater abundance and diversity of microbial communities in pond aquaculture group induced better growth condition in American shad. The results of the microbial community are consistent with growth indicators.

The metabolomic and transcriptomic analysis showed the metabolic disruption of the gut of American shad. CDP-ethanolamine, L-proline, sulfuric acid, L-valine, L-tryptophan, creatinine, uric acid, and L-isoleucine increased in the pond aquaculture group compared to the tank aquaculture group. The integration data at the pathway level showed the affected metabolic routes in the gut of American shad after the pond aquaculture, including glycerophospholipid metabolism, arginine and proline metabolism, purine metabolism, valine, leucine and isoleucine degradation, and glycine, serine and threonine metabolism. The total energy of fish meets the basic metabolic needs, including growth, development and reproduction (Bunnell et al. 2003; Ye et al., 2011). Amino acids and glycerophospholipid have a crucial role in the development of fish, including providing essential fatty acids, essential

protein and enough energy for muscle, tissue and gonad development (Masrizal et al., 2015). The results of both transcriptomic and metabolomic are in agreement with growth indicators.

Fish is the source of the essential protein and fatty acids for humans. At present, aquaculture is the main source for fish production and consumption (FAO, 2020). Bjrnevik et al., (2017) revealed that the improper aquaculture pattern affected the quality and the taste of fish flesh, including reducing protein content. Ostbye et al., (2018) reported that the quality of fish, including nutritional composition, was primarily affected by environmental factors. This conclusion is in agreement with our study, indicating the changes of metabolites in a different aquaculture environment, involving DP-ethanolamine, L-proline, sulfuric acid, L-valine, L-tryptophan, creatinine, uric acid, and L-isoleucine. The transcriptome data revealed four genes (As23G026314, As04G005148, As21G024434, and As04G005193) related to these metabolisms (valine, leucine and isoleucine degradation, and glycerophospholipid) significantly up-regulated. In comparison, the four genes (As23G026314, As13G016035, As02G001872 and As07G009244) related to metabolisms (glycine, serine and threonine metabolism, purine metabolism, and arginine) significantly down-regulated in pond aquaculture group. Based on our analysis, numerous genes and metabolites differentially expressed between the tank and pond aquaculture groups, in agreement with earlier investigations. For example, the gene, lipid metabolism and amino acid metabolism are the most direct manifestation of the response of plants changes in the external environment (Sun et al., 2020; Yang et al., 2019). Xu et al., (2016) also reported that glycine, serine, arginine, proline metabolism and threonine metabolism of oriental river prawn *Macrobrachium nipponense* were significant differences in response to acute and chronic environment factor stress. This survey provides valuable information on the diversity of microbiomes in different aquaculture. In addition, the potential effects of microbiomes on the growth of these fishes are also mentioned.

Conclusion

Our research analyzed the differences in the distributions of microbial communities between the tank aquaculture and the pond aquaculture environment. We found that the dominant phyla were Bacteroidetes, Actinobacteria, Fusobacteria, Gemmatimonadetes, Firmicutes, Acidobacteria, Nitrospirae and Epsilonbacteraeota in both aquaculture environment, by comparing microorganisms in different aquaculture ponds from the same location. The bacterial abundance significantly increased in the pond aquaculture than that in tank aquaculture. Some identified differential genes and metabolites were associated with the response of American shad to different aquaculture environments. In conclusion, the body weight, amino acid metabolism, and glycerophospholipid metabolism of American shad significantly changed due to the pond aquaculture environment. Taken together, during popular aquaculture, the interaction effect of the aquaculture environment on cost benefits should be carefully evaluated. Besides, meat quality during aquaculture should be seriously considered. This result could potentially serve as microbiome indicators in different aquaculture environments. Our study highlights the necessity to incorporate microbiome toxicology research in the aquaculture industry.

Declarations

Availability of data and materials All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Authors' contributions Jia Du conceived and designed the experiments. Qinhua Liu supported the organization of the project and performed the experiments. Keqin Zhang, Chengxiang Zhang and Gaohua Yao analyzed the data. All contributing authors have read and approved the final version of the manuscript.

Ethics approval Not applicable

Consent to participate Not applicable

Consent of publication Not applicable

Competing interests The authors declare that they have no conflict of interest.

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Tables

Table 1

Biological processes and molecular functions of the most differentially expressed genes (top-twenty) after culture different cultured environments.

Gene id	Gene name	Gene description	Base Mean	pValue	Fold Change	Molecular Function
Up-regulated						
As04G004894	Rnh1	Ribonuclease inhibitor	15.45035579	0.00364084	102.2612438	protein binding
As23G026860	SDHC	Succinate dehydrogenase cytochrome b560 subunit	78.17066854	5.82E-16	61.00085809	succinate dehydrogenase activity
As07G008885	mogat2-a	2-acylglycerol O-acyltransferase 2-A	7.283084764	0.000139609	43.74880818	transferase activity, transferring acyl groups other than amino-acyl groups
As11G013878	PGC	Gastricsin	173251.9722	0.046261289	38.22168004	aspartic-type endopeptidase activity
As04G004598	Unknown	Cystatin	30708.22391	0.042029946	34.39259952	cysteine-type endopeptidase inhibitor activity
As20G023043	SBF1	Myotubularin-related protein 5	7.503540635	0.017474453	22.05166777	phosphatase regulator activity
As08G010278	cyp1a1	Cytochrome P450 1A1	12.52643903	0.001242407	19.99463312	iron ion binding
As21G023933	UMAD1	UBAP1-MVB12-associated (UMA)-domain containing protein 1	3.200192741	0.035681065	18.66265885	
As15G019128	tim23	Mitochondrial import inner membrane translocase subunit Tim23	26.86638894	1.75E-09	18.64867257	TIM23 mitochondrial import inner membrane translocase complex
As02G001904	Unknown	L-rhamnose-binding lectin CSL3	93.35504268	0.028271521	17.97440459	carbohydrate binding
Down-regulated						
As24G026970	ATP2A1	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	95.17048306	0.034741487	0.496868494	nucleotide binding
As15G018679	Unknown	Unknown	170.1634723	0.005104726	0.496303833	Unknown
As13G016569	SSH1	Protein phosphatase Slingshot homolog 1	34.07582779	0.047482077	0.493473838	protein tyrosine phosphatase activity
As14G017421	Dusp8	Dual specificity protein phosphatase 8	146.4822989	0.032029515	0.49267269	protein tyrosine phosphatase activity
As02G001872	PLA2G1B	Phospholipase A2	3692.414228	0.001021302	0.491257297	phospholipase

						A2 activity
As08G010690	SLC4A4	Electrogenic sodium bicarbonate cotransporter 1	49.49409386	0.021895553	0.490846315	inorganic anion exchanger activity
As13G016353	SLC27A4	Long-chain fatty acid transport protein 4	37.70459613	0.034515932	0.486819323	catalytic activity
As19G022184	Tnni1	Troponin I, slow skeletal muscle	3809.149612	0.033993754	0.486635234	troponin complex
As16G019505	Myh7	Myosin-7	2666.635533	0.027133197	0.485618664	motor activity
As22G025262	PCDHA8	Protocadherin alpha-8	74.02863914	0.032156152	0.483550399	calcium ion binding

Table 2

Biological processes and molecular functions of the commom differentially expressed genes

Gene id	Gene name	Gene description	Regulation	Molecular Function
As13G016035	Unkown	Phospholipase A2 GL16-1	Down-regulated	glycerophospholipid metabolism
As21G024434	lpcat1	Lysophosphatidylcholine acyltransferase 1	Up-regulated	calcium ion binding
As04G005193	Pla2g4c	Cytosolic phospholipase A2 gamma	Up-regulated	phospholipase activity
As04G005148	PNPLA6	Cytosolic phospholipase A2 gamma	Up-regulated	Unkown
As02G001872	PLA2G1B	Phospholipase A2	Down-regulated	phospholipase A2 activity
As07G009244	SAT2	Diamine acetyltransferase 2	Down-regulated	pN-acetyltransferase activity
As15G018635	PDE10A	cAMP and cAMP-inhibited cGMP 3',5'-cyclic phosphodiesterase 10A	Down-regulated	3',5'-cyclic-nucleotide phosphodiesterase activity
As13G016748	HMGCS1	Hydroxymethylglutaryl-CoA synthase, cytoplasmic	Up-regulated	catalytic activity
As23G026314	TDH	L-threonine 3-dehydrogenase, mitochondrial	Down-regulated	catalytic activity

Figures

Figure 1
Body weight in tank aquaculture group and pond aquaculture group.

Figure 2

(A) Heatmap of the statistically significant tentatively identified metabolites whose concentrations changed between tank aquaculture group and pond aquaculture group, (a) down-regulated metabolites; (b) up-regulated metabolites; (B) OPLS-DA score plot shows clear separation of metabolic profiles between the two groups; (C) Top-20 metabolic pathways enrichment map (the red line indicates $P = 0.01$, and the blue line indicates $P = 0.05$) (D) Bubble diagram of metabolic pathways, the ordinate indicates the name of metabolic pathways and the abscissa indicates the enrichment factors (Rich factor, Rich factor=significantly different number of metabolites/total number of metabolites in the pathway).

Figure 3

(A) Heatmap of the DEGs in two groups; (B) The distribution comparison diagram of DEGs and all genes by GO enrichment analysis at level 2; (C) GO enrichment analysis: the horizontal axis represents Go entry name and the vertical axis represents $-\log_{10}(p \text{ value})$; (D) Bubble diagram of KEGG enrichment analysis TOP-20 of DEGs.

Figure 4

Figure 6

(A) Microbiome composition at the phylum level samples from tank aquaculture group and pond aquaculture group. The top fifteen phyla were reported for each sample, and all other phyla were grouped into "Other" ; (B) The cluster tree of two groups. Left represents the clustering of species; Above cluster branch Group represents samples from different groups; Orange indicates relatively high species abundance and blue indicates relatively low species abundance; Chao 1 (C) and Shannon (D) indexes of sediment samples in GH and WH groups.

Figure 8

(A) LefSe analysis of microbial abundance, the cladogram of microbial communities; (B) LDA score identified the size of differentiation between GH and WH groups. Data presented as the mean $\pm$ SEM of three replicates and an asterisk indicates the statistical significance (p -value < 0.05) between TET treated and control groups; (C) The relative abundance of top-ten phylum in two groups; (D) The species correlation network map at phylum levels.

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