Cell Wall Components of *Bacillus pumilus* SE5 Improved the Growth, Digestive and Immunity of Grouper (*Epinephelus coioides*)

Hong-Ling Yang1†, Xi Hu1†, Ji-Dan Ye1, Vijayaram Seerengaraj1, Wei Yang2, Chun-Xiang Ai3 and Yun-Zhang Sun1,*

1Fisheries College, Jimei University, Xiamen 361021, China; 2Xiamen Jiakang feed Co., Ltd., Xiamen 361021, China; 3College of Ocean and Earth Science, Xiamen University, Xiamen 361000, China

Abstract: **Background:** Probiotic cellular components could be an interesting alternative to live probiotics, which could potentially cause safety problems in open aquatic environments.

**Objective:** The cell wall (CW), peptidoglycan (PG) and lipoteichoic (LTA) were extracted from probiotic strain of *Bacillus pumilus* SE5, and these biomolecules were used to develop the possible application in fish aquaculture.

**Methods:** Grouper (*Epinephelus coioides*) juveniles were fed with either a basal control diet or the basal diet supplemented with CW, PG and LTA respectively for 60 days, and the growth performance, digestive enzymes activities, serum immune responses and immune genes expression in head kidney were determined.

**Results:** Dietary supplement PG and LTA significantly improved final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR) and protein efficiency ratio (PER), while significantly decreased feed conversion ratio (FCR) was noticed in all the treatments compared with the control group. Dietary supplements of CW, PG and LTA enhanced the activities of trypsin, lipase and amylase in the liver. Serum complement C3 and IgM levels as well as, AKP, ACP and SOD activities elevated significantly in fish fed the PG and LTA containing diets. Furthermore, PG and LTA containing diets, significantly up-regulated expression of TLR2, NOD2, IL-8, IgM and three antibacterial peptides genes (epinecidin-1, hepcidin-1 and β-defensin) in the head kidney.

**Conclusion:** PG and LTA originated from probiotic *B. pumilus* SE5 could effectively enhance the growth performance, feed utilization, digestive ability and boost innate and adaptive immune system of *E. coioides*.

**Keywords:** Probiotics, *Bacillus pumilus*, peptidoglycan, lipoteichoic acid, growth, immunity, *Epinephelus coioides*.

1 INTRODUCTION

Groupers of the genus *Epinephelus* are economically important mariculture fish species in China and Southeast Asian countries and the annual production in China reaches as high as 1,83,127 tons in 2019. Due to intensive culture, groupers are vulnerable to diseases caused by pathogenic bacteria, like *Vibrio* species [1]. In the past decades, antibiotics have been applied extensively in fish aquaculture to control bacterial disease. However, the widespread use of antibiotics as a preventive measure may alter the gut microbiota of fish and induce resistant bacteria populations, with unpredictable long-term effects on public health [2, 3].

Therefore, there is a growing interest in the use of functional feed additives, such as probiotics, as a sustainable alternative for improving the general health and welfare of fish [3-6].

It has been proposed that probiotic applications for aquaculture can be a substance of a microbial cell whose benefits to the hosts are achieved at least in part via improving the microbial balance of hosts or the ambient environment [7, 8]. It has been extensively reported that probiotics could improve immune responses, disease resistance and growth performance in fish [2, 3, 9]. Viability has been suggested as an important characteristic of any probiotics, which help them to adhere and subsequently colonize the intestinal tract of the host [6]. However, numerous reports are demonstrated that inactivated probiotics and even probiotic cellular components are benefits to the fish host [6, 10, 11]. For example, Sharifuzzaman *et al.* (2011) reported that probiotic strain of...
Probiotic Bacillus pumilus SE5 is a dominant bacterium in the gut of fast growing grouper Epinephelus coioides [9], both the viable and heat-inactivated B. pumilus SE5 could modulate the intestinal immunity and microbiota [12], and improve the growth performance and systemic immunity in E. coioides [13]. Currently, probiotic strain of B. pumilus SE5 cell wall (CW), peptidoglycan (PG) and lipoteichoic acid (LTA) were extracted and their effects on intestinal immune related genes expression and microbiota were evaluated in a 60 days feeding trial in E. coioides [14]. In this study, the effects of CW, PG and LTA from B. pumilus SE5 on growth performance, digestive ability and systemic immunity in E. coioides were evaluated.

2. MATERIALS AND METHODS

2.1. Probiotic Strain and Cell Wall Components Extraction

Probiotic strain of Bacillus pumilus SE5 was isolated from the intestine of juvenile grouper Epinephelus coioides and cultured and prepared as previously described [9]. After incubation, the cells were harvested and re-suspended in PBS. The number of SE5 in the suspension was approximately 1.0×10^9 cells ml^-1, which was determined by plate counting on Tryptone Soya Agar (TSA) at 28 °C for 48 h. The live bacterial suspension was heat-inactivated in a water bath at a temperature 95 °C for 60 min, and the non-viability was checked by plating on TSA. Cell Wall (CW), peptidoglycan (PG) and Lipoteichoic Acid (LTA) were extracted from probiotic strain of B. pumilus SE5 as we previously described [14]. Briefly, to extract CW, bacterial culture was centrifuged and the harvested cells were suspended in a lysis solution and submitted to sonication, the target product was harvested by centrifugation and washed with sterile water for 3–4 times, then lyophilized and stored at -80 °C. To extract PG, bacterial sludge was dissolved in 10% TCA, incubated in a boiling bath for 1 h, and then centrifuged. The sediment was treated with a special solvent, chloroform, methanol mix for 24 h. After centrifugation, the insoluble residue was incubated in Tris-HCl containing 0.15% trypsin, and the mixture was centrifuged and the sediment was harvested and washed in sterile water, then lyophilized and stored at -80 °C. To extract LTA, bacteria suspension was mixed with n-butanol in a shaking bath, after centrifugation, the aquatic phase was lyophilized, resuspended with chromatography start buffer and centrifuged. The supernatant was subjected to Hydrophobic Interaction Chromatography (HIC) on octyl-Sepharose, the target product was collected and confirmed as previously described [14].

2.2. Diet Preparation and Sample Collection

Probiotic strain of B. pumilus SE5 at dose of 1.0×10^8 CFU g^-1 has been confirmed to be effective in modulating the intestinal immunity and microbiota in grouper E. coioides [9, 12]. In this study, heat-inactivated SE5 (HK) was supplemented to the basal diet with the dose of 1.0×10^8 CFU g^-1, and CW, PG and LTA were extracted from the same amount of B. pumilus SE5 and added to the basal diet. The experimental diets were formulated as previously described [14]. Briefly, experiment diets were prepared by gently spraying the required amount of heat-inactivated SE5 (HK), CW, PG or LTA suspensions on the control diet and mixed in a three-dimensional drum mixer. Dietary ingredients were mixed with the required amount of water and then cold press extruded (CD4XITS extruder, South China University of Technology, Guangzhou, China) to produce 5 mm pellets.

The animal trial was conducted in the Haikang Aquaculture Research Base of Dabeilong Aquaculture group (Zhaoan, China). The feeding trial with five groups was conducted in 20 fibreglass tanks, each connected to an open circulating system with 300-l seawater. Each tank was randomly stocked with 30 fish (initial weight 7.85±0.21 g) and each treatment has four tanks. Fish were fed to apparent satiation at 08:30 and 18:30 h with one of the five diets for 60 days. Uneaten feed was recovered, then dried and weighed, to determine the amount of feed consumed. The growth performance and feed utilization were determined as described in Yan et al. (2016) [13]. The survival rate (SR), weight gain rate (WGR), feed conversion ratio (FCR), and specific growth rate (SGR), protein efficiency ratio (PER) were calculated using the following formulae: WGR (%) = (W_t - W_0)/ W_0 × 100; SGR (%/d) = (ln W_t - ln W_0) × 100/t; FCR = FI/(W_t - W_0); PER = (W_t - W_0)/(FI × P) ×100; SR (%) = N_t/N_0 × 100. Where W_0 is the weight of fish at day t, W_t is the initial weight of fish, t is the duration of feeding (in days), FI is feed intake, P is protein content of feed, N_0 is the initial number of fish, N_t is the number of fish at day t.

For serum immune parameters analysis, two fish were taken randomly from each tank (i.e. 6 fish per treatment) at day 60. Blood was individually withdrawn from the caudal vein and the serum was collected by centrifugation at 1500 ×g for 10 min at 4 °C and stored at -80 °C until analysis. For digestive enzymes activities and immune genes analysis, two fishes were taken randomly from each tank (i.e. 6 fish per treatment) at day 60, fish liver and head kidney was aseptically excised and collected as described in Yan et al. (2016) [13]. Then, the samples were stored at -80°C in TRizol reagent (Invitrogen, Carlsbad, USA) for RNA extraction and immune genes analysis.

2.3. Digestive Enzymes Activities Assays

The total soluble protein content, protease, amylase and lipase activities in liver were measured using a commercial kit (Jiancheng, Nanjing, Jiangsu, China) as previously described [15]. Enzyme activities were measured as the change in absorbance using a spectrophotometer (UV-2802S, Unico, Shanghai, China) and expressed as specific activity (U/mg^-1 protein) [15].
2.4. Serum Immunological Assays

2.4.1. Acid Phosphatases (ACP) and Alkaline Phosphatase (AKP) Assay

The Acid Phosphatases (ACP) and alkaline phosphatase (AKP) activities in serum were measured by its ability to break down di-sodium phenyl phosphate using a reagent kit (Jiancheng, Nanjing, Jiangsu, China) [16]. One unit of ACP or AKP activity was defined as the amount of enzyme that reacted with the matrix and produced 1 mg phenol in 15 min at 37 °C.

2.4.2. Superoxide Dismutase (SOD) Assay

Serum SOD activity was measured by its ability to inhibit superoxide radical-dependent reactions using a commercial kit (Jiancheng, Nanjing, Jiangsu, China) as previously described [9, 13]. One unit of SOD activity was defined as the amount of enzyme necessary to produce a 50% inhibition of the Nitro Blue Tetrazolium (NBT) reduction rate measured at 550 nm.

2.4.3. Complement C3 Assay

The serum complement C3 level was determined by using a commercial kit (Jiancheng, Nanjing, Jiangsu, China). Analysis of the complement level included measurement of the increase in turbidity after immunity response of complement and its increased antibody [9, 13]. Results of complement C3 are presented as mg/mL-1.

2.4.4. Serum IgM level

The serum IgM level was determined by an enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Jianchen, Nanjing, Jiangsu, China) as previously described [9, 13]. ELISA plates were read at 450 nm using a plate reader. Negative controls consisted of samples without the biotin antibody. The mean absorbance of the negative controls for each plate was subtracted from the optical density at 450 nm.

2.5. Immune Related Genes Expression Analysis

Head kidney tissues (six per treatment) were homogenized and total RNA was extracted from each homogenized tissue sample using TRIzol™ reagent (Invitrogen, California, USA). First-strand cDNA was synthesized using a TIANscript RT Kit (Tiangen, Beijing, China). The expression level of genes determined in this study included TLR2, NOD2, IL-8, epinecidin-1, hepcidin-1, β-defensin and IgM. The cDNA was used to perform real-time PCR with specific primers as previously described [14]. The RT-qPCR was performed with the SYBR Green Real-time PCR Master Mix (Toyobo, Shanghai, China) in an ABI 7500 real-time PCR Detection system (Applied Biosystems, California, USA). All RT-qPCR were performed at least three times.

2.6. Statistical Data Analysis by SPSS Method

Data of expression of immune genes from eight samples are presented as fold increase (mean ± standard error, SE). Data were examined by one-way analysis of variance (ANOVA). When ANOVA identified differences among groups, a multiple comparison (Duncan’s) test was conducted to examine significant differences among treatments using Statistical Package for Social Science (SPSS), release 22.0 (SPSS Inc., Chicago, IL, USA). Significant differences were declared at P ≤ 0.05.

3. RESULTS

3.1. Effect of Cellular Components on the Growth Performance and Feed Utilization

After 60 days of feeding trial period, fish biomass (initial weight 7.85±0.21 g) increased by 201.21%, 200.72%, 249.07% and 235.90% in the groups HK, CW, PG and LTA, respectively, but only 177.26% in the control. Significantly improved final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR) and protein efficiency ratio (PER) were observed in groups PG and LTA (P < 0.05), while there are no significant difference between the groups HK, CW and the control. Compared with the group HK, significantly increased FBW, WGR and SGR was observed in the group PG (P < 0.05). Significantly decreased feed conversion ratio (FCR) was noticed in all the treatments compared with the control (P < 0.05). The survival rate (SR) showed no significant difference between the treatments and control (Table 1).

3.2. Effect of Cellular Components on the Digestive Enzymes Activities

At the end of the trial, trypsin and lipase activities were significantly increased in all treatments compared to the control group (P < 0.05), while the improvement of trypsin and lipase activities were observed in the group PG (Fig. 1A, 1B). Amylase activities in groups CW, PG and LTA were significantly higher than that in the control group (P < 0.05) and the enhancement of amylase activity was observed in the group PG, which was significantly higher than that in groups HK and LTA (Fig. 1C).

3.3. Effect of Cellular Components on the Serum Immune Parameters

At the end of the trial, serum ACP and AKP activities in groups PG and LTA were higher significantly than those in control (P < 0.05), while the groups HK and CW had shown no significant differences with the control group (Fig. 2A, 2B). Serum SOD activity in groups CW, PG and LTA were significantly increased than those in the control group and group HK (P < 0.05) (Fig. 2C). Serum complement C3 and IgM levels in groups CW, PG and LTA increased significantly than those in the control group (P < 0.05), while the group HK, showed no significant difference with the control group (Fig. 2D, 2E).

3.4. Effect of Cellular Components on Immune Genes Expression

As showed in Fig. (3), significantly upregulated expression of TLR2 and NOD2 was observed in groups CW, PG and LTA compared with the control group, and the expression of TLR2 in the group PG was significantly higher than that in the group HK (P < 0.05) (Fig. 3A, 3B). Significantly upregulated gene expression of IL-8 was observed in the groups PG and LTA when compared with the control group,
Fig. (1). Digestive enzymes activities (U mg\(^{-1}\) protein) in the liver of *Epinephelus coioides* fed the control diet, or diets containing heat-inactivated *B. pumilus* SE5 (HK), cell wall (CW), peptidoglycan (PG) and lipoteichoic acid (LTA) from SE5. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 1. **Effects of cellular components of *B. pumilus* SE5 on growth performance, feed utilization and survival of *Epinephelus coioides***.

<table>
<thead>
<tr>
<th>Groups</th>
<th>FBW (g)</th>
<th>WGR (%)</th>
<th>SGR (% /d)</th>
<th>FCR</th>
<th>PER</th>
<th>SR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.44±0.97(^a)</td>
<td>177.26±10.65(^c)</td>
<td>1.66±0.08(^c)</td>
<td>1.23±0.04(^a)</td>
<td>1.72±0.05(^b)</td>
<td>99.17±0.83</td>
</tr>
<tr>
<td>HK</td>
<td>23.77±0.73(^bc)</td>
<td>201.21±5.87(^bc)</td>
<td>1.84±0.03(^bc)</td>
<td>1.10±0.05(^b)</td>
<td>1.94±0.09(^ab)</td>
<td>97.50±2.50</td>
</tr>
<tr>
<td>CW</td>
<td>24.20±0.56(^bc)</td>
<td>200.72±9.04(^bc)</td>
<td>1.83±0.05(^bc)</td>
<td>1.09±0.02(^b)</td>
<td>1.94±0.03(^ab)</td>
<td>100.00±0.0</td>
</tr>
<tr>
<td>PG</td>
<td>27.00±0.97(^a)</td>
<td>249.07±11.27(^a)</td>
<td>2.06±0.04(^a)</td>
<td>0.96±0.06(^b)</td>
<td>2.22±0.14(^a)</td>
<td>99.74±2.28</td>
</tr>
<tr>
<td>LTA</td>
<td>26.27±0.58(^ab)</td>
<td>235.90±8.45(^ab)</td>
<td>2.01±0.04(^ab)</td>
<td>1.07±0.01(^b)</td>
<td>1.97±0.02(^a)</td>
<td>99.17±0.83</td>
</tr>
</tbody>
</table>

Note: Different superscripts in the same column data indicate significant differences (\(^P<0.05\)); HK- heat killed SE5; CW- cell wall; PG- peptidoglycan; LTA- lipoteichoic acid; FBW- final body weight; WGR- weight gain rate; SGR- specific growth rate; FCR- feed conversion rate; PER- protein efficiency ratio; SR- survival rate.
Fig. (2). Acid phosphatases (ACP), alkaline phosphatase (AKP), superoxide dismutase (SOD) activities, and complement C3 and IgM levels in the serum of *Epinephelus coioides* fed the control diet, or diets containing heat-inactivated *B. pumilus* SE5 (HK), cell wall (CW), peptidoglycan (PG) and lipoteichoic acid (LTA) from SE5. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).
Fig. (3). The expression of immune genes in the head kidney of *Epinephelus coioides* fed the control diet, or diets containing heat-inactivated *B. pumilus* SE5 (HK), cell wall (CW), peptidoglycan (PG) and lipoteichoic acid (LTA) from SE5. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
groups HK and CW \((P < 0.05)\) (Fig. 3C). Significantly up-regulated gene expression of IgM was observed in groups CW, PG and LTA compared with the control and the gene expression of IgM in the groups PG and LTA was significantly higher than that in the groups HK and CW \((P < 0.05)\) (Fig. 3D).

The gene expression of three antibacterial peptides genes (epinecidin-1, hepcidin-1 and \(\beta\)-defensin) was determined by RT-qPCR (Fig. 3E). Compared with the control, significantly increased gene expression of hepcidin-1 was observed in all treatments, while significantly increased gene expression of epinecidin-1 and \(\beta\)-defensin was observed in groups PG and LTA \((P < 0.05)\). In addition, the gene expression of epinecidin-1 and \(\beta\)-defensin in groups PG and LTA were significantly higher than those in groups HK and CW \((P < 0.05)\).

4. DISCUSSION

The effects of probiotics on growth and immunity have been extensively studied in fish [2, 17]. However, little information is available about the application of cellular components of probiotics in fish aquaculture [10, 11]. In the present study, growth performance and immunostimulatory effects of diets containing cell wall (CW), peptidoglycan (PG) and lipoteichoic acid (LTA) from probiotic strain of \(B.\) \(pumilus\) SE5 were evaluated in grouper (\(E.\) \(coioides\)). The results showed that PG and LTA from \(B.\) \(pumilus\) SE5 could significantly improve the growth performance and feed utilization in \(E.\) \(coioides\). Interestingly, the growth performance of fish fed diet containing PG was even better than those fed diet containing heat inactivated whole probiotic cell. The precise mechanisms under these effects remain unclear. Probiotics have been shown to enhance the digestive enzymes activities, improve the growth performance and/or feed utilization in fish [18]. Interestingly, to the best of our knowledge, this study showed for the first time that PG and LTA originated from a probiotic strain of \(B.\) \(pumilus\) SE5 significantly increased the digestive enzymes activities in fish; this may be one possible reason why the growth and feed utilization improved in fish fed diets containing probiotic cellular components. Recently, in vitro study demonstrated that LTA from probiotic \(Lactobacillus\) \(plantarum\) could inhibit pathogenic organism of \(Vibrio\) \(anguillarum\)-induced inflammation and apoptosis in intestinal epithelial cells of silvery pomfret (\(Pampus argenteus\)) [19]. In accord with the results, our previous study has shown that PG and LTA of \(B.\) \(pumilus\) SE5 shaped the intestinal microbiota and mucosal immunity in \(E.\) \(coioides\) [14]. Therefore, this maintenance of intestinal homeostasis by cellular components of probiotics was postulated to benefit the digestion and metabolism of the nutrients in the intestine and thus promote the growth performance and feed utilization.

It has been extensively reported that dietary supplementation of whole probiotic cells, live or inactivated, could improve the immune response in fish, but the mechanisms are largely unclear [12]. It has been proposed that cell components shed by commensal microbes/probiotics can activate toll-like receptors (TLRs) signaling cascades that finely tune the production of immune effectors [20]. In this study, significantly up regulated genes expression of TLR2 and NOD2 was observed in the head kidney of grouper fed PG and LTA containing diets, which is in line with an in vitro study which demonstrated that LTA from probiotic \(L.\) \(plantarum\) significantly increased the gene expression of TLR2 in intestinal epithelial cells from silvery pomfret (\(Pampus argenteus\)) [21]. The activation of TLR2 and NOD2 usually indicated the inducing gene expression of downstream cytokines. In fact, a significantly increased gene expression of IL-8 was observed in fish fed PG and LTA containing diets in this study. Similarly, MacKenzie et al. (2010) observed that both LTA (from \(Bacillus subtilis\)) and PG (from \(Staphylococcus aureus\) and \(B.\) \(subtilis\)) hold an equal potency to induce cytokine gene expression in rainbow trout macrophages. Moreover, they proposed that the induction of cytokines in trout by crude LPS was primarily due to the contaminating PG and nucleic acids, since ultrapure LPS were inactive [22]. Induction of pro-inflammatory cytokines may indicate that cell components from probiotic strains may stimulate downstream immune responses.

It has been reported that innate immune factors such as complement, lysozyme and phagocytic activity were upregulated in fish by the administration of bacterial PG [23, 24]. In line with the previous study, the data in the present study demonstrated that diets contain CW, PG and LTA could enhance serum SOD activity and complement C3 level in grouper, and diets contain CW and PG significantly improve the ACP and AKP activities. In addition, both PG and LTA from \(B.\) \(pumilus\) SE5 could significantly increase the expression of three AMPs (epinecidin-1, hepcidin-1 and \(\beta\)-defensin) in the head kidney of \(E.\) \(coioides\). AMPs are part of fish innate immune system and clear pathogens effectively by interacting directly with their negatively charged membrane, disrupting the osmotic balance of the microbial membrane [25]. Based on these data, PG and LTA from \(B.\) \(pumilus\) SE5 could induce an improved innate defense mechanism and inhibit the growth of pathogenic organisms, as our previous study have shown that PG and LTA from SE5 significantly decreased the abundance of common pathogenic \(Vibrio\) and increased the abundance of beneficial \(Lactobacillus\) in the intestine of \(E.\) \(coioides\) [14].

In teleost fish, different types of immunoglobulin were characterized such as, IgM, IgD, IgT and IgZ, while IgM is the main type and mediates antigen-specific opsonization and phagocytosis of bacterial pathogens [26]. Several studies have shown that probiotics, live or heat-killed, could improve the total IgM level in the serum of fish [27]. In this study, CW, PG and LTA of \(B.\) \(pumilus\) SE5 could effectively enhance serum IgM level and the expression of IgM gene in the head kidney of grouper. This study suggested that cell components from indigenous probiotic \(B.\) \(pumilus\) SE5 may not only induce the innate immunity and also the acquired immunity in grouper.

CONCLUSION

PG and LTA from indigenous probiotic strain \(B.\) \(pumilus\) SE5 could improve the growth performance, feed utilization, digestive enzymes activities and induce the expression of TLR2 and NOD2 signaling genes and also tune the innate and acquired immunity of \(E.\) \(coioides\).
ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The procedures for care and use of marine animals were approved by the animal care and use committee of Jimei University, China.

HUMAN AND ANIMAL RIGHTS

All experiments are in accordance with the guidelines of Jimei University, China.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

FUNDING

This work was financially supported by the national natural science foundation of China (Grant No. 32072990, No.31772861), the Industry-University Cooperation Project of Fujian Province (Grant No. 2018N5011), Xiamen Marine and Fisheries Development Fund (Grant No. 19CZP018HJ04) and Research Foundation of Education Bureau of Fujian Province (Grant No. JAT190351).

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

We thank the technical assistance in cellular components characterization from The Third Institute of Oceanography, State Oceanic Administration, People's Republic of China.

REFERENCES


Yang et al.
http://dx.doi.org/10.1016/j.fsi.2018.12.026 PMID: 30553887
Gao, Q.; Gao, Q.; Min, M.; Zhang, C.; Peng, S.; Shi, Z. Ability of Lactobacillus plantarum lipoteichoic acid to inhibit Vibrio anguillarum-induced inflammation and apoptosis in silvery pomfret (Pampus argenteus) intestinal epithelial cells. Fish Shellfish Immunol., 2016, 54, 573-579. http://dx.doi.org/10.1016/j.fsi.2016.05.013 PMID: 27179425


