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Short communication

Effects of *Vibrio harveyi* infection on serum biochemical parameters and expression profiles of interleukin-17 (IL-17) / interleukin-17 receptor (IL-17R) genes in spotted sea bass

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ABSTRACT

Vibrio harveyi is regarded as serious pathogen for marine fishes. To evaluate the physiological responses of spotted sea bass (*Lateolabrax maculatus*) after *V. harveyi* infection, four biochemical biomarkers including alanine amino transferase (ALT), albumin (ALB), total protein (TP) and glucose (GLU) were measured in serum. Our results showed that *V. harveyi* infection significantly influenced the concentration of ALT, ALB and GLU. Additionally, five interleukin-17 (IL-17) and five IL-17 receptors (IL-17R) genes were identified in spotted sea bass and their gene structures were characterized. Furthermore, the expression patterns of IL-17 and IL-17R genes were determined by qPCR in liver, intestine, spleen and head kidney after *V. harveyi* infection. All IL-17 and IL-17R genes exhibited time- and tissue-dependent expressions. Several tested genes were dramatically induced by *V. harveyi* treatment, particularly IL-17A/F1 in liver and head kidney, IL-17A/F2 in head kidney, IL-17RC in spleen with more than 10-fold increases, which suggested their potential essential roles against bacterial infection.

1. Introduction

With the rapid development of intensive farming technology, aquaculture is currently the fastest growing food producer in the world. However, diseases caused by bacterial infections keep erupting and spreading, which constraints to the sustainable development of aquaculture (Ige, 2013). Spotted sea bass (*Lateolabrax maculatus*) is one of the most popular mariculture fish species in China, and the annual production has exceeded 150 thousand tons (B. Chen et al., 2019; Fan et al., 2019). However, *Vibrio harveyi*, a gram-negative bacterium which is frequently reported as a serious pathogen of marine fish and invertebrates, could cause mass mortalities in cultured spotted sea bass (Fan et al., 2019; Tian et al., 2019). Despite this, the immune system and underlying molecular mechanism of spotted sea bass remains largely unknown.

Changes in serum biochemical parameters, including alanine amino transferase (ALT), albumin (ALB), total protein (TP) and glucose (GLU) are generally regarded as useful biomarkers to evaluate physiological status under stresses (Charlie-Silva et al., 2019). For example, ALT is one of the most important aminotransferases in liver (Sheikhzadeh et al., 2012). Liver injury would alter the transport function and membrane permeability of ALT, leading to leakage of ALT from the cells to serum and causing the increase ALT levels in serum (Raja et al., 2007). Hence, ALT levels in serum could reflect the liver injury status under stresses. Additionally, liver is the sole source of the bulk of ALB, and changes of ALB levels in serum could be closely associated with ability of protein synthesis in liver (Sherlock and Dooley, 1964). TP levels are generally used to understand the general state of fish health and metabolism (Martinez et al., 2004). GLU, as the main physiological fuel, directly reflects the energy metabolism of organisms (Lee et al., 2003). It has been well documented that the levels of ALT, ALB, TP and GLU were significantly changed in red hybrid tilapia (*Oreochromis .sp*) (Alsaid et al., 2015) and crucian carp (*Carassius auratus*) after bacterial infection (Tang et al., 2019).

The function of the immune system relies heavily on interleukin (IL), which is a group of cytokines generated by a variety of innate immune cells. In teleost, it has been proved that IL plays essential roles in antimicrobial immunity (Secombes et al., 2011). The subset of IL, named interleukin-17 (IL-17), is a pro-inflammatory ancient cytokine family that mediate multiple pro-inflammatory mediators in various

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types of cells (fibroblasts, endothelial cells, macrophages and epithelial cells), playing an important role in induction of inflammation and clearance of extra-cellular bacteria (Kumari et al., 2009). Up to now, six ligands in IL-17 gene family including IL-17A, B, C, D, E and F have been found in mammals (Wang et al., 2014). IL-17A and IL-17F, sharing the highest homology, play essential roles in promoting the release of immune-related elements including pro-inflammatory cytokines, chemokines and antimicrobial peptides to accelerate the pro-inflammatory responses (Wang et al., 2014). In addition to having the similar function with IL-17A in enhancing the expression of diverse inflammatory cytokines, IL-17B and IL-17C can significantly stimulate the release of TNF-α, IL-1β from monocytic cell line (Li et al., 2000). IL-17D is the most evolutionary conserved IL-17 family member (Yoshitomi et al., 2005), which regulate cytokine production to indirectly modulate the immune response in various tissue (Starnes et al., 2002). IL-17E is mainly expressed in Th2 cells and is directly involved in Th2-associated allergic inflammation by stimulating Th2 cytokines (Kono et al., 2011). In teleosts, IL-17 gene family had been firstly reported in zebrafish (*Danio rerio*), which possess five variants (IL-17A/F1-3, IL-17C and IL-17D) (Gunimaladevi et al., 2006). As more genomic resources were available in the past few years, IL-17 gene family has been annotated for several fish species, with genes number ranging from 4 to 7 (Kono et al., 2011; Wang et al., 2014). Comparing with higher vertebrates, IL-17B and IL-17E homologue genes has not been identified in the available fish databases; moreover, IL-17N is a unique IL-17 ligand that has only been identified in fish (Kono et al., 2011).

For signal transduction, IL-17 family cytokines must rely on cell surface IL-17 receptors (IL-17Rs) (Wang et al., 2014). In mammals, IL-17Rs contain five molecules: IL-17RA, IL-17RB, IL-17RC, IL-17RD and IL-17RE (Jiang et al., 2017). Although each IL-17R member has affinity for more than one IL-17, it prefers to bind with one IL-17 than others. For example, IL-17RA preferentially binds to IL-17A than IL-17F (Kramer et al., 2007), whereas IL-17RB has higher affinity to IL-17E than IL-17B, although the IL-17RB has been reported as monomer receptor for IL-17B signal (Song and Qian, 2013). Moreover, IL-17Rs can assemble into different homodimeric or heterodimeric complex to bind of IL-17 family members (Kono et al., 2011). For example, the homodimer formed by IL-17RE has been demonstrated be used by IL-17C (Song et al., 2011). It has been common reported that IL-17RA can serve as shared receptor for IL-17 cytokines and form heterodimeric complex with other IL-17 receptors including IL-17RB, IL-17RC, IL-17RD or IL-17RE (Kramer et al., 2007). In human (*Homo sapiens*), IL-17RA and IL-17RC can form heterodimer to bind with IL-17A and IL-17F (Wright et al., 2008). IL-17RA can pair with IL-17RB for IL-17E signaling, or pair with IL-17RE for IL-17C signaling, as well as form a heterodimer with IL-17RD for unknown ligand (Jiang et al., 2017). After IL-17 binding to IL-17Rs, the complex interact with Act1 adaptor or ubiquitin ligase, which subsequently initiate signaling cascades and activate other inflammatory cytokines like IL-1β, IL-6, IL-8, TNF-α and chemokines for promoting innate immune responses (Sonder et al., 2011). In teleost, the IL-17Rs orthologs have been reported in only a few species. For example, the conserved five IL-17Rs (IL-17RA-RE) were identified in large yellow croaker (*Larimichthys crocea*) and four IL-17Rs (IL-17RA, IL-17RC, IL-17RD and IL-17RE) were characterized in channel catfish (*Ictalurus punctatus*) (Wang et al., 2014). Two or more copies of IL-17RA (IL-17RA1 and IL-17RA2) arising from tandem gene duplication were confirmed in grouper (*Epinephelus coioides*) (Jiang et al., 2017) and zebrafish (Gunimaladevi et al., 2006). However, the gene information and immune functions of IL-17Rs in teleost remain largely unknown.

This study aims at evaluating the changes of serum biochemical characters and determining the expression patterns of immune-related IL-17/IL-17R genes in spotted sea bass after *V. harveyi* infection. Firstly, four biochemical biomarkers, including ALT, ALB, GLU and TP, were examined in serum after infection. Then, the complete set of IL-17/IL-17R gene were identified and characterized in spotted sea bass. To further investigate their immune function, the expression patterns of IL-17 and IL-17R genes were determined in four immune-related tissues (liver, spleen, intestine and head kidney) at 0h, 12h, 24h, 48h and 72h after *V. harveyi* infection. This study would enhance the understanding of physiological reaction and roles of IL-17 and IL-17R genes in immune response in spotted sea bass and other teleosts.

2. Materials and methods

2.1. Bacteria challenge experiments and fish samples

*V. harvey*i (strain number: EcGY020401) were obtained from laboratory of immunology and pathology of aquatic animals (Ocean University of China, Shandong, China), and further confirmed by the phylogenetic analysis of 16s rRNA and Sanger sequencing of the PCR products (Supplementary Fig. 1, Supplementary Fig. 2). The concentration of the bacteria was determined using colony forming unit (CUF) per ml by plating 10 μl of 10-fold serial dilutions onto BHI agar plates.

Spotted sea bass were obtained from Shuangying Aquaculture Company, Dongying, Shandong Province, China. 90 spotted sea bass adults (body weight: 178.25 ± 18.56g, body length: 48.76 ± 4.26 cm) were randomly selected and acclimated for a week in a square tank [5 × 5 × 1 m (L × W × H)]. After the acclimation, the individuals were transferred to three tanks at the density of 30 per tank. Then, individuals were treated with intraperitoneal injection of 0.1 mL *V. harveyi* solution at a concentration of 3×10^6 CFU/mL. During the entire experiment, other environments conditions (water temperature: 17–18 °C, pH: 7.4–7.9, salinity: 27–30 ppt and DO: 6.0–7.2 mg/L) kept stable3 individuals per tank were euthanized with MS-222 and sampled at 0h (before infection), 12h, 24h, 48h, 72h after infection for blood, liver, spleen, intestine and head kidney. Blood samples were stored at 4 °C and the other tissue samples were quickly frozen in liquid nitrogen for the following analyses.

2.2. Biochemical parameters assays

Blood samples were immediately centrifuged at 5,000r/min for 10 min to obtain serum, which were then stored at −20 °C until measurement of biochemical parameters. The concentration of serum ALT, ALB, TP and GLU were assayed with respective commercial assay kit according to their manufacturer's instructions using BS-180 Automated Biochemistry Analyzer (Shenzhen Mindry Bio-Medical Electronicsco, LTD, Guangzhou, China).

2.3. Data mining and bioinformatics analysis of IL-17/IL-17R family genes

To identify IL-17/IL-17R genes in the spotted sea bass, the reference genome (PRJNA408177) (J. Chen et al., 2019) and transcriptome databases (PRJNA515783, PRJNA515986) (Zhang et al., 2017; Tian et al., 2019) of the spotted sea bass were searched using TBLASTN program (1e-5) with all available amino acid sequences of human and zebrafish IL-17/IL-17R genes as queries. The identified IL-17 and IL-17R genes in the spotted sea bass were translated into amino acid sequences using Open Reading Frames (ORF) finder (https://www.ncbi.nlm.nih.gov/orffinder/). Additionally, online ProtParam tool (https://web.expasy.org/protparam/) was utilized to predict the molecular weight (MW) and theoretical isoelectric point (pI) of IL-17/IL-17R protein.

Phylogenetic analysis was conducted to confirm the annotation of the identified IL-17/IL-17R genes in spotted sea bass. The amino acid sequences of IL-17/IL-17R from selected vertebrates, including human, mouse (*Mus musculus)*, chicken (*Gallus*), spotted gar (*Lepisosteus oculatus*), zebrafish, Atlantic salmon (*Salmo salar*), fugu (*Takifugu rubripes*), Japanese medeka (*Oryzias latipes*) and Nile tilapia (*Oreochromis niloticus*), were downloaded from NCBI or ENSEMBLE databases for the construction of phylogenetic tree. Multiple amino acid sequences were

aligned using ClustalW2 program (https://www.ebi.ac.uk/Tools/msa/clustalw2/) with default parameters (Edgar, 2004). The phylogenetic tree was built using MEGA 7.0 software based on the neighbour-joining (NJ) method and Jones-Taylor-Thornton (JTT) model with 1000 bootstrap replicates.

Syntenic analysis was conducted by comparing genome regions that surrounding IL-17/IL-17R genes in spotted sea bass, Nile tilapia and zebrafish. The neighboring genes of IL-17/IL-17R in spotted sea bass were identified from the reference genome and further confirmed by BLASTP against Non-Redundant Proteins Sequence Database. The conserved syntenic regions of IL-17/IL-17R in tilapia and zebrafish were determined by searching the Genomicus databases (v 95.01).

The three-dimensional protein structures and the functional domains of spotted sea bass IL-17/IL-17R were predicted by the Swiss-Model (http://swissmodel.expasy.org/) and the online tool (http://smart.embl-heidelberg.de/), respectively. The corresponding spatial images were depicted by PyMOL software (Kumari et al., 2009).

2.4. RNA extraction and quantitative real-time PCR analyses (qPCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer's protocol. RNase-free DNase I (Takara, Otsu, Japan) was treated for avoiding the contamination of genomic DNA. The integrity and concentration of RNA samples were tested by 1.0% agarose gel electrophoresis and NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), respectively. Then, RNA of each samples were reversed transcribed into cDNA by PrimeScript™ RT reagent Kit (Takara, Otsu, Japan). All cDNA products were adjusted to 1000 ng/ul for following qPCR experiment. The primers of IL-17/IL-17R genes of the spotted sea bass were designed using Primer 5 software (Supplementary Table 1). The primer specificity amplification was verified by melting curve and agarose gel electrophoresis, and the amplification efficiency was calculated as $E(\%) = (10^{(-1/\text{slopes})} - 1) \times 100$. 18S rRNA of spotted sea bass was set as an internal reference gene (Wang et al., 2018). qPCR experiment was performed on Applied Biosystems 7300 machines (Applied Biosystems, CA, USA). The reaction volume was 20 μl, containing 2 μl cDNA, 10 μL SYBR®FAST qPCR Master Mix (2 ×), 0.4 μl forward and reverse primers, 0.4 μl ROX Reference Dye, 6.8 μl ddH_2O. The qPCR analysis was repeated in triplicate (technical replicates) and qPCR amplification was carried out using the following conditions: 95 °C for 30s, 40 cycles of 95 °C for 5s and Tm for 30s, followed by 72 °C for 30s. The relative expression levels of RNA were calculated using the comparative $2^{-\Delta\Delta CT}$ method.

2.5. Statistics analysis

Data were presented as mean ± standard error (SE). SPSS 22.0 software (SPSS Inc., Chicago, USA) was employed for statistical analyses. One-way analysis of variance (ANOVA) followed by Duncan's multiple range tests were used to analyze the experimental data. Differences were considered to be significant at $P < 0.05$.

3. Results & discussion

3.1. Effects of V. harveyi infection on serum biochemical biomarkers

Biochemical parameters are generally regarded as useful biomarkers to evaluate health condition and physiological status of organisms (Canli et al., 2018). Hence, in this study, it was of great importance to examine the concentration of ALT, ALB, GLU and TP in serum of spotted sea bass after *V. harveyi* infection. Our results indicated that ALT, ALB and GLU concentration of spotted sea bass were significantly influenced by bacterial infection, especially ALT and GLU. ALT concentration was significantly decreased at 12h after infection, then sharply increased to the peak at 24h and gradually decreased until 72h (Fig. 1A). As the indicators of liver function, rapid changes of ALT concentration suggested that liver functions were damaged at the beginning of *V. harveyi* infection, and then gradually recovered over time. In agreement with our results, similar expression trends were also found in red hybrid tilapia after *Streptococcus agalactiae* infection (Alsaid et al., 2015). As shown in Fig. 1B, ALB levels were significantly decreased at 12h and 24h, and returned to the normal levels at 72h. This results may be closely linked with the effect of *V. harveyi* infection on ability of protein synthesis in liver, which was seriously limited at the beginning *V. harveyi* infection, and recovered over time, being consistent with the results of ALT. GLU concentration was sharply decreased at 12h and then a significant time-dependent increase of GLU was observed from 24 to 72h after infection (Fig. 1C). The results suggested that *V. harveyi* infection was able to influence the energy metabolism of spotted sea bass by altering GLU concentration, the main energy fuel in fishes. Similar observations were previously reported in Nile tilapia (Zeng et al., 2017) and Korean catfish (*Silurus asotus*) (Yu et al., 2010). In addition, the change trends of GLU further confirmed the severe effect of *V. harveyi* infection on physiological conditions at the beginning, and then gradually recovered to normal. No significant differences were detected in TP levels among different time points (Fig. 1D).

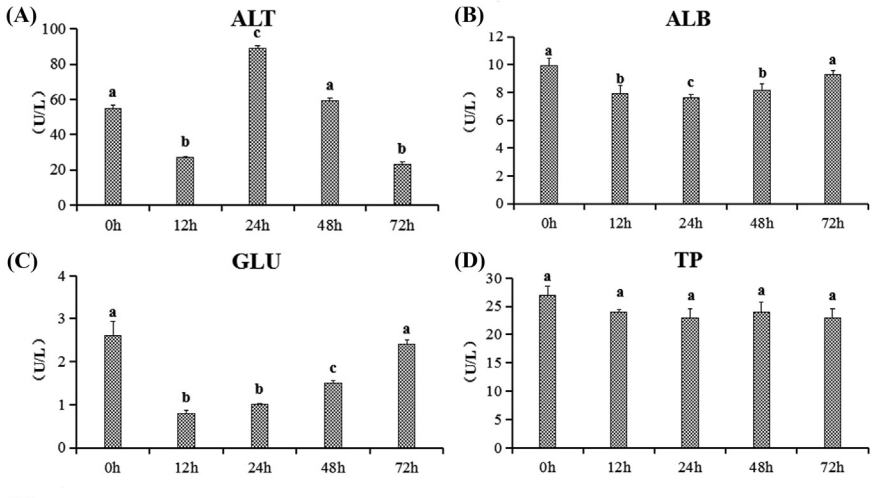
3.2. Characterizations of IL-17/IL-17R genes in spotted sea bass

IL-17 cytokines were critical players in host defense and inflammatory diseases, which were emerging as key players in immune responses (Pappu et al., 2011). IL-17R gene family comprises receptor molecules that are responsible for IL-17 signal transduction (Okamura et al., 2020). Despite their importance, IL-17/IL-17R gene families have not been systematically studied in spotted sea bass. In the present study, a total of 5 IL-17 (IL-17A/F1, IL-17A/F2, IL-17A/F3, IL-17C, IL-17D) and 5 IL-17R (IL-17RA, IL-17RB, IL-17RC, IL-17RD, IL-17RE) genes were identified from genomic and transcriptomic databases in spotted sea bass. The characteristics of these IL-17/IL-17R genes and their GenBank accession numbers were summarized in Fig. 1E. Every IL-17 and IL-17R gene in spotted sea bass contained the complete open reading frame. The CDS, exon number and predicted molecular weight of IL-17R genes were much larger than these of IL-17 genes in spotted sea bass (Fig. 1E). The pI of IL-17 gene family were ranged from 5.55 to 10.46, while pI of IL-17R gene family were between 5.41 and 8.70.

3.3. Phylogenetic and syntenic analysis

Phylogenetic and syntenic analysis was conducted to confirm the annotation and investigate the evolution relationships of IL-17 and IL-17R gene families. As shown in Fig. 1F, the IL-17 genes of spotted sea bass were clustered with teleost counterparts as expected and four clusters of sub-family were generated, including IL-17A/F, IL-17B, IL-17C and IL-17D. For IL-17R genes of spotted sea bass, IL-17RA, IL-17RB, IL-17RC, IL-17RD and IL-17RE were grouped with their corresponding teleost counterparts, respectively (Fig. 1G). As a result, the phylogenetic relationships of IL-17 and IL-17R genes were conserved between spotted sea bass and other tested vertebrates, which wdeir annotation in our study.

As shown in Supplementary Fig. 3, similar neighboring genes surrounding the tested IL-17/IL-17R genes were observed between spotted sea bass, tilapia and zebrafish. In details, genomic distribution of IL-17/IL-17R genes was divided into two types: individual and tandem arrangements. As shown in Supplementary Fig. 4, IL-17C, IL-17D and IL-17A/F3 were separately distributed on chr6, chr11 and chr18, while IL-17RA, IL-17RB and IL-17RD genes were located on chr22, chr9 and chr4, respectively. In contrast, two IL-17 genes (IL-17A/F1 and IL-17A/F2) and two IL-17R genes (IL-17RC and IL-17RE) were tandemly arranged on chr1 and chr9 of spotted sea bass genome (Supplementary Fig. 4), which may be caused by gene duplication events, homologous recombination or other complicated evolutionary processes (Liu et al.,



(E)

Gene name	Chromosome location	CDS (bp)	Predicted amino acid size (aa)	Exon number	Molecular weight (kDa)	pI*	Accession number
IL-17A/F1	Chr1: 2672492-2673992	414	137	2	15.48	5.55	MT129784
IL-17A/F2	Chr1: 2681531-2684238	429	142	3	15.36	9.03	MT129785
IL-17A/F3	Chr18: 1035983-1037528	489	162	4	17.86	10.46	MT129786
IL-17C	Chr6: 395027-399308	573	190	3	21.65	9.05	MT129787
IL-17D	Chr11: 3128703-3134090	633	210	3	23.46	9.67	MT129788
IL-17RA	Chr22: 29887-40242	2445	814	11	91.47	5.41	MT129789
IL-17RB	Chr9: 239145-242293	954	317	5	34.92	6.82	MT129790
IL-17RC	Chr9: 224844-232125	2274	757	15	83.15	5.83	MT129791
IL-17RD	Chr4: 686951-714180	2469	822	15	90.18	7.16	MT129792
IL-17RE	Chr9: 424331-426510	2481	826	17	92.14	8.70	MT129793

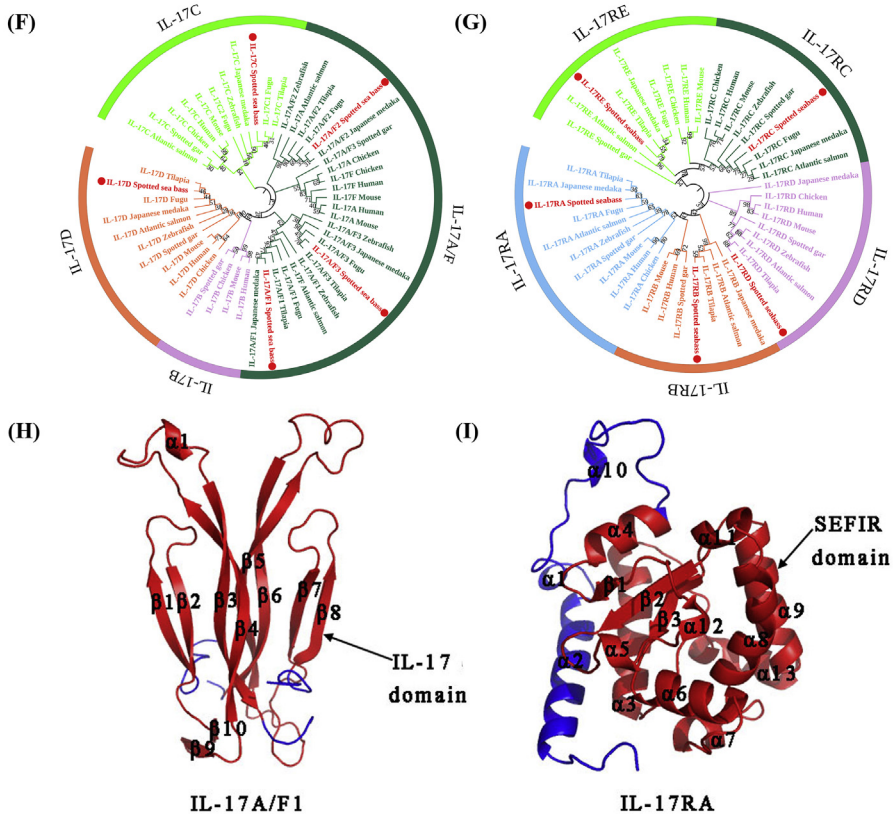


Fig. 1. (A–D) The concentration of ALT, ALB, GLU and TP in spotted sea bass serum at 0h, 12h, 24h, 48h and 72h after *V. harveyi* infection. The concentration of ALT, ALB, GLU and TP were presented as mean ± SE (n = 9). Various letters indicated significant differences ($P < 0.05$). **(E)** Characteristics of IL-17/IL-17R genes in spotted sea bass. pI* stands for theoretic isoelectric points of proteins. **(F–G)** Phylogenetic relationships of IL-17 and IL-17R genes in spotted sea bass and other selected vertebrate species. The phylogenetic tree was constructed by amino acid sequences of representative mammals and teleosts based on the neighbour-joining (NJ) method and Jones-Taylor-Thornton model with 1000 bootstrap replications in MEGA7 software. Different subfamily genes were marked with different colors. IL-17 and IL-17R genes of spotted sea bass were labeled by red dots. **(H–I)** The tertiary structures and functional domains of IL-17A/F1 and IL-17RA in spotted sea bass. The functional domains of tertiary structure were marked by red color, including IL-17 domain in IL-17 and SEFIR (IL-17R) domain in IL-17R. The α helices and β sheets were labeled in black fonts.

2019). Overall, the syntenic analysis provided additional supporting evidence for the annotations of IL-17 and IL-17R in spotted sea bass.

3.4. Gene copy numbers of IL-17/IL-17R genes

The copy numbers of IL-17/IL-17R genes in tested representative vertebrates and spotted sea bass were summarized in Supplementary Table 2. The numbers of IL-17 genes varied from 4 to 6, while IL-17R gene numbers ranged from 3 to 5 among different species. Almost all IL-17 and IL-17R genes possessed single copy, while the duplicated IL-17C genes were discovered only in fugu. Moreover, IL-17B were absent in most examined teleosts except for spotted gar, IL-17E gene was mammal-specific and IL-17N gene was only discovered in Japanese medaka and fugu (Supplementary Table 2). The results indicated that the gene number of IL-17 and IL-17R were relatively conserved during evolution.

3.5. Predicted tertiary structure and functional domains of IL-17/IL-17R genes

The predicted tertiary structures indicated that the main component of IL-17 genes in spotted sea bass was β-sheets, the number of which were ranged from 8 to 12 (Fig. 1H, Supplementary Fig. 5A-D). Besides, 1 or 2 α-helices existed in the IL-17 genes except for IL-17A/F2. The predicted tertiary structure of IL-17R were composed of both α-helices and β-sheets, their numbers were varied from 8 to 13 and 3 to 5, respectively (Fig. 1I, Supplementary Fig. 5E-H). In addition, the function domain region in predicted tertiary structures of IL-17 and IL-17R were relatively conserved among different gene members (Fig. 1H and I, Supplementary Fig. 5). Normally the intracellular SEFIR domain of IL-17R can bind to the IL-17 domain to initiate signaling cascades and activate other inflammatory cytokines for promoting innate immune responses in fishes (Kumari et al., 2009; Wang et al., 2014).

3.6. Expression patterns of IL-17 and IL-17R genes after V. harveyi infection

It has been generally acknowledged that liver, intestine, spleen and head kidney are the primary tissues responsible for innate immunity in teleosts, playing important roles in host defenses against invading bacteria (Press and Evensen, 1999). Therefore, qPCR analysis was conducted to determine the expression patterns of the complete sets of IL-17 and IL-17R genes in liver, intestine, spleen and head kidney of spotted sea bass at 0h, 12h, 24h, 48h and 72 h after *V. harveyi* infection. In general, all the IL-17 and IL-17R genes in spotted sea bass were significantly differentially expressed after infection in the tested tissues. However, the expression patterns after infection varied among different genes and tissues.

In details, the mRNA expression level of IL-17A/F1 was significantly up-regulated after infection in all the four tested immune related tissues. Accumulating evidence demonstrated that IL-17A/F1 plays important roles in host defense and autoimmunity via induction of the pro-inflammatory gene expression and release of chemokine CXCL-8 (Du et al., 2015), which has been proven in several teleosts, such as carp (*Cyprinus carpio*) (Dong et al., 2019) and large yellow croaker (Ding et al., 2016). It was notable that the expressions of IL-17A/F1 were dramatically induced at 24h in liver (21-fold) and head kidney (13-fold), reflecting its important roles in immune responses in liver and head kidney of spotted sea bass. In line with our findings, IL-17A/F1 in liver and head kidney were significantly up-regulated and reach the peak at 24h after *Aeromonas salmonicida* stimulation in turbot (*Scophthalmus maximus*) (Costa et al., 2012). Our results showed that IL-17A/F2 were also significantly differentially expressed in all the four examined tissues (Fig. 2B), revealing its potential function in host defense against bacterial infection. In agreement with our results, the expressions of IL-17A/F2 were significantly influenced in head kidney and gill tissues of rainbow trout (*Oncorhynchus mykiss*) after *Yersinia ruckeri* infection (Monte et al., 2013). Compared with IL-17A/F1, the highest expression levels of IL-17A/F2 were occurred at 24h in intestine and 12h in head kidney with greater than 8-fold increases (Fig. 2B). As shown in Fig. 2C, the expression of IL-17A/F3 also reached the peak at 12h in head kidney with more than 5-fold increases. Tissue- and time-dependent expression patterns of IL-17A/F1 and IL-17A/F2, IL-17A/F3 genes in spotted sea bass may be related to their unique biological roles. In addition, both IL-17C and IL-17D genes showed up-regulated expression levels since 12h in intestine and head kidney tissues (Fig. 2D and E). This observation was paralleled with the findings in large yellow croaker, where IL-17C and IL-17D expression was triggered rapidly by *Aeromonas hydrophila* infection in spleen and head kidney (Ding et al., 2017). IL-17C and IL-17D genes may promote the inflammatory response and host defense via activating NF-κB signalling (Ding et al., 2017).

With respect to IL-17R genes, the expression levels of IL-17RA were significantly up-regulated by 4.8-fold at 24h in liver, 1.9-fold at 24h in intestine and 1.9-fold at 12h in head kidney (Fig. 2F). Consistent with our observations, IL-17RA was found to be up-regulated in Japanese medaka after injected with *Edwardsiella tarda* (Okamura et al., 2020). As the co-receptor, IL-17RB could pair with IL-17RA to form a functional receptor complex for signaling in response to bacterial infection (Gaffen, 2009). Accordingly, as shown in Fig. 2G, IL-17RB displayed similar expression patterns with IL-17RA in intestine and head kidney. Additionally, IL-17RA could form a heteromeric complex with IL-17RC for the initiation of IL-17A/F1-3 signal transduction to enhance inflammatory response toward *V. harveyi* infection (Wright et al., 2008), which may be responsible for the similar expression trends between IL-17A/F1-3 and IL-17RA in our study (Fig. 2A, B, C and F). However, the expression pattern between IL-17RA and IL-17RC were quite different except in liver after *V. harveyi* infection (Fig. 2F and H), indicating the functional differences between IL-17RA and IL-17RC among tissues. In addition, significant up-regulation of IL-17RD was detected in head kidney after *V. harveyi* infection (Fig. 2I). Previous studies have demonstrated that members of the IL-17 cytokine family and their receptors generally synergize with cytokines and Toll-like receptors (TLRs) to augment the inflammatory response to bacterial and fungal infection (Gaffen, 2009). However, dysregulation of TLR signaling would lead to chronic inflammatory disease (Mellett et al., 2015). IL-17RD could negatively regulate TLR signaling to avoid the presence of chronic inflammatory disease (Mellett et al., 2015). Our results also showed that IL-17RE was dramatically induced in liver, intestine and head kidney, especially in intestine with more than 7-fold increase at 24h after infection in spotted sea bass (Fig. 2J). IL-17RE is the least understood member of the IL-17R family and little is known about its signal transduction and functional aspects until now (Gaffen, 2009). However, dramatically up-regulated IL-17RE in intestine suggested that it may be of crucial importance for the host defense against *V. harveyi* infection. Previous reported that IL-17RE had the highest expression level in intestine of large yellow croaker (Ding et al., 2016).

In summary, the significant variations of serum biochemical parameters including ALT, ALB and GLU indicated that the physiological status of spotted sea bass was significantly influenced by *V. harveyi* infection. Additionally, a total of five IL-17 and five IL-17R genes were systematically identified in spotted sea bass. Phylogenetic and syntenic analyses not only suggested the conservation of IL-17 and IL-17R in evolution, but also provide additional supporting evidence for the annotation of IL-17 and IL-17R of spotted sea bass. All IL-17 and IL-17R genes were differentially expressed after *V. harveyi* infection, and showed different expression patterns among various genes and tissues, suggesting their important but distinct roles in the immune responses to bacterial infection.

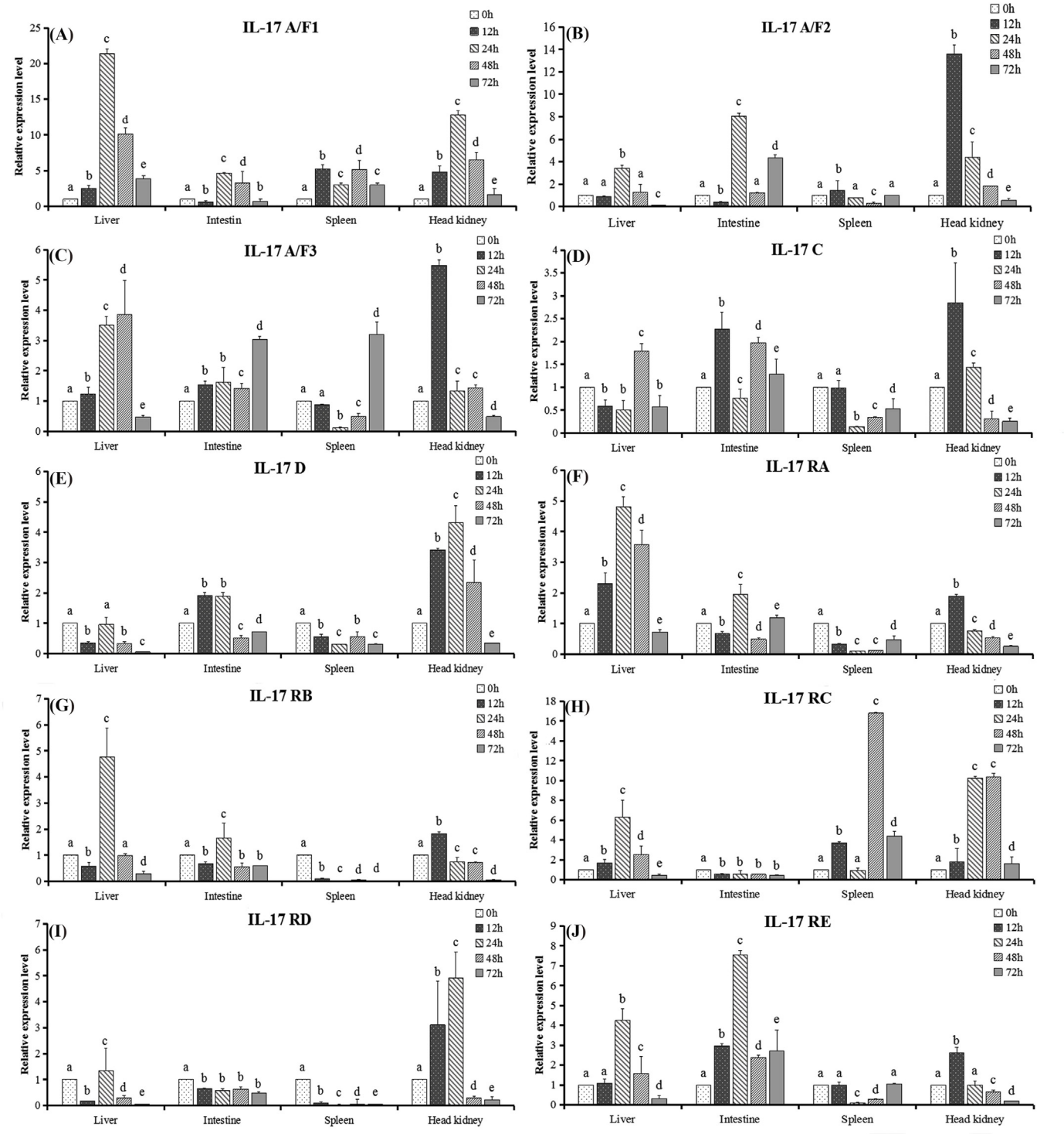


Fig. 2. Expression profiles of IL-17 genes and IL-17R genes in the liver, spleen, intestine and head kidney of spotted sea bass at 0h, 12h, 24h, 48h and 72h after *V. harveyi* infection. Gene expressions were calculated in relative to control group (0h) and presented as mean ± SE (n = 9). 18S rRNA was set as the internal reference gene. Various letters represented the significant differences ($P < 0.05$).

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.dci.2020.103731.

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