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Short communication

Molecular characterization and immune response to lipopolysaccharide (LPS) of the suppressor of cytokine signaling (SOCS)-1, 2 and 3 genes in Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Suppressor of cytokine signaling (SOCS) proteins are inverse feedback regulators of cytokine and hormone signaling mediated by the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway that are involved in immunity, growth and development of organisms. In the present study, three *SOCS* genes, *SOCS-1*, *SOCS-2* and *SOCS-3*, were identified in an economically important fish, Nile tilapia (*Oreochromis niloticus*) referred to as *NtSOCS-1*, *NtSOCS-2* and *NtSOCS-3*. Multiple alignments showed that, the three SOCS molecules share highly conserved functional domains, including the SRC homology 2 (SH2) domain, the extended SH2 subdomain (ESS) and the SOCS box with others vertebrate counterparts. Phylogenetic analysis indicated that NtSOCS-1, 2 and 3 belong to the SOCS type II subfamily. Whereas NtSOCS-1 and 3 showed close evolutionary relationship with Perciformes, NtSOCS-2 was more related to Salmoniformes. Tissue specific expression results showed that, *NtSOCS-1*, *2* and *3* were constitutively expressed in all nine tissues examined. *NtSOCS-1* and *3* were highly expressed in immune-related tissues, such as gills, foregut and head kidney. However, *NtSOCS-2* was superlatively expressed in liver, brain and heart. *In vivo*, *NtSOCS-1* and *3* mRNA levels were up-regulated after lipopolysaccharide (LPS) challenge while *NtSOCS-2* was down-regulated. *In vitro*, LPS stimulation increased *NtSOCS-3* mRNA expression, however it inhibited the transcription of *NtSOCS-1* and *2*. Collectively, our findings suggest that, the *NtSOCS-1* and *3* might play significant role(s) in innate immune response, while *NtSOCS-2* may be more involved in metabolic regulation.

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1. Introduction

Cytokines are pleiotropic proteins that have extensive effects on vertebrate growth, homeostasis and immune system [1]. Most of the cytokines act as regulators by combining the cytokine receptors at the cell membrane and activating the Janus kinase (JAK) – signal transducers and activators of the transcription (STAT) signaling pathway, ultimately eliciting downstream effects in responsive cells [2,3]. Moreover, the regulatory model of JAK/STAT signaling pathway is also effective to important hormones such as growth hormone, insulin and leptin [4–6]. However, under- or over-expression of cytokines or hormones would interrupt the normal homeostasis and cellular functions [7]. The suppressor of cytokine signaling (SOCS) family members serve as one of the most important feedback inhibitors of JAK/STAT signaling pathway that have received much attention since being discovered [8,9].

In mammals, eight SOCS family members, ranging from SOCS-1 to SOCS-7 and SH2 domain containing protein (CISH) have been identified. The identified mammalian SOCS are involved in a wide range of biological processes, such as immunological processes [10], atherosclerosis [11], metabolism [12] and cancer [13]. *SOCS-1* knockout (*SOCS-1*$^{-/-}$) mice exhibited more association with inflammation, such as uncontrolled interferon-gamma (IFN-γ)

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signaling and excessively activated inflammatory response [14]. In addition, overexpression of *SOCS-3* in mice cardiac myocytes resulted in increased sensitivity to coxsackievirus B3 infection [15]. However, the *SOCS-2*-null mutant mice had inability to down-regulate growth hormone (GH) signaling and were characterized by overgrowth phenotype [16]. A recent study has shown that, *SOCS-2* knockout (*SOCS-2*$^{-/-}$) mice fed with high-fat-diet acquired alleviative hepatic steatosis but worsens insulin resistance [17]. Moreover, constitutive production of *SOCS-2* in beta cells leads to hyperglycaemia and glucose intolerance [4].

Compared to mammals, studies on SOCS family in teleost fishes are still limited. The teleost *SOCS-1*, *2* and *3* genes have been identified in five model fish species (zebrafish, tetraodon, fugu, medaka, and stickleback) [18–20] and rainbow trout [21]. The *SOCS-1* and *SOCS-3* with two copies each have been identified in channel catfish [22]. In addition, the *SOCS-3* gene has also been cloned in common carp [23]. Several studies have explored the functions of fish SOCS via stimulants, as well as by using transgenic and gene knock-out technologies revealing various results depending on fish species and cell lines examined. In rainbow trout, all the *SOCS-1*, *2* and *3* were up-regulated post recombinant trout IFN-γ and interleukin-1β (IL-1β) stimulation in the fibroid cell line RTG-2, but only IFN-γ could up-regulate these genes in the cell line RTS-11 [21]. Homozygous GH-transgenic zebrafish has been shown to possess significantly higher expression level of *SOCS-1* and *SOCS-3* genes compared to non-transgenic zebrafish [24]. In channel catfish, the *SOCS-1a* and *SOCS-3a* were significantly up-regulated after four hours challenge with *Flavobacterium columnare* bacteria [22]. More recently, the deletion of *SOCS-1a* in zebrafish elucidated its physiological roles. The *SOCS-1a*-deficient zebrafish exhibited decreased adiposity, hepatic steatosis and insulin resistance [25]. Based on these observations, SOCS presumably have various functions in different fish species. However, it is unclear whether the SOCS functions are conserved or different among teleost fishes.

Nile tilapia (*Oreochromis niloticus*) is one of the most economically important fish species worldwide. Recently, diseases and metabolic disorders have been very common in cultured Nile tilapia leading to extensive studies on the mechanisms involved in its immunity process and metabolism [26–29]. However, as important regulators of both immune response and metabolic activities in mammals and other teleost fishes, the SOCS family members have not been widely studied in Nile tilapia. More specifically, the molecular characteristics of SOCS family and their potential correlations among the different SOCS members and immune response or metabolic process should be identified.

Therefore, the purpose of the present study was to determine the full-length cDNA sequences coding for *SOCS-1*, *2* and *3* genes and characterize their structure in Nile tilapia. Furthermore, tissue specific distribution pattern of the three genes was explored. In order to evaluate the potential functions of the three genes in immune regulation, the expression response of *SOCS-1*, *2* and *3* challenged with bacterial lipopolysaccharide (LPS) were examined *in vivo* and *in vitro*. The results obtained in our study provide insights into the molecular structure, evolution, organization and the potential functions of the SOCS family in teleost fishes.

2. Materials and methods

2.1. Animals and immune challenge experiment in vivo

Nile tilapia (with average weight of 8 ± 2 g) were obtained from Bairong Fish Farm in Qingyuan (Guangdong province, China) and maintained in 300 L opaque polyethylene tanks in a recirculation system. They were kept at 28 ± 2 °C on a 12D:12L photoperiod and fed twice daily to visual satiation with commercial diet (Dajiang, China). Fish were acclimatized for one week prior to the experiment. After acclimatization period, six healthy fish were collected for evaluating mRNA expression profile in nine different tissues (gills, skin, muscle, liver, spleen, heart, head kidney, foregut and brain). Fish were sacrificed by treating them with MS-222 (0.1 g/L) before dissection. Tissue samples collected were immediately frozen in liquid nitrogen, followed by storage at −80 °C until needed for further analysis. Twenty healthy fish weighing 11 ± 2 g were randomly selected from acclimatized fish. Selected fish were divided into two groups (ten fish each group); one group was intraperitoneally injected with LPS (*Escherichia coli* 055:B5, Sigma; 2.5 mg/kg wt in saline) while the other group was injected with saline as a control. Liver and gills samples from a random sample of five fish were collected in both groups 12 h post injection and immediately processed as described above. The untreated Nile tilapia were raised in the same conditions as described above for ten weeks. Liver samples from them were sampled for preparing primary cell culture.

2.2. Primary culture of Nile tilapia hepatocytes and immune challenge in vitro

The isolation and primary culture of Nile tilapia hepatocytes were performed as previously described by Liu et al. [30] with some modifications. The liver was carefully excised and rinsed twice with phosphate buffered saline (PBS), then dissected into small pieces and digested with PBS containing 0.1% collagenase IV (Sigma, USA) for 50 min at 28 °C water bath on a shaker. The softened liver tissue was agitated and filtered through 70 μm nylon mesh (Falcon, NJ, USA). The cell suspension was centrifuged (800 rpm for 10 min) at room temperature. The resulting supernatant was discarded while the pellet obtained was re-suspended in enough red blood cell lysis buffer (CWBIO, China) for 5 min, centrifuged and washed twice with the PBS. The harvested cell pellet was re-suspended in a medium (DMEM with 10% FBS, 2% penicillin-streptomycin) (Gibco, USA). The isolated hepatocytes were seeded at a density of 10^6 mL^{-1} in PRIMARIA 6-well tissue culture plates (Falcon, USA), 2 mL/well. After 24 h of culture, half medium was carefully replaced by the same medium with the LPS (50 μg/mL) (Sigma, USA) or the PBS as a control. Cells from the culture plates with LPS and PBS were collected after 12 h for RNA isolation.

2.3. RNA isolation and synthesis of cDNA

The tissues and cell samples were homogenized for extraction of total RNA using TransZol™ RNA isolation reagent (TransGen, China). NanoDrop 1000 spectrophotometer (Hach, America) was used to determine the quality and quantity of total RNA. The cDNA was synthesized using a PrimerScript RT reagent kit (Takara, Japan) and used as the template for gene cloning. PrimeScript™ RT reagent Kit with a gDNA Eraser (Perfect Real Time) (Takara, Japan) was used to synthesize cDNAs for quantitative real time-PCR (qPCR) [31].

2.4. Cloning of the sequences and molecular characterization analysis

Database sequence searches were performed using the BLAST package (NCBI, http://www.ncbi.nlm.nih.gov/BLAST) and BLAT (UCSC genome browser, http://genome.ucsc.edu) to get the putative Nile tilapia *SOCS-1*, *SOCS-2* and *SOCS-3* (*NtSOCS-1*, *NtSOCS-2*, *NtSOCS-3*). Specific primers were designed for cloning the *NtSOCS-1*, *NtSOCS-2* and *NtSOCS-3* genes (Table 1). The PCR conditions were 35 cycles at 94 °C for 1 min, 58 °C for 30 s, and 72 °C for 1 min, with an additional 10 min final extension at 72 °C [32]. Multiple amino acid sequence alignments were generated using Bioedit 3.1 and

Table 1
Primers used in the present study.

Usage	Gene	Primer name	Sequence (5′–3′)	Size(bp)
Genes cloning	*NtSOCS-1*	S1F	CCATACGGATCCTTAAACCGCTAC	857
		S1R	AATCTCCATTGCGTCGCAGA	
	NtSOCS-2	S2F	GTGCCCTGCAGACCTTGTACAT	754
		S2R	ACTCACAGAGCGCATCGACAAT	
	NtSOCS-3	S3F	CTTCTTGCCTACTCGAACAACCCT	811
		S3R	GCTTCATTACTCCTCAAATGCAGC	
qRT-PCR	*EF1α*	E1F	CTACGTGACCATCATTGATGCC	106
		E1R	AACACCAGCAGCAACGATCA	
	β-actin	ActF	CAGGATGCAGAAGGAGATCACA	92
		ActR	CGATCCAGACGGAGTATTTACG	
	NtSOCS-1	qS1F	TTCTTCACGCTGTCCTACCACG	113
		qS1R	GCAAAGAGTGTTTGGAAAGACCG	
	NtSOCS-2	qS2F	AACAACACCGGAGCTGTGGAA	119
		qS2R	TGCAGGATCTCTTTGGCTTCA	
	NtSOCS-3	qS3F	ACCCTCAGTGTCAAGACAGCCTC	121
		qS3R	AGAACGCAGTCAAAGTGGGGAA	

CLUSTALW programs. The presumed tertiary structures were established using SWISS-MODEL prediction algorithm (http://swissmodel.expasy.org/). The MEGA5 software was applied to construct a maximum likelihood (ML) tree [33]. Jones–Taylor–Thornton and Gamma distributed with Invariant sites (JTT + G + I) models were chosen based on the alignment results. During phylogenetic analysis, 1000 bootstrap replicates were performed.

2.5. Real-time PCR

The mRNA levels of *NtSOCS-1*, *NtSOCS-2* and *NtSOCS-3* genes were determined by real-time quantitative PCR (qRT-PCR) using SYBR green master mix (KWBIO, China) on a CFX96 real-time PCR system (Bio-Rad, USA). The PCR reaction volume was 25 μL containing 500 ng of total cDNA as template, 12.5 μL of SYBR Mix, 2.0 μL of PCR primers (5 μM) and nuclease-free water. PCR reactions were run with the following conditions: 95 °C for 30 s; 38 cycles of 95 °C for 5 s; and 60 °C for 20 s. The relative expression of *NtSOCS-1*, *NtSOCS-2* and *NtSOCS-3* genes were calculated using the $2^{-\Delta Ct}$ method, thereof, $\Delta Ct = Ct_{target} - (Ct_{EF1\alpha} + Ct_{\beta\text{-actin}})/2$. Elongation factor 1 alpha (EF1α) and β-actin were amplified to confirm the steady-state level of expression and used as reference genes [31]. Prior to the analysis, results from a preliminary study proved that, EF1α and β-actin were stably expressed in various tissues and different nutritional treatments in Nile tilapia.

2.6. Statistical analyses

Results are presented as mean ± standard error of the mean (SEM) and data were tested for homogeneity of variances using Levene's test to safeguard against violation of parametric statistics. After confirming homogeneity of variances, one-way analysis of variance (ANOVA) was used to determine statistical differences in the qRT-PCR data and tissue expression pattern among the different tissues examined followed by Tukey's post hoc test for specific differences. In the immune challenge assay, the differences between two groups were assessed by using a *t*-test. Differences were considered significant at $P < 0.05$ and extremely significant at $P < 0.01$. All statistical analyses were performed using SPSS Statistics 19 (IBM, USA) for Windows.

3. Results

3.1. Gene cloning and homologous analysis of NtSOCS

We identified the putative *SOCS-1*, *2* and *3* sequences in Nile tilapia by searching the genome browsers and cloned these genes from the liver cDNA library of Nile tilapia using PCR with specific primers (Table 1). The cDNAs of the *NtSOCS-1*, *2* and *3* (GenBank accession numbers: KR149237, KR149238 and KR149239, respectively) were 648 bp, 606 bp and 618 bp in length, encoding proteins with 215 amino acid, 201 amino acid and 205 amino acid residues, respectively.

The results of amino acid sequences multiple alignments showed that, all the NtSOCS-1, 2 and 3 contained a SH2 domain, a SOCS box and the extended SH2 subdomain (ESS). The latter acts as an additional functional domain for binding to phosphopeptides (Fig. 1A, B and C). Another additional functional domain: the kinase inhibitory region (KIR) located at the upstream of the SH2 domain exists in NtSOCS-1 and 3 but not found in NtSOCS-2 (Fig. 1A and C). NtSOCS-1 shared only 36.6% identity with human SOCS-1 and also has a lower identity compared with other fish species SOCS-1. NtSOCS-2 and 3 protein sequences shared a better homology with other known SOCS-2 and 3 from other organisms compared to NtSOCS-1. The former have an approximately 70% identity with other teleost fish species (Fig. 1A, B and C).

3.2. The potential tertiary structures of NtSOCS2

The tertiary configuration of mature NtSOCS-2 (residues 28 to 201) was built with reference to human SOCS-2 (PDB ID: 4jgh). However, tertiary configurations for the NtSOCS-1 and NtSOCS-3 were not constructed because there were no apposite templates to enable their constructions. Based on a SWISS-MODEL rendering (Fig. 2), the structure of SOCS box was highly conserved. The SH2 domain of Nile tilapia contained three α-helices and eight β-sheets, one more α-helix and two additional β-sheets than the SH2 domain in human SOCS-2.

3.3. Phylogenetic analysis of NtSOCS

To verify the identity of NtSOCS-1, 2 and 3 sequences and understand the evolutionary relationships among the SOCS genes, forty full protein sequences of SOCS-1, 2 and 3 from different species were used to construct a phylogenetic tree using the MEGA5 software. The species used included mammals, birds, amphibians and bony fishes. Results revealed that, SOCS-1, 2 and 3 are well

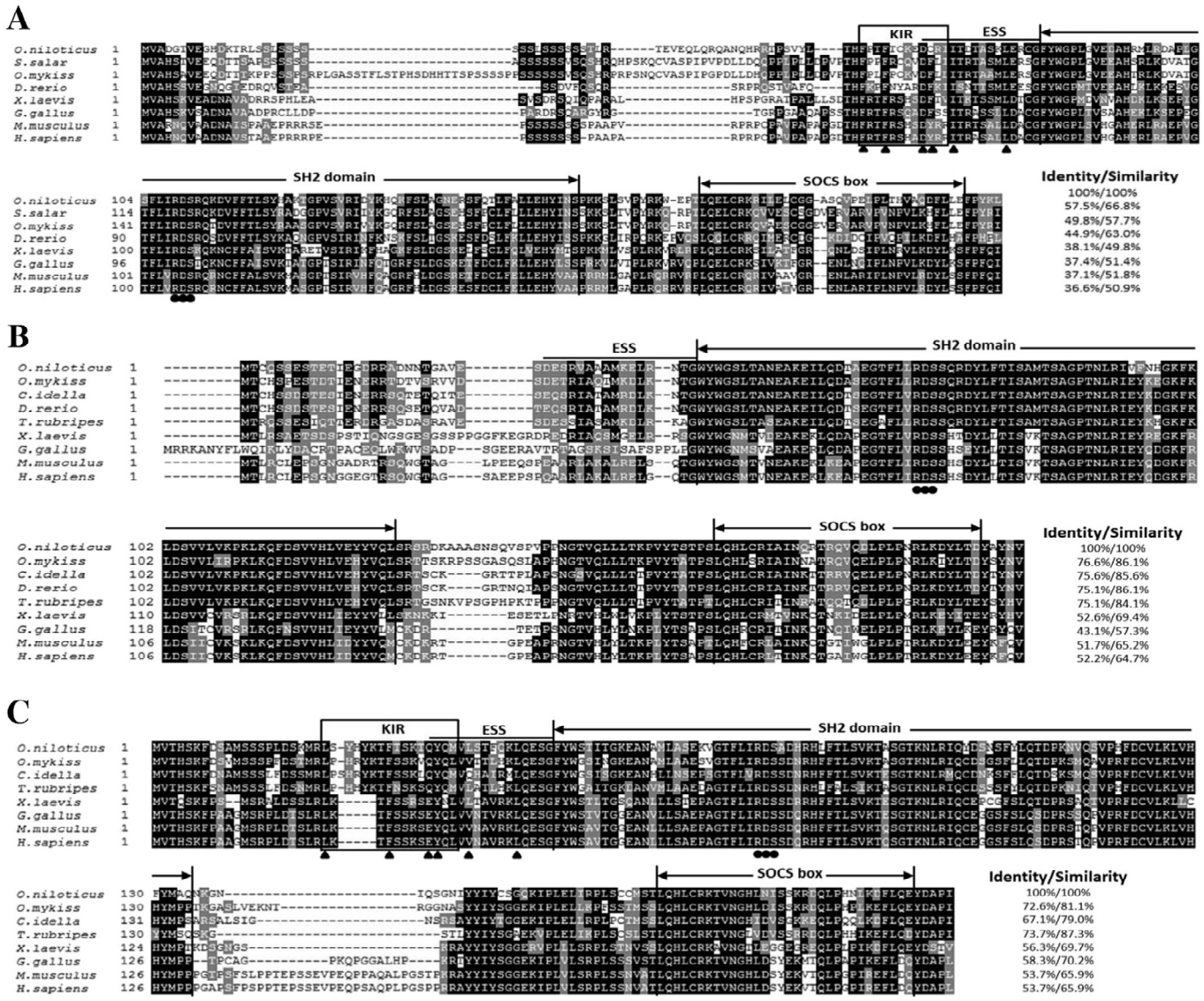


Fig. 1. Amino acid sequence alignments and homology of NtSOCS-1, 2 and 3 in mammals, birds, amphibians, and fish. The SH2 domain and SOCS box are pointed out with two arrows above the alignment. The ESS is indicated by solid lines above the alignment. The positions of mutations that disrupt the SOCS1–JAK2 and SOCS-GHR interactions are indicated by triangles and filled circles. (A) NtSOCS-1, the KIR is boxed. The accession numbers of the amino acid sequences are AHC08716 (*Salmo salar*), NP_001139638 (*Oncorhynchus mykiss*), NP_001003467 (*Danio rerio*), NP_001153160 (*Xenopus laevis*), NP_001131120 (*Gallus gallus*), NP_034026 (*Mus musculus*), NP_003736 (Homo sapiens). (B) NtSOCS-2, the accession numbers of the amino acid sequences are NP_001139639 (*Oncorhynchus mykiss*), ACV85622 (*Ctenopharyngodon idella*), NP_001108022 (*Danio rerio*), NP_001116332 (*Takifugu rubripes*), NP_001089229 (*Xenopus laevis*), NP_989871 (Gallus *gallus*), NP_001162128 (*Mus musculus*), NP_001257397 (Homo sapiens). (C) NtSOCS-3, the KIR is boxed. The accession numbers of the amino acid sequences are NP_001139640 (*Oncorhynchus mykiss*), ACD82060 (*Ctenopharyngodon idella*), NP_001072096 (Takifugu rubripes), NP_001084581 (*Xenopus laevis*), NP_989931 (Gallus *gallus*), NP_031733 (*Mus musculus*), NP_003946 (Homo sapiens).

classified and each has a single clade (Fig. 3). Apparently, the NtSOCS-1 and 3 were closely related to the Perciformes, while the NtSOCS-2 shared similarity to the Salmoniformes.

3.4. Tissue distribution of the Nile tilapia SOCS genes

The expression of the *NtSOCS-1, 2* and *3* genes were examined in nine different tissues; gills, skin, muscle, liver, spleen, heart, head kidney, foregut and brain from Nile tilapia by real-time PCR. All the three *NtSOCS* genes mRNA expression were detectable in the nine tissues examined. The *NtSOCS-3* gene was highly expressed in almost all tissues, except in the liver where the expression of *NtSOCS-2* gene was the highest (Fig. 4). The expression of *NtSOCS-1* gene was the lowest in most tissues, but it had relatively higher expression in gills and spleen. Both *NtSOCS-1* and *NtSOCS-3* were highly expressed in gills among all tissues examined.

3.5. Expression analysis of SOCS post LPS challenge in vivo and in vitro

LPS was used to investigate the immune regulation of *NtSOCS-1, 2* and *3* transcriptions *in vivo* and *in vitro*. Gills and liver were chosen in this LPS challenge test as representative tissues because they had higher expression of *NtSOCS-1, 3* and *NtSOCS-2* genes, respectively. *In vivo* mRNA expression level of *NtSOC-1 and 3* genes were significantly up-regulated in gills ($P < 0.05$) after LPS challenge compared to the saline control, whereas the *NtSOCS-2* levels in gills was significantly down-regulated ($P < 0.01$; Fig. 5A). In liver, LPS injected group had significantly higher expression of the *NtSOCS-1* and *3* genes compared to the saline control ($P < 0.01$),

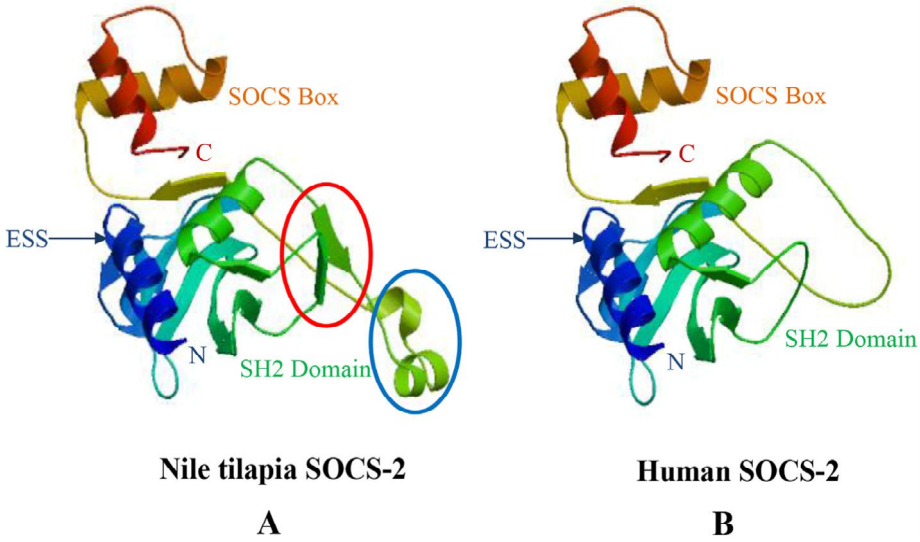


Fig. 2. The predicted three dimensional structure of NtSOCS-2 protein. The template PDB ID: 4jgh. (A) NtSOCS-2, the red oval and blue oval indicates the additional α-helix and β-sheet compared to human SOCS-2. (B) Human SOCS-2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

while the mRNA expression of *NtSOCS-2* gene was reduced but not significant ($P > 0.05$; Fig. 5B). In the hepatocyte culture, both *NtSOCS-1* and *2* genes were significantly reduced ($P < 0.05$ and $P < 0.01$, respectively) with LPS challenge while the *NtSOCS-3* gene was significantly increased ($P < 0.05$; Fig. 5C) relative to the control group.

4. Discussion

Amino acid multiple alignments of the NtSOCS-1, 2 and 3 showed that all the three SOCS in Nile tilapia have the same functional domains with mammalian SOCS. The KIR domain in NtSOCS-1 and the ESS domains in NtSOCS-1 and 2 have a lower similarity compared to mammals. In mammals, the ESS domain has been demonstrated as a necessary additional functional domain for binding to phosphopeptides and the KIR domain has been shown to be responsible for the high-affinity binding to the kinase domain of JAK2 [34]. The impact of the specific ESS and KIR domains in Nile tilapia is still unclear, but their divergence may indicate discrepancy in the pattern of regulation.

The predicted three-dimensional (3D) structure of NtSOCS-2 was established using the SWISS-MODEL prediction algorithm based on the template 4jgh [35]. In general, the 3D structure of NtSOCS-2 was homologous to that of humans. The SH2 domain in NtSOCS-2 possessed one more α-helix and two additional β-sheets compared to the SH2 domain in humans SOCS-2. The SH2 domain in mammals is deemed to be the main functional domain. The 3D structure of NtSOCS-2 containing one more α-helix and two additional β-sheets might be more structurally stable. However, further investigation is needed to reveal the biological function(s) of additional helix and two β-sheets in NtSOCS-2. Previous studies have demonstrated that, SOCS are clustered into two groups, with the SOCS4, SOCS5, SOCS6, SOCS7 forming the type I subfamily and the CISH, SOCS1, SOCS2 and SOCS3 forming the type II subfamily [20]. Our phylogenetic analysis revealed that, NtSOCS-1, 2 and 3 are well classified, each has a single clade and they all belong to the type II subfamily.

Expression pattern analysis in various tissues showed that, the *NtSOCS-1*, 2 and *3* mRNA were constitutively expressed in all the nine tissues examined. The expression levels of *NtSOCS-1* and *NtSOCS-3* genes were higher in gills compared to muscle and liver. The *NtSOCS-1* expression in gills was 102.3 and 36.4 fold compared to muscle and liver, respectively whereas that of *NtSOCS-3* was 10.2 and 19.6 fold compared to the same tissues, respectively. This is in agreement with previous result in rainbow trout whereby high expression of *SOCS-1* and *3* genes was found in gills and other immune-related tissues, such as the head kidney, spleen, intestine and skin [21]. Moreover, the *SOCS-3* gene was also highly expressed in gills in common carp and pufferfish compared to its expression in liver and muscle [23]. These expression characteristics indicate that the *SOCS-1* and *3* genes may play a momentous role in host immune responses of teleost fishes.

Interestingly, the highest expression of *NtSOCS-2* gene was obtained in liver (1.3-fold compared to gills). These results are contrary to those obtained in rainbow trout, in which *SOCS-2* gene had the lowest expression in liver among all the tissues examined [21]. It is noteworthy that, in mammals the *SOCS-2* gene is tightly linked to the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) signaling such that the *SOCS2* − deficiency ($SOCS\text{-}2^{-/-}$) or GH transgenesis have been verified to increase body weight [16]. It is known that, liver plays a critical role in intermediary metabolism. Thus, the high expression of *NtSOCS-2* gene in the liver of Nile tilapia suggests that this gene might be playing an important role in metabolic regulation.

The expression of *NtSOCS-1*, *2* and *3* genes in response to LPS challenge both *in vivo* and *in vitro* revealed increase and decrease of the *NtSOCS*. *In vivo*, LPS increased the *NtSOCS-1* and *3* genes while decreased the *NtSOCS-2* gene in liver and gills. The present results on *NtSOCS-1* and *3* are consistent with those obtained by Shepherd et al. [36] in juvenile yellow perch whereby the expression of *SOCS-1* and *3* were increased after LPS challenge. However, the results on expression of *NtSOCS-2* gene are contrary to those obtained by Jin et al. [20] in tetraodon fish in which the expression of *SOCS-2* gene increased in head kidney (Hdk) following LPS injection. *In vitro*, LPS elevated the expression of *NtSOCS-3* gene and reduced the expression of *NtSOCS-1* and *2* genes. The findings on *NtSOCS-3* gene are corroborated to those obtained by Wang et al. [37] in RTS-11 cells, in which LPS up-regulated the expression of rainbow trout *SOCS-3* in time-dependent manner. However, in the same species LPS reduced the expression of *SOCS-3* in hepatocytes but had no effect on *SOCS-1* [38]. In murine macrophages, LPS increased the expression of *SOCS-3* at various period intervals, but had no effect

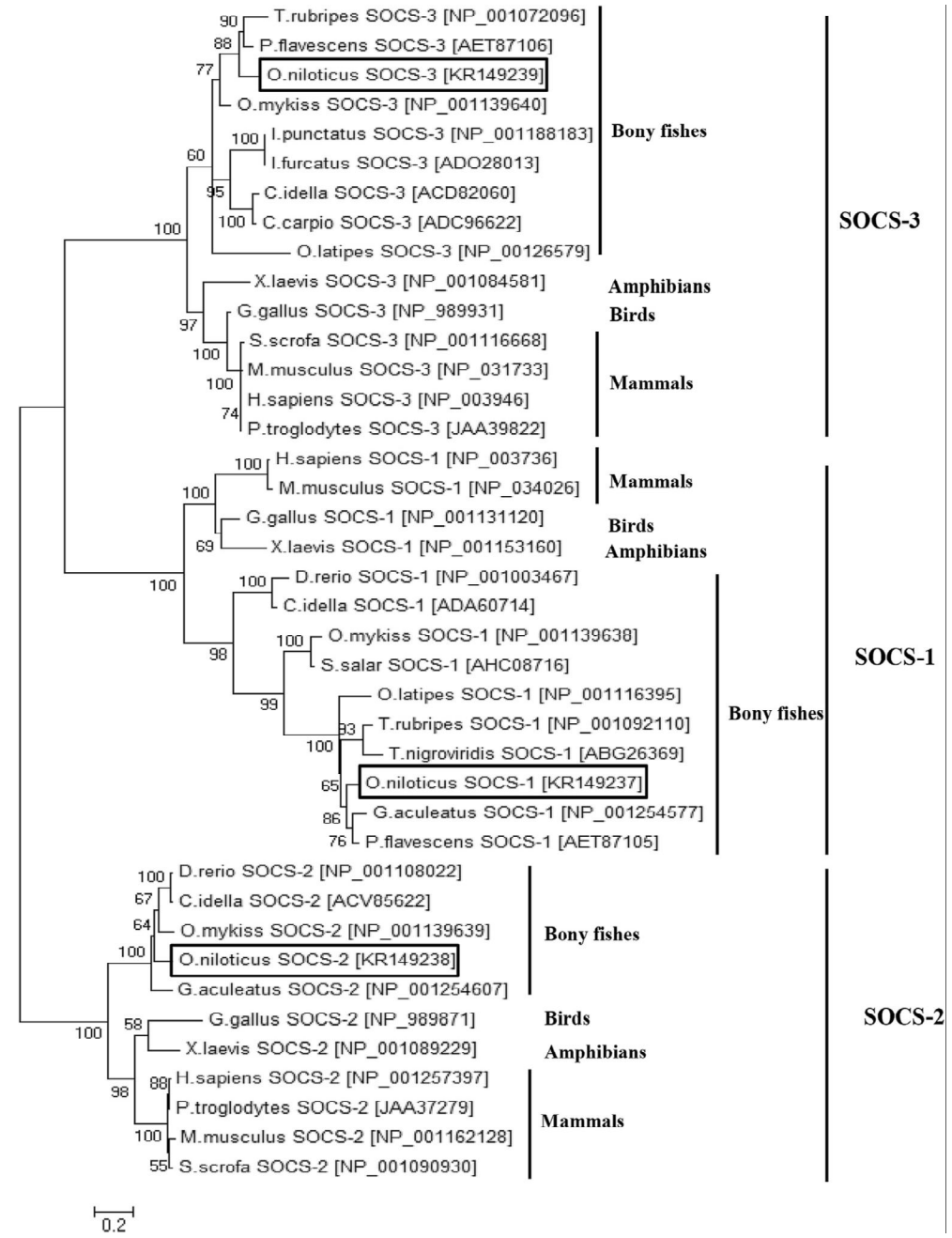


Fig. 3. Phylogenic analysis of the NtSOCS-1, 2 and 3 with full-length proteins SOCS-1, 2 and 3 of others species. Number at each node indicate the bootstrap confidence values of 1000 replicates.

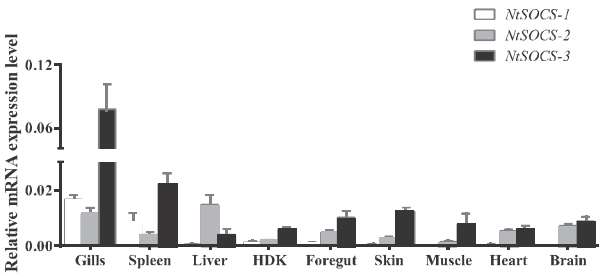


Fig. 4. Tissue distribution of NtSOCS-1, 2 and 3 genes in Nile tilapia. The HDK represents Head kidney. The data were showed as mean ± SEM (n = 6).

on the expression of *SOCS-1* and *SOCS-2* [39]. Furthermore, Nakagawa and his colleagues also found that *SOCS-1* expression was promptly induced upon LPS stimulation in mice macrophages [40]. These results indicate that the response of *SOCS* genes in vertebrates might varies with species, growth stage and stimulant concentration as well as exposure time and cell type examined.

In conclusion, three *SOCS* genes (*SOCS-1*, *2* and *3*), were discovered in Nile tilapia and the identified genes are highly conserved among vertebrates. Tissue-specific expression analysis showed that *NtSOCS-1* and *3* genes are highly expressed in gills and other immune-related tissues, while *NtSOCS-2* gene is highly expressed in liver. Moreover, LPS stimulation induced high expression of *NtSOCS-1* and *3* genes, while it caused low expression

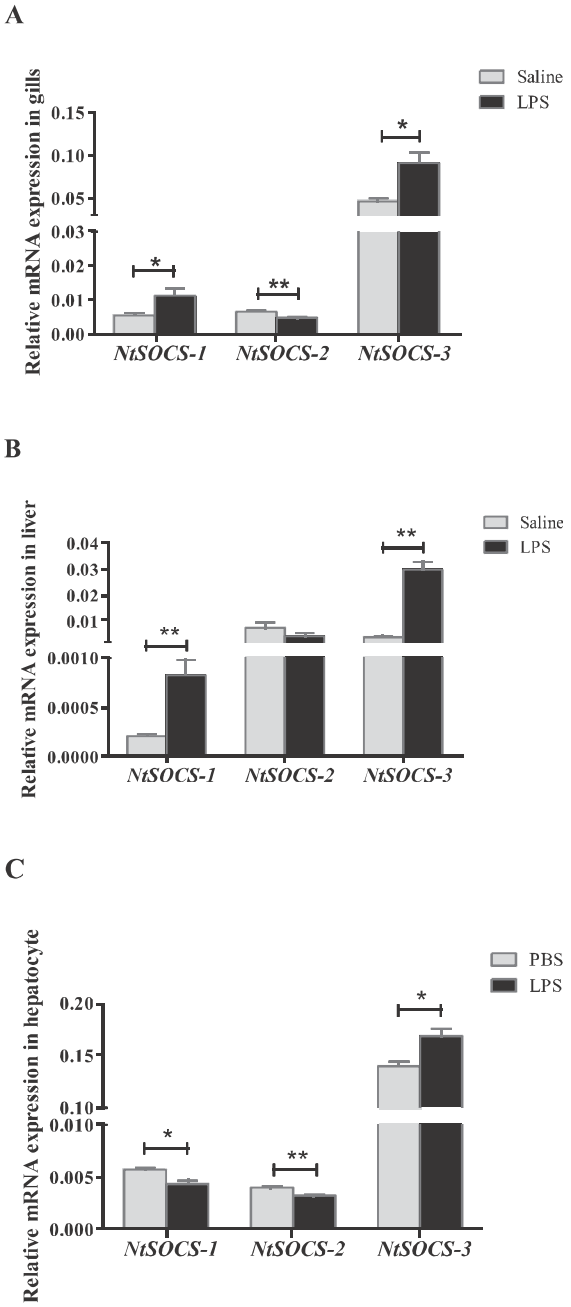


Fig. 5. Transcriptional regulation of NtSOCS-1, 2 and 3 post LPS challenge. (A) The mRNA expression level of NtSOCS-1, 2 and 3 in gills. (B) The mRNA expression level of NtSOCS-1, 2 and 3 in liver. (C) LPS stimulation in Nile tilapia hepatocytes. Hepatocytes were exposed to either control (PBS) or LPS (50 μg/ml). The data were showed as mean ± SEM (n = 3).*P < 0.05 and **P < 0.01.

of *NtSOCS-2* gene. Taken together, our results provided evidence that the *NtSOCS-1* and *NtSOCS-3* genes are more likely to play role(s) in innate immune regulation, while *NtSOCS-2* gene is more involved in metabolic regulation in Nile tilapia.

Acknowledgments

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