Turk. J. Fish.& Aquat. Sci. 20(7), 531-540
http://doi.org/10.4194/1303-2712-v20_7_03

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Immune Responses of Three SOCS Genes in Yellow Catfish (*Pelteobagrus fulvidraco*) Challenged with *Aeromonas hydrophila* or *Edwardsiella ictaluri*

Hongyan Zhang[1,2], Xueying Pei[1,2], Yadong Hu[1,2], Zhaowen Wu[1,2], Xiang Zheng[1,2], Guosong Zhang[1,3], Tao Wang[1,2,*], Shaowu Yin[1,2,*]

[1]Nanjing Normal University, College of Marine Science and Engineering, College of Life Sciences Nanjing, Jiangsu 210023, China
[2]Co-Innovation Center for Marine Bio-Industry Technology of Jiangsu Province, Lianyungang, Jiangsu 222005, China
[3]Heze University, School of agriculture and bioengineering, Key laboratory for physiology biochemistry and application, Heze, Shandong 274015, China.

Article History
Received 17 July 2019
Accepted 24 December 2019
First Online 30 December 2019

Corresponding Author
Tel.: +862585891840
E-mail: seawater88@126.com

Keywords
SOCS
Pelteobagrus fulvidraco
Aeromonas hydrophila
Edwardsiella ictaluri
Immune responses

Abstract

Suppressors of cytokine signaling (SOCS) are a family of intracellular proteins emerging as key physiological regulators of cytokine responses in fish innate immune system. In this study, the tissue-specific distribution indicated that *PfSOCS1*, 2 and 3 were expressed ubiquitously and differentially in eight examined tissues. The highest transcript levels of *PfSOCS1* and 3 respectively expressed in gill and liver, while that of the *PfSOCS2* appeared mainly in muscle, followed by head kidney. The temporal patterns of *PfSOCSs* were assessed through the experimental challenge of *A. hydrophila* or *E. ictaluri*, and their expressions were altered in liver, gill and head kidney. Concretely, western blotting and quantitative real-time PCR analyses showed that *PfSOCSs* were significantly up-regulated in the early stage and then decreased after *A. hydrophila* or *E. ictaluri* challenge. Furthermore, the innate immune response of *PfSOCSs* in gill was more sensitive than that in liver and head kidney. *PfSOCSs* played vital roles in response to *A. hydrophila* or *E. ictaluri* challenge in three essential immune-related tissues. Our findings suggested that *PfSOCSs* played crucial roles in innate immunity of *P. fulvidraco*, and provided useful evidence for further understanding on the modulation mechanism of *PfSOCSs* in the innate immune system of *P. fulvidraco*

Introduction

As pleiotropic secreted proteins, cytokines have extensive effects on many biological processes including vertebrate growth and development, homeostasis and immune response (Croker, Kiu, & Nicholson, 2008). Cytokines play significant roles in the immune system by binding to cytokine receptors on the cell membrane to regulate related genes in the nucleus. The cytokine receptors transduce signals by activating the Janus kinase (JAK) and transcriptional (STAT) signaling pathways, leading to trans-phosphorylation of JAKs and exciting downstream cells (Ilangumaran, Ramanathan, & Rottapel, 2004; Shepherd, Rees, Binkowski, & Goetz, 2012). It is worth mentioning that the suppressor of cytokine signaling (SOCS) is one of the most important feedback inhibitors of cytokine receptor signaling in JAK/STAT signaling pathway (Gadina *et al*., 2001). In mammals, SOCS family proteins can be divided into three regions, a central SH2 domain, a carboxyl terminal SOCS box and a N-terminal region with various length and primary sequence (Starr *et al*., 1997; Tiehui Wang & Secombes, 2008). Previous reports have demonstrated that SOCS proteins contain three major regulatory mechanisms. Firstly, SOCS proteins can interact with the JAKs domains, inhibiting the kinase activity of the latter (Skjesol *et al.,* 2014). Secondly, SOCS proteins can block the recruitment of STAT to the cytokine receptor by binding phosphotyrosines residues on the receptor through SH2 domain (Lesinski *et al.,* 2010). Thirdly, the

SOCS box can attach to the Elogin BC complex, recruiting ubiquitin E3 ligase, ultimately degrading SOCS and signaling proteins through the proteasome (Sun, Lan, Xu, Niu, & Wang, 2016). Furthermore, due to the difference in N-terminal region, the SOCS family has been divided into two sub-families as follows: type I containing SOCS4-7, and type II consisting of CISH, SOCS1-3 (Tanja, Vecino, Simon, Tiehui, & Secombes, 2014).

To date, the functions of *SOCS1*, 2 and 3 have been initially documented in mammals. *SOCS1*-knocked mice have severe pancreatic inflammation, indicating that *SOCS1* probably regulates the body's immune and metabolic system (Kentaro *et al*., 2008). Similarly, it has been documented that *SOCS2* and *SOCS3* are involved in immune responses in mice (Akihiko, Tetsuji, & Masato, 2007; Machado *et al*., 2006). Nowadays, identifications and analyses of *SOCS1*, 2 and 3 have been reported in several species, while the studies on *SOCS* genes in aquatic animals are still limited compared with mammals. Recent studies have illustrated that *SOCS* genes play a vital role in combating bacterial infection and pathogen stimulation in aquatic animals. For example, Jun Li *et al*. have shown that in *Paralichthys olivaceus*, eight *SOCS* genes are involved in immune responses after injection with poly (I:C) and formalin-killed cells (FKC) of *Edwardsiella tarda* (Thanasaksiri, Hirono, & Kondo, 2016). Jun Yao *et al*. have documented that most *CsSOCS* genes display distinctly different expressions after challenged by bacterial pathogens in *Cynoglossus semilaevis* (Hao & Sun, 2016). Moreover, the expressions of *SOCS* genes are both tissue-specific and time-dependent after *Flavobacterium columnare* or *Edwardsiella ictaluri* challenge in *Ictalurus punctatus* (Yao *et al*., 2015). Besides, studies in *Oreochromis niloticus* (C. Z. Liu *et al*., 2016), *Oncorhynchus mykiss* (Kotob *et al.,* 2018; Anju M. Philip, Kim, & Vijayan, 2012; Tiehui Wang & Secombes, 2008), *Scophthalmus maximus* (Tan *et al*., 2017; M. Zhang, Xiao, & Sun, 2011) and so on have also demonstrated that *SOCS* genes act as key physiological regulators of the immune system that can be exploited by pathogens to circumvent host responses (Sobhkhez *et al.,* 2017). However, it is unclear that the functions of *SOCS1*, 2 and 3 are conserved or different in teleost fish such as the yellow catfish (*Pelteobagrus fulvidraco*).

Yellow catfish (*P. fulvidraco*), belonging to *Siluriformes*, *Bagridae* and *Pelteobagrus*, is an omnivorous and commercial freshwater teleost. At present, due to its high nutritional, medicinal and market values, naturally cultured fish has difficulty to the market demand. Consequently, the artificial breeding of *P. fulvidraco* has quietly emerged in East and South Asia (Cheng, Jie, Da, & Jian-Fang, 2013; X. T. Zhang *et al*., 2017). With the rapid expansion of breeding scale and the promotion of breeding density, *P. fulvidraco* has suffered diverse diseases such as ichthyophthiriasis (Wei, Li, & Yu, 2013), bacterial septicemia (X. T. Zhang *et al*., 2017), ascites (Ding, 2008) and red-head disease (RHD) (Zhu *et al.,* 2017), which have restricted the sustainable development of the industry, resulting in huge economic losses. Among them, RHD is a severe disease with high mortality, high infectiousness and difficulty of controlling. Such disease is incited by a class of Gram-negative bacteria (G-) named *Edwardsiella ictaluri* (*E. ictaluri*), and the mortality may be up to 100% in serious cases (J. Y. Liu, Li, Zhou, Wen, & Ye, 2010). Another lethal serious disease is bacterial septicemia, which is caused by a class of Gram-negative bacteria (G-) called *Aeromonas hydrophila* (*A. hydrophila*) (Kong *et al*., 2017). However, it remains unclear how SOCS proteins affect the *P. fulvidraco* under the challenge of bacterial pathogen. Therefore, further effort is required to understand the mechanism of immune defense against pathogens in *P. fulvidraco*, which may contribute to the disease management as well as the sustainability of its culture.

It is generally accepted that liver, gill and head kidney are important immune-related tissues in keeping fish steady and maintaining normal physiological functions (Xin *et al*., 2017). Specifically, liver acts as an immune-responsive tissue, playing an essential role in intermediary metabolism, oxidation defense, detoxification (A. M. Philip, Jorgensen, Maule, & Vijayan, 2018). Gill contains numerous gill-associated lymphoid tissues and large mucosal surfaces, and the immune function includes immune barriers (such as immune response and inflammatory response) and physical barriers (such as tight junctions) (Rebl *et al.,* 2014). Head kidney is a critical lymphoid and hematopoietic organ in teleost, containing multiple immune cells such as B lymphocytes and various granulocytes (Kotob *et al.,* 2018). However, the immune regulations of *PfSOCS* genes in these three tissues after *A. hydrophila* or *E. ictaluri* challenge in *P. fulvidraco* have not been explored.

In the present study, we aimed to investigate the expression patterns of *PfSOCS1*, 2 and 3 in the innate immunity system to prevent bacterial infection in *P. fulvidraco*. Our study not only offered useful evidence to clarify the underlying mechanism of *PfSOCSs* in *P. fulvidraco*, but also laid the foundation for disease management and development of sustainable culture of *P. fulvidraco*.

Materials and Methods

Experiment Animals and Microbes

Healthy *P. fulvidraco* (4-month-old, 9.82 ± 0.61 cm in length, 15.4 ± 1.45 g in weight) were gathered from Nanjing Fisheries Research Institute, China. The collected individuals were randomly transferred into nine 200-L aquaria, which were equipped with bio-filtered water recirculation systems (equipped with cooling and heating functions and a flow rate of 5 L/min) and a normal photoperiod (14 L: 10D). Fish were fed with granulated feed containing 40.0% protein

(Yangzhou Hongda Feed Co., Ltd., China) twice daily (8:00-9:00 h and 20:00-21:00 h). After acclimated at 25-26 °C for 3 weeks, they were applied to the challenge experiments.

A. hydrophila were acquired from Microbial Culture Collection Center, Beijing, China (ATCC 7966) (L. Wang *et al*., 2016). *E. ictaluri* were received from Zhejiang Institute of Freshwater Fisheries, Huzhou, China (J. Li *et al*., 2019). The culture, identification and concentration of bacteria were adjusted based on the methods described in the reference (L. Wang *et al*., 2016; J. Zhang *et al*., 2019).

Immune Challenge

All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals in China. This study was also approved by the Ethics Committee of Experimental Animals at Nanjing Normal University (grant No. SYXK 2015-0028, Jiangsu).

After acclimation, the 225 *P. fulvidraco* with good physiological condition and uniform specifications were selected for formal experiment, and they were randomly distributed into 9 aquaria (25 tails / aquarium). According to the previous study (J. Zhang *et al*., 2019), fish were randomly divided into three experimental groups. The first group was intraperitoneally injected with *A. hydrophila* at a concentration of 1×10^6 CFU/ml (0.01 ml/g; the dosage was 1×10^4 CFU/g). The second group was intraperitoneally administered with *E. ictaluri* at a concentration of 5×10^5 CFU/ ml (0.01 ml/g; the dosage was 5×10^3 CFU/g). In the third group, the individuals were inoculated with an equal volume of PBS as a control group. The injection volume was proportionally adjusted according to the body weight of the individuals. Three repetitions were set for each treatment group. At 0, 6, 12, 24, 48 and 72 h after bacterial challenge, three fish per group were anesthetized with MS-222 (100 mg/l), and then their tissues were quickly dissected, frozen in liquid nitrogen and stored at -80 ℃ prior to further analyses.

Total RNA Extraction and cDNA Synthesis

Total RNA was extracted from each organ by using High Purity RNA Fast Extract Reagent (Bioteke, Beijing, China) following the manufacturer's protocol. The quality of extracted RNA was determined by spectrophotometer (ND-1000, NanoDrop, optical density 260/280 ratio, 1.9–2.05). The quality of RNA integrity was evaluated with 1% (w/v) agarose gel electrophoresis. The single-stranded cDNA was synthesized by using HiScript™ QRT SuperMix for qPCR (+gDNA wiper) (Vazyme, Shanghai, China). Subsequently, freshly synthesized cDNA was 1:10 diluted and stored at −20 °C prior to quantitative real-time PCR (qRT-PCR) (H. Zhang *et al*., 2018). Table 1 lists all primers used in this study

qRT-PCR

The tissue distribution and temporal expression profiles of *PfSOCS1*, 2 and 3 in different tissues of healthy and challenged *P. fulvidraco* were assessed by qRT-PCR. Samples were measured with a SYBR Green Master kit according to the manufacturer's instructions (Roche, Basel, Switzerland). *PfSOCS1*, 2 and 3 at the mRNA level were determined by qRT-PCR and β-actin was selected as a housekeeping gene (X. T. Zhang *et al*.) .The experiments were carried out in triplicate with a total volume of 20 μL using ABI StepOnePlus™ (Applied Biosystems,USA), containing 10 μL of SYBR green master, 4μL of cDNA (500 ng),and 3 μL of forward and reverse primers (2 mmol/L). The conditions were as follows: 95 °C for 10min, followed by 40 cycles at 95 °C for 15 s and 55 °C for 1min. The relative expressions of genes were calculated using the $2^{-\Delta\Delta Ct}$ method. The expression levels of *PfSOCSs* in the PBS group, *A. hydrophila* group and *E. ictaluri* group were compared at the corresponding time points.

Western Blotting Analysis

The frozen samples were homogenized, prepared with a total protein extraction kit (KeyGen BioTech, Nanjing, China). Samples (10 μg) were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with the BioRad MiniProtean III system (BioRad) and then transferred onto PVDF membranes (Millipore, Bedford, MA, USA). The membranes were blocked with TBS containing 0.05% Tween-20 and 5% albumin bovine V (Solarbio, Beijing, China) for 2 h. The mouse antibody against β-actin was selected as internal references to

Table 1. Primer sequences used in this study

Name	Primer sequences (5′-3′)	Application
SOCS1-F	AGAACCGTCAATGTTCCCCC	qRT-PCR
SOCS1-R	CTGAAGTGCCTGACGTGTCT	
SOCS2-F	TGAGTGCCGCAGTGCTTCAT	
SOCS2-R	AGCCTGCGCTCCTTTAGTCC	
SOCS3-F	TTCCCGACAGCACCACTTTT	
SOCS3-R	ATCAAGGATGGCTCAACGGG	
β-actin-F	TCCCTGTATGCCTCTGGTCGT	
β-actin-R	AAGCTGTAGCCTCTCTCGGTC	

F forward primer, R reverse primer.

obtain the relative expression of the SOCSs at protein level. Subsequently, the membranes were incubated with different antibodies overnight at 4 °C, and antibodies including SOCS1 (1:1000, D160748, Sangon Biotech, Shanghai, China), SOCS2 (1:1000, BS2914, Bioworld Technology, Minnesota, USA), SOCS3 (1:500, D221242, Sangon Biotech, Shanghai, China) and β-actin (1:2600; A5441; Sigma, St. Louis, MO, USA), which have been used in fish and are capable of stable amplification (D. Wang *et al*., 2019; D. Wang *et al*., 2018). The molecular weight of PfSOCS1, PfSOCS2, PfSOCS3 and β-actin proteins was approximately 21, 27, 24 and 42 kDa, respectively (Genbank accession number of PfSOCS1, PfSOCS2, PfSOCS3: MK335757, MK335758 and MK335759, Table S1,S2, Figure S1-S3).Afterwards, the membranes were washed three times with TBST and incubated with secondary antibody (goat anti-rabbit IgG or goat anti-mouse IgG, SAB, Baltimore, MD, USA) for 2 h at 25°C. Immunoreactive bands were visualized with ECL Reagent and densitometry analysis was performed using ImageJ software.

Statistical Analysis

Results are presented as mean ± standard error of deviation (SD) of triplicates experiments. One-way analysis of variance (ANOVA), followed by post-hoc comparison with Tukey's test, were used to determine statistical differences in terms of relative mRNA and protein expression at single time point. Significance was defined as $P < 0.05$.

Results

Tissue Distribution of PfSOCS1, 2 and 3

PfSOCSs were examined in eight healthy tissues from *P. fulvidraco* by qRT-PCR, including gill, muscle, liver, spleen, heart, head kidney, intestine and brain. The result (Figure 1 A-C) illustrated that the expressions of all three *PfSOCS* at the mRNA level were detectable in all examined tissues. The highest expression of *PfSOCS1* was found in gill, followed by heart, while its expression was low in other tissues. *PfSOCS2* had the highest expression in muscle, followed by head kidney and brain. *PfSOCS3* exhibited the highest expression in liver and heart, and it was relatively abundant in other tissues except for head kidney and intestine.

Transcription Patterns of PfSOCS1, 2 and 3 After Bacterial Infection

The temporal expressions of *PfSOCSs* were evidently altered after bacterial challenge even though there was no mortality of *P. fulvidraco* during the experiment. The expressions of *PfSOCSs* at the mRNA level in liver, gill and head kidney were significantly altered after challenged with *A. hydrophila* or *E. ictaluri* (Figure 2).

In liver (Figure 2 A-C), *PfSOCSs* exhibited an upward trend after *E. ictaluri* infection and peaked at 12 h, and then decreased gradually. In addition, similar findings were observed from *PfSOCS2* under *A. hydrophila* challenge, while *PfSOCS1* and *PfSOCS3* reached the highest expressions at 24 h. At this time point (24 h), *PfSOCSs* challenged with *A. hydrophila* were equal to or greater than the expressions after *E. ictaluri* challenge.

In gill (Figure 2 D-F), *PfSOCSs* were first increased and peaked at 6 h after *A. hydrophila* or *E. ictaluri* challenge, and then a declining trend was observed. At 6 h, *PfSOCS1* and *PfSOCS3* challenged with *A. hydrophila* were significantly higher than the expressions of *E. ictaluri* induced, which was opposite to *PfSOCS2.*

In head kidney (Figure 2 G-I), *PfSOCSs* were gradually up-regulated and then declined after challenged by *A. hydrophila* or *E. ictaluri*. *PfSOCS1* and *PfSOCS3* challenged with *E. ictaluri* were significantly higher than the expressions of *A. hydrophila* challenged at most sampling time point, which was opposite to *PfSOCS2*.

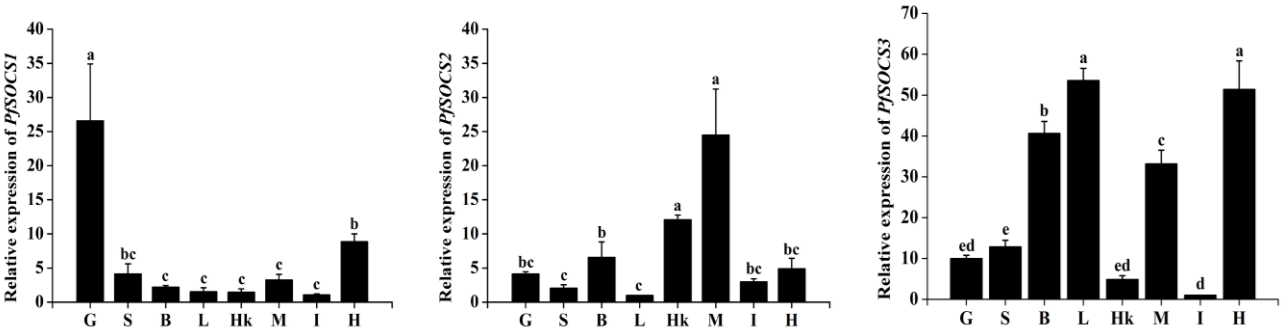

Figure 1 Tissue distribution analysis of *PfSOCS1* (A), *PfSOCS2* (B) and *PfSOCS3*(C) transcripts in *P. fulvidraco* using qRT-PCR. The relative expression of *PfSOCS* genes at the mRNA level in each tissue was calculated by the $2^{-\Delta\Delta Ct}$ method using *P. fulvidraco* β-actin as an internal reference gene. The tissues include the gill (G), spleen (S), brain (B), liver (L), head kidney (Hk), muscle (M), intestine (I) and heart (H). Values were presented as the mean and vertical bars represent a standard deviation. Significant differences between different tissues are identified with different letters ($P < 0.05$, One-way ANOVA).

Temporal Expressions of PfSOCS1, 2 and 3 at the Protein Level After Bacterial Infection

Western blotting analysis demonstrated that the temporal expressions of PfSOCSs at the protein level after challenged with *A. hydrophila* or *E. ictaluri* (Figure 3).

In liver (Figure 3 A-C), PfSOCS1 significantly increased at 6h after *E. ictaluri* infection, but it significantly decreased in *A. hydrophila* infection at the same time point. Also, it significantly declined in *E. ictaluri* infection at 24 h. PfSOCS2 increased at 6, 12, and 24 h after *A. hydrophila* infection. Additionally, it up-regulated in the early stage and peaked at 6 h after *E. ictaluri* challenge. PfSOCS3 under the *A. hydrophila* challenge or *E. ictaluri* challenge was gradually increased and peaked at 48 h and 24 h, respectively. PfSOCS2 and 3 challenged with *A. hydrophila* had higher expressions than challenged with *E. ictaluri* at most sampling time point.

In gill (Figure 3 D-F), PfSOCS1 and PfSOCS2 up-regulated in the early stage and peaked at 6 h after *A. hydrophila* challenge or *E. ictaluri* challenge, and then their expressions were tardily decreased. PfSOCS3 increased at 6 and 12h after *A. hydrophila* challenge, while PfSOCS3 increased slowly and peaked at 48h after *E. ictaluri* challenge.

In head kidney (Figure 3 G-I), PfSOCS1 was significantly increased and peaked at 6 h after *A. hydrophila* challenge, and then gradually baked to the normal level. Under the same challenge, PfSOCS2 and PfSOCS3 were initially increased slowly and finally reached the maximum at 48 h. Moreover, PfSOCS1, 2 and 3 after *E. ictaluri* challenge respectively peaked at 6 h, 6 h and 12 h, and then gradually decreased to the lowest value at 72 h. At 12 h, *PfSOCS3* challenged with *E. ictaluri* were significantly higher than the expressions of *A. hydrophila* challenged, which was opposite to *PfSOCS2*.

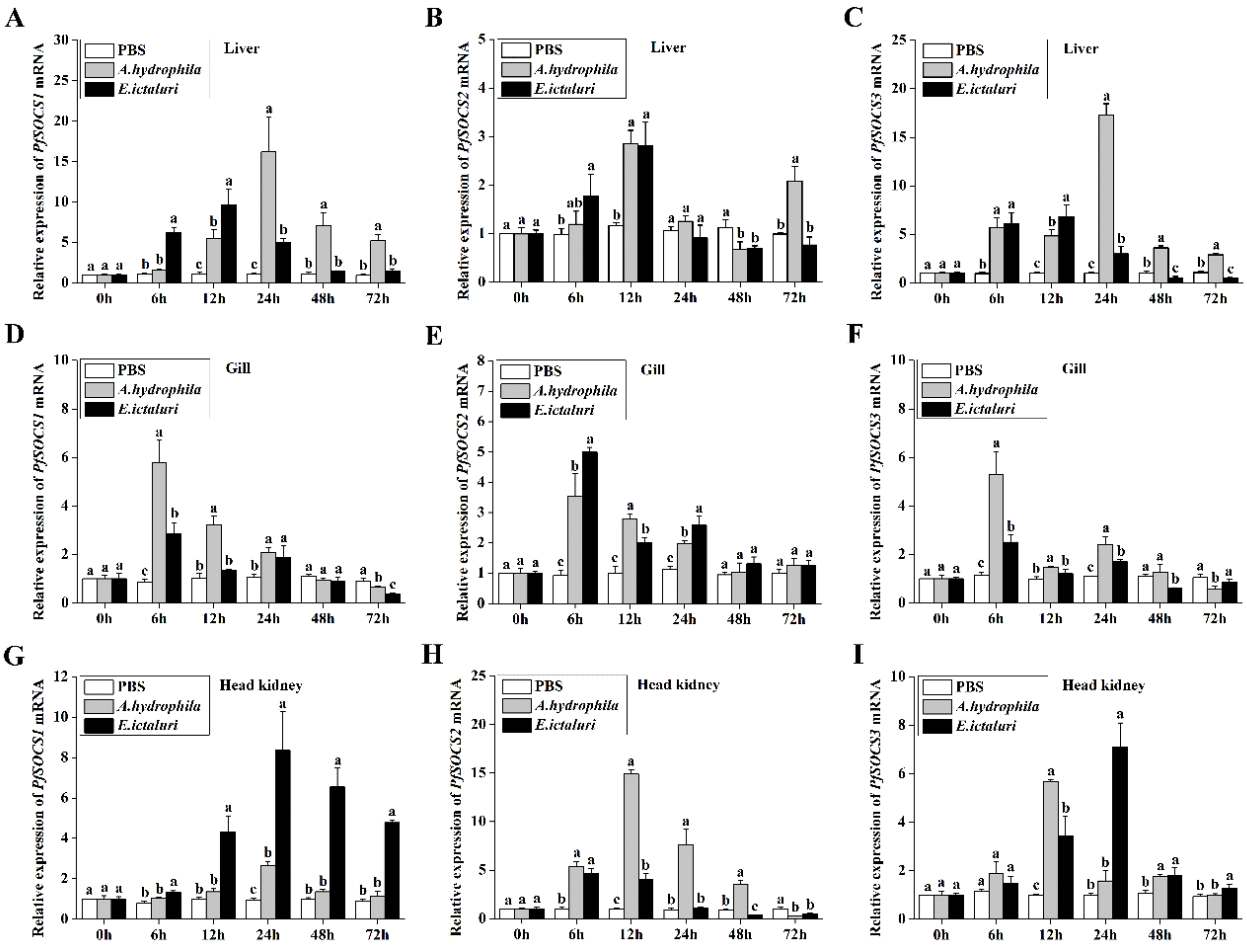

Figure 2. qRT-PCR analysis of *P. fulvidraco* in liver (A-C), gill (D-F), head kidney (G-I) after *A. hydrophila* or *E. ictaluri* challenge. The relative expression of *PfSOCS* genes at the mRNA level in each tissue was calculated by the $2^{-\Delta\Delta Ct}$ method using *P. fulvidraco* β-actin as an internal reference gene. Letters (a, b and c) were used to indicate the significant differences between PBS, *A. hydrophila* challenge and *E. ictaluri* challenge at the same sampling time point ($P < 0.05$, One-way ANOVA).

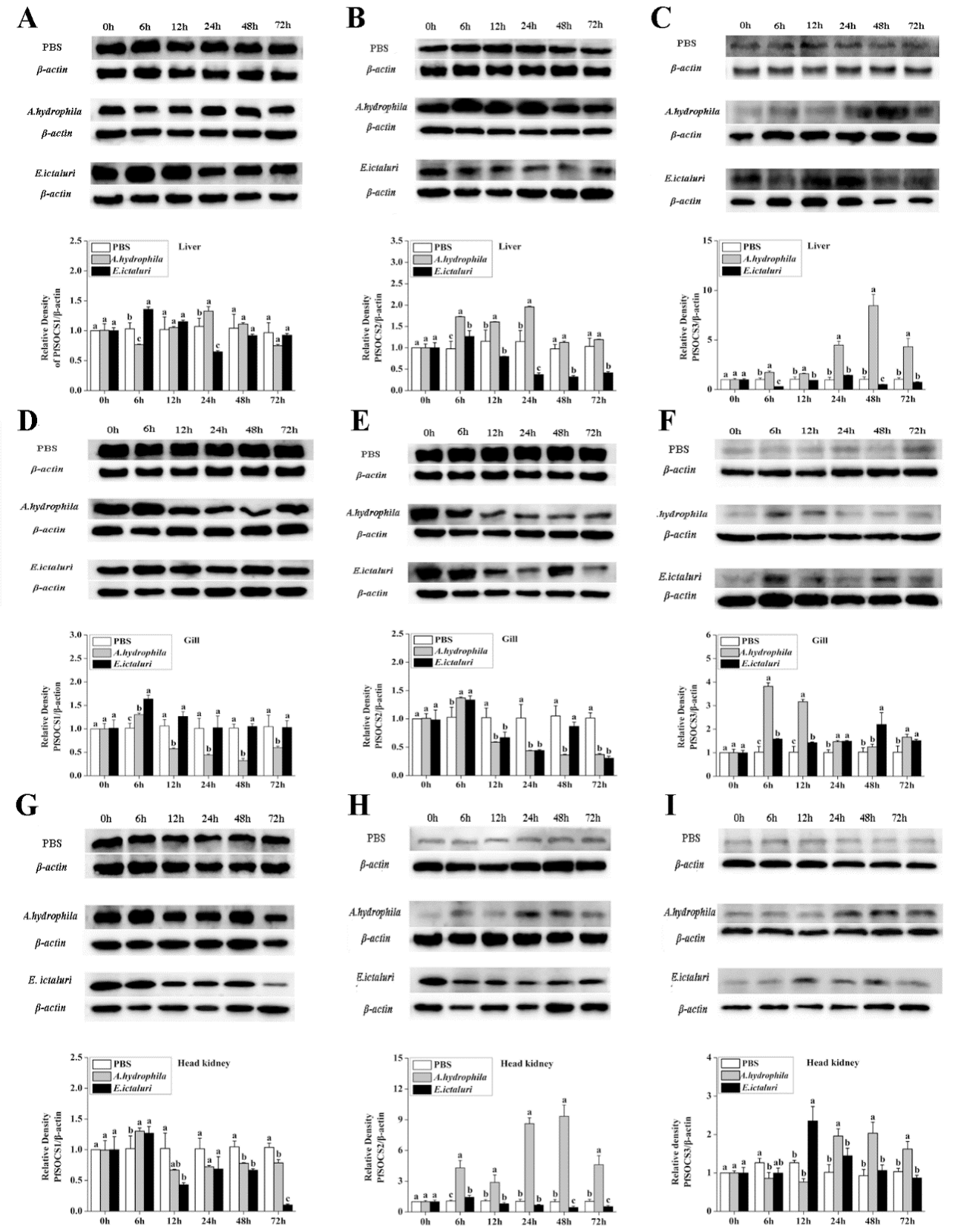

Figure 3 Western blotting analysis of *P. fulvidraco* in liver（A-C）, gill（D-F） and head kidney（G-I）after *A. hydrophila* or *E. ictaluri* challenge. The molecular weight of PfSOCS1, PfSOCS2, PfSOCS3 and β-actin proteins was approximately 21, 27, 24 and 42 kDa, respectively. ImageJ software was used to perform densitometry analysis. Letters (a, b and c) were used to indicate the significant differences between PBS, *A. hydrophila* challenge and *E. ictaluri* challenge at the same sampling time point (P<0.05, One-way ANOVA).

Discussion

In order to obtain a comprehensive understanding of *SOCS1*, 2 and 3 in *P. fulvidraco* after bacterial injection, we examined the tissue distribution patterns of *PfSOCSs* in healthy *P. fulvidraco* and found that they were extensively expressed in all examined tissues (Figure 1). The expression of *PfSOCS1* in gill was significantly higher than that in other tissues, which was consistent with the data from *O. niloticus* and *Takifugu fasciatus* (C. Z. Liu *et al*., 2016; D. Wang *et al*., 2018). Gill is an immune-competent organ characterized by gill-associated lymphoid tissues, which can secrete lysozyme and acid phosphatase (ACP) to kill pathogens (Dezfuli, Pironi, Giari, & Noga, 2010). *PfSOCS2* was highly expressed in muscle and head kidney, followed by heart and brain, which was similar to those found in *Atlantic salmon* (Skjesol *et al*., 2014).Concretely, *PfSOCS2* was highly expressed in head kidney, which is a fish-specific immune organ with numerous lymphocytes and phagocytic cells (Elena *et al*., 2005). Interestingly, the expression of *PfSOCS2* in the constitutive tissues, such as muscle, heart and brain, was relatively higher, which might be attributed to growth stage, immunological status, genetic background and species variation (C. Z. Liu *et al*., 2016). *PfSOCS3* had the highest expression in liver and heart, which was similar to those obtained from *T. fasciatus* (D. Wang *et al*., 2018). The liver is the largest reticuloendothelial phagocytic system of fish, which can phagocytize, isolate, and eliminate various invading and endogenous antigens (Bory, 2010). Furthermore, we noticed another article related to *SOCS* genes of *P. fulvidraco* (Ye, Zhao, Wu, Cheng, & Tan, 2019). We found that there are some differences in tissues expression results, which may be related to their different living environments (Nanjing and Wuhan population). Above studies have shown that the distribution profiles of *PfSOCSs* are not consistent, which may be related to their special functions in mediating immune response. Besides, a comparative study of *PfSOCSs* displayed that a lower expression was always found in intestine, indicating that the innate immunity function of intestine in *P. fulvidraco* was not prominent. These results indicated that *PfSOCSs* were highly expressed in immune tissues (such as liver, gill and head kidney) and played vital roles in innate immune response of *P. fulvidraco*.

In teleosts, it has been extensively demonstrated that *SOCSs* can be induced by LPS or Gram-negative bacteria through immunological modulations, such as *O. niloticus* (C. Z. Liu *et al*., 2016), *I. punctatus* (Yao *et al*., 2015), *O. mykiss* (Shepherd *et al*., 2018), *Perca flavescens* (Shepherd *et al.,* 2012), Ayu *Plecoglossus altivelis*(Minami, Suzuki, Watanabe, Sano, & Kato, 2018), *Crassostrea gigas* (Jun *et al*., 2015) and *Eriocheir sinensis* (Qu *et al*., 2017; Y. Zhang *et al*., 2010), which is consistent with our current findings. After *P. fulvidraco* was stimulated by *A. hydrophila* or *E. ictaluri*, the expressions of *PfSOCSs* in liver, gill and head kidney were significantly increased during different stages. These results revealed that *SOCSs* were inducible multi-factors involved in the regulation of immune defense in fish.

In liver, after *E. ictaluri* challenge, the time reaching the peak of PfSOCS1, 2 and 3 was mostly earlier compared with *A. hydrophia* challenge both at the protein and mRNA levels (Figure 2 A-C, Figure 3 A-C). Similar result has been described in *C. semilaevis*, and the bacterium of *Edwardsiella tarda* is more sensitive than *Vibrio harveyi* (Hao & Sun, 2016), illustrating that *PfSOCSs* were more sensitive to *E. ictaluri* challenge in liver. Additionally, we compared the expression profiles of *PfSOCSs* under different pathogen infections, and found that the induction folds that *A. hydrophia* enhanced were equal to or greater than *E. ictaluri* both at the protein and mRNA levels (Figure 2 A-C, Figure 3 A-C), indicating that *PfSOCSs* were more inducible after *A. hydrophia* challenge in liver. Although both *A. hydrophila* and *E. ictaluri* belong to gram-negative bacteria, they have different infection mechanisms, causing the induction folds and patterns were markedly different (C. Li *et al*., 2013; Yang *et al*., 2013). Meanwhile, the different expressions of *PfSOCSs* after bacterial infection were also related to the tissue types, infection stages and pathogen species (Hao & Sun, 2016).

In gill, we found that PfSOCS1, 2 and 3 were highly expressed in the early phase, and most of them peaked at 6 h with the stimulation of *A. hydrophila* both at mRNA and protein level (Figure 2 D-F, Figure 3 D-F). Relatively, the expression of *EsSOCS6* is not significantly changed during the first 24 h, while it sharply increased at 48 h after *A. hydrophila* challenge in *E. sinensis* (Qu *et al*., 2017) Among them, SOCS1, 2 and 3 belong to type II subfamily, and SOCS6 is classified into type I subfamily. These results suggest that different types of *SOCS* genes may exhibit different gene expression patterns under the same challenge. Moreover, the immune characterization of *PfSOCSs* was closely related to gene types and species. On the other hand, compared to liver and head kidney, gill responded quickly to bacterial infections (Figure 2, Figure 3), reflecting that it might have more sensitive immune response to prevent bacteria at the early stage. It has been reported that fish gill can take up fish pathogens as well as killed and viable cells of *Aeromonas salmonicida*, which may be the reason why gill can respond quickly after pathogens infections (Rebl *et al.,* 2014).

In head kidney, like what is observed with *O. niloticus* and *C. semilaevis SOCS1* and 3, *PfSOCS1* and 3 were significantly induced after bacterial challenge (Figure 2 G, 2 I, 3 G, 3 I), which suggests that *PfSOCS1* and 3 are likely to be involved in host immune response against bacterial infection (Hao & Sun, 2016; C. Z. Liu *et al.,* 2016). *PfSOCS2* peaked at 6 h after *E. ictaluri* challenge and it significantly expressed for a long period of time after *A. hydrophia* challenge (Figure 2 H, Figure 3 H), which indicated that *PfSOCS2* in head kidney is more persistent in response to *A. hydrophila* than *E.*

ictaluri and *PfSOCS2* may play a momentous role in the immune system of head kidney. However, a recent study has displayed that *NtSOCS2* is more involved in the metabolic regulation of *O. niloticus* (C. Z. Liu *et al*., 2016), and *LvSOCS2* is more sensitive to viral infection instead of bacterial stimulation in *Litopenaeus vannamei* (S. Wang *et al*., 2016). These findings suggest that the fish *SOCS* genes are not only affected in a species-specific manner, but also the differential expression may be induced by cytokines, viruses and bacteria (T. Wang *et al*., 2011). Moreover, we compared the expression profiles and induction folds of the same *PfSOCS* gene under different pathogen infections (Figure 2 G-I, Figure 3 G-I). The conclusion is that consistent with *PfSOCSs* in liver, the immune response of *PfSOCSs* was more sensitive to *E. ictaluri* in *P. fulvidraco* head kidney. In addition, *PfSOCS1* and 3 were more induced after *E. ictaluri* challenge in head kidney, which is opposite to *PfSOCS2*. *PfSOCSs* can be highly induced by *E. ictaluri* challenge in head kidney and it might be related to the special roles of SOCS proteins in mediating innate immune responses for host defense against bacteria-induced tissue damages.

In the present study, we found that most of *PfSOCSs* tended to be up-regulated early with challenge, but down-regulated at later stages of pathogenesis (Figure 2, Figure 3), which may be correlated with massive expression of chemokines after bacterial challenge. As a superfamily of cytokines, chemokines are responsible for regulating normal physiological functions under both inflammatory and physiological conditions (Yao *et al*., 2015). Additionally, although the expression profiles of PfSOCSs presented similar trend at protein and mRNA levels, a slight difference still existed between them. For example, the peak times of PfSOCSs after bacterial challenge were not completely consistent between the mRNA and protein levels. Above results revealed that the relationship between mRNA and protein was not strictly linear, and the number of two molecules is mainly determined by translation and protein degradation(Abreu, Penalva, Marcotte, & Vogel, 2009). Meanwhile, the complex post-transcriptional mechanisms are responsible for the inconsistency between mRNA and protein expression (Palash, Michael, & Hamilton, 2005).

Conclusion

PfSOCS1, 2 and 3 possessed distinct characteristics in different tissues of *P. fulvidraco*. Meanwhile, their transcription and translation were able to respond to the stimulation of pathogenic bacteria. Our study explored the underlying regulatory mechanisms in the innate immune system of *P. fulvidraco*, laying a foundation for further functional studies on teleost SOCSs.

Acknowledgements

This study was supported by the Key Research and Development Program of Jiangsu Province (BE2017377), The Creation Project of Major New Species of Agriculture in Jiangsu Province (PZCZ201742) , Jiangsu Agriculture Science and Technology Innovation Fund [CX(19)2034], Natural Science Foundation of Shandong Province of China (ZR2019QC002), Heze university doctoral fund (XY18BS08)

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