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Effects of brewer's yeast hydrolysate on the growth performance and the intestinal bacterial diversity of largemouth bass (*Micropterus salmoides*)

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ABSTRACT

To investigate the effects of brewer's yeast hydrolysate (BYH) on the growth and intestinal bacteria diversity of largemouth bass (*Micropterus salmoides*)sthe fish were fed with basal diet supplemented with or without BYH (0, 0.1%, 0.2%) for 8 weeks. The results showed that the weight gain rate, survival rate, hepatosomatic index, organ coefficient and condition factor were not significantly different among the groups, except intraperitoneal fat ratio was significantly reduced in 0.2% BYH group. Totally 25 phyla, 56 classes, 103 orders, 155 families, 216 genera and 1051 operational taxonomic units (OTUs) of bacteria were identified from the 2,057,871 sequences obtained by Illumina MiSeq 16S rRNA sequencing. Among them, Fusobacteria, Firmicutes and Proteobacteria were the most three dominant phyla in the intestines regardless of diets. Furthermore, the richness of Fusobacteria, Cyanobacteria, Tenericutes and Actinobacteria in 0.2% BYH group was significantly lower than those of the control group. Interestingly, the ratio of Bacteroides/Firmicutes was decreased in the groups fed with BYH, this might be related to the modulations of energy absorption and storage, resulting in the decrease of fat deposition in fish body. At the genus level, several bacterial genera (*Plesiomonas*, *Mycoplasmas*, *Synechococcus* and *Peptostreptococcus*) which were potential pathogens of fish were significantly decreased in the groups fed with BYH. By contrast, a significant increase of *Cetobacterium* which could ferment peptides and carbohydrates to produce vitamin B_{12} was observed in fish fed with BYH, indicating that dietary BYH was helpful in propagation of some probiotic bacteria. Taken together, the overall intestinal bacterial diversity was decreased in fish fed with BYH which might promote the growth of some probiotics and inhibit some pathogenic bacteria, suggesting that BYH may serve as feed additive in modulation of the gut bacterial community of largemouth bass to decrease the potential pathogens.

1. Introduction

Largemouth bass (*Micropterus salmoides*) is an important freshwater fish species native to North America (Coyle et al., 2000). It was firstly introduced into Guangdong Province, China in 1983. Thereafter, it was quickly disseminated through other provinces, and has become one of the most commercially important fresh water species worldwide. In the past decades, researchers have focused their studies on the macronutrient requirements, alternative protein or lipid sources for largemouth bass (Andrews et al., 2011; Chen et al., 2012; Chen et al., 2015; Subhadra et al., 2006; Tidwell et al., 1996), which promoted the development of formulated feed. However, recently improper components of the formulated feed can cause the nutrition metabolism disturbance of largemouth bass, such as intestinal dysfunction, fatty liver, resulting in the poor growth or impaired immunity of the fish (Li and Chen, 2011). Therefore, optimizing feed formula or developing functional feed additives are highly needed for the sustainable culture of largemouth bass.

Brewer's yeast (*Saccharomyces cerevisiae*) is a rich source of immune-stimulating compounds such as β-glucans, nucleotides, mannan oligosaccharides, selenium and some microbial nutrients such as enzymes, B-complex vitamins and amino acids, which have been proved to have positively influence on growth performance, immune responses or anti-stress status of various fish species (Andrews et al., 2011; Führ et al.,

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2016; Gatesoupe, 2007; Hoseinifar et al., 2011; Li and Gatlin, 2005). However, the cell walls of yeast can hardly be digested by fish, attempts have been made to break the cell walls and release the cell contents for better utilization. Hydrolyzed yeast is a hydrolysate of yeast cells derived by acid, enzyme or other method of hydrolysis (Amorim et al., 2016). It has been successfully applied in aquatic feed to achieve better growth, enhance immunity, or relieves stress of fish (Führ et al., 2016; Yar Ahmadi et al., 2014).

In various fish species, diets has been proved to affect intestinal microflora which reflecting health status of fish such as immune responses, growth performance or physiological functions (Afrilasari et al., 2016; Reveco et al., 2014; Tian et al., 2017; Torrecillas et al., 2017). However, the impact of yeast hydrolysate on fish intestinal microbiota are still unknown, except a few studies have investigated some individual bacterial features, such as lactic acid bacteria population, viable aerobic bacterial count, microbial populations (Adel et al., 2017; Batista et al., 2016), which provided very limited information for the comprehensive understanding of bacterial diversity in the intestine induced by BYH. The present study was conducted to investigate the effects of a commercial dietary brewer's yeast (*S. cerevisiae*) hydrolysate on growth and intestinal bacterial features of largemouth bass, so as to evaluate the effectiveness and functions of the yeast product.

2. Materials and methods

2.1. Experimental animals and diets

Largemouth bass juveniles were obtained from a commercial fish farm in Guangdong Province, China. The fishes were maintained and fed with the commercial diet (crude protein 42%, crude lipid 8%) for at least 2 weeks so that they acclimated to the experimental conditions before experiments. A basal diet was formulated to contain 44.3% protein and 7.5% lipid (Table 1). This diet served as the control diet and the experimental diets were produced using the same basal diet supplemented with either 0.1% or 0.2% commercial brewer's yeast *S. cerevisiae* hydrolysate (purchased from by Sintun Aquatic Technology Co. Ltd.). The pelleted diets were dried at 50 °C and stored at − 20 °C until use. Chemical analyses of feeds and fish were performed according to standard AOAC methods (AOAC, 1990).

2.2. Growth trial

600 fish individuals with an average weight of 34.0 g (initial weight) were randomly divided into 12 net cages (1.0 m × 1.0 m × 1.5 m/cage) with 50 individuals per cage, four replicate groups of fishes were randomly assigned as groups 0.1% BYH, 0.2% BYH and the control groups. All the assigned groups were fed twice per day (about 3% of body weight, fed at 09:00 and 16:00 h, respectively) for a period of 8 weeks. Continuous aeration was provided to each cage through an air stone connected to a central air compressor.

Table 1
Formulation and proximate composition of the basal diet.

Ingredients	Content (g/100 g diet)
Fish meal	65.0
Soybean meal	10.0
Wheat	20.0
Fish oil	1.0
$Ca(H_2PO_3)_2$	2.0
Compound premix	2.0
Composition of the experimental diets (% dry matter)	
Dry matter	91.5
Crude protein	44.3
Crude lipid	7.5
Crude ash	13.5

Water temperature, dissolved oxygen and ammonia levels were monitored daily and maintained at 31 ± 3 °C, 7.1 ± 0.3 mg/L and 0.04 ± 0.01 mg/L, respectively.

2.3. Collection and preparation of samples

All fish were weighed at the end of the feeding trial for estimation of growth performance and physical indicators. Six fish from each treatment group were randomly sampled from each tank. The entire intestinal contents were removed aseptically into 2 mL Eppendorf tubes followed by storage at − 80 °C until they were used for bacterial analysis.

2.4. DNA extraction

Total bacterial community DNA was extracted using a PowerFecal™ DNA Isolation kit (MO BIO Laboratories, Carlsbad, CA, USA). DNA quality and quantity were measured by a Fluorometer Qubit® (Invitrogen, US). The obtained DNA was stored at − 20 °C until they were used for the PCR amplification of the bacterial 16S rRNA genes.

2.5. 16S rDNA sequence amplification and MiSeq sequencing

Bacterial 16S rRNA gene was amplified by PCR using the conventional primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 806R (5′-GGACTACHVGGGTWTC TAAT-3′) which targeted the V4 region (Whiteley et al. 2012). Ask company for these sequences. The PCR reaction were carried out in a total volume of 25 μL containing 2.5 μL 10 × AccuPrime buffer II, 0.1 μL AccuPrime Taq DNA Polymerase, 1 μL forward and 1 μL reverse primer (10 μmol/L), 5 μL template DNA (10 ng), and adding ultra-pure water up to 25 μL. The reaction conditions were 94 °C for 3 min; 28 cycles of 94 °C for 20 s, 53 °C for 25 s, 68 °C for 45 s and followed by a final extension at 68 °C for 10 min. All reactions was performed in triplicate.

The PCR products were detected and quantified using QuantiFluor™-ST Blue Fluorescence Quantitative System (Promega) according to preliminary quantitative electrophoresis results by following the manufacturers' instruction. Then the corresponding proportions were mixed, based on the sequencing requirements of each sample to form a sequencing sample library. The sequencing was completed on an Illumina MiSeq sequencer using the MiSeq Reagent Kit v2 (500-cycle) in the Beijing Genomics Institute (Shenzhen, China).

2.6. Bacterial 16S rDNA sequence analysis

The original sequences were processed by BLAST program to remove PhiX sequence and sample reads were allocated by barcode. The forward and reverse reads with low quality were removed by the Btrim program (Kong, 2011). Using the FLASH program, forward and reverse sequences were spliced with ≥ 15 bp overlap and < 10% error rates. The quality of the reads was assessed using FastQC Software, and the reads were then filtered by quality and with a length of 252 ± 0 bp were obtained as operational taxonomic units (OTUs) representative sequences, by using the usearch_global program. The OTU table without singletons and the corresponding sequence files were generated at the 97% similarity level using UPARSE (version 7.1) program (Edgar, 2013). The OTUs representative sequences were blasted against the database using the Ribosomal Database Project (RDP classifier –Version 2.2) (Cole et al., 2009). Chimeric sequences generated in the PCR amplification were detected by UCHIME algorithm (version 4.2.40). The non-bacterial OTUs that could not be correctly aligned were also removed. Venn diagrams were employed to characterize the number of common/unique OTUs in group's shared bacterial communities among all samples.