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Cloning, expression and molecular characterization of a *14-3-3* gene from a parasitic ciliate, *Cryptocaryon irritans*

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ABSTRACT

Cryptocaryon irritans is a parasitic ciliate and responsible for cryptocaryosis of ocean teleostean. In this paper, one gene homologous to *14-3-3* was isolated from cDNA library of *C. irritans* trophont/protomont stage and designated as *Ci14-3-3*. The full-length cDNA of the gene was 892 bp with an open reading frame of 744 bp, which encoded a polypeptide of 247 amino acids with a predicted molecular weight of 28.4 kDa. After modification of the non-universal genetic codes, the open reading frame of *Ci14-3-3* was inserted into plasmid pGEX-4T-1, transformed into *Escherichia coli* DH5α strain and then expressed as a glutathione *S* transferase fusion protein (rCi14-3-3). The result of western blot analysis showed that the rCi14-3-3 had antigenicity and the *Ci14-3-3* gene in *C. irritans* was expressed at all stages of life cycle. The endogenous Ci14-3-3 not only distributed in cytoplasm, but also presented on the plasma membrane and the front end of cytostome in newly hatched theronts. However, when theronts were dying the protein appeared as dot-like aggregates around the nucleuses. The murine anti-rCi14-3-3 sera were capable of causing agglutination/immobilization of theronts, suggesting its potential for vaccine development.

1. Introduction

Cryptocaryon irritans is a ciliate that parasitizes tropical and subtropical ocean teleostean (Wright and Colorni, 2002). The parasite whose life cycle includes four stages, theront, trophont, protomont, and tomont, proliferates quickly (Khoo et al., 2012). The theronts mainly invade skin, gills, and eyes of ocean teleostean and in hosts they develop into the parasitic stage (trophonts) which feed on the whole cells and tissue debris of hosts, causing localized infiltration of lymphocytes, focal necrosis and variation of epithelial proliferation (Colorni and Diamant, 1993; Khoo et al., 2012). After leaving hosts, the mature trophonts develop into the protomonts and tomonts and then another life cycle begins (Colorni and Diamant, 1993). This ectoparasitic protozoan can cause cryptocaryosis, an infective and deadly disease of fish, which is known as 'white spot disease' (Colorni and Burgess, 1997). In wild marine fish, cryptocaryosis does not happen seriously (Diggles and Adlard, 1997). However, in recent years, with the rapid development of the intensive aquaculture, cryptocaryosis outbreaks frequently with high mortality, which has been reported in numerous parts of the Asia regions and caused substantial economic losses heavily retarding the development of the mariculture industry (Bondad-Reantaso et al., 2005; Huang et al., 2012; Luo et al., 2008; Seng et al., 2006). To solve the problem, many physical and chemical methods have been used (Kawano and Hirazawa, 2012; Picon-Camacho et al., 2011). But not all of these methods are effective and they have brought some bad influence,

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such as environmental pollution, harm for fish and so on. Therefore, immunoprophylaxis as a potential and safer method is considered to bring the disease under control (Dan et al., 2013; Luo et al., 2007; Misumi et al., 2012). It has become a focus of the study on the etiology of cryptocaryosis in molecular aspects and the development of an effective vaccine (Huang et al., 2012).

The conserved members of 14-3-3 protein family are basically intracellular acidic polypeptides with molecular weights of 28–33 kDa and ubiquitous in all eukaryotic organisms in a given species (Aitken, 2006; Siles-Lucas et al., 2008; van Hemert et al., 2001). 14-3-3 family contains several isoforms which can self-assemble into homo- and hetero-dimers with the capacity to interact with a wide range of functional proteins (14-3-3 target proteins) (Aitken, 2006; Gardino et al., 2006; Morrison, 2009). By means of recognizing and binding to two motifs, RSXpSXP and RXXXpSXP (pS represents phospho-serine) in 14-3-3 target proteins, the 14-3-3 dimers can change the conformation of the proteins and may alter the stability and/or catalytic activity as well as subcellular localization of the proteins (Gardino et al., 2006; Li and Dhaubhadel, 2012; Morrison, 2009; Porter et al., 2006; Rajagopalan et al., 2008; Rezabkova et al., 2012). Hence, 14-3-3 proteins have been identified as adaptor molecules or regulatory proteins modulating protein–protein interactions. To date, these proteins have been found to play a crucial role in a diverse array of essential cellular processes including signal transduction, cell-cycle regulation, cell apoptosis, metabolism, host–pathogen interactions, protein trafficking, stress response and malignant transformation (Clapp et al., 2012; De et al., 2012; Ottmann, 2013; Porter et al., 2006; van Hemert et al., 2001). Because of the multiple functions and characteristics of 14-3-3s, they have been considered to be candidates for diagnosis, treatment and prevention of some diseases (Li et al., 2012; Luo et al., 2009; Schechtman et al., 2001). Currently, 14-3-3s have being deep studied in the fields of plant, animal, and fungal species. Concerning parasites, the information of this protein family like their subcellular distribution and functions is still not very clear.

A full-length cDNA library of *C. irritans* has been constructed in our lab, from which a novel gene homologous to *14-3-3* was isolated and termed as *Ci14-3-3*. In the study, the molecular characterization of *Ci14-3-3* was analyzed to provide basis for further study in pathogen biology of cryptocaryosis and strategy for the disease control.

2. Materials and methods

2.1. Parasite and experimental animals

The parasite, *C. irritans*, was obtained from infected *Pseudosciaena crocea* that were bred in the coastland of Xiapu county, Fujian province, China. Judged from the alignment of the ribosomal DNA internal transcribed spacer sequences, the parasite was consistent with PYH4.12 or Chiayi strain (Diggles and Adlard, 1997; Sun et al., 2006). Then this strain of the parasite was steadily cultured and passaged with *Sebastiscus marmoratus* as a host in aquariums. The parasites of the four stages, trophonts, pretomonts, tomonts and theronts were collected respectively, washed in sterile seawater and stored in no time at −80 °C for further investigation.

2.2. Cloning of Ci14-3-3 gene

The recombinant phages from the cDNA library of *C. irritans* constructed as described previously (Huang et al., 2012) were mixed with *Escherichia coli* X-1 blue strain for plaque lifting. Then the plaques formed were picked one by one. The phages from each plaque were respectively transduced into *E. coli* BM25.8 strain with Cre recombinase expression so as to transfer the recombinant phage DNA λTriple into plasmid. The bacterial monoclones were picked randomly and verified to be recombinant by PCR. Then the inserted genes in the plasmids prepared from the bacteria were sequenced in Invitrogen China Limited. After sequence analysis, a gene with high homologous to *14-3-3* was obtained and designated as *Ci14-3-3*.

2.3. Modification and subcloning of the Ci14-3-3 gene

In order to express the open reading frame (ORF) of *Ci14-3-3* gene in bacteria, TAA and TAG in the ORF, which are two genetic codons for the glutamine of ciliates but the termination codons universally, were modified into CAA and CAG respectively, two universal genetic codons for glutamine (Salim et al., 2008; Wuitschick and Karrer, 2007). The modified ORF was synthesized artificially and then inserted into plasmid pUC57 (Invitrogen Ltd. Shanghai, China). A set of oligonucleotide primers, 5′-ACGGATCCATGTCATTAAATCGTGAA-3′ and 5′-ACGAATTCTCAATTGTTCTGATCATC-3′ (*Bam*H I and *Eco*R I recognition sites were indicated by underlining in each primer, respectively) and the pUC57/*Ci14-3-3* as template were used to amplify the modified *Ci14-3-3* ORF by PCR. *Bam*H I and *Eco*R I (TaKaRa, Otsu, Japan) were used to doubly digest the purified PCR product and pGEX-4T-1, respectively. Then the two products were ligated together and transformed into *E. coli* DH5α.

2.4. Expression, extraction and purification of recombinant Ci14-3-3 protein

The selected bacteria containing plasmid pGEX-4T/*Ci14-3-3* were cultured in liquid LB medium at 37 °C until OD_{600nm} was between 0.3 and 0.5, and then divided into two groups. After the addition of isopropylthio-b-D-galactoside (IPTG) to induce the foreign gene expression, one group was cultured at 37 °C for a further 4 h, while, the other group was at 25 °C. According to the instructions from the manufacturer, Glutathione Sepharose 4B (GE Healthcare Life Sciences, Uppsala, Sweden) was used to purify recombinant Ci14-3-3 protein (rCi14-3-3). After being dialysed in PBS at 4 °C, the protein product was analyzed by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).