Fish & Shellfish Immunology 50 (2016) 127–141

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Full length article

Transcriptome and analysis on the complement and coagulation cascades pathway of large yellow croaker (*Larimichthys crocea*) to ciliate ectoparasite *Cryptocaryon irritans* infection

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ARTICLE INFO

Article history:
Received 17 September 2015
Received in revised form
15 January 2016
Accepted 19 January 2016
Available online 21 January 2016

Keywords:
Larimichthys crocea
Cryptocaryon irritans
Transcriptome
Immune system
Complement and coagulation cascades pathway

ABSTRACT

Large yellow croaker (*Larimichthys crocea*) is one of the most valuable marine fish in southern China. Given to the rapid development of aquaculture industry, the *L. crocea* was subjected to ciliate ectoparasite *Cryptocaryon irritans*. It therefore is indispensable and urgent to understand the mechanism of *L. crocea* host defense against *C. irritans* infection. In the present study, the extensively analysis at the transcriptome level for Cryptocaryoniasis in *L. crocea* was carried out. These results showed that 15,826,911, 16,462,921, and 15,625,433 paired-end clean reads were obtained from three cDNA libraries (A: 0 theronts/fish, B: 12,000 theronts/fish, and C: 24,000 theronts/fish) of the *L. crocea* immune-related tissues by Illumina paired-end sequencing technology. Totally, 30,509 unique transcript fragments (unigenes) were assembled, with an average length of 1715 bp. In B/A, C/A, and C/B pairwise comparison, 972, 900, and 1126 genes showed differential expression respectively. Differently expressed immune-related genes (DEIGs) were scrutinized, in B/A pairwise comparison, 48 genes showed differential expression, including 26 up-regulated genes and 22 down-regulated genes in B; in C/A pairwise comparison, there were 39 DEIGs, including 7 up-regulated genes and 32 down-regulated genes in C; in C/B pairwise comparison, 40 genes showed differential expression, including 11 up-regulated genes and 29 down-regulated genes in C. There were 16 DEIGs enriched KEGG pathways, in which the complement and coagulation cascades pathway was the top most DEIGs enriched pathway (B:A = 42; C:A = 28; C:B = 42). The coagulation and fibrinolytic system was in a highly active state after infected by *C. irritans* with non-lethal concentration; the alternative complement pathway may play an important role in the early stages of *C. irritans* infection. These results demonstrated that low-concentration infection can significantly induce the immunological response in fishes, however, when fishes were in fatal conditions, the immunity was suppressed.

1. Introduction

Cryptocaryon irritans, a species of obligatory ciliate ectoparasite, can infect various species of marine teleost fishes. Large yellow croaker (*Larimichthys crocea*) is an important breeding species and one of the fish species that are most vulnerable to *C. irritans* infection in coastal regions of southeast China [1]. Cryptocaryoniasis occurs in April and May when the water temperature reaches 18°C in major producing areas of *L. crocea*. Especially in high-density culture, *C. irritans* proliferates very fast. Thus the fishes are under continuous attack by myriads of new-born *C. irritans* theronts which can lead to stress response or even fatality.

In order to investigate the pathogenic mechanism of *C. irritans* in fishes and to find out effective control methods, fish–parasite interactions have been intensively investigated [2]. In artificial infection experiments, stress, tissular and organic injury, and

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http://dx.doi.org/10.1016/j.fsi.2016.01.022
1050-4648/

Table 1
Summary of *de novo* assembly of transcriptomic profiles of *L. crocea*.

	Total Length(bp)	No.	Max Length(bp)	Ave Length(bp)	N50	GC %
Contig	115,670,417	262,328	32,977	440.94	704	46.24%
Transcript	135,413,136	172,798	33,141	784	1471	47.30%
unigene	52,328,062	30,509	33,141	1715	2550	49.92%

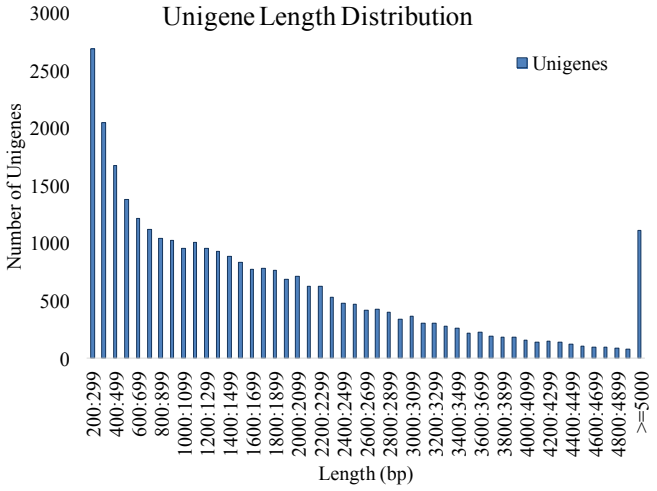

Fig. 1. Transcriptome sequence length distributions of *L.crocea* unigenes. The x-axis indicates unigene size and the y-axis indicates the number of unigenes with different lengths.

immune responses in fishes caused by the infection have been clarified. The results showed that *C. irritans* infection led to elevate plasma COR and GLU contents, accelerate respiratory rate, and inhibit ingestion in marbled rockfish *Sebastiscus marmoratus* [3,4], and gill tissue necrosis and granular hyperplasia in orange-spotted grouper *Epinephelus coioides* [5]. In non-lethal infections, the expression levels of immunity-related genes, e.g. IL-34/MCSF2, MCSFR1/MCSFR2 [6], NCCRP-1 [7], TRAF6 [8], IRAK-4 [9], interleukin-1b (IL-1b), TNF-a, MHC I/II, CCR6 [10], and TGF-b1 [11] significantly changed in fish tissues; antibody titer in fish surface mucus and immunoprotective rate significantly increased; the amount of infecting trophonts decreased [12–15]. Studies on Cryptocaryoniasis in *L. crocea*, demonstrated that *C. irritans* infection caused up - regulated activities or contents of LZM, AKP, IgM, complement C3 and other immunologic factors in fish surface mucus [16]; expression level of Pc-pis [17], Nrdp1 [18] and Interferon-gamma [19] significantly changed. However, the patterns of immunologic responses in infected fishes are barely known currently.

A transcriptome represents all RNA transcripts in cell or tissue and reflects genes expressed in specific tissues in different life stages, physiological states, and environments. Transcriptome studies can holistically exhibit functions and structures of genes and reveal the molecular mechanism of biological process and pathogenesis, thus transcriptomics has been widely applied in fundamental research, clinical diagnosis, drug development, etc. In recent years, RNA-sequencing has become a widely used approach in investigating on fish–parasite interactions, e.g. Atlantic *Salmo salar*, chum *Oncorhynchus keta*, and pink salmon *Oncorhynchus gorbuscha* infected with salmon lice *Lepeophtheirus salmonis* [20], Pacific bluefin tuna *Thunnus orientalis* infected by blood fluke *Cardicola orientalis* and *C. opisthorchis* [21], gilthead sea bream *Sparus aurata* infected with myxosporean *Enteromyxum leei* [22], turbot *Scophthalmus maximus* infected by myxozoan *Enteromyxum scophthalmi* [23], brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* infected by *Tetracapsuloides bryosalmonae* [24]. For studies on Cryptocaryoniasis, Khoo et al. (2012) reported the utilization of cDNA microarray in analyzing stress reactions of sea bass *Lates calcarifer* to *C. irritans* infection which is the only report so far [25]. The understanding of the molecular mechanism against *C. irritans* infection is still deficient.

Although RNA-seq has been used in the studies on immune response of *L. crocea* [26–31], no investigation was conducted regarding transcriptomic changes in immune tissues of *C. irritans* infected *L. crocea*. This study has investigated the changes in the transcripts of infected fishes by RNA-seq and artificial infection of *L. crocea* by *C. irritans* theronts. Results have shown that complement and coagulation cascades pathway is activated, thus analysis on the variation tendency of immune genes related to this pathway has been subsequently conducted, aiming at obtaining more intensive information about the interactions between the parasite and the host.

2. Materials and methods

2.1. Cryptocaryon irritans and experimental fish

C. irritans were derived from naturally infected *L. crocea*, and *L. crocea* (100 ± 10 g) were then used as the model to establish the passage system. Propagation, collection of tomonts and theronts were conducted using a method from Yin et al. (2015) [16]. The animal model *L. crocea* were infected with a non-lethal concentration of theronts (≤10,000 theronts/fish), in 5 L of seawater per fish; 2 h after infection, fresh seawater was added. Three days post infection, the white trophonts on fin, skin and gill of the fish could be seen. Four days post infection, a large number of tomonts were found to adhere to the bottom of aquarium. The fish were then

Table 2
Annotation of unigenes of transcriptomic profiles of *L.* crocea.

Database	Number of annotated unigenes	Percentage of annotated unigenes in NR top hit
Swiss-Prot	26,643	87.33%
eggNOG	29,249	95.87%
GO	20,163	66.09%
KO	10,965	35.94%
KEGG	22,683	74.35%
NR top hit (Total)	30,509	100%