Fish & Shellfish Immunology 49 (2016) 110–121

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Septin genes in channel catfish (*Ictalurus punctatus*) and their involvement in disease defense responses

Qiang Fu [a, b], Yun Li [b], Yujia Yang [b], Chao Li [c], Jun Yao [b], Qifan Zeng [b], Zhenkui Qin [b], Shikai Liu [b], Daoji Li [a], Zhanjiang Liu [b, *]

[a] State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai, 200062, China
[b] The Fish Molecular Genetics and Biotechnology Laboratory, Aquatic Genomics Unit, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, AL, 36849, USA
[c] Marine Science and Engineering College, Qingdao Agricultural University, Qingdao, 266109, China

ARTICLE INFO

Article history:
Received 30 September 2015
Received in revised form
9 December 2015
Accepted 12 December 2015
Available online 15 December 2015

Keywords:
Septin
Catfish
Infection
Innate immunity
Expression

ABSTRACT

Septins are an evolutionarily conserved family of GTP-binding proteins. They are involved in diverse processes including cytokinesis, apoptosis, infection, neurodegeneration and neoplasia. In this study, through thorough data mining of existed channel catfish genomic resources, we identified a complete set of 15 septin genes. Septins were classified into four subgroups according to phylogenetic analysis. Extensive comparative genomic analysis, including domain and syntenic analysis, supported their annotation and orthologies. The expression patterns of septins in channel catfish were examined in healthy tissues and after infection with two major bacterial pathogens, *Edwardsiella ictaluri* and *Flavobacterium columnare*. In healthy channel catfish, most septin genes were ubiquitously expressed and presented diversity patterns in various tissues, especially mucosal tissues, proposing the significant roles septin genes may play in maintaining homeostasis and host immune response activities. After bacterial infections, most septin genes were regulated, but opposite direction in expression profiles were found with the two bacterial pathogens: the differentially expressed septin genes were down-regulated in the intestine after *E. ictaluri* infection while generally up-regulated in the gill after *F. columnare* infection, suggesting a pathogen-specific and tissue-specific pattern of regulation. Taken together, these results suggested that septin genes may play complex and important roles in the host immune responses to bacterial pathogens in channel catfish.

1. Introduction

Septins are cytoskeletal GTP-binding proteins that work as dynamic regulatable scaffolds for recruitment of other proteins [1,2]. The septin gene family members possess a conserved GTP-binding domain and fall into the large superclass of P-loop GTPases [3]. All septins have a P-loop containing a G1 motif (GxxGxGKST), G3 motif (DxxG) and G4 motif (xKxD) [1,4]. Within the GTPase domain of septins, a polybasic region is located in the N-terminal, which has been shown to bind phosphoinositides [5], and a 53-amino-acid element is located in the C-terminal [6]. While all septins have a GTP-binding domain, the length and constituents of N and C termini vary among septins. Noteworthy is the coiled-coil domain in the C-terminal of many septins, characteristic of 50–100 residues in length, which was postulated to be a protein–protein interaction domain [5]. In addition, septins typically assemble into oligomeric complexes and highly ordered polymers to fulfill their functions [7,8]. Septins were first discovered in yeast [9,10], and later have been found from all animals and fungi studied to date, but are absent in plants [3,11,12]. The numbers of septin genes vary widely among organisms; for instance, *Caenorhabditis elegans* has two genes, *Saccharomyces cerevisiae* has seven, zebrafish has 15, frog and chicken each has 11, mouse has 13, and humans have 14 genes [12,13]. The gene number difference was caused by various levels of duplication in various species, which also leads to the functional complexities of septin genes in vertebrates [14].

In mammals, septins are localized not only to the plasma membrane, but also throughout the cytoplasm together with the microtubule and actin cytoskeletons [2]. They are involved in cytokinesis [15,16] and DNA damage-related checkpoint response

* Corresponding author.
E-mail address: liuzhan@auburn.edu (Z. Liu).

http://dx.doi.org/10.1016/j.fsi.2015.12.022
1050-4648/

[17], as initially reported in yeast. Mammalian septins are progressively recognized for other functions including roles in cell membrane dynamics [18,19], vesicle trafficking [18,19], and interaction with microtubules and actins [20,21]. Previous studies revealed that several human diseases were associated with septin overexpression or mutation [1,22,23]. In the past decade, septin genes, as key participants and regulators in actin-based innate immune processes such as autophagy, inflammation and exocytosis, attracted much greater attention, and several studies were conducted in human, mouse, and horse [24–27]. In spite of extensive studies in mammal species, nothing is known about fish septins. The objective of this study was to characterize septin genes from catfish.

Catfish is the leading species in the United States [28], but its sustainable production is threatened by frequent and severe disease outbreaks. In particular, the enteric septicemia of catfish (ESC) and columnaris disease, caused by *Edwardsiella ictaluri* and *Flavobacterium columnare* respectively [29,30], are the two major bacterial diseases that cause huge economic losses to the catfish industry. For the purpose of generating effective strategies to better manage fish diseases, it is of great significance to understand the immune-related functional genes and their expression in the process of bacterial infection. In channel catfish, many important innate immune genes have been characterized including pathogen recognition receptors [31–37], chemokines [38,39], antimicrobial peptides [40–44], lysozymes [45], lectins [46–48], NOS genes [49], protease inhibitors [50,51] and complement regulatory protein genes [52]. Here we report the identification and characterization of a complete set of 15 septin genes of channel catfish, their expression patterns in healthy tissues and after bacterial infections.

2. Materials and methods

2.1. Gene identification and sequence analysis

In order to identify septin genes of channel catfish, TBLASTN was conducted firstly against the catfish transcriptome database [53–55], using human and zebrafish septin protein sequences retrieved from the NCBI (http://www.ncbi.nlm.nih.gov/) and Ensembl (http://www.ensembl.org) as queries, with a cutoff E-value of $1e^{-5}$. After that, ClustalW2 (https://www.ebi.ac.uk/Tools/msa/clustalw2/) was used to generate an initial septin candidate sequences pool in channel catfish. Then, ORF (opening reading frames) finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) was used to predict coding sequence, which were further validated by BLASTP against NCBI non-redundant (nr) protein database. For the purpose of verifying copy number and the sequence accuracy of septin genes in channel catfish, BLASTN was performed against channel catfish whole genome database (unpublished), using the initial pool of septin transcriptome sequences as queries, with a cutoff E-value of $1e^{-10}$. Fgenesh program of Molquest software (Softberry Int.) was used to predict the genes from retrieved genomic scaffold sequences [56]. The simple modular architecture research tool (SMART http://smart.embl-heidelberg.de) was used to identify the characteristic functional septins domains and further endorsed by conserved domain predicted through BLASTP. The amino acid sequences acquired above were used in the phylogenetic analysis.

2.2. Phylogenetic analysis

Phylogenetic analysis was conducted using septin protein sequences from various organisms along evolutionary spectrum including human (*Homo sapiens*), mouse (*Mus musculus*), cattle (*Bos taurus*), chicken (*Gallus gallus*), frog (*Xenopus laevis*) and several fish species with inclusion of channel catfish (*Ictalurus punctatus*), zebrafish (*Danio rerio*), tilapia (*Oreochromis niloticus*), guppy (*Poecilia reticulata*), tongue sole (*Cynoglossus semilaevis*), fugu (*Takifugu rubripes*), medaka (*Oryzias latipes*) and et al. Multiple amino acid sequences were aligned by MUSCLE with default parameters [57]. The phylogenetic analysis was conducted using MEGA 6 [58] with the maximum likelihood method. Based on the alignment results, JTT (Jones-Taylor-Thornton) + I (invariant sites) + G (gamma distribution for modeling rate heterogeneity) model was selected [59]. The bootstrapping with 1000 replications was performed to test the phylogenetic tree and gaps were removed by complete deletion.

2.3. Syntenic analysis

To better support the orthologies for the annotation, combined with the phylogenetic analysis, synteny analysis was conducted on the basis of comparing the flanking genes of septins in channel catfish with zebrafish and human. Briefly, to obtain the genome scaffolds containing channel catfish septin genes, the catfish septin coding sequences were used as queries to search against the catfish genome sequence database (unpublished) using BLASTN with a cutoff E-value of $1e^{-10}$. The neighboring genes of catfish septins were predicted from the catfish genomic scaffolds using Fgenesh program Molquest software (Softberry Int.) [56] and validated by running BLASTP against NCBI non-redundant database. The genomic pattern of septin genes and their neighboring genes in human and zebrafish were obtained from NCBI, Ensembl and Genomicus [60] database. For the nomenclatures of channel catfish septins, we named them after zebrafish whenever possible, based on orthologues which determined by phylogenetic and syntenic analysis.

2.4. Expression analysis of septin genes in healthy channel catfish tissues

Quantitative real-time PCR was performed to determine the expression patterns of septin genes in channel catfish. All procedures involved in handing and treatment of fish during this study were approved by the Auburn University Institutional Animal Care and Use Committee (AU-IACUC) prior to the initiation of the study. After treated with MS-222 (200 mg/L), eight tissues including skin, spleen, head kidney, liver, gill, trunk kidney, intestine and muscle from nine healthy catfish (Marion strain) (3 replicates of 3 fish) were collected. All samples were flash frozen in liquid nitrogen and stored at −80 °C ultra-low freezer until RNA extraction.

Total RNA from 8 tissues were extracted using RNeasy Plus Universal Mini kit (Qiagen, USA) following the manufacturer's protocol. RNA concentration and integrity were measured using a Nanodrop 2000 Spectrophotometer (NanoDrop Technologies, USA). A uniform quantity of DNA-free RNA was used to synthesize first-strand cDNA using the iScript™ cDNA Synthesis Kit (Bio-Rad, USA) following manufacturer's protocol. All the cDNA products were diluted to 250 ng/μL for real-time PCR reaction.

Quantitative real-time PCR was performed using SYBR Green PCR Master Mix on a CFX96 real-time PCR detection system (Bio-Rad, USA) to determine mRNA expression of septin genes in healthy channel catfish tissues. Gene specific primers were designed using Primer3 software (Table 1). The channel catfish 18s rRNA gene was used as reference gene because this gene has been demonstrated to be stably expressed under various physiological and environmental conditions including various stresses [61]. In addition, this gene has been used as an internal control for RT-PCR analysis in various conditions including various tissues and after infection with Edwardsiella ictaluri and Flavobacterium columnare [45,49,50,53,55]. Three biological replicate RNA samples of