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# A novel acute phase reactant, serum amyloid A-like 1, from *Oplegnathus fasciatus*: Genomic and molecular characterization and transcriptional expression analysis

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ABSTRACT

Acute phase response is a significant component of innate immunity, playing a vital role in the signaling processes and elimination of invading pathogens. Acute phase proteins are synthesized in liver and secreted into the blood for transportation to an infection site, where the defense function is exerted. Serum amyloid A (SAA) and C-reactive proteins are the major positive acute phase proteins. In this study, we have identified and characterized a novel SAA related gene from rock bream (*Oplegnathus fasciatus*), designated OfSAAL1. Genomic characterization revealed the presence of 13 exons and 12 introns, similar to SAAL1 in zebrafish. Multiple protein sequence alignment revealed high conservation with other SAAL1 homologues. Phylogenetic analysis showed that OfSAAL1 clustered with another fish homologue, and pairwise alignment revealed highest identity and similarity at the amino acid level with zebrafish SAAL1. Promoter region analysis revealed the presence of immunologically significant transcription factor binding sites. Tissue distribution profiling to indicate physiological relevance showed the highest levels occur in blood, followed by liver, suggesting a positive immune role in rock bream. Transcriptional analysis by reverse transcription polymerase chain reaction to understand OfSAAL1 responsiveness to immune challenge with poly I:C, *Edwardsiella tarda*, *Streptococcus iniae* and rock bream iridovirus, revealed a significant level of elevation from 12 h to 48 h post-infection in blood, spleen, head kidney, and liver. To our knowledge, OfSAAL1 is the first characterized SAAL1 homologue from teleosts. We anticipate that its identification will prove inspiring for further studies of SAAL1 homologues as biomarkers of the acute phase response.


## 1. Introduction

The immune system in fish is comprised of two functionally distinct yet interacting arms, namely innate and adaptive immunity. Innate immunity is made up of cellular and humoral components. The cellular component involves physical barriers, such as mucus, skin and gills, and is capable of preventing infectious pathogens from entering the body. In addition, immunomodulatory cells, such as monocytes, macrophages and non-specific cytotoxic cells, come into play when a pathogen breaches the physical barriers and enters the body. Humoral components, on the other hand, include proteins and glycoproteins, such as proteases, complement factors, lectins, cytokines, chemokines and antibacterial peptides, which are capable of annulling or arresting the growth and development of infectious microorganisms (Medzhitov and Janeway, 2000). As such, the germ line-encoded innate immune system is the immediate response to pathogen invasion preceding the adaptive response and is responsible for the activation and determination of downstream adaptive responses that are essential for maintaining homeostasis (Fearon and Locksley, 1996; Medzhitov and Janeway, 1997). Therefore, the two arms of immunity interact with one another to enhance the organism's defense mechanisms and promote their survival (Tort et al., 2003; Schenten and Medzhitov,

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2011). Fish, like other higher-order organisms, possess adaptive immunity molecules, including immunoglobulins, T cell receptors, cytokines, and major histocompatibility complex, yet they rely extensively on innate immunity, specifically the pathogen recognition receptor (PRR) molecules, Toll-like receptors (TLRs), β-1,3 glucan recognition protein, RIG-I-like receptors, NOD-like receptors, and C-type lectin receptors (Cuesta et al., 2006; Fujiki et al., 2003; Grimholt and Lie, 1998; Nakanishi et al., 2002; Nam et al., 2003; Takeuchi and Akira, 2010; Warr, 1997).

Acute phase response (APR), a part of innate immunity, comprises a series of systemic changes to counter the homeostasis imbalance caused by injury or infection (Gruys et al., 2005a). During an acute phase reaction, a variety of plasma proteins, known as acute phase proteins (APPs), are synthesized in liver; this immediate response is accompanied by an increase in the plasma concentration of APPs, such as C-reactive protein (CRP), serum amyloid A (SAA), complement factors, fibrinogen, mannose binding proteins, and ferritin, by as much as 1000-fold. Acute phase proteins may elicit positive or negative effects on the APR, with increases or decreases in serum levels during infection or injury corresponding respectively. For example, SAA is a positive acute phase protein, whose level increases during inflammation (Uhlar and Whitehead, 1999). Inflammation occurs when the immune system sends signaling molecules to recruit white blood cells to the site of infection or injury to fight against invading pathogens and aid in tissue repair. The inflammation process and duration is carefully regulated so as to prevent damage to healthy cells and tissues. SAA, as an endogenous ligand, is also known to stimulate cytokine expression, in particular that of the interleukin (IL)-23 (He et al., 2006), an apolipoprotein known to play a significant role in cholesterol transport during inflammation. The serum amyloid family includes constitutive (C-SAAs) and inducible acute phase SAAs (A-SAAs). These proteins are widely distributed among evolutionarily diverse organisms, and oftentimes several isoforms are present in a particular species. For example, four different forms of serum amyloid A have been identified in humans (SAA1–4), five in mouse, three each in dog, mink, horse and rabbit, and two in hamster (Uhlar and Whitehead, 1999).

The increase in global population has highlighted the aquaculture industry as a major food source and important economic factor. Research attention has focused on the health and productivity of aquaculture species, in order to combat the enormous setbacks caused by pathogenic infections and environmental hazards. To this end, it is essential to gain a detailed and comprehensive understanding of the immune mechanisms that fight disease and maintain homeostasis. In fish, the acute phase response plays a vital role in defense against infection, and several of the APPs characterized in mammals, including SAA, have been also been identified in various fish species (Bayne and Gerwick, 2001). Like other APPs, precise regulation of SAA expression and activity is necessary for proper immune function and normal physiologic processes. As a precursor of amyloid A, increased expression or accumulation of SAA due to chronic inflammation can lead to amyloidosis, which is characterized by extracellular, fibrillar deposits of amyloid A protein (Pepys, 2006). Earlier studies of human amyloid A-related diseases, such as Alzheimer's and prion diseases, have correlated consumption of animal-derived foods containing amyloid aggregates with disease onset and severity. These findings have raised significant alarm of the potential risks to human health posed by consumption of fish skeletal muscle, which contains amyloid aggregates and amyloid-enhancing factor (Cui et al., 2008; Gruys et al., 2005c; Lundmark et al., 2002). Hence, SAA transcript and/or amyloid AA could be useful biomarkers for appraising fish quality and safety as a human food source.

Considering the above-mentioned features of SAA and biomarker potential we designed an investigation of the economically important rock bream (*Oplegnathus fasciatus*) genome and transcriptome to search for a probable acute phase protein. We identified a serum amyloid A-like 1, designated as OfSAAL1. While the mammalian SAA1 isoform has been previously characterized in cheetah (Zhang et al., 2008), transcriptional elevation and regulation of other SAA family members have been reported in various teleosts upon a variety of immune and chemical challenges, including *Aeromonas salmonicida* and *Staphylococcus aureus* infection in zebrafish (*Danio rerio*) (Lin et al., 2007), *Flavobacterium psychrophilum* in rainbow trout (*Oncorhynchus mykiss*) (Villarroel et al., 2009), *Lepeophtheirus salmonis* in Atlantic salmon (*Salmo salar*) (Tadiso et al., 2011), and sodium alginate, scleroglucan (Fujiki et al., 2000), *Ichthyophthirius multifiliis* infection (Gonzalez et al., 2007), *Trypanoplasma borreli* infection and tissue injury in common carp (*Cyprinus carpio*) (Fujiki et al., 2001; Huising et al., 2003; Saeij et al., 2003). Among teleosts, serum amyloid A-like 1 has been identified from *D. rerio* and *O. fasciatus*. Since the function of SAAL1 from *D. rerio* is yet to be studied, SAAL1 from *O. fasciatus* could be the first serum amyloid A-like 1 gene characterized from teleosts.

## 2. Materials and methods

### 2.1. Rock bream cDNA library construction and isolation of OfSAAL1 cDNA

We have constructed a rock bream cDNA sequence database by the GS-FLX™ genome sequencing technique (Droege and Hill, 2008). Animal rearing, multi-tissue collection, and cDNA synthesis were performed as previously described (Umasuthan et al., 2011). To account for over-representation by the most commonly expressed transcripts, synthesized cDNA was normalized with the TRIMMER-DIRECT cDNA normalization kit (Evrogen, Russia). A cDNA GS-FLX shotgun library was created from the sequencing data obtained by using the GS-FLX titanium system (DNA Link, Republic of Korea). A single putative cDNA, homologous to known serum amyloid A-like 1, was identified by homology screening using the basic local alignment sequencing tool (BLAST; http://www.blast.ncbi.nlm.nih.gov/Blast), and was designated as OfSAAL1.

### 2.2. BAC library construction, screening, and genomic characterization

Rock bream were obtained from the Jeju Special Self-Governing Province Ocean and Fisheries Research Institute (Jeju, Republic of Korea). Blood was harvested aseptically from the caudal fin using a sterile 1 mL syringe with 22 gauge needle, and a BAC library was constructed from the isolated blood cells (Lucigen Corp., USA). Briefly, genomic DNA obtained from blood cells was randomly sheared and the blunt ends of large inserts (>100 kb) were ligated to obtain an unbiased, full coverage library. Around 92160 clones, possessing an average insert size of 110 kb, were arrayed in 240 microtiter plates with 384 wells. BAC clones inoculated into the 384-deep well plates were grown individually.

Aliquots of the grown cultures were pooled with other clones from the same plate, row or column pools for DNA preparation. Then, they were further combined to form Super Pools ($n = 20$). A two-step polymerase chain reaction (PCR) screening procedure with gene-specific primers followed to identify the Super Pools and the individual plate, row, and columns. Each Super Pool had a corresponding 96-well plate containing its Plate-pools, Row-pools, and Column pools (P-R-C pools). The Super Pools were screened during the first round of PCR, and plates, rows, and columns were screened during the second round of PCR to determine the exact well containing the clone of interest. Finally, the identi-