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Expression analysis of immune-relevant genes in the spleen of large yellow croaker (*Pseudosciaena crocea*) stimulated with poly I:C

Wenbiao Zheng [a,b], Guozhu Liu [b], Jingqun Ao [b], Xinhua Chen [b,*]

[a] *Department of Biology, School of Life Science, Xiamen University, Xiamen 361005, PR China*
[b] *Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, Daxue Road 178, Xiamen, Fujian 361005, PR China*

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Abstract

A SMART cDNA library from spleen of large yellow croaker (*Pseudosciaena crocea*) stimulated by poly I:C was constructed. A total of 1039 clones from the library were single-pass sequenced and compared with known sequences in the GenBank database. Of those expressed sequence tags (ESTs), 607 were identified as orthologs of known genes in the GenBank databases by Blast X search. Four hundred and thirty-two did not show significant homology with any known sequences in the public databases. These identified ESTs represented at least 252 different genes, which were categorised into nine groups according to their function. Of the identified genes, 159 genes (63.1%) shared homology with fish genes while 93 (36.9%) showed the highest homology to the genes from other species. Forty-six genes were identified to be involved in immune functions, including complement system components, immunoglobulins, antigen processing and presentation proteins, interferon system proteins, cytokines, and some innate defence molecules. The most frequently occurring genes in this spleen cDNA library were hepcidin precursors represented by 46 ESTs, which were divided into five groups based on their putative amino acid sequences. The expression analysis of selected genes during polyI:C induction was performed by reverse transcription-PCR (RT-PCR), including Mx protein, beta2-microglobulin (β_2m), CD2 binding protein 1(CD2BP1), placenta-specific 8 genes, MHC class II associated invariant chain (li) and cytochrome b-245 alpha peptide (Cyba). The results revealed that expression levels of Mx protein, β_2m, placenta-specific 8 genes, and Cyba were significantly upregulated at 30 h after induction with poly I:C, and the CD2BP1 expression was also induced by polyI:C, suggesting that these genes may be involved in an immune response induced by poly I:C in large yellow croaker.

Keywords: Large yellow croaker; Expressed sequence tag; Immune-relevant gene; Poly I:C; Antiviral immune response

1. Introduction

Large yellow croaker (*Pseudosciaena crocea*), is a species of jewfish and is found mainly in the coast in the temperate zone. Large yellow croaker is an economically important marine fish species in China, and also represents

* Corresponding author. Tel. +86 592 2195297; fax: +86 592 2085376.
E-mail address: chenxinh@tom.com (X. Chen).

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the largest yield for a single species in Chinese marine net-cage farming. In recent years, with the rapid development of the large yellow croaker culture industry, the infectious diseases caused by viruses, bacteria, and parasites are becoming more and more severe, resulting in great economic losses. At present little is known about the genetic and immunological basis of this fish. This lack of knowledge may represent a major obstacle that hinders the establishment of effective measures in disease control and genetic improvement.

The sequencing of the whole genome of zebrafish and *fugu* has recently been completed, which provides a rich resource for identifying immune-relevant genes in these species. In the last few years, many important immunity genes such as IL-10 [1], CD4 [2], Toll-like receptors [3], and interferon [4] and interferon-gamma [5], have been identified in these model fish by analysing their genome databases. But compared to their mammalian counterparts, a large number of immune-relevant genes remain to be identified. As a compensation of genomic resources, EST analysis is a powerful approach for identifying new genes and profiling gene expression in tissues and cells. In regard to this, EST resources of these model fish and other fish with great economical interests such as Japanese flounder [6,7], Atlantic salmon [8], rainbow trout [9], and red seabream [10] have grown quickly in recent years. Many immune-relevant genes have been successfully identified in bony fish by EST analysis. Discovery of these immune-relevant genes will be helpful not only to study the molecular mechanisms of fish immunity, but also to develop effective ways to enhance resistance of cultured fish to diseases.

At present, little is known regarding the molecular immunology of the large yellow croaker. The aim of the present study is to increase genomic resources in cultured fish and analyse the expressed genes in the immune system during virus infection. To this end, we constructed a cDNA library from mRNA isolated from the spleens of large yellow croaker stimulated with a viral micmic, polyinosinic polycytidynic acid (poly I:C). A total of 1039 ESTs from the library were sequenced and compared with sequences in GenBank, and 252 genes were identified by EST analysis, of which 46 genes may be implicated in the immune functions. And the expression analysis of interest genes during poly I:C induction was performed by RT-PCR.

2. Materials and methods

2.1. Fish

Large yellow croaker (mean weight 200 g) were purchased from mari-culture farm at Tongan, Xiamen, China. After 1 day of acclimatising in an aerated seawater tank, fish were injected with 0.5 mg of poly I:C each. A spleen sample was collected from three fish at various time points after induction (0 h, 30 h, 72 h), and frozen immediately in liquid-nitrogen until RNA extraction.

2.2. cDNA library construction

Poly $(A)^+$ RNA was directly isolated from a spleen sampled at 30 h post-induction using a PolyATtract® mRNA isolation kit (Promega, USA) according to the manufacturer's instructions. The obtained mRNA was used to construct the cDNA library with a SMART cDNA construction kit (Clontech, USA) with a slight modification. Briefly, cDNA synthesis, *sfi*I digestion and size fractionation were performed according to the manufacturer's instructions, but the obtained cDNA was cloned into *Sfi*I-linearised pcDNA3.0 vector [11], and transformed to *Escherichia coli* JM109, by electroporation.

2.3. Plasmid preparation and sequencing

The DNAs were extracted using Vitagene 96-easy plasmid Miniprep kit (Vitagene Biochemical Technique). Single-pass sequencing of the 5′-termini of each cDNA clone was conducted in an automated ABI PRISM 3700 sequencer (Perkin-Elmer), using ABI PRISM Big-Dye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) and T7 primer.