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Abundant members of Scavenger receptors family and their identification, characterization and expression against *Vibrio alginolyticus* infection in juvenile *Larimichthys crocea*

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ABSTRACT

Scavenger receptors (SRs) are crucial pattern recognition receptors (PRRs) to defense pathogen infection in fish innate immunity. In this paper, some members in SRs family of *Larimichthys crocea* were identified, including eight genes in the class A, B, D and F families. (G + C) % of all SRs members held 51% ~ 59%, and these genes were no obvious codon bias by analyzing the distribution of A-, T-, G- and C-ended codons. The order of Enc for all SRs members by sequencing was *LycCD68* > *LycSCARA5* > *LycSCARB1* > *LycCD163* > *LycMARCO* > *LycSREC1* > *LycSCARA3* > *LycSREC2*. Moreover, different lengths and numbers of exons and introns led to the diverse mRNAs and respective functional domains or motifs, for example, an optional cysteine-rich (SRCR) domain in *LycMARCO* and *LycSCARA5*, an epidermal growth factor (EGF) and EGF-like domain in *LycSREC1* and *LycSREC2*. The subcellular localization demonstrated SRs members mainly located in plasma membrane or extracellular matrix. Further, all of the SRs members in *L. crocea* were almost low expressed in heart, gill and intestine, whereas high in spleen and liver. After stimulation by *Vibrio alginolyticus*, the class A and F families were induced significantly, but the class B and D families expressed less even none after pathogenic infection. All the findings would pave the way to understand not only the evolution of the SR-mediated immune response, but also the complexity of fish immunity.

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1. Introduction

Fish, which is regarded as a potential and crucial model in comparative immunology studies due to the classification and the early vertebrate evolution [1], provides the crucial resource of natural proteins for human beings due to its fine palatability and high nutritional quality, meanwhile its farming in the marine environment has brought high income for individuals as well as the whole country in modern society. However, the increasingly serious diseases breaking out in the fish farming every year all over the world, contribute to the tremendous economic losses. The pathogenic threats including bacteria [2,3], microorganisms [4] and viruses [5,6]. Bacterial infections are generally considered as the main reasons for the frequent outbreak and high mortality in large

aquatic farms and cultural system with high density [7,8]. *Vibrio* genus are the common bacterial species in marine system, and some members of them are pathogenic for the vertebrate and invertebrate animals [9], even human beings, such as *Vibrio parahaemolyticus*, *Vibrio Harveyi*, *Vibrio vulnificus*, and *Vibrio alginolyticus* [10]. Many previous studies [11–13] have demonstrated *V. alginolyticus* is a motile gram negative bacteria challenging fish, shellfish, mammal and human being [14,15]. The innate immune response (non-specific immunity) and adaptive immune response (specific immunity) [16,17] are two sorts of immune systems for defending pathogen infection in fish. Although the immunity of fish is generally poorer than mammals, the innate immune response is necessary mechanism for fish to defend pathogenic threats from survival and aquatic environment.

Larimichthys crocea (large yellow croaker), marine teleost [18], belongs to order Perciformes, family Sciaenidae, genus *Larimichthys*, which is cultured widely in the eastern and southern parts of China sea and had high production from 1960s [19]. After several years of prosperity, the production is diminishing for genetic disease, overfishing, high cultural density and mortality partly

Abbreviations

Ac-LDL	acetylated low-density lipoprotein
AK7	Adenylate kinase 7
APC	antigen-presenting cells
ARV1	Protein ARV1
Axis	the principal axis of principle component analysis
BAZ1A	Bromodomain adjacent to zinc finger domain protein 1A
Bola2	BolA-like protein 2
CAI	codon adaptation index
CAPN9	Calpain-9
CBI	codon bias index
Clusterin	Coturnix coturnix japonica
CRP	C-reactive protein
CUP	codon usage pattern
DAMPs	damage associated molecular patterns
DC	activate the dendritic cells
ECE2	Endothelin-converting enzyme 2
EGF	Epidermal growth factor
EIF4H	Eukaryotic translation initiation factor 4H
ENc	effective number of codons
ESCO2	N-acetyltransferase esco 2
Exoc8	Exocyst complex component 8
Fop	frequency of optimal codons
ING2	Inhibitor of growth protein 2
JNK	c-Jun N-terminal kinase
MRs	mannose receptors
MS4A4A	Membrane-spanning 4-domains subfamily A member 4A
NF-κB	transcription factor κB

one-way ANOVA	one-way analysis of variance
ORF	open reading frame
Ox-LDL	oxidized low-density lipoprotein
PAMPs	pathogen-associated molecular patterns
PBK	Lymphokine-activated killer T-cell-originated protein kinase homolog
PRODH	Proline dehydrogenase 1
PRPF8	Pre-mRNA-processing -splicing factor 8
PRRs	pattern recognition receptors
qRT-PCR	quantitative real-time PCR
Rffl	E3 ubiquitin-protein ligase rififylin
RILPL1	RILP-like protein 1
RSCU	relative synonymous codon usage
Sarm1	Sterile alpha and TIR motif-containing protein 1
SCARAs	the class A of Scavenger receptors
SCARBs	the class B of Scavenger receptors
SCARDs	the class D of Scavenger receptors
SCAREs	the class E of Scavenger receptors
SCARFs	the class F of Scavenger receptors
Sepm	Selenoprotein M
Slc46a1	Proton-coupled folate transporter
Slx1a	Structure-specific endonuclease subunit slx1
SRs	Scavenger receptors
TLRs	Toll-like receptors
Tm2d1	TM2 domain-containing protein
TNFR	tumor necrosis factor receptor
TRAF2	major tumor necrosis factor receptor 2
TRAFs	tumor necrosis factor receptor-associated factors
VLDL	very Low Density Lipoprotein
VTN	Vitronectin
ZFP106	Zinc finger protein 106

caused by pathogens (especially *V. alginolyticus*), though artificial marine culture have made since 1986. The immunity of *L. crocea* was very important for defending pathogenic threats, but its adaptive immunity of was poorer than innate immunity because some important genes linked with adaptive immunity (such as CD4, CD8) were partly incomplete [20]. Generally, the innate immune system of fish is divided into three forms: physical barriers, cellular and humoral components [16]. Physical barriers, also named epithelial barriers or epidermal mucus, provide the first line to defense against pathogens. Cellular and humoral components are considerably complicated, including non-specific cytotoxic cells [21], Phagocytic cells, Neutrophils cells, Lytic enzymes, complement pathways [22], C-reactive protein (CRP) [23], Chemokines [24], pattern recognition receptors (PRRs) [25], and antibacterial peptides [26], etc. Among them, more and more literature focus on PRRs, which play an extraordinary role in innate immune system. These PRRs rely on recognizing pathogen-associated molecular patterns (PAMPs) [27] and damaging associated molecular patterns (DAMPs) [28] to eliminate the invading germs [16,29]. Until now, PRRs are functionally divided into three types: signaling PRRs, soluble bridging PRRs and endocytic PRRs. Generally, signaling PRRs are responsible for cell activation, as well soluble bridging PRRs and endocytic PRRs are in charge of microbe internalization by phagocytes [30,31]. The endocytic PRRs further are divided into three subtypes, Mannose receptors (MRs) [32], Scavenger receptors (SRs) [18,33] and C-type lectin receptors [34] for microbe internalization.

In the 1970s, the first member of SRs was discovered as the important pattern recognition receptors in the innate immune system. From then on, more and more members in SRs family had been found and now have categorized into eight classes (class A-H) according to their structural characteristics [35]. Now almost all the members of SRs have been studied in different species and every family has their special function during participating in the different immune process against bacteria [18] or virus [36]. They mainly recognized and bound some relevant ligands other than native LDL to eliminate pathogen through three different pathways, eg. the acetylated low-density lipoprotein (Ac-LDL), oxidized low-density lipoprotein (Ox-LDL) and very Low Density Lipoprotein (VLDL) [37]. In many cases, SRs-dependent method always displays a faster or more robust process than the SRs-independent response, such as dsRNA uptake via SR-As [38]. SRs associating with LDL or other modified forms have large influences on treating viral infection or diseases. The scientists have adopted some antibody against SRs as a treatment strategy, for example, Meuleman et al. [36] artificially generated a human monoclonal antibody against SCARB1 (the class B type I of SRs) to efficiently prevent infection of Huh-7.5 hepatoma cells and primary hepatocytes by cell-culture-derived HCV. Cellular immune process is a crucial stage in cleaning pathogenic bacteria, fortunately, SRs could transfer the signals to activate some cells [16], such as antigen-presenting cells (APC) and the dendritic cells (DC). On the other hand, SRs play the key roles in transporting signals to other cellular surface receptors or other signaling PRRs, for instance, the class E and F of SRs (SCAREs and SCARFs) were relevant to HSP70 or HSP90 [39,40] and CD36 (a member of the class B of SRs, SCARBs) to activate some Toll receptors (TLR4/TLR6) as co-receptors [41]. Due to the significant roles in the immune process, there are increasing studies concentrating on interaction and regulation of SRs in some marine fish (eg. Japanese sea bass (*Lateolabrax japonicus*) [42], Atlantic cod (*Gadus*