Fish and Shellfish Immunology 94 (2019) 697–704

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Regulation of the disease resistance and mTOR and NF-kB signaling pathway of *Tilapia mossambica* by *Rhodopseudomonas capsulatus* wastewater treatment

Pan Wu[a,b], Yuqiao Hu[a], Yanling Wang[c], Yuan Wu[a], Ning Li[a], Yuying Dong[a,b,*], Ying Zhang[a,b,**]

[a] School of Environment and Resources, Dalian Minzu University, Dalian, 116600, China
[b] School of Resources and Environment, Northeast Agricultural University, Harbin, 150030, China
[c] Department of Anesthesiology, The Third Affiliated Hospital of SunYat-Sen University, Guangzhou, 510630, China

ARTICLE INFO

Keywords:
Tilapia mossambica
Wastewater
Effluent
R. capsulatus
Yield
Disease resistance

ABSTRACT

The employment of traditional bait and medicament in freshwater aquaculture causes the frequent occurrence of environmental pollution and disease. Effluent collected after *Rhodopseudomonas capsulatus*-mediated wastewater treatment could be re-utilized as microbial feeds, medicament, and aquaculture water to culture *Tilapia mossambica*. Therefore, a novel integrated system of wastewater treatment using effluent containing *R. capsulatus* that improves yield, increases disease resistance, and enhances the quality of aquaculture water for *Tilapia mossambica* culture was proposed and investigated. *Tilapia mossambica* can grow well in effluent containing *R. capsulatus* (ER). The survival rate, yield, and whole body composition of the ER group were all increased compared to the control group (CK). The biochemical (B vitamin) and other substances in the effluent of *R. capsulatus* enhanced the activity of AKP, ACP, phagocytic, SOD and CAT by upregulating the expression of *AKP, ACP, SOD* and *CAT* genes. Theoretical analysis showed that biochemical molecules regulate the expression of these gene and enzyme activities by acting as a signal that stimulates the active center. Moreover, biochemical molecules present in *R. capsulatus* enhanced the activity of the mTOR and NF-kB signaling pathways. Furthermore, *R. capsulatus* inhibited *Aeromonas hydrophila* that increases resistance against fish disease. Meanwhile, *R. capsulatus* in the effluent also improved the aquaculture water quality. This technology would save the aquaculture water, reduce water pollution and wastewater discharge, and increase the output and disease resistance of *Tilapia mossambica*, simultaneously.

1. Introduction

Tilapia mossambica meat is fine and tender, and was rich in the protein, fat, carbohydrate, vitamin, nicotinic acid and inorganic components such as calcium, phosphorus, iron [1,2]. Therefore, it is one of the most important and popular freshwater aquaculture species in China. The total output is over three million tons per year. But, the large-scale freshwater aquaculture and the application of commercial bait and medicament (antibiotics and chemotherapeutics) cause the pollution of water environment and the frequent occurrence of disease [3]. These directly reduce the production performance of aquatic animals [4]. Thus, it has become necessary to exploit the natural bait and fish disease drugs instead of chemical substances methods, like probiotic. Safari and Paolucci et al. [5] used *galactooligosaccharide* and *Enterococcus faecalis* to improve the growth and survival of juvenile crayfish fed. Nguyen et al. [6] used probiotic *Lactococcus lactis* to enhance growth rate in olive flounder. Rahimnejad et al. [7] found the improvement of growth performance in rock fish with *Pediococcus acidilactici*. Now, the probiotics are garnering increasing scientific and commercial interest, and were quite common in health-promoting functional feeds as well as therapeutic, prophylactic, and growth supplements [8].

Rhodopseudomonas capsulatus (R. capsulatus) is a kind of photosynthetic bacteria and probiotic, widely distributing in rivers, lakes, seas [9]. *R. capsulatus* are rich in high value biochemicals such as single cell proteins, carotenoids, vitamins, folic acid, antiviral substances and antigens [10]. Moreover, *R. capsulatus* are non-toxic and harmless, and do not secrete toxic or harmful substances. Thus, *R. capsulatus* is very suitable for the improvement of *Tilapia mossambica* yield as microbial feeds and medicament. Similarly, Chumpol et al. [11] demonstrated the single cell protein in photosynthetic bacteria to enhance growth and survival in white shrimp cultivation. Chiu and Liu [12] found the

* Corresponding author. School of Environment and Resources, Dalian Minzu University, Dalian, 116600, China.
** Corresponding author. School of Environment and Resources, Dalian Minzu University, Dalian, 116600, China.
E-mail addresses: 17866237777@163.com (Y. Dong), 13786823379@163.com (Y. Zhang).

https://doi.org/10.1016/j.fsi.2019.09.001
Received 31 January 2019; Received in revised form 29 August 2019; Accepted 1 September 2019
Available online 24 September 2019
1050-4648/ © 2019 Elsevier Ltd. All rights reserved.

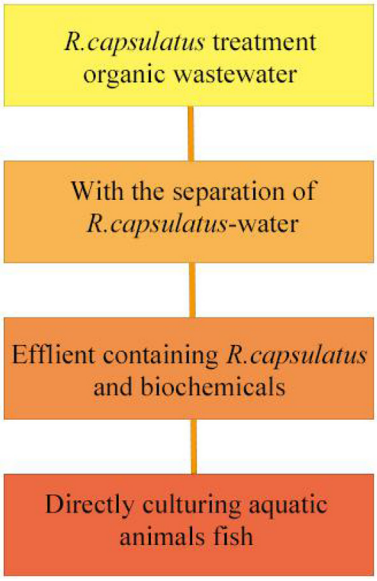

Fig. 1. The diagrammatic sketch of effluent and *R. capsulatus* after wastewater treatment culturing *Tilapia mossambica*.

extract of photosynthetic bacteria enhanced the growth performance and innate immune responses of seawater red tilapia. Meanwhile, they are capable of handling a variety of highly concentrated organic wastewater. Idi et al. [13] described photosynthetic bacteria removed high amount of nitrate and ammonia without nitrite accumulation. Ponsano et al. [14] used photosynthetic bacteria to treat the poultry slaughterhouse wastewater. Wu et al. [15] treated the soybean protein wastewater with photosynthetic bacteria. Hülsen et al. [16] described photosynthetic bacteria treated poultry processing wastewater. To sum up, *R. capsulatus* have such potential as Fig. 1 shown. Firstly, *R. capsulatus* are used to treat organic wastewater. Afterward, the effluent without the separation of *R. capsulatus*-water is reused directly to culture *Tilapia mossambica* as aquaculture water and feeds, and to purify aquaculture water quality simultaneously.

Therefore, a novel integrated system of the wastewater treatment, the improvement of yield, disease resistance and aquaculture water quality of *Tilapia mossambica* by effluent containing *R. capsulatus* is proposed in this work. The effluent is directly re-utilized to culture *Tilapia mossambica* as microbial feeds, medicament and aquaculture water. The new strategy owns the advantage of wastewater treatment, the reduction of aquaculture water consumption, the improvement of yield, disease resistance and aquaculture water quality at the same time.

Soybean processing wastewater is non-toxic and harmless, do not contain heavy metals and is rich in nutrients that was required by the growth of *Tilapia mossambica* and *R. capsulatus* [17]. Thus, soybean protein wastewater is used in the above strategy. The strategy will not cause two pollution for aquaculture water as both *R. capsulatus* and wastewater were non-toxic harmless.

To our the best knowledge, the enhancement of yield, disease resistance and aquaculture water quality of *Tilapia mossambica* by effluent containing *R. capsulatus* is not researched. Studies on the increased of yield in aquaculture by photosynthetic bacteria have focused on their extracts and nutrients [11,12]. Moreover, the mechanism also is not clear that *R. capsulatus* regulate the growth, immunity, antioxidation and disease resistance of *Tilapia mossambica*. Therefore, the purpose of the work is to investigate the feasibility of the effluent culturing *Tilapia mossambica* and enhancing its yield and disease resistance; to explain the mechanism of the effluent affecting *Tilapia mossambica* yield and disease resistance in terms of nonspecific immunity, antioxidation and mTOR and NF-kB signaling pathways.

2. Materials and methods

2.1. Wastewater treatment

R. capsulatus have the advantage of wastewater treatment, the improvement of yield, disease resistance and aquaculture water quality for aquaculture at the same time. As Fig. 1 shown, *R. capsulatus* are firstly use to treatment soybean processing wastewater (SPW) in this work. Afterward, the effluent without separation of *R. capsulatus* and water is recycled directly to feed *Tilapia mossambica* as microbial feeds, medicament and aquaculture water.

SPW was adopted for *R. capsulatus* treatment and *Tilapia mossambica* growth due to it being non-toxic and harmless. SPW was obtained from soybean soak process in Dalian Soybean Products Machining Factory (Dalian, China). The wastewater was diluted using deionized water. The basic characteristics of the diluted SPW were shown in Table S1. The pH of SPW was adjusted to 7 before treatment.

Separation and inoculation programs of *R. capsulatus* were reported in our previous study [15]. *R. capsulatus* strain were kept at 4 °C in a fridge and grown in the improved medium in a thermostat shaker (120 rpm, 32 ± 2 °C) for approximately 48 h before the experiment.

Photo-bioreactor used was shown in Fig. S1. The working volume of reactor was 1 L. The reactor was a complete mixed type and hydraulic retention time was kept about 6 days. Both SPW and reactor were sterilized 30 min by a sterilizer at 121 °C before addition of *R. capsulatus*. The SPW at a fixed quantity of 600 mL was treated in bioreactors. The original consistence of *R. capsulatus* were 190 mg/L and the original pH on inoculation was about 7.0. The wastewater/bacteria mixtures were placed in a 30 ± 2 °C thermostat shaker (Leyan, Tianjin, China) under a rotating speed of 120 rpm. In this work, light-aerobic condition was used during 1-3 days, and light-anaerobic was used during 4-6 days. The light intensity was kept at around 3000 lux by adjusting the distance between the bioreactor and the bulbs. Aerobic condition was realized by aeration and the DO concentration in the bioreactor was kept around 2.0 mg/L. Anaerobic was achieved by nitrogen with a purity of 99%.

After 6 days treatment of *R. capsulatus*, the quality of SPW effluent as aquaculture water was inspected. The COD, ammonia nitrogen and metal ions in effluent were determined. The result is shown in Table S1. *R. capsulatus* had very good treatment effective for SPW. *R. capsulatus* biomass reached 4000 mg/L, the residual COD, ammonia nitrogen and metal ions in effluent were 200, 1 and 0.1 mg/L. Although the effluent still contained COD and *R. capsulatus*, it could not cause harm to *Tilapia mossambica*. This was because SPW, effluent and *R. capsulatus* were innocuous and harmless, and did not contain heavy metals. Ammonia nitrogen were high for aquaculture water although they complied with national wastewater emission standards. Therefore, the effluent was diluted by six times. After, the ammonia nitrogen was below 0.2 mg/L, which reached the aquaculture water quality standards of China. In addition, 2 mL 1% sodium alginate (Leyan, Tianjin, China). was added to 10 mL effluent to immobilize *R. capsulatus* for *Tilapia mossambica* ingestion.

2.2. Fish rearing by the effluent

The experiments were carried out at May to August 2018. *Tilapia mossambica* (30 ± 5 g and 11 ± 3 cm) were bought from the local fish farming plant (Zhongshan District, Dalian). A total of 150 fish were acclimated in tank at least 7 days. During the acclimatization, the fish were fed every day with commercial fish feed.

After acclimatization, 120 fish was selected from 150 *Tilapia mossambica* and assigned to triplicate four groups with 12 tanks (10 fish per 80 L tank containing 60 L water) randomly. Four processing groups were set and as follows: CK represented the control group of water and commercial fish feed; WR represented the water and *R. capsulatus*; EO represented the effluent without *R. capsulatus*; ER represented the

effluent containing *R. capsulatus* was used to breed *Tilapia mossambica*. Each processing group (CK, WR, ER and EO) was repeated three times. The original water was renewed daily and the feces was removed. *Tilapia mossambica* were fed once daily at a rate of 10-15% of body weight during the experiment. In this work, *R. capsulatus* in effluent were immobilized and used as bait under WR and ER processing groups. The *R. capsulatus* biomass were 40 g in 60 L water (Table S1). ER processing group also contained some residual (COD and TOC) organic matter (Table S1). Thus, food and bait were sufficient for *Tilapia mossambica* in WR and ER groups. Fish feces was removed with a siphon once daily during culturing. Water temperature (25.0 ± 1.0 °C), dissolved oxygen (6.0 ± 1.0 mg/L), and pH (7.0 ± 1.0) were respectively determined daily using a thermometer (T-2000, Hangzhou, China), DO meter (LY-800, Shanghai, China), and pH meter (TD-500, Guangzhou, China). Afterward, in this work, all fish were administered in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (China).

2.3. Analysis and measurement

Two wastewaters (SPW and *Tilapia mossambica* aquaculture) were collected respectively from bioreactors after 6 days treatment or tanks after 123 days of feeding. Triplicate samples were centrifuged at 9000 × *g* for 10 min (4 °C) before analysis. The supernatants were collected to detect chemical oxygen demand (COD), total organic carbon (TOC), total nitrogen (TN) and ammonium (NH_4^+) in SPW and *Tilapia mossambica* aquaculture water at the end of 6 days treatment or 123 days cultivation. The sediments (*R. capsulatus*) were used to measure the dry cell weight at the end of 6 days treatment.

The COD and TOC were determined respectively using COD analyzer (JY-203, Tianjin, China) and TOC analyzer (multi N/C 3100, AnalytikJena). The ammonium (NH_4^+) and TN were determined at 420, 538, 220, 220 and 270 nm wavelength respectively using ultraviolet visible spectrophotometer (UV-7500, Shanghai, China) according to the American Public Health Association (APHA) Standard Methods for the Examination of Water and Wastewater and the National Standard Method of China.

2.3.1. Determination of yield and quality of Tilapia mossambica

After 123 days feeding, each *Tilapia mossambica* was individually weighed (± 0.01) on an electronic scale (AND, Japan). The survival was calculated as follows:

$$\text{Survival (\%)} = (\text{final number of fish/initial number of fish}) \times 100$$

At the end of the feeding, three individuals per tank were collected in each processing group. *Tilapia mossambica* were anesthetized in ice water prior to euthanasia. The fish were then decontaminated with 70% ethanol and dissected immediately with sterile scissors. Small pieces of liver and head kidney were immersed in TRIzol reagent (Cwbio, Beijing, China) and stored in −80 °C until RNA extraction.

Meanwhile, the dorsal muscle were evenly removed. The analysis of crude protein, crude lipids and ash were analyzed by standard procedures as per the Association of Official Analytical Chemists (AOAC). Moisture content was estimated by gravimetric analysis after oven drying at 105 °C for 12 h. Crude protein was determined by Kjeldahl method (Kjeltec 2100, FOSS, Tecator, Sweden) after acid hydrolysis [18]. Crude lipids were extracted based on the method of Folch et al. [19]. Total ash was determined gravimetrically by ignition at 600 °C for 6 h in muffle furnace.

2.3.2. Determination of various enzyme activities

The superoxide dismutase (SOD) and catalase (CAT) activities in liver; the alkaline phosphatase (AKP) and acid phosphatase (ACP) in head kidney were measured at 550, 240, 510 and 440 nm using the assay kit (Nanjing Jiancheng Bioengineering Institute, China) by a UV/Vis spectrophotometer (Pharmacia Biotech Ultrospec 2000) respectively and according to Kong et al. [20]; Lin et al. [8]; Cooper et al. [21]; Yuan et al. [22]. The head kidney macrophages (HKM) were isolated and prepared according to Secombes [23]. The phagocytic activity of macrophages was determined by the following Sakai et al. [24] and Houwen [25].

2.3.3. Immune enzymes-related genes and antioxidant enzymes-related genes expression

According to Kong et al. [20]; Qi et al. [26], total RNA was extracted from tissue samples by TRIzol Reagent (Cwbio, Beijing, China) and treated with 4 × gDNA wiper Mix to minimize the contamination of genomic DNA. The quality and purity of RNA were verified by electrophoresis on ethidium bromide staining 1.0% agarose gels and by A260 nm/A280 nm ratio. Complementary DNA was then synthesized using the HiScript® Reverse Transcriptase Kit (Vazyme, Jiangsu, China) following the instructions. The real-time quantitative PCR (RT-qPCR) was performed using AceQ™ qPCR SYBR ® Green Master Mix kit and CFX96 Real-Time PCR Detection System (Bio-Rad, USA). The β-actin gene was used as a house keeping gene. The PCR primer sequences and the reaction conditions used for real-time quantitative PCR are listed in Table S2, and the cycleindex was 30. The PCR efficiency of each primer was between 95.6 and 99.2%. RNA extracted from the head kidney was performed to detect the expression of alkaline phosphatase gene (*AKP*), acid phosphatase gene (*ACP*), *TOR* gene and *4E-BP* gene in mTOR and *NF-kB p65* gene and *IkB* gene in NF-kB signaling pathway genes. RNA extracted from the livers was performed to detect the expression of superoxide dismutase gene (*SOD*) and catalase gene (*CAT*). Each individual sample was run in triplicate wells [27–30]. The RT-qPCR data were analyzed by the $2^{-\triangle\triangle Ct}$ method [31].

2.4. Challenge test

The challenge test was carried out separately according to Lin et al. [8]; Chiu et al. [32]. After the 60 days, fish were randomly selected for pathogen injection. *Aeromonas hydrophila (A. hydrophila, strain number:ATCC7966)*, selected as the pathogen, was cultured on tryptic soy agar for 24 h at 28 °C and transferred to 50 mL of tryptic soy broth for 24 h at 28 °C as the stock test culture. Broth cultures were centrifuged at 7000 *g* for 10 min at 4 °C. The supernatant was discarded, and bacterial pellets were re-suspended in saline solution (0.85% NaCl) as the stock bacterial solution. The challenge test was carried out in triplicate by an intraperitoneal injection of 60 μL of the stock bacterial solution, resulting in 4×10^6 cfu/g body weight. The mortality was observed after 14 days of challenge. The cumulative mortality was calculated.

In addition, the concentration of *A. hydrophila* was also measured using selective media. Each dissected intestine sample (5 g) was put into 50 mL of sterile distilled water and incubated in a rotary shaker (160 rpm) at 28 °C for 30 min. To assess the populations of *A. hydrophila*, the suspensions (200 μl) were smeared on Rimler-shotts and AHM culture mediums according to the National standard law of China GBT18652-2002 (Methods for detection of pathogenic *Aeromonas hydrophila*).

2.5. Statistical analyses

All data in this study were analyzed by Statistical Product and Service Solutions (SPSS 18.0) and were expressed as mean ± SE. Statistical analyses were performed using one-way ANOVA. Tukey's multiple-comparisons test (SAS Institute, Cary, NC, USA) was conducted to examine differences among four groups. Significant difference is set at the level of $P < 0.05$.

Table 1
The survival rate, yield and whole fish body composition of *Tilapia mossambica* after three months under CK, WR, ER and EO groups Values (mean ± S.E.) in the same column with different superscript letters significantly differ from each other ($P < 0.05$).

Group	Yield (g)	Survival rate	Moisture	Ash	Crude protein	Crude fat (%)
CK	57.65 ± 2.99[a]	94.87 ± 0.33[a]	71.03 ± 1.15	1.80 ± 1.11	14.87 ± 0.49[a]	8.16 ± 0.03[a]
WR	57.31 ± 3.85[a]	96.13 ± 0.68[b]	71.13 ± 0.75	1.85 ± 1.06	16.48 ± 0.41[b]	8.64 ± 0.19[b]
ER	57.49 ± 4.85[a]	96.67 ± 0.88[b]	71.43 ± 0.87	1.97 ± 1.28	16.58 ± 0.43[b]	8.87 ± 0.13[b]
EO	53.18 ± 2.50[b]	93.33 ± 0.75[c]	71.18 ± 1.49	1.80 ± 1.09	14.37 ± 0.18[a]	8.13 ± 0.53[a]

3. Results

3.1. The feasibility of culturing Tilapia mossambica with effluent containing R. capsulatus

To research the effect of effluent containing *R. capsulatus* on *Tilapia mossambica* growth, the survival rate, yield and whole fish body composition were determined. The results were showed in Table 1.

It was observed from Table 1 that *Tilapia mossambica* could survive and grow well under all given processing groups (CK, WR, ER and EO). The survival rate and yield in EO group were the lowest due to lack of food. The yield did not show significant difference between CK, WR and ER groups ($P > 0.05$). The survival rate was higher under WR and ER group than CK group, and showed significant difference ($P < 0.05$). The ER group was the best for the survival rate and yield under all given groups. The increased of survival rate and yield in ER group might be associated with *R. capsulatus* and residual organic matter in effluent through the analysis for the composition of the aquaculture water and bait. Table 1 indicated that it had very good feasibility to culture *Tilapia mossambica* using the effluent containing *R. capsulatus*.

Meanwhile, the moisture and ash content in whole fish body composition did not show significant difference between all given groups ($P > 0.05$). Compared with CK and EO groups, the crude protein and crude fat of *Tilapia mossambica* were improved under ER and WR groups, and presented significant difference ($P < 0.05$).

3.2. Affecting the disease resistance of Tilapia mossambica

To investigate the mechanism by which the effluent affected *Tilapia mossambica* growth from the point of nonspecific immunity and antioxidant, the alkaline phosphatase (AKP), acid phosphatase (ACP), phagocytic, superoxide dismutase (SOD) and catalase (CAT) activities of *Tilapia mossambica* were determined. The results were showed in Table 2.

The AKP, ACP, phagocytic, SOD and CAT activities of *Tilapia mossambica* in other three groups were the better than the control group, and ER and WR groups presented significant difference for CK group ($P < 0.05$). Among, the ER group was the best and showed significant difference for other three groups ($P < 0.05$).

To further investigate the molecular biological mechanism of the effluent regulating nonspecific immunity and antioxidant, *AKP, ACP, SOD* and *CAT* gene expression levels were determined. These results were showed in Table 3. The *AKP, ACP, SOD* and *CAT* gene expression levels in other three given groups were the better than the control group, and ER and WR groups presented significant difference for the control group ($P < 0.05$). Among, ER group had the best *AKP, ACP, SOD* and *CAT* gene expression levels, and showed significant difference for other three groups ($P < 0.05$). Tables 2–3 indicated that *R. capsulatus* and remainder biochemicals in effluent improved AKP, ACP, SOD and CAT activities through up-regulating *AKP, ACP, SOD* and *CAT* genes expression levels.

Table 3
The relative expression levels of *AKP, ACP, SOD, CAT* genes under CK, WR, ER and EO groups Values (mean ± S.E.) in the same column with different superscript letters significantly differ from each other ($P < 0.05$).

Group	SOD	CAT	AKP	ACP
CK	34 ± 2.61[a]	3.22 ± 0.45[a]	5.6 ± 1.32[a]	6.3 ± 1.23[a]
WR	45 ± 3.61[b]	4.36 ± 0.87[b]	8.9 ± 1.64[b]	8.9 ± 1.60[b]
ER	66 ± 3.92[c]	4.46 ± 1.02[c]	10.4 ± 1.54[c]	9.4 ± 1.16[c]
EO	36 ± 2.49[a]	3.24 ± 0.47[a]	6.2 ± 1.77[a]	6.8 ± 1.38[a]

Furthermore, the *Aeromonas hydrophila (A. hydrophila)* challenge test was also conducted. Table 4 showed the number of pathogenic bacteria and cumulative mortality of *Tilapia mossambica* in ER and WR groups were the lower than CK and EO groups, and presented significant difference ($P < 0.05$). Among, ER group was the best.

3.3. Regulation of mTOR and NF-kB signal transduction pathways

The relative expression levels of mTOR and NF-kB signaling pathway genes in head kidney were exhibited in Table 5. Compared with the control group, the relative expression level of *TOR gene* and *4E-BP gene* in mTOR and *NF-kB p65*, *IkBa* in NF-kB was increased in ER, WR and EO groups, and ER and WR groups presented significant difference for CK and EO groups ($P < 0.05$). Among, ER group was the best, and showed significant difference for other three groups ($P < 0.05$). Table 5 indicated that *R. capsulatus* promoted mTOR and NF-kB signaling pathway through up-regulating *TOR gene*, *4E-BP gene* in mTOR and *NF-kB p65*, *IkBa* in NF-kB gene expression levels.

In addition, to clarify the effect of *R. capsulatus* in effluent on *Tilapia mossambica* aquaculture water quality, the ammonia nitrogen, COD were determined in *Tilapia mossambica* aquaculture water. It was found from Table 6 that the *Tilapia mossambica* aquaculture water quality in WR and ER groups were significantly improved comparing with CK and EO groups. The ammonia and COD contents were reduced under WR and ER groups.

Table 2
The nonspecific immune related enzyme and antioxidant related enzyme activities of *Tilapia mossambica* under CK, WR, ER and EO groups Values (mean ± S.E.) in the same column with different superscript letters significantly differ from each other ($P < 0.05$).

Group	SOD	CAT	AKP	ACP (U/mg protein)	Phagocytic (%)
CK	7.5 ± 2.02[a]	4.8 ± 1.52[a]	2.8 ± 2.72[a]	23.9 ± 9.18[a]	25.7 ± 2.31[a]
WR	9.6 ± 1.82[b]	5.7 ± 1.75[b]	3.6 ± 2.26[b]	34.0 ± 7.37[b]	49.1 ± 2.79[b]
ER	10.7 ± 1.93[c]	6.4 ± 1.97[c]	5.0 ± 2.55[c]	31.5 ± 8.13[c]	49.9 ± 2.58[c]
EO	7.8 ± 1.70[a]	4.9 ± 1.99[a]	2.9 ± 3.54[a]	29.4 ± 10.43[a]	26.4 ± 2.49[a]

Table 4
The number of pathogenic bacteria and cumulative mortality of *Tilapia mossambica* under CK, WR, ER and EO groups Values (mean ± S.E.) in the same column with different superscript letters significantly differ from each other ($P < 0.05$).

Group	Number of *A.hydrophila* (CFU/g)	Cumulative mortality (%)
CK	4.5×10^{11a}	80.43 ± 3.41^{a}
WR	3.3×10^{4b}	16.87 ± 5.49^{b}
ER	3.1×10^{4b}	14.41 ± 6.75^{b}
EO	4.0×10^{11a}	79.67 ± 4.56^{a}

Table 5
The relative expression levels of *TOR*, *4E-BP*, *NF-kB p65*, *IkBa* genes in mTOR and NF-kB signaling pathway under CK, WR, ER and EO groups Values (mean ± S.E.) in the same column with different superscript letters significantly differ from each other ($P < 0.05$).

Group	TOR	4E-BP	NF-kB p65	IkBa
CK	163.9 ± 2.3^{a}	71.88 ± 6.55^{a}	33.67 ± 0.32^{a}	16.5 ± 2.3^{a}
WR	177.6 ± 2.9^{b}	87.04 ± 3.45^{b}	53.84 ± 0.18^{b}	40.1 ± 2.3^{b}
ER	189.0 ± 3.6^{c}	96.31 ± 5.48^{c}	54.11 ± 0.74^{c}	49.9 ± 2.3^{c}
EO	167.4 ± 2.7^{a}	74.69 ± 6.54^{a}	33.59 ± 0.32^{a}	16.9 ± 2.3^{a}

Table 6
The ammonia nitrogen, COD contents in *Tilapia mossambica* aquaculture water after three months under CK, WR, ER and EO groups Values (mean ± S.E.) in the same column with different superscript letters significantly differ from each other ($P < 0.05$).

Group	Ammonium	COD (mg/L)
CK	6.85 ± 0.66^{a}	160 ± 14.31^{a}
WR	4.43 ± 0.41^{b}	50.17 ± 5.89^{b}
ER	4.14 ± 0.51^{b}	45.65 ± 5.78^{b}
EO	6.33 ± 0.71^{a}	155 ± 13.15^{a}

4. Discussion

Current research showed that effluent containing *R. capsulatus* (ER group) had better promoting effect on the growth and the crude protein, crude fat contents than other three groups under the same breeding conditions (Table 1). The main reason was that the effluent, as aquaculture water, contained *R. capsulatus* and residual organic matter. Compared with chemical bait or water (CK), *R. capsulatus* or effluent provided more abundant and diverse nutrients for *Tilapia mossambica*. *R. capsulatus* were rich in and secreted diverse biochemicals such as amino acid (arginine), B vitamins, lipids, pigments and trace element (copper) [9,10]. These biochemicals contributed to the growth, metabolism and the synthesis of substances in *Tilapia mossambica*. Pereira et al. [33] found that arginine affected on growth and whole-body composition for *Nile tilapia*. Yuan et al. [22] studied that the copper supplementation enhanced the growth for juvenile *Litopenaeus vannamei*.

Meanwhile, an interesting phenomenon was observed from Table 1. *Tilapia mossambica* could also grow well and the yield also was enhanced under EO group compared with initial weight. Moreover, the yield and the crude protein, crude fat contents were also different between ER and WR groups under the same amount of *R. capsulatus*. It was found by analysis and comparison that the residual organic components in effluent was only different between ER and WR groups. SPW also was rich in some biochemicals (isoflavone, saponin, sterols and oligosaccharides) and nutrient substance [17]. The effluent without *R. capsulatus* still contained a certain amount of protein, peptide, amino acid, monosaccharide or biochemicals secreted by *R. capsulatus*.

These residual organic compounds could serve as food (protein, amino acid) even healthcare substance (isoflavone) [17]. For example, soy isoflavones could enhance the non-specific immunity and antioxidant capacities of cells according to Cao et al. [34]. Thus, Zhang et al. [29] used soybean meal (protein, isoflavone) to improve the growth of Japanese seabass (*Lateolabrax japonicus*). *Tilapia mossambica* could directly absorb and utilize these residual substances in effluent. Moreover, *R. capsulatus* could degrade unceasingly the residual organic matter into monosaccharide, amino acids and fatty acids. They also could secrete continually biochemicals using the residual organic matter as substrate. The residual substances as food promoted the crude protein, crude fat accumulations and growth of *Tilapia mossambica*. As healthcare substance, they improved the survival rate of *Tilapia mossambica*.

Table 1 indicated that it was feasible to promote *Tilapia mossambica* production, survival rate and whole fish body composition by effluent containing *R. capsulatus* and residual biochemicals. Similar studies had been reported on probiotics and biochemicals. Safari and Paolucci et al. [5] observed the maximum specific growth rate (2.32% day^{-1}) and survival rate (93.67%) in the juvenile crayfish fed with *galactooligosaccharide* + *Enterococcus faecalis* diet. Nguyen et al. [6] used the probiotic *Lactococcus lactis* WFLU12 to improve growth rate in olive flounder. Rahimnejad et al. [7] found the significant improvement in rock fish growth performance with *Pediococcus acidilactici MA18/5 M*, *galactooligosaccharide* and their synbiotic. Zhang et al. [29] used soybean meal (protein, isoflavone) to improve the growth of Japanese seabass (*Lateolabrax japonicus*). Although numerous studies have examined the effects of probiotics in aquatic animals, currently there was no consensus regarding the usefulness of probiotics, and the individual probiotic strains greatly differed in their effects on many aspects of host functions [8]. Moreover, there was no studies on *Tilapia mossambica* aquaculture and the improvement of yield using the effluent containing *R. capsulatus* and residual biochemicals.

Meanwhile, Tables 2–5 showed that the effluent improved the survival rate and production of *Tilapia mossambica*, which is closely related to the regulatory for the nonspecific immunity and antioxidant capabilities, mTOR and NF-kB signaling pathways and *A. hydrophila* inhibition by *R. capsulatus*. For nonspecific immunity systems, both AKP and ACP were the marker enzyme of macrophage lysosome in organism, and were also the important hydrolytic enzyme in nonspecific immunity [35,36]. They could kill invading pathogens, and also accelerate the phagocytosis of phagocytes and the degradation rate of foreign bodies. Moreover, AKP was closely related to the growth of aquatic animals, and played an important role in the absorption and utilization of nutrition, even the synthesis of protein. Thus, higher AKP and ACP activities had a positive effect on defense against external pathogens and microbial invasions. Meanwhile, it was also observed from Table 2 that phagocytic activity was significantly increased. Leukocyte had the function of the phagocytosis for pathogenic bacteria and bactericidal, which was an important aspect of non-specific immunization [8,20]. As Table 2 shown, ER group significantly increased the AKP and ACP, phagocytic activities of *Tilapia mossambica*, which improved the nonspecific immunity ability, disease resistance, survival rate and growth (Table 1).

As for antioxidant systems, the SOD and CAT were the vital enzymes in antioxidant defense system [20,26]. They were able to scavenge reactive oxygen species (Ros) and alleviate its damage to cells. Moreover, SOD could enhance the defense function of macrophages, and was closely related to the immune system. As Table 2 shown, ER group significantly increased SOD and CAT activities. This finding indicated that *R. capsulatus* could enhance the antioxidant ability of *Tilapia mossambica*, protected cells from damage, improved the survival rate and promoted the growth of *Tilapia mossambica* (Table 1).

With increased of ACP, AKP, SOD and CAT activities, Table 4 indicated that *A. hydrophila* was inhibited. For one thing, the remainder biochemicals and *R. capsulatus* in effluent enhanced the antioxidant and non-specific immune ability of *Tilapia mossambica* (Table 2), which inhibited or killed *A. hydrophila*. For another, *R. capsulatus* owned or

secreted some bio-active substances as inhibitors to inhibit or kill directly pathogenic bacteria *A. hydrophila*. Jagielo et al. [37] described that *R. capsulatus* cell wall contained 3-hydroxy-sebacic acid and diphosphonic acid lipoid A. Baker et al. [38] observed that *R. capsulatus* contained antiviral, antigen Ag1, Ag2, Ag3 and immune factors, even secreted antibiotics. Above bio-active substances could inhibit or kill pathogenic bacteria [37,38]. Moreover, *R. capsulatus* with absolute dominance also competed the nutrition and space with pathogenic microbes. Besides, *R. capsulatus* also might destroy the pathogenic bacteria by activating the specific immune response of *Tilapia mossambica*. Fečkaninová et al. [39] summarised the use of probiotic bacteria against *Aeromonas* infections in salmonid aquaculture. Yi et al. [40] studied the antimicrobial activity against fish pathogenic bacteria of probiotic *Bacillus velezensis JW*. Zhou et al. [30] resisted to *Aeromonas hydrophila* of juvenile yellow catfish with different arginine levels.

Meanwhile, these findings of Table 2 showed the effluent enhanced AKP, ACP, SOD and CAT activities simultaneously. Wu et al. [41]; Yu et al. [17]; Mujahid et al. [9] showed that both *R. capsulatus* and SPW effluent contained the biochemical substances (carotenoids, PHB, pantothenic acid, riboflavin, citric and sorbic acid) and a few metal ions. Further, Liu et al. [42] observed the effects of carotenoids on the immune related enzymes of yellow catfish. Duan et al. [43] studied the effect of dietary poly-β-hydroxybutyrate (PHB) on digestive enzymes of *Litopenaeus vannamei*. Li et al. [44] found that dietary pantothenic acid deficiency and excess depressed the digestive enzymes activities of grass carp. Chen et al. [45] found that dietary riboflavin deficiency decreases antioxidant related enzymes activities in the gills of *Ctenopharyngodon idella*. Zhang et al. [29] used isoflavone to improve the digestive enzymes activity of *Lateolabrax japonicus*. Zhou et al. [30] found that arginine enhanced non-specific immune system enzyme activity of juvenile yellow catfish. He et al. [46] observed that the citric and sorbic acid increased the alkaline phosphatase, phenoloxidase, glutathione peroxidase activities in shrimp. To sum up, *R. capsulatus* and SPW effluent regulated AKP, ACP, SOD and CAT activities by containing biochemical substances and metal ions.

With regard to the mechanism of biochemical substances and metal ions regulating these enzymes activities, there might be two reasons. Firstly, the biochemical substances and metal ions constituted enzymes or regulated enzyme synthesis pathway. *R. capsulatus* contained a variety of amino acids, which were the basic components of enzymatic proteins [10]. At the same time, they contained co-enzyme Q_{10}, 5-aminolevulinic acid and a large number of vitamins B (riboflavin, pantothenic acid, folic acid), which constituted a variety of co-enzymes (flavin mononucleotide, coenzyme A, transmethylase) [10]. Iron, magnesium and zinc were the active center of AKP, SOD and CAT in this work. Moreover, 5-aminolevulinic acid regulated the synthesis of cytochrome C, myoglobin, cytochrome, peroxidase and catalase by affecting porphyrin, ferrous hemoglobin and vitamin B_{12}. Besides, *R. capsulatus* might also enhance the enzyme activity through degrading enzyme inhibitors [10].

Secondly, *R. capsulatus* and the remainder biochemicals also might induce or stimulate the expression of *AKP, ACP, SOD* and *CAT* gene as stimulation signal or activation factor (Table 3). This view was explained by some researches. *R. capsulatus* were rich in carotenoid [10]. Chiu and Liu [12] observed that the carotenoid product enhanced the gene expression levels of *GHR1*, *IGF-1* and ACH_{50} in tilapia. Meanwhile, the composition of SPW and effluent was consistent with that of soybean meal [17]. Zhang et al. [29] used soybean meal (protein, isoflavone) to improve the expression of gut transporter genes of *Lateolabrax japonicus*. He et al. [46] observed that the organic acids and essential oils (thymol and vanillin) increased the gene expression levels of *TNF-α*, *LITAF* and *RAB6A* in shrimp.

As a signal transduction pathway, the mTOR signaling pathway, which plays a vital role in nutrition regulation and has complex impact on cell growth [47], widely exists in eukaryotes [48], food intake and environmental stresses [49,50]. TOR pathway is a key regulator of the balance between protein synthesis and degradation in response to nutrition quality and quantity [51,52], and the protein synthesis is essential for cell growth, proliferation, apoptosis, and autophagy [53]. Moreover, immune protein synthesis and nutrient transport are also each related to mTOR [54]. In this study, higher genes expression in mTOR signaling pathway was induced in ER and WR groups. The yield, immune-related enzymes were enhanced in ER and WR groups.

As for NF-kB signaling pathway, it regulated the congenital and acquired immunity, inflammation, stress response and the formation of B cell and lymphoid organ [55]. It was closely related to the differentiation of immune cells [56]. In this study, higher genes expression in NF-kB signaling pathway was induced in ER and WR groups, and then immune-related enzymes activities were enhanced in ER and WR groups. Jagielo et al. [37]; Baker et al. [38] thought that *R. capsulatus* contained the antigen Ag1, Ag2, Ag3 and the non-methylation dinucleotide sequence (CpG-DNA). Further, Baker et al. [38]; Morales-Nebreda et al. [57] observed that antigen Ag1, Ag2, Ag3 and CpG-DNA had very strong immune stimulation and induced the release of immune factors and differentiation of immune cells. Therefore, phagocytic activity was enhanced under ER and WR groups in this work. These results suggested that *R. capsulatus* promoted the differentiation of immune cells and the activities of immune-related enzymes by regulating NF-kB signaling pathway. Besides, *R. capsulatus* as stimulation signal also might activate the specific immune response to destroy pathogenic bacteria by regulating signal transduction pathway. Thus, the number of pathogenic bacteria and cumulative mortality of *Tilapia mossambica* was decreased under WR and ER groups, and thus survival rate was increased (Tables 1 and 5).

In addition, the ammonia and COD contents in aquaculture water were reduced under WR and ER groups (Table 6). According to Idi et al. [13], *R. capsulatus* had good removal effect on ammonia nitrogen due to they could use them as substrates nitrogen source. Similarly, Luo et al. [58] found that purple non-sulfur bacteria cleaned up aquaculture water quality during shrimp cultivation. Table 6 and Idi et al. [13]; Luo et al. [58] expressed that *R. capsulatus* can removed ammonia nitrogen and promoted fish growth as feed at the same time. A closed loop was formed that *R. capsulatus*-*Tilapia mossambica*-feces-*R. capsulatus*.

To the best of researchers' knowledge, the present study is the first one addressing to culturing *Tilapia mossambica* with the effluent containing *R. capsulatus*. Tables 1 and 6 indicated that effluent containing *R. capsulatus* could be reused directly to culture *Tilapia mossambica* as aquaculture water, microbial feeds and medicament, and to purify aquaculture water quality simultaneously. *R. capsulatus* improved the yield and survival rate, the nonspecific immunity, antioxidant and disease resistance capacities, the mTOR, NF-kB signaling pathway, and purified aquaculture water quality. The technology reduced the use of chemical feeds, medicament and aquaculture water in freshwater aquaculture, and completed the recycle and reuse of wastewater effluent and *R. capsulatus*. It also simplified the subsequent treatment process, burden, cost and energy consumption of *Tilapia mossambica* aquaculture wastewater.

5. Conclusion

Improvement of yield, disease resistance and aquaculture water quality of *Tilapia mossambica* by effluent containing *R. capsulatus* was feasible. Survival rate, yield, whole fish body composition were increased under ER group. Biochemical and remainder substances in effluent and *R. capsulatus* improved nonspecific immunity, antioxidant, mTOR and NF-kB signaling pathway through up-regulating related genes expression levels. Theoretical analysis showed biochemical substances regulated these genes expressions and enzyme activities as stimulus signal, component, active center. Moreover, *R. capsulatus* and biochemical substances inhibited *Aeromonas hydrophila*. Meanwhile, *R. capsulatus* in effluent purified aquaculture water quality. This technology would saved the water of aquaculture, reduced the water

pollution and wastewater discharge, and increased the output and disease resistance of *Tilapia mossambica* simultaneously.

Acknowledgments

The authors gratefully acknowledge the National Nature Science Fund for Distinguished Young Scholars of China (Grant No. 41625002), the National Natural Science Foundation Young of China (Grant No. 31700432), the National Natural Science Foundation of China (Grant No. 31700432; 31470550; 81500493; 31400386; 51778114), Post-doctoral Science Foundation Project of China and Heilongjiang Province (Grant No. 2018M641797; 2018HLJ6578), Opening Project of Key Laboratory of Soil Conservation and Rehabilitation in Heilongjiang Province (Grant No. SPR2018001), Natural Science Foundation of Liaoning Province (Grant No. 2019-ZD-0172), Natural Science Foundation of Guangdong Province (Grant No. 2015A030313098), Application Technology Research and Development Projects of Harbin (Grant No. 2016RAXXJ103), the MOA Modern Agricultural Talents Support Project for valuable financial support and Basic scientific research service fees of the Cairo University and Dalian Nationalities University (0113-20000101).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.09.001.

References

[1] E.S. Nunes, S.N. Desai, P.V. Desai, Effect of ferrous sulphate on aspartate and alanine aminotransferases of brain of *Tilapia mossambica*, Food Chem. Toxicol. 48 (2010) 490–494.
[2] Suhendrayatna, A. Ohki, T. Nakajima, S. Maeda, Studies on the accumulation and transformation of arsenic in freshwater organisms II. Accumulation and transformation of arsenic compounds by *Tilapia mossambica*, Chemosphere 46 (2002) 325–331.
[3] H.K. Miandare, S. Farvardin, A. Shabani, S.H. Hoseinifar, S.S. Ramezanpour, The effects of galactooligosaccharide on systemic and mucosal immune response, growth performance and appetite related gene transcript in goldfish (*Carassius auratus gibelio*), Fish Shellfish Immunol. 55 (2016) 479–483.
[4] P. Samanta, H. Im, J. Na, J.H. Jung, Ecological risk assessment of a contaminated stream using multi-level integrated biomarker response in *Carassius auratus*, Environ. Pollut. 233 (2018) 429–438.
[5] O. Safari, M. Paolucci, Modulation of growth performance, immunity, and disease resistance in narrow-clawed crayfish, Astacus leptodactylus leptodactylus (*Eschscholtz, 1823*) upon synbiotic feeding, Aquaculture 479 (2017) 333–341.
[6] T.L. Nguyen, C.I. Park, D.H. Kim, Improved growth rate and disease resistance in olive flounder, Paralichthys olivaceus, by probiotic *Lactococcus lactis WFLU12* isolated from wild marine fish, Aquaculture 471 (2017) 113–120.
[7] S. Rahimnejad, F.A. Guardiola, E. Leclercq, M.A. Esteban, S.M. Lee, Effects of dietary supplementation with *Pediococcus acidilactici MA18/5M*, galactooligosaccharide and their synbiotic on growth, innate immunity and disease resistance of rockfish (*Sebastes schlegeli*), Aquaculture 482 (2018) 36–44.
[8] H.L. Lin, Y.L. Shiu, C.S. Chiu, S.L. Huang, C.H. Liu, Screening probiotic candidates for a mixture of probiotics to enhance the growth performance, immunity, and disease resistance of Asian seabass, Lates calcarifer (Bloch), against *Aeromonas hydrophila*, Fish Shellfish Immunol. 60 (2017) 474–482.
[9] M.D. Mujahid, C.H. Sasikala, C.H.V. Ramana, Production of indole-3-acetic acid and related indole derivatives from L-tryptophan by *Rubrivivax benzoatilyticus JA2*, Appl. Microbiol. Biotechnol. 89 (2011) 1001–1008.
[10] P. Wu, Y. Zhang, Z.B. Chen, Y.L. Wang, F.F. Zhu, B. Cao, Y. Wu, N. Li, The organophosphorus pesticides in soil was degradated by *Rhodobacter sphaeroides* after wastewater treatment, Biochem. Eng. J. 141 (2019) 247–251.
[11] S. Chumpol, D. Kantachote, T. Nitoda, H. Kanzaki, Administration of purple nonsulfur bacteria as single cell protein by mixing with shrimp feed to enhance growth, immune response and survival in white shrimp (*Litopenaeus vannamei*) cultivation, Aquaculture 489 (2018) 85–95.
[12] K.H. Chiu, W.S. Liu, Dietary administration of the extract of Rhodobacter sphaeroides WL-APD911 enhances the growth performance and innate immune responses of seawater red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*), Aquaculture 418–419 (2014) 32–38.
[13] A. Idi, Z. Ibrahim, S.E. Mohamad, Z.A. Majid, Biokinetics of nitrogen removal at high concentrations by *Rhodobacter sphaeroides ADZ101*, Int. Biodeterior. Biodegrad. 105 (2015) 245–251.
[14] E.H.G. Ponsano, C.Z. Paulino, M.F. Pinto, Phototrophic growth of *Rubrivivax gelatinosus* in poultry slaughterhouse wastewater, Bioresour. Technol. 99 (2008) 3836–3842.
[15] P. Wu, J.Z. Li, Y.L. Wang, X.S. Liu, C. Du, Q.Y. Tong, N. Li, Promoting the growth of *Rubrivivax gelatinosus* in sewage purification by addition of magnesium ions, Biochem. Eng. J. 91 (2014) 66–71.
[16] T. Hülsen, K. Hsieh, S. Tait, E.M. Barry, D.J. Batstone, White and infrared light continuous photobioreactors for resource recovery from poultry processing wastewater-A comparison, Water Res. 144 (2018) 665–676.
[17] H.Q. Yu, F. Wilson, J. Tay, Kinetic analysis of an anaerobic filter treating soybean wastewater, Water Res. 32 (1998) 3341–3352.
[18] A.H. Simonne, R.R. Simonne, H.A. Eitenmiller, C.P. Mills III, Cresman, Could the Dumas method replace the Kjeldhal digestion for nitrogen and crude protein determinations in foods, J. Sci. Food Agric. 73 (1997) 39–45.
[19] J. Folch, M. Lees, C.H. Sloane-Stanley, A simple method for the isolation and purification of total lipids from animal tissue, J. Biol. Chem. 226 (1957) 497–509.
[20] X.H. Kong, D. Qiao, X.L. Zhao, L. Wang, H.X. Zhang, The molecular characterizations of Cu/ZnSOD and MnSOD and its responses of mRNA expression and enzyme activity to *Aeromonas hydrophila* or lipopolysaccharide challenge in Qihe crucian carp *Carassius auratus*, Fish Shellfish Immunol. 67 (2017) 429–440.
[21] R.U.L.M. Cooper, Clough, M.A. Farwell, T.L. West, Hypoxia-induced metabolic and antioxidant enzymatic activities in the estuarine fish *Leiostomus xanthurus*, J. Exp. Mar. Biol. Ecol. 279 (2002) 1–20.
[22] Y. Yuan, M. Jin, J. Xiong, Q.C. Zhou, Effects of dietary dosage forms of copper supplementation on growth, antioxidant capacity, innate immunity enzyme activities and gene expressions for juvenile *Litopenaeus vannamei*, Fish Shellfish Immunol. 84 (2019) 1059–1067.
[23] C.J. Secombes, Isolation of salmonid macrophage and analysis of their killing activity, in: T.C. Fletcher, D.P. Anderson, B.S. Roberson, W.B. Van Muiswinkel (Eds.), Techniques of Fish Immunology, vol. 1, SOS Publications, Fair Haven. NJ, 1990, pp. 137–154.
[24] M. Sakai, M. Kobayashi, T. Yoshida, Activation of rainbow trout, *Oncorhynchusmykiss*, phagocytic cells by administration of bovine lactoferrin, Comp. Biochem. Physiol. 110B (1995) 755–759.
[25] B. Houwen, Blood film preparation and staining procedures, Clin. Lab. Med. 22 (2002) 1–14.
[26] X.Z. Qi, M.Y. Xue, S.B. Yang, J.W. Zha, F. Ling, Ammonia exposure alters the expression of immune-related and antioxidant enzymes-related genes and the gut microbial community of crucian carp (*Carassius auratus*), Fish Shellfish Immunol. 70 (2017) 485–492.
[27] R. Meidong, K. Khotchanalekha, S. Doolgindachbaporn, T. Nagasawa, S. Tongpim, Evaluation of probiotic *Bacillus aerius B81e* isolated from healthy hybrid catfish on growth, disease resistance and innate immunity of *Pla-mong Pangasius bocourti*, Fish Shellfish Immunol. 73 (2018) 1–10.
[28] Y.J. Park, M. Moniruzzaman, S.H. Lee, J.W. Hong, S.C. Bai, Comparison of the effects of dietary single and multi-probiotics on growth, non-specific immune responses and disease resistance in starry flounder, *Platichthys stellatus*, Fish Shellfish Immunol. 59 (2016) 351–357.
[29] C. Zhang, S. Rahimnejad, Y.R. Wang, K.L. Lu, K.S. Mai, Substituting fish meal with soybean meal in diets for Japanese seabass (*Lateolabrax japonicus*): effects on growth, digestive enzymes activity, gut histology, and expression of gut inflammatory and transporter genes, Aquaculture 483 (2018) 173–182.
[30] Q.C. Zhou, M. Jin, Z.C. Elmada, X.P. Liang, K.S. Mai, Growth, immune response and resistance to *Aeromonas hydrophila* of juvenile yellow catfish, *Pelteobagrus fulvidraco*, fed diets with different arginine levels, Aquaculture 437 (2015) 84–91.
[31] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta DeltaC(T)) Method, Methods 25 (2001) 402–408.
[32] S.T. Chiu, Y.L. Shiu, Tsung-Meng Wu, Y.S. Lin, C.H. Liu, Improvement in non-specific immunity and disease resistance of barramundi, Lates calcarifer (Bloch), by diets containing *Daphnia similis* meal, Fish, Shellfish Immunol 44 (2015) 172–179.
[33] R.T. Pereira, P.V. Rosa, D.M. Gatlin, Glutamine and arginine in diets for *Nile tilapia*: effects on growth, innate immune responses, plasma amino acid profiles and whole-body composition, Aquaculture 473 (2017) 135–144.
[34] Z.H. Cao, J.M. Green-Johnson, N.D. Buckley, Q.Y. Lin, Bioactivity of soy-based fermented foods: a review, Biotechnol. Adv. 37 (2019) 223–238.
[35] E. Gisbert, H. Nolasco, M. Solovyev, Towards the standardization of brush border purification and intestinal alkaline phosphatase quantification in fish with notes on other digestive enzymes, Aquaculture 487 (2018) 102–108.
[36] Z.Q. Xia, S.J. Wu, Effects of glutathione on the survival, growth performance and non-specific immunity of white shrimps (*Litopenaeus vannamei*), Fish Shellfish Immunol. 73 (2018) 141–144.
[37] P.J. Jagielo, T.J. Quinn, N. Qureshi, D.A. Schwartz, Grain dust-induced lung inflammation is reduced by *Rhodobacter sphaeroides* diphosphoryl lipid A, Am. J. Physiol. 274 (1998) 26–31.
[38] P.J. Baker, C.E. Taylor, P.W. Stashak, M.B. Fauntleroy, K. Hasløv, N. Qureshi, K. Takayama, Inactivation of suppressor T cell activity by the nontoxic lipopolysaccharide of *Rhodopseudomonas sphaeroides*, Infect. Immun. 58 (1990) 2862–2868.
[39] A. Fečkaninová, J. Koščová, D. Mudroňová, P. Popelka, J. Toropilová, The use of probiotic bacteria against *Aeromonas* infections in salmonid aquaculture, Aquaculture 469 (2017) 1–8.
[40] Y. Yi, Z. Zhang, F. Zhao, H. Liu, L. Yu, J. Zha, G. Wang, Probiotic potential of *Bacillus velezensis JW*: antimicrobial activity against fish pathogenic bacteria and immune enhancement effects on *Carassius auratus*, Fish Shellfish Immunol. 78 (2018) 322–330.
[41] P. Wu, Z.B. Chen, Y. Zhang, Y.L. Wang, F.F. Zhu, B. Cao, Y. Wu, N. Li, *Rhodopseudomonas palustris* wastewater treatment: cyhalofop-butyl removal, biochemicals production and mathematical model establishment, Bioresour. Technol. 282 (2019) 390–397.

[42] F. Liu, Y.K. Qu, A.M. Wang, Y.B. Yu, Q. Nie, Effects of carotenoids on the growth performance, biochemical parameters, immune responses and disease resistance of yellow catfish (*Pelteobagrus fulvidraco*) under high-temperature stress, Aquaculture 503 (2019) 293–303.
[43] Y.F. Duan, Y. Zhang, H.B. Dong, X.T. Zheng, J.S. Zhang, Effect of dietary poly-β-hydroxybutyrate (PHB) on growth performance, intestinal health status and body composition of Pacific white shrimp *Litopenaeus vannamei (Boone, 1931)*, Fish Shellfish Immunol. 60 (2017) 520–528.
[44] L. Li, L. Feng, W.D. Jiang, J. Jiang, Y. Liu, Dietary pantothenic acid deficiency and excess depress the growth, intestinal mucosal immune and physical functions by regulating NF-κB, TOR, Nrf2 and MLCK signaling pathways in grass carp (*Ctenopharyngodon idella*), Fish Shellfish Immunol. 45 (2015) 399–413.
[45] L. Chen, L. Feng, W.D. Jiang, J. Jiang, Y. Liu, Dietary riboflavin deficiency decreases immunity and antioxidant capacity, and changes tight junction proteins and related signaling molecules mRNA expression in the gills of young grass carp (*Ctenopharyngodon idella*), Fish Shellfish Immunol. 45 (2015) 307–320.
[46] W.Q. He, S. Rahimnejad, L. Wang, K. Song, C.X. Zhang, Effects of organic acids and essential oils blend on growth, gut microbiota, immune response and disease resistance of Pacific white shrimp (*Litopenaeus vannamei*) against *Vibrio parahaemolyticus*, Fish Shellfish Immunol. 70 (2017) 164–173.
[47] D. Cota, E.K. Matter, S.C. Woods, R.J. Seeley, The role of hypothalamic mammalian target of rapamycin complex 1 signaling in diet-induced obesity, J. Neurosci.: Off. J. Soc. Neurosci. 28 (2008) 7202–7208.
[48] N. Takei, K. Furukawa, O. Hanyu, H. Sone, H. Nawa, A possible link between BDNF and mTOR in control of food intake, Front. Psychol. 5 (2014) 1093–1095.
[49] P. Wu, W.G. Yang, Y.Y. Dong, Y.L. Wang, Y. Zhang, X.J. Zou, H. Ge, D.X. Hu, Y.B. Cui, Z.B. Chen, Feasibility of cultivation of *Spinibarbus sinensis* with coconut oil and its effect on disease resistance (nonspecific immunity, antioxidation and mTOR and NF-kB signaling pathways), Fish Shellfish Immunl. 93 (2019) 726–731.
[50] H.D. Li, X.L. Tian, K. Zhao, W.W. Jiang, S.L. Dong, Effect of clostridium butyricum in different forms on growth performance, disease resistance, expression of genes involved in immune responses and mTOR signaling pathway of *Litopenaeus vannamai*, Fish Shellfish Immunol. 87 (2019) 13–21.
[51] P. Wu, Z.B. Chen, Y. Zhang, Y.L. Wang, F.F. Zhu, B. Cao, L.M. Jin, X.Y. Hou, Y. Wu, N. Li, Carbaryl waste-water treatment by *Rhodopseudomonas sphaeroides*, Chemosphere 233 (2019) 597–602.
[52] M. Wu, S. Lu, X. Wu, S. Jiang, Y. Luo, W. Yao, et al., Effects of dietary amino acid patterns on growth, feed utilization and hepatic *IGF-I, TOR* gene expression levels of hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂*)* juveniles, Aquaculture 468 (2017) 508–514.
[53] P. Wu, W.T. Mo, Z.B. Chen, Y.L. Wang, Y.B. Cui, Y. Zhang, Y.T. Song, L.M. Jin, X.Y. Hou, F.F. Zhu, B. Cao, N. Li, The removal of cyhalofop-butyl in soil by surplus *Rhodopseudanonas palustris* in wastewater purification, J. Environ. Manage 245 (2019) 168–172.
[54] M.E. Cardenas, N.S. Cutler, M.C. Lorenz, C.J.D. Como, J. Heitman, The TOR signaling cascade regulates gene expression in response to nutrients, Genes 13 (1999) 3271–3279.
[55] P. Wu, B. Cao, Y. Zhang, W.B. Li, Y.L. Wang, Y. Wu, N. Li, The bio-mitigation of acetochlor in soil using *Rhodopseudomonas capsulata* in effluent after wastewater treatment, J. Soil. Sediment 2 (2019) 1–7.
[56] P. Wu, P.C. Huo, Y.L. Wang, Y.Y. Dong, Y.B. Cui, Z.B. Chen, Y. Zhang, X.Z. Wu, Practicality of effluent containing *Rubrivivax gelatinosus* culturing the crucian carp, Aquaculture 514 (2020) 734418.
[57] L. Morales-Nebreda, F.S. McLafferty, B.D. Singer, DNA methylation as a transcriptional regulator of the immune system, Transl. Res. 204 (2019) 1–18.
[58] W. Luo, X. Deng, W. Zeng, D. Zheng, Treatment of wastewater from shrimp farms using a combination of fish, photosynthetic bacteria, and vegetation, Desalin. Water Treat. 47 (2012) 221–227.