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Research Article

Molecular Characterization of Immunoglobulin M (IgM) and Polymeric Immunoglobulin Receptor (pIgR) and Expression Response to *Vibrio harveyi* Challenge in Leopard Coral Grouper (*Plectropomus leopardus*)

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Immunoglobulin M (IgM) is the primary antibody in fish, which is transported from epithelial cells into the external secretion system by polymeric immunoglobulin receptor (pIgR). In this study, the full length of IgM and pIgR complementary DNA sequences from leopard coral grouper (*Plectropomus leopardus*) was characterized, and their expression levels under *Vibrio harveyi* infection were studied. cDNA sequence analysis showed that the pl-IgM heavy chain cDNA contained an open reading frame (ORF) of 1797 bp encoding a polypeptide of 597 amino acids consisting of a signal peptide, a variable region, and 4 constant regions. pl-pIgR (1041 bp) encodes 346 amino acids with 2 Ig-like domains (ILDs). The expression levels of pl-IgM and pl-pIgR in healthy fish were mainly in the intestine and spleen of fish, which indicates the relationship between the mucosal-associated lymphoid tissue and system lymphoid tissue. Messenger RNA (mRNA) expression profiles of pl-IgM and pl-pIgR were significantly increased in the intestine, head kidney, spleen, and liver after *V. harveyi* infection. The cDNA sequences of two ILDs were cloned and expressed in *Escherichia coli* BL21 (DE3). The recombinant pl-pIgR protein had antibacterial function against *V. harveyi* and *Vibrio alginolyticus* at 200 ng/μl. Our results indicate that pl-IgM and pl-pIgR are involved in the mucosal-associated immune and systemic immune response in the antibacterial immunity of leopard coral grouper.

1. Introduction

Leopard coral grouper (*Plectropomus leopardus*) is an important fish for the marine aquaculture industry [1]. Artificial breeding technology has made a breakthrough in recent years. The breeding and cultivation of leopard coral grouper were observed in Hainan, Guangdong, and Fujian Provinces, and northern areas. Skin ulceration is a severe disease in leopard coral grouper. The major pathogen is *Vibrio harveyi* based on morphological, physiological, and biochemical characteristics, leading to surface ulceration and visceral lesion during the growth period [2, 3]. A high-quality chromosome-level genome of leopard coral grouper has been assembled using 10 × Genomics, high-throughput

chromosome conformation capture (Hi-C), and PacBio long-read sequencing technologies. The genome assembly has a length of 881.55 Mb with a scaffold N50 of 34.15 Mb [4–6]. The gene families enriched in immune response were immunoglobulin heavy chain (IGH), β-2 microglobulin, PYRIN-containing Apaf1-like (PYPAF1), and nucleotide-binding oligomerization domain (NLRP) [6].

Immunoglobulin heavy chain (IGH) included IgM, IgD, and IgT in teleost. Immunoglobulin M (IgM) is the main compound of antibodies and found in the mucosal secretions on the surface of the intestines, skin, and gills of teleost. IgM has been discovered in many fish species, including common carp (*Cyprinus carpio* L.) [7], rainbow trout (*Oncorhynchus mykiss*) [8], turbot (*Scophthalmus maximus*) [9], sea bream (*Sparus aurata*) [10], and olive flounder (*Paralichthys olivaceus*) [11]. IgM is tetrameric in most teleost and the monomer has two heavy (H) chains of molecular weight 70 kDa and two light (L) chains of 25 kDa. IgM protects the host against pathogens and its concentration in lymphoid organs was significantly upregulated under bacterial infection [12, 13], whereas the research of IgM function in leopard coral grouper requires further investigation.

Polymeric immunoglobulin receptor (pIgR) is a highly conserved single transmembrane glycoprotein with three domains, including extracellular Ig, transmembrane, and intracellular domains [14, 15]. The mammalian pIgR consists of 5 Ig domains. D1 and D5 domains can bind pentameric immunoglobulin M (IgM) or dimeric immunoglobulin A (IgA) [14, 16]. Teleost pIgR contains only 2 Ig domains. pIgR protein is highly found in mucosal tissues of fish and transports polymeric immunoglobulins across epithelial cells into external secretion [17, 18]. pIgR is cleaved and the extracellular part is known as the secretory component (SC). SC is free or bound to polymeric IgM (or IgA) and contributes as a microbial scavenger to protect the epithelial surface [19, 20]. Previous studies reported the gene sequences of pIgR in fugu (*Takifugu rubripes*) [21], common carp [17], orange-spotted grouper (*Epinephelus coioides*) [22], tlantic salmon (*Salmo salar*) [23], olive flounder [24], turbot [25], sea bass (*Lateolabrax japonicus*) [26], crucian carp (*Carassius auratus*) [27], dojo loach (*Misgurnus anguillicaudatus*) [28], grass carp (*Ctenopharyngodon idellus*) [29], half-smooth tongue sole (*Cynoglossus semilaevis*) [30], and largemouth bass (*Micropterus salmoides*) [31]. Previously written articles demonstrated that the pIgR transported tetrameric IgM into the skin mucus in fugu and oliver flounder [18, 21]. pIgR in the liver can mediate mucus IgM transport from the liver into the biles and intestines of fish [32].

In this study, the IgM and pIgR gene sequences were characterized in leopard coral grouper. The immune responses of pl-IgM and pl-pIgR at the transcription level were investigated under *V. harveyi* infection to elucidate protective mechanism of fish IgM and pIgR. In addition, we found the antimicrobial function of recombinant pl-pIgR protein in Oxford cup assay *in vitro*, which will provide novel insights into the immune defense mechanism of leopard coral grouper and contribute to the disease control in aquaculture.

2. Materials and Methods

2.1. Ethical Statement. Experiments were performed according to the guidelines and ethical standards of Regulations for the Administration of Affairs Concerning Experimental Animals of the State Science and Technology Commission of Shandong Province, China. This study was approved by the Ethics Committee of the Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China.

2.2. V. harveyi Infection and Sample Collection. A total of 75 juvenile fish (Average body weight of 15.0 ± 3.3 g) were obtained from Mingbo Aquatic Company (Laizhou, China). Fish were maintained in tanks with recirculation water for a week, continuous aeration, and regular feed in the disconnecting test area. Eight tissues (liver, spleen, kidney, intestine, gill, skin, brain, and muscle) were collected from 5 healthy fish, transferred into liquid nitrogen, and stored at −80°C. *V. harveyi* was isolated from skin ulcer diseased leopard coral grouper and was shaking-grown at 28°C for 16 h [33]. Thirty-five fish were randomly selected and intraperitoneally injected with *V. harveyi* at 1×10^5 colony forming unit (CFU)/ml at 0.1 ml/100 g fish weight as the experiment group. The remaining 35 fish were intraperitoneally injected with phosphate-buffered saline (PBS) at 0.1 ml/100 g fish weight as the control group. Tissue samples, including the spleen, kidney, liver, and intestine, were collected from 5 fish at 0 h, 6 h, 12 h, 24 h, 48 h, 72 h, and 96 h after injection from the experiment group and control group after anesthesia in 5 mg/L MS-222 (tricaine methane sulfonate).

2.3. Molecular Cloning of Complementary DNA (cDNA) Sequences of pl-IgM and pl-pIgR. Total RNA was extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and stored at −80°C. The RNA quality and integrity were assayed at 1% agarose gel electrophoresis and NanoDrop spectrophotometer. cDNA was synthesized from the total RNA with the FastQuant RT Kit (Tiangen, Beijing, China), according to the manufacturer's instructions. The predicted pl-IgM and pl-pIgR sequences were obtained from the genome data of the leopard coral grouper (Bioproject: PRJNA622646) [4]. Open reading frame (ORF) sequences of pl-IgM and pl-pIgR were obtained using polymerase chain reaction (PCR) with specific primers (IgM-ORF-F/IgM-ORF-R and pIgR-ORF-F/pIgR-ORF-R). All primers used in this study are listed in Table 1. The PCR products were purified using a Gel Extraction Kit (CWBio, Beijing, China) after 1% agarose gel electrophoresis and subcloned into the pEASY-T1 simple cloning vector (Transgen Biotech, Beijing, China) for sequencing.

2.4. Sequence Analysis, Homology, and Phylogenetic Analysis. The predicted protein was analyzed by the Basic Local Alignment Search Tool (BLAST) program of the National Center for Biotechnology Information (NCBI) database

Table 1: Primers used for amplification, quantitative real-time polymerase chain reaction RT-PCR (qRT-PCR), and protein expression.

Gene	Name	Sequence (5′–3′)	Purpose
pl-IgM	IgM-ORF-F IgM-ORF-R	ATGTTCTCTGTAGCTCTGATACTG CTATTGGGGCTTGCACTTTTCAGG	Amplification
pl-pIgR	pIgR-ORF-F pIgR-ORF-R	ATGCAGCAGCTCTTTATATTTG TCAGTACATATGTACTTCCTCA	Amplification
pl-IgM	IgM-RT-F IgM-RT-R	AGTGTTTCCTCTGAAGCCAT AATGGAGCCTGTCAAGGCAA	qRT-PCR
pl-pIgR	pIgR-RT-F pIgR-RT-R	GAGGGTCAGCCCCTCACAGT TGGTGCAGAATTCCTTCATC	qRT-PCR
Actin	Actin-RT-F Actin-RT-R	GAGTAGCCACGCTCTGTC GCTGTGCTGTCCCTGTA	qRT-PCR
pl-pIgR	pIgR-exF1 pIgR-exR1	GATAACCACCGAGGGAGAGCTA ATGTCACAGACACTGGGGCTGG	Recombinant protein

(https://www.ncbi.nlm.nih.gov/blast). The putative immunoglobulin domain was identified using conserved domains search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) by comparing it with IgM and pIgR of other species as listed in Table 2. The putative amino acid sequence alignment was performed using ClustalX and ESPript 3.0 (https://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi). Using the neighbor-joining method, the phylogenetic trees of IgM and pIgR were constructed with the sequences listed in Table 2 using Molecular Evolutionary Genetic Analysis (MEGA) 7.0 software. The reliability of the branching was tested through bootstrap resampling with 1000 replications.

2.5. Expression Profile Assay by Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). The qRT-PCR was performed to examine the expression levels of pl-IgM and pl-pIgR in healthy fish and bacteria infected fish. Total RNA and tissue cDNA were synthesized according to the manufacturer's instructions. The qRT-PCR conditions were as follows: 95°C for 5 min, followed by 40 cycles at 95°C for 5 s and 60°C for 34 s. Relative fold changes of genes were calculated by the methods of $2^{-\Delta\Delta Ct}$. β-actin was used as a control for the normalization of expression.

2.6. Expression, Purification, and Antibacterial Function of Recombinant pl-pIgR. The secretory component region of the pl-pIgR cDNA was amplified by PCR using the primers listed in Table 1, which is inserted into the pET-his expression vector and transformed into *Escherichia coli* BL21 (DE3) cells (Transgen, Beijing, China). The positive clones were cultured in 200 ml LB medium with 50 μg/ml ampicillin. When the optical density at 600 nm (OD600) reached 0.5-0.6, a final concentration of 0.1 mM isopropyl-bD-thiogalactopyranoside (IPTG) was added, and the protein was induced for another 6 h. The bacteria were centrifuged at 6000 rpm for 5 min at 4°C and resuspended in lysis buffer (50 mM Tris-Cl pH 8.0, 150 mM NaCl) with 1 mM phenylmethanesulfonyl fluoride (PMSF). After sonicating for 6 min, the sediment was dissolved in precooled solution. The recombinant pl-pIgR protein was purified using the HisTrap FF crude purification system for six-His-tagged proteins (Invitrogen, Shanghai, China) and renatured by gradient urea dialysis. The supernatant was collected and stored at −80°C. In this assay, 12% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to detect the recombinant protein. The extracellular antibacterial function of the recombinant pl-pIgR protein was determined with the Oxford cup method. Three trypticase soy agar plates (TSA) were inoculated with 10^5 CFU/ml of *V. harveyi*, *Vibrio alginolyticus*, and *Edwardsiella tarda*. Then, 100 μl aliquots of protein (200 ng/μl), ampicillin (5 ng/μl), and PBS buffer were added to each Oxford cups. The plates were incubated at 28°C overnight, and the inhibition zones around the cup were observed and measured.

2.7. Statistical Analysis. The statistic P values were calculated by one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) 19.0 software, and P value <0.05 was considered statistically significant.

3. Results

3.1. Nucleotide and Protein Sequence Analysis of pl-IgM and pl-pIgR. Sequence analysis revealed the full ORF of pl-IgM cDNA of 1797 bp, encoding the protein with 598 amino acids. The heavy chain of pl-IgM was constituted by a signal peptide, variable domain, and four constant domains (CH1: 21–140, CH2: 146–234, CH3: 248–340, CH4: 375–453) (Figure 1(a)). The full ORF cDNA of pl-pIgR was 1041 bp, encoding the protein with 346 amino acids. A signal peptide, an extracellular region, a transmembrane region, and an intracellular region were observed in pl-pIgR. The extracellular region had 2 Ig-like domains: ILD1 (26–129) and ILD2 (138–228) (Figure 1(b)).

3.2. Sequence Analysis and Phylogenetic Relationship of pl-IgM and pl-pIgR. The pl-IgM shared higher amino acid identities with the grouper IgM. There were conserved cysteine residues in each CH domain (CH1-CH2-CH3-CH4), and inter- and intrachain disulfide bridges were conserved in the teleosts. Comparative analysis showed that ILD1 and ILD2 of pl-pIgR shared homology with other fish ILD1 and ILD2 (Figure 2).

Table 2: The protein ID of IgM and pIgR in fish and other species.

Protein name	Protein ID
Epinephelus coioides IgM	AAX78206.1
Epinephelus akaara IgM	AEK82140.1
Paralichthys olivaceus IgM	BAC99314.1
Scophthalmus maximus IgM	AGE84011.1
Larimichthys crocea IgM	ACF22093.1
Ctenopharyngodon idella IgM	ABD96.1
Danio rerio IgM	AAK69167.1
Tachysurus fulvidraco IgM	AER10487.1
Ictalurus punctatus IgM	XP_47016287
Silurus meridionalis IgM	AJL46903.1
Homo sapiens IgM	AAS01769.1
Xenopus laevis IgM	AAH84123.1
Paralichghys olivaceus pIgR	ADK91435.1
Scophthalmus maximus pIgR	AGN54539.1
Epinephelus coioides pIgR	ACV91878.1
Lates calcarifer pIgR	XP_018533069.1
Cynoglossus semilaevis pIgR	XP_024921865.1
Takifugu rubripes pIgR	NP_001266911.1
Oncorhynchus mykiss pIgR	ADB81776.1
Larimichthys crocea pIgR	XP_027139465.1
Sebastes umbrosus pIgR	XP_022303838.1
Sandwer lucioperca pIgR	XP_031145332.1
Epinephelus lanceolatus pIgR	XP_033481159.1

The distance tree showing the relationship between the amino acid sequences of the constant regions of IgM heavy chain for different fish species was constructed. The pl-IgM and pl-pIgR were clustered into one branch with other groupers (Figure 3).

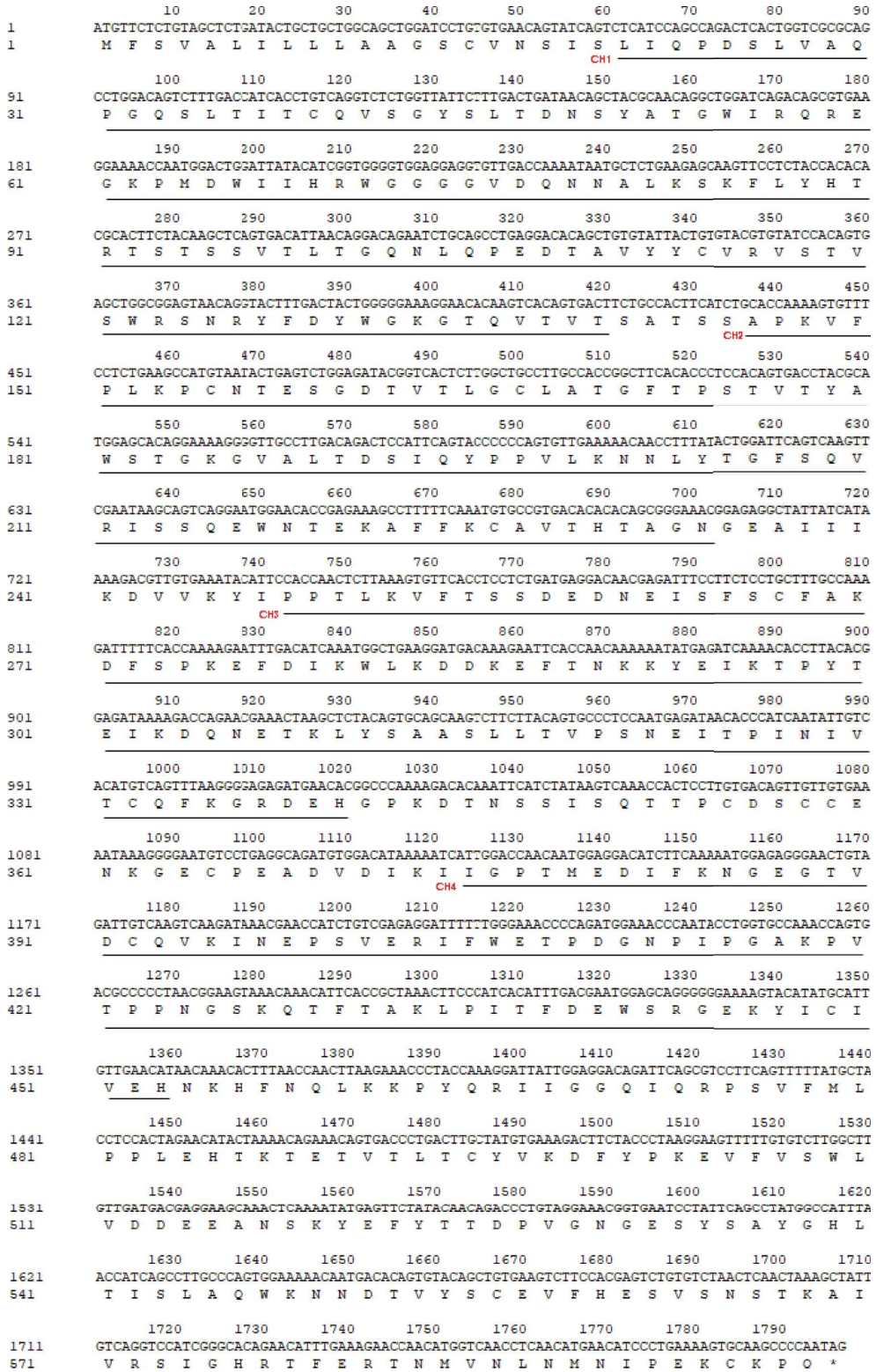
3.3. Expression Profiles of pl-IgM and pl-pIgR. The pl-IgM messenger RNA (mRNA) was detected in immune-related tissues, with higher expression levels in the intestine, kidney, and spleen and middle expression levels in the gill, liver, skin, and brain of fish (Figure 4(a)). The pl-pIgR mRNA was highly expressed in the intestine and spleen and weakly expressed in the skin, gill, and kidney, which was similar to pl-IgM mRNA (Figure 4(b)).

3.4. Expression Levels of pl-IgM and pl-pIgR in Immune-Related Tissues after V. harveyi Infection

3.4.1. pl-IgM. The expression levels of pl-IgM increased significantly in immune-related tissues after *V. harveyi* infection. pl-IgM mRNA expression pattern in 4 tissues of infected fish showed a significant increase compared with uninfected fish. The pl-IgM expression fluctuated in the intestine, head kidney, and spleen from 6 h. The highest expression of pl-IgM was observed in the intestine at 6 h (~4 fold) and 24 h (~7 fold) after bacterial challenge (Figure 5(a)). In the kidney and spleen, the expression levels were upregulated at 2 peak times (6/12 h and 72 h). The expression levels were 5~6 fold compared to 0 h (Figures 5(b) and 5(c)). In the liver, the expression level of pl-IgM was up to the peak time at 72 h, and the expression level was up to 20-fold postinfection (Figure 5(d)).

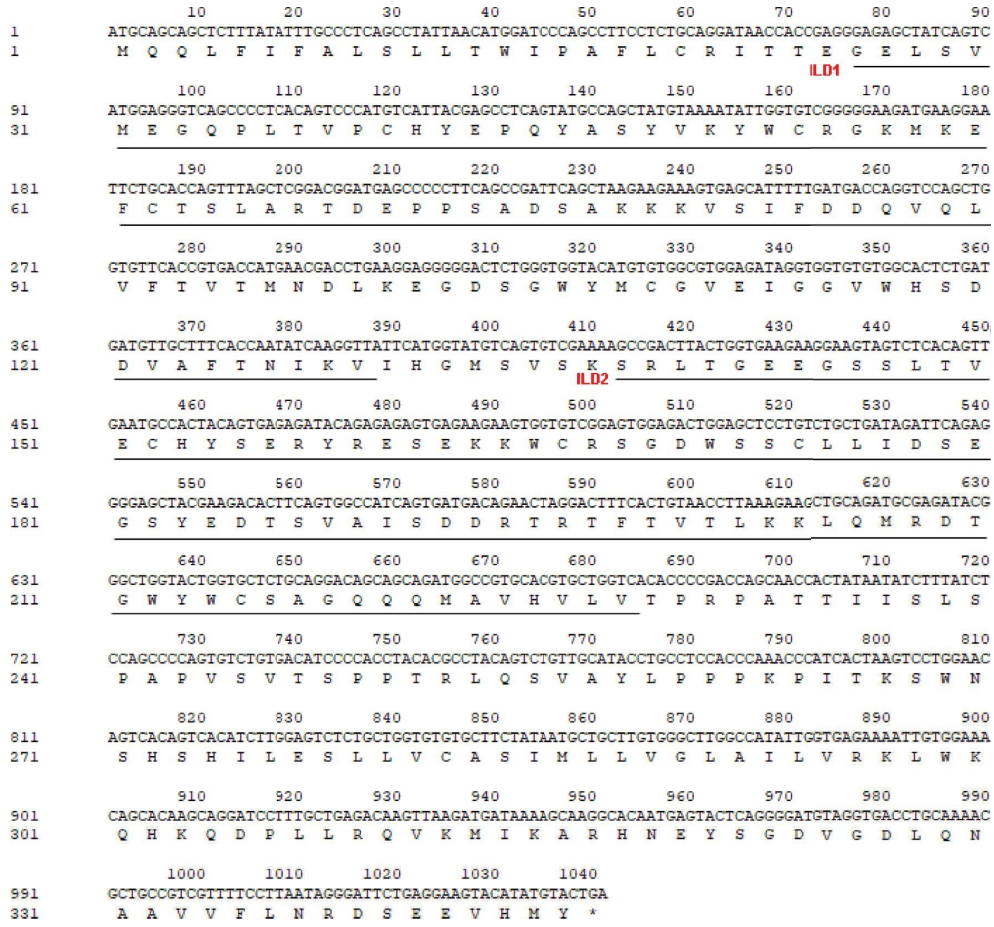
3.4.2. pl-pIgR. Experimental infection with *V. harveyi* caused significant induction of pl-pIgR expression level in infected fishes compared with uninfected fishes. The peak time point of pl-pIgR expression level was 12 h after infection in the intestine and head kidney (Figures 6(a) and 6(b)). The pink time point of pl-pIgR expression levels in the spleen and liver were 24 h and 48 h, respectively (Figures 6(c) and 6(d)). The peak expression levels of pl-pIgR in the intestine, kidney, spleen, and liver were 8, 5, 3, and 5 times higher than those in 0 h, respectively (Figure 6).

3.5. Recombinant Expression and Antibacterial Function of the pl-pIgR Protein. The recombinant pl-pIgR protein was obtained using the pET-His vector. After sonicating, the supernatant and sediment of uninduced and IPTG-induced *E. coli* DE3 (BL21) were assessed separately. As shown in Figure 7(a), a specific band of approximately 42 kDa was identified in the lane of sediment of IPTG-induced *E. coli* DE3 (BL21). This protein was consistent with the predicted molecular mass of the recombinant protein of pl-pIgR with His-tag. The recombinant protein was purified and dissolved in a 2 mg/ml concentration with neutral Tris buffer. On the Oxford cup assay, each plate contained 200 ng/μl pl-pIgR protein as experiment group, 5 ng/μl ampicillin as a positive control, and PBS was used as negative controls. The recombinant pl-pIgR protein displayed antibacterial activity against *V. harveyi* and *V. alginolyticus* according to the



(a)

Figure 1: Continued.



(b)

FIGURE 1: The nucleotide and deduced amino acid sequences of the heavy chain of pl-IgM (a) and pl-pIgR (b). The number on the left showed the positions of nucleotide and amino acid. The stop codon is represented with an asterisk (*).

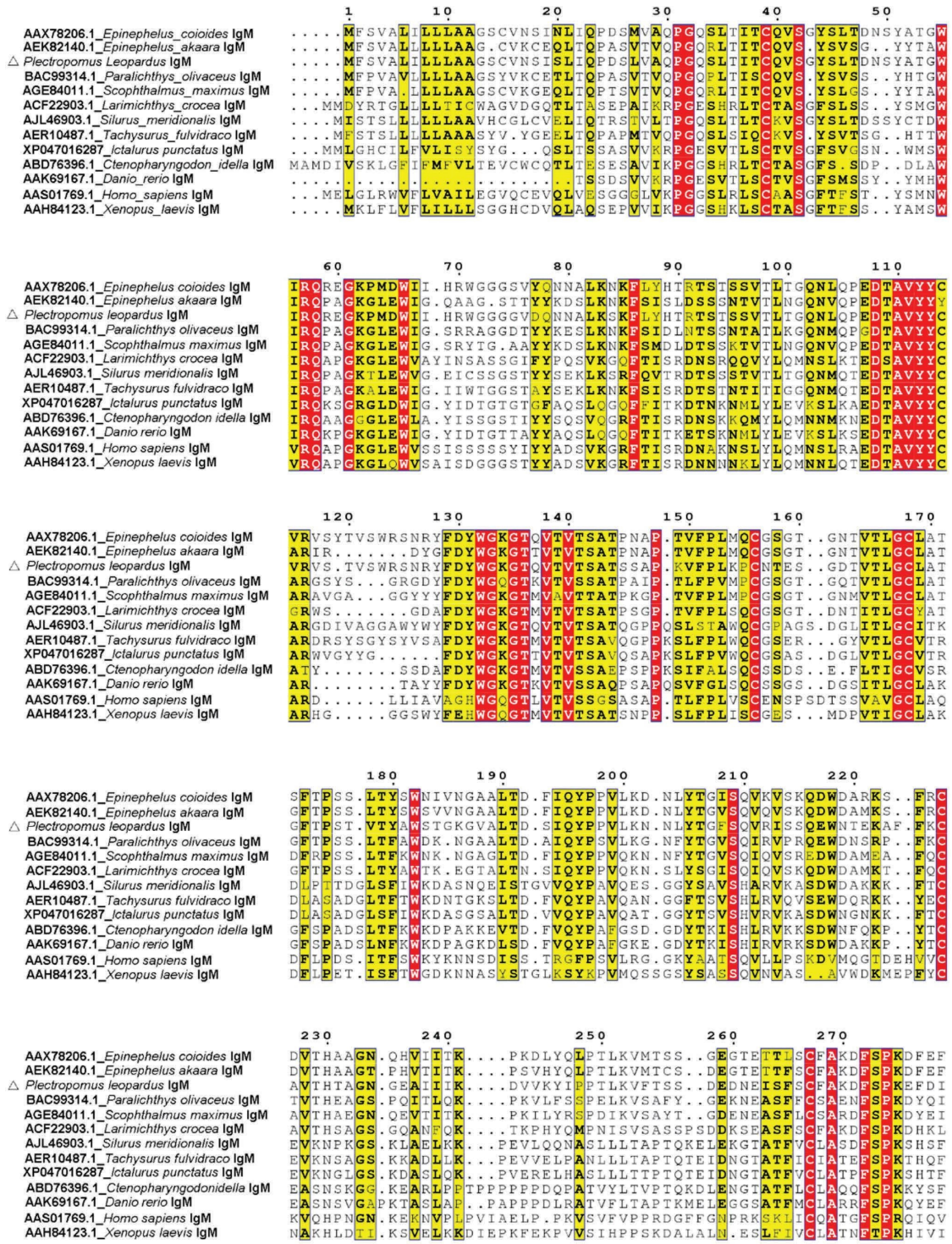
Oxford cup and without antibacterial activity against *E. tarda* (Figure 7(b)).

4. Discussion

In this study, the pl-IgM and pl-pIgR genes were characterized in leopard coral grouper, and the transcriptome profiles of these two genes were analyzed in healthy fish and after *V. harveyi* infection. The SC part of pl-pIgR was expressed in the *E. coli* system. The recombinant protein had an antibacterial function against two tested Vibrio (*V. harveyi* and *V. alginolyticus*).

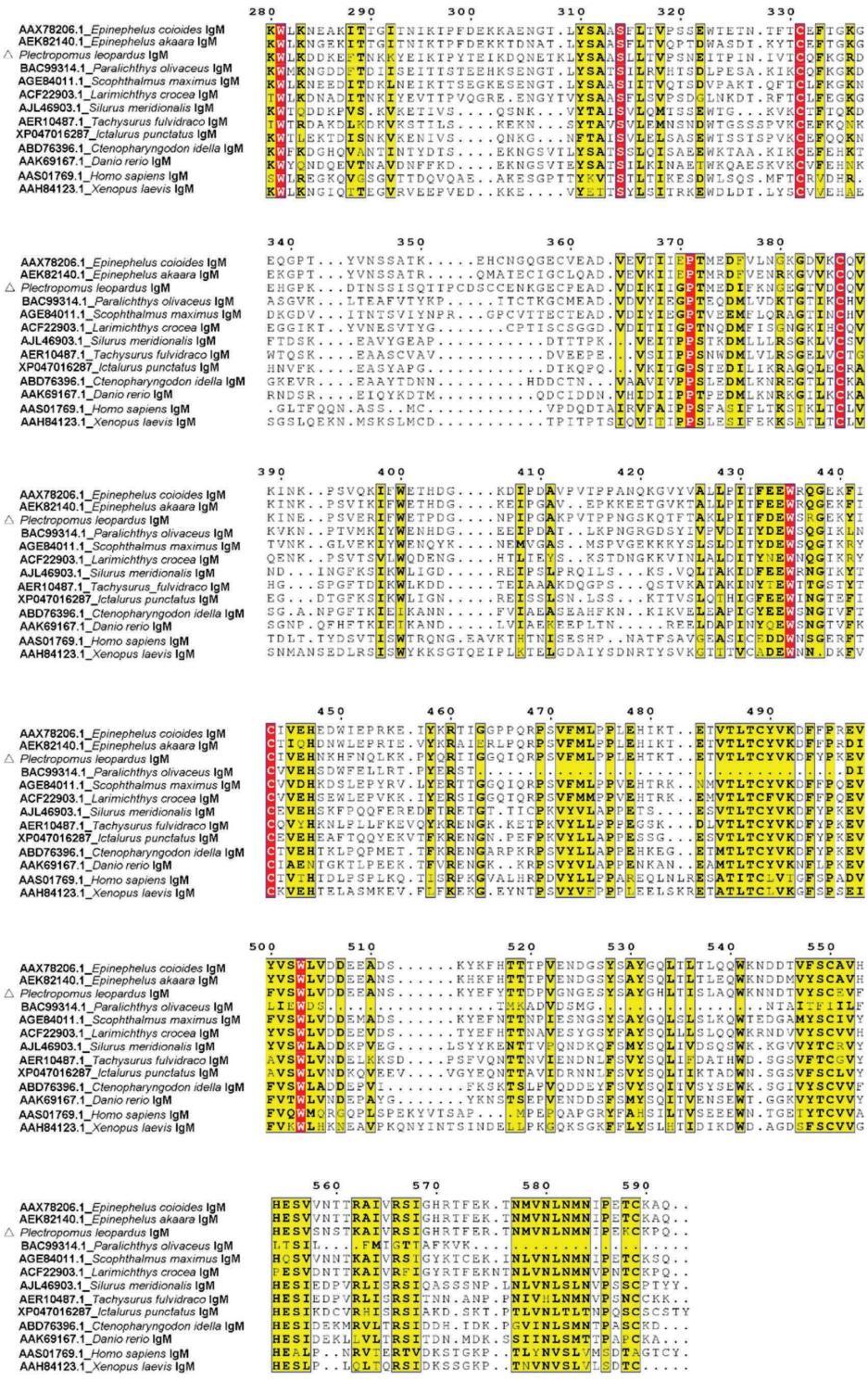
Several conserved amino acid residues of the pl-IgM and pl-pIgR were found in the multiple sequence alignment, such as the conserved cysteine in the Ig-like domains, which suggests the stability of protein structure. The pIgR homologs in teleost have 2 ILDs, which are homologous with the ILD1 and ILD5 of the mammalian pIgR. These 2 ILDs are sufficient for interaction with tetrameric IgM, and there should be further investigation on the binding sites. pIgR mediates IgM across the intestinal epithelium into gut mucus and the epithelia in Japanese flounder [18, 34]. The protein structure and phylogenetic analysis showed that the evolution of leopard coral grouper IgM and pIgR genes had the nearest neighbor-joining with other groupers, such as *Epinephelus coioides* and *Epinephelus lanceolatus*, due to the same origin of the grouper family of teleost.

IgM is the principal immunoglobulin in humoral and mucosal immunity in teleost. IgM activates the complement system and can cause pathogen lysis [35]. The tissue distribution of pl-IgM and pl-pIgR demonstrated the vital function in immune-related tissues such as intestine, spleen, and kidney. The highest expression levels of pl-IgM and pl-pIgR were observed both in the mucosal-associated tissues and systemic immune tissues, including the intestine, spleen, kidney, gill, liver, and skin. The present result is consistent with the previous studies on Japanese flounder, turbot, and half-smooth tongue sole [13, 24, 30]. The expression levels of IgM and pIgR were affected in fish under infection with bacteria, parasites, and viruses. In the orange-spotted grouper, the transcript level of IgM was upregulated in the head, kidney, and spleen, followed by *V. alginolyticus* infection [36]. After exposure to *Flavobacterium columnare*, grass carp IgM and pIgR mRNA levels were significantly upregulated and downregulated to control levels [37]. We focused on the changes in the expression levels of pl-IgM and pl-pIgR in



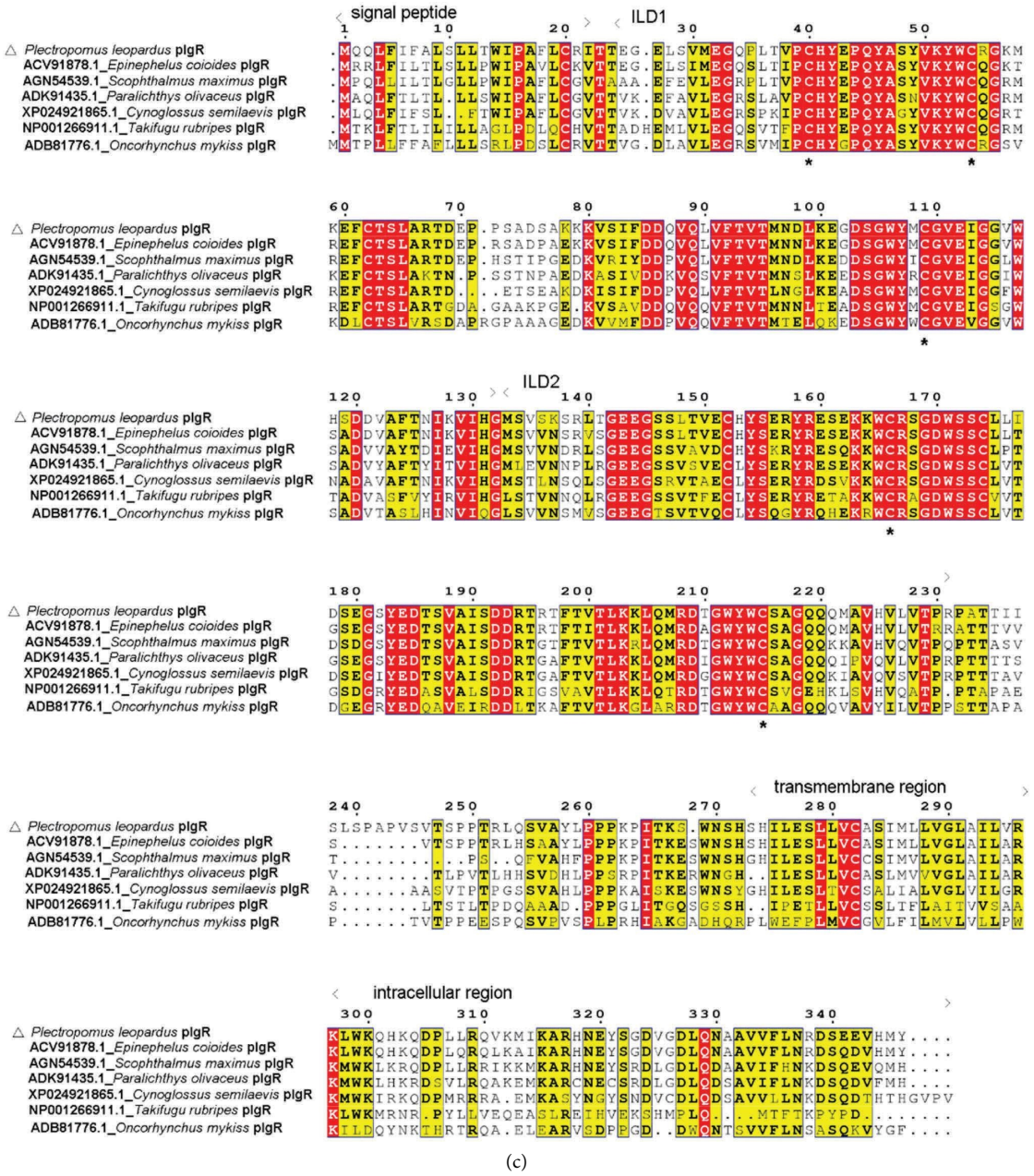
(a)

Figure 2: Continued.



(b)

FIGURE 2: Continued.



(c)

Figure 2: Multiple sequence alignment of pl-IgM (a,b) and pl-pIgR (c) deduced amino acid sequences with the corresponding sequences of other species. Sequences corresponding to signal peptide, immunoglobulin-like domain (ILD), transmembrane region, and extracellular region are noted in the brackets at the top of the sequence matrix.

mucosal and systemic tissues of the infected fish. Under infection of *V. harveyi*, the expression changes of pl-IgM and pl-pIgR in mucosal tissue (intestine) were more evident than in systemic immune tissues (spleen, head kidney, and liver), and the peak time of pl-IgM in these tissues was earlier than pl-pIgR. Comparative expression levels of pl-IgM and pl-pIgR under pathogen infection indicated their contribution to the clearance of pathogenic organisms.

pIgR is a core gene in bridging innate and adaptive immune responses, and its extracellular ligand-binding region (SC part) has intrinsic antimicrobial properties [14]. In this study, an antibacterial activity analysis based on the inhibition zone proved that the recombinant SC part protein of pl-pIgR exhibited antibacterial function against two tested *Vibrio* species in the observed inhibition zones, which indicates its potential usage in anti-vibriosis.

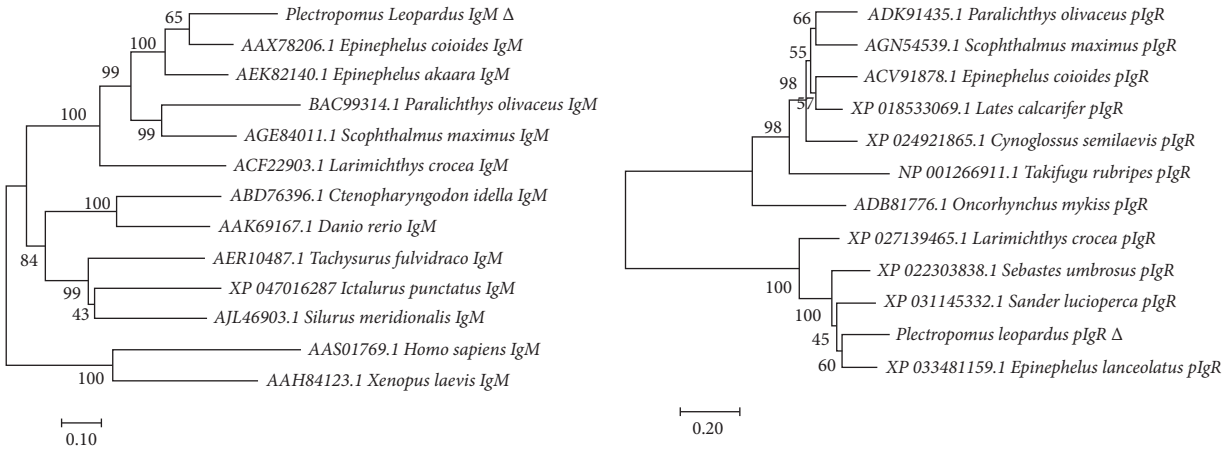


FIGURE 3: Phylogenetic trees were constructed with the neighbor-joining method based on the amino acid sequences of pl-IgM (a) and pl-pIgR (b) from leopard coral grouper and other species. The reliability of each node is estimated by bootstrapping with 1000 replications implemented in MEGA 7.0. The scale bar (0.1) represents the genetic distance. The numbers marked near the nodes indicate the bootstrap test scores.

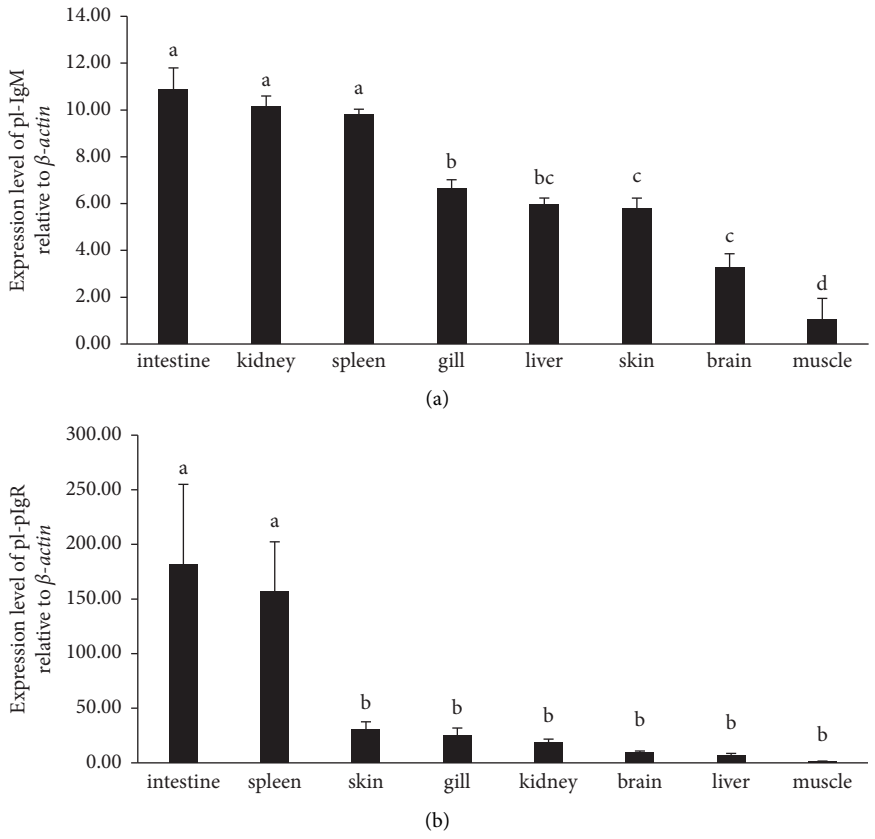


FIGURE 4: The distribution patterns of the pl-IgM (a) and pl-pIgR (b) in various tissues using quantitative real-time polymerase chain reaction (qRT-PCR) analysis with specific primers. Different letters above bars represented significant differences at $P < 0.05$.

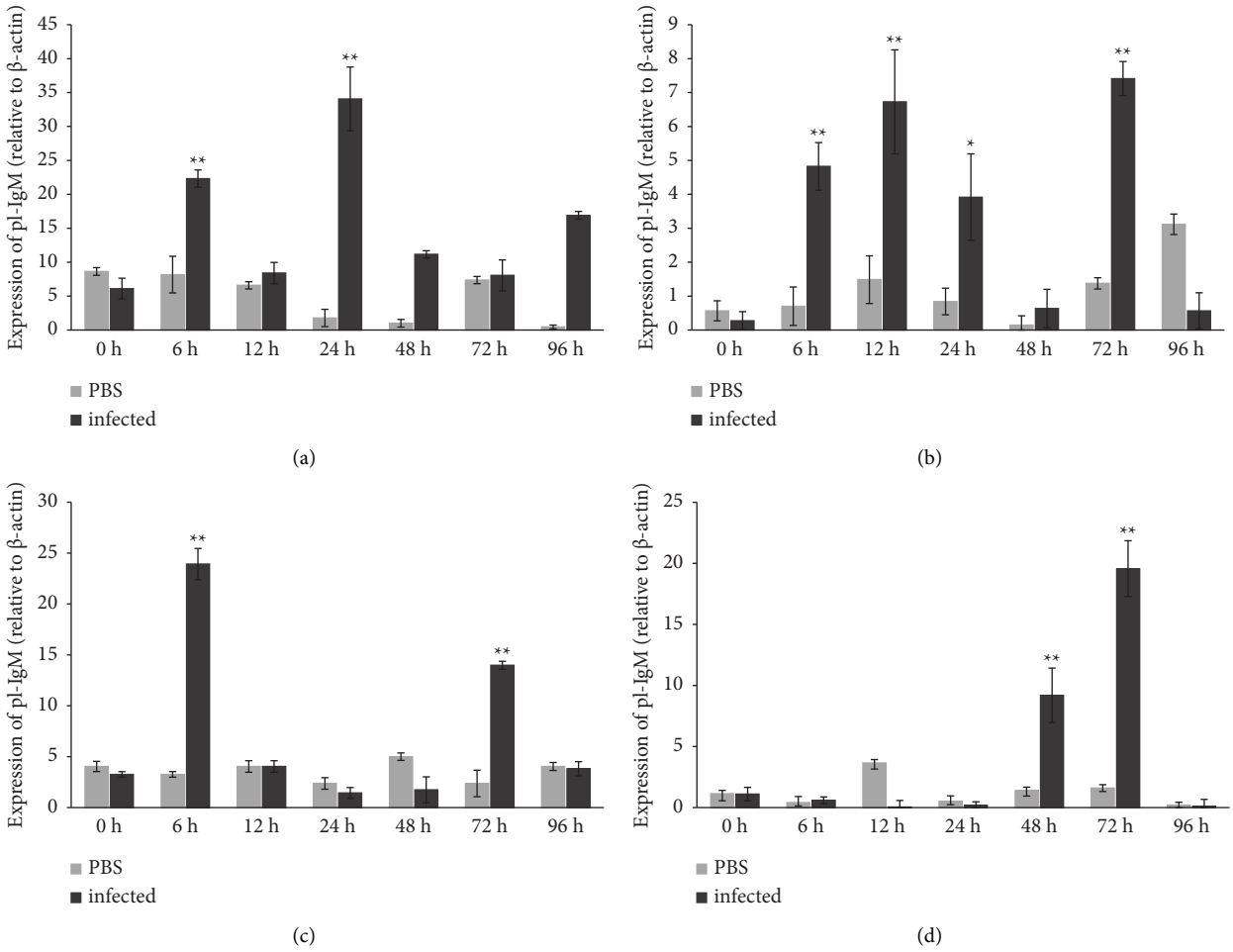
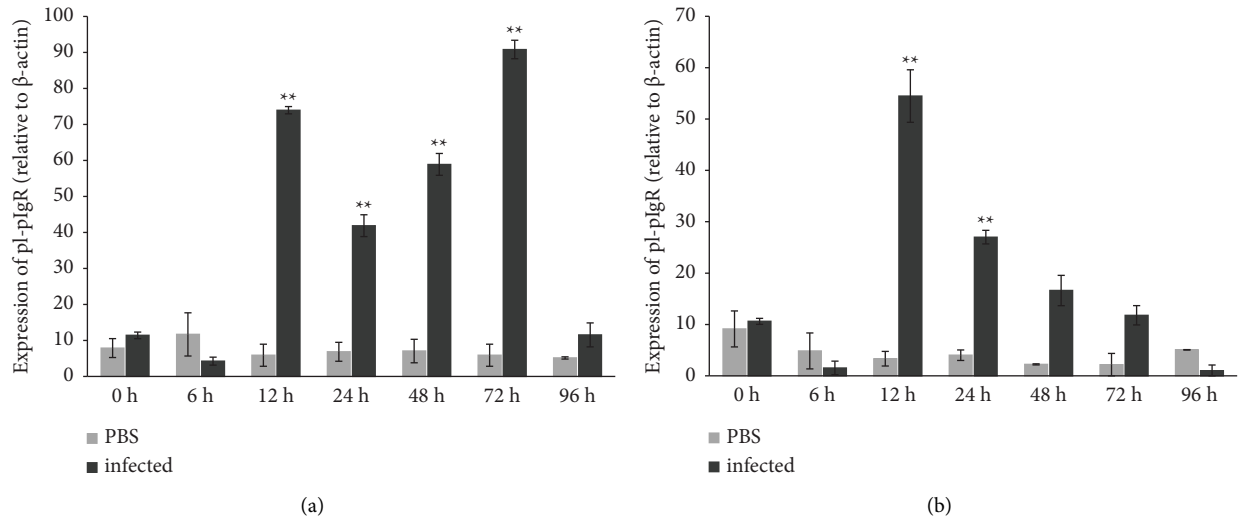


Figure 5: Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of expression of pl-IgM messenger RNA (mRNA) after being infected with *V. harveyi*. The asterisk on the bars indicates the statistical significance of pl-IgM compared to the 0 h group ($^{*}P < 0.05$, $^{**}P < 0.01$). The mRNA levels of pl-IgM were determined by normalized relative to β-actin. (a) Intestine. (b) Kidney. (c) Spleen. (d) Liver.



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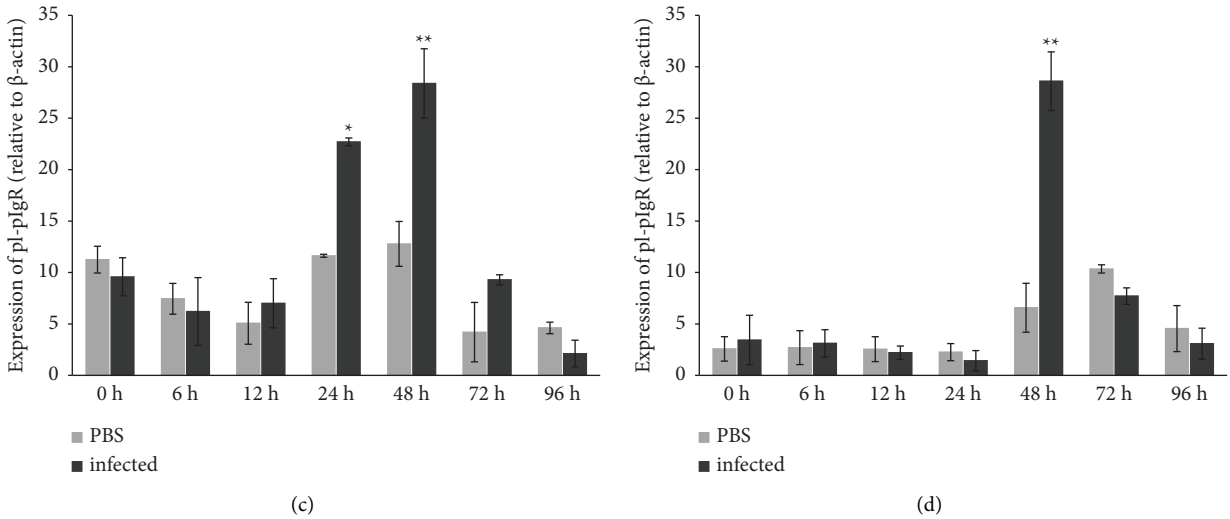

Figure 6: Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of expression of pl-pIgR messenger RNA (mRNA) after being infected with *V. harveyi*. The asterisk on the bars indicates the statistical significance of pl-pIgR compared to the 0 h group ($^{*}P < 0.05$, $^{**}P < 0.01$). The mRNA levels of pl-pIgR were determined by normalized relative to β-actin.

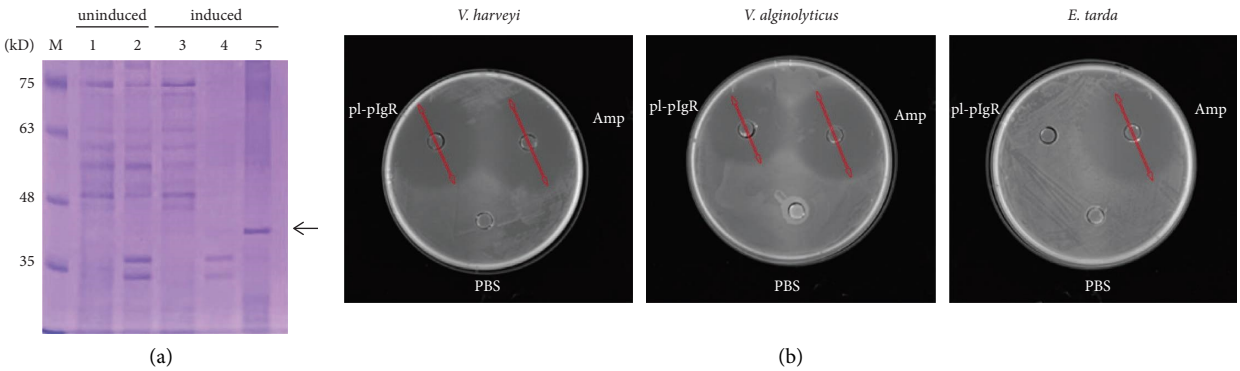

Figure 7: Recombinant expression and antibacterial activity analysis of recombinant pl-pIgR protein. (a) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of recombinant pl-pIgR protein. M protein marker; lane 1: uninduced *E. coli* total bacteria; lane 2: supernatant of uninduced *E. coli*; 3: induced *E. coli* total bacteria; 4: supernatant of induced *E. coli*; 5: sediment of induced *E. coli*. The arrow indicates the recombinant pl-pIgR protein. (b) Antibacterial activity of the recombinant pl-pIgR protein in Oxford cup assay.

5. Conclusion

Our study characterized the IgM and pIgR genes in leopard coral grouper and analyzed mRNA expression profiles of pl-IgM and pl-pIgR in healthy fishes and in response to *V. harveyi* infection. The protein sequences and pl-IgM and pl-pIgR structures had the highest similarity with other groupers. Under *V. harveyi* infection, pl-IgM and pl-pIgR were upregulated in immune-related tissues with similar expression trends. The recombinant pl-pIgR protein has the antibacterial function against two tested vibrio species, indicating the potential value in replacing antibiotics.

Data Availability

The data used to support the findings of this study are included within the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

LW contributed to the design of the study, performed most of the experiments and statistical analysis, and drafted and revised the manuscript. ZZ, YL, and KL participated in collecting the experimental samples. QZ, CZ, and SC revised the manuscript. All the authors read and approved this version of the final manuscript and confirmed the integrity of this study.

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