Fish & Shellfish Immunology 30 (2011) 182–188

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Changes in complement responses in Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) under crowding stress, plus viral and bacterial challenges

I. Mauri [a,*], A. Romero [a,d], L. Acerete [a], S. MacKenzie [a], N. Roher [a], A. Callol [a], I. Cano [b], M.C. Alvarez [c], L. Tort [a,*]

[a] Departament de Biologia Cellular, Fisiologia Animal i Immunologia, Universitat Autònoma de Barcelona, 08193 Cerdanyola. Catalunya, Barcelona, Spain
[b] Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Campus Teatinos, 29071 Málaga, Spain
[c] Departamento de Genética, Facultad de Ciencias, Universidad de Málaga, Campus Teatinos, 29071 Málaga, Spain
[d] Instituto de Investigaciones Marinas, CSIC, Eduardo Cabello 6, 36208 Vigo, Spain

ARTICLE INFO

Article history:
Received 4 June 2010
Received in revised form
20 September 2010
Accepted 7 October 2010
Available online 15 October 2010

Keywords:
Complement
Density
Stressor
Pathogen
Suppressor

ABSTRACT

Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) were subjected to either experimental infection with *Photobacterium damselae* subsp. *piscicida* or Nodavirus after a period of 2 weeks of crowding in which fish were subjected to a 5-fold increase in density (10–50 kg/m^3). Samples were obtained before the crowding period (0 h or control) and at 24 h and 72 h after crowding from both groups of infected fish. The Complement haemolytic activity and the expression of the C3 gene were evaluated in blood and liver samples respectively. The bacteriolytic and lysozyme activities were also assessed. The results showed that Complement haemolytic activity was reduced at 72 h with both bacteria and virus in high density Gilthead seabream, and a similar increase was observed at low density. Bacteriolytic activity under both bacterial and viral challenges for both species was increased at 24 h, under low density. At high density, the bacterial challenge did not induce significant changes. C3 mRNA abundance was substantially increased after pathogen treatments in low density groups at 24 h but no significant changes were detected at high densities.

These results support the idea of the suppressor effect of stressors on the immune system since a reduction of Complement activity under virus and high density, or lack of response in C3 expression under high density were observed.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) the two most important fish species in the Mediterranean aquaculture and studies on their immune system are important for scientific research and applied aquaculture [1]. Under intensive production conditions fish may be disturbed due to husbandry practices such as handling, transportation or confinement; they may experience stress [2] and become more susceptible to disease [3]. Fish respond to maintain homeostasis after stress and an array of changes are developed including molecular, cellular, metabolic, physiological and behavioral acclimation [4]. Poor husbandry management may facilitate disease outbreaks which impose a significant constraint in fish and shellfish production [5]. In commercial farms, unfavorable environmental conditions (low water quality, temperature changes) or poor management practices (overcrowding, frequent handling or inadequate nutrition) become stressors for the fish thus causing growth rate reduction and immune suppression leading to increased infections due to associated pathogens. *Photobacterium damselae* subsp. *piscicida* and Nodavirus are the causative agents of pseudotuberculosis or pasteurellosis, and the viral encephalopathy and retinopathy (VER) or viral nervous necrosis (VNN), respectively. Pasteurellosis and VNN have been reported in wild and cultured marine fish species in Europe, mainly affecting Gilthead seabream and European seabass [6]. Both diseases are responsible for relevant economic losses in Mediterranean fish aquaculture [7].

The vertebrate immune response is characterized by an innate non-specific response and an acquired specific response [1]. The Complement System is one of the major components of the innate immune response in vertebrates and participates in several defense mechanisms such as the formation of the membrane attack complex, opsonization and also in the development of antibodies [8,9]. The Complement System is composed of more than 30 soluble proteins

* Corresponding authors.
E-mail addresses: israel.mauri@uab.cat (I. Mauri), lluis.tort@uab.es (L. Tort).

1050-4648/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fsi.2010.10.006

working in cascade that are acting through three different pathways: the classical, alternative and lectin pathways [8,10,11]. The main functions involved in the complement response are microbial killing, phagocytosis, inflammatory reactions, clearance of immune complexes and antibody production [12]. Less is known about Complement regulatory proteins and Complement receptors in fish, and it is unclear whether all the Complement functions that have been identified in mammals also occur in fish. Fish appear to possess activation pathways similar to those in mammals, and fish Complement proteins identified thus far show significant homologies to their mammalian counterparts [13]. It has been clearly demonstrated that fish Complement can lyse foreign cells and opsonise foreign organisms to be destroyed by phagocytes. There are also indications that Complement fragments participate in inflammatory reactions [12]. Fish also possess multiple isoforms of several Complement proteins, such as C3 and factor B [14,15]. It has been hypothesized that the function of this diversity in Complement proteins serves to expand their innate immune recognition capacity and response [16]. The key protein of the Complement System, the C3 protein, is synthesized in the liver but is also expressed in other tissues such as gills, intestine or skin [17,18]. This protein is a conserved molecule that is present in many organisms from echinoderms and tunicates to mammals [19,20], thus demonstrating the persistence of this defense mechanism. Understanding the functions of Complement in fish and the roles that the individual proteins play in host defense, including various isoforms, is important not only for understanding the evolution of Complement System [21] but also for the development of new strategies in fish health management. Complement action in serum displays bactericidal and haemolytic capacities [22]; these activities have been used as indicators of Complement action [8,9]. However, few studies have been done on the expression of C3 mRNA under different conditions, mainly due to no sequence availability in fish species. Thus, few work has been done up to now on measuring in parallel the C3 expression in the liver and its activity as a protein in the plasma.

Thus, the aim of this work was to evaluate the Complement activity at gene expression level and functional activity in the Gilthead seabream, *S. aurata* and the European seabass, *D. labrax*, held under different stocking densities and subsequently challenged with *P. damselae* subsp. *piscicida* or Nodavirus.

2. Material and methods

2.1. Fish and treatments

Adult Gilthead seabream (*S. aurata*) and adult European seabass (*D. labrax*) of 30–40 g body weight were obtained from the Centro de Investigación y Formación Pesquera y Acuícola El Toruño (Puerto de Santa María, Cádiz, Spain). The fish were acclimatized to laboratory conditions for 15 days before experiments. Fish were held in tanks with recirculated water under a photoperiod of 12 h light/12 h dark, natural conditions of temperature 19–21 °C, and fed twice a day with a commercial diet (Skretting). For density experiments, fish were exposed to a crowding stress by increasing the density of the tanks from 8–10 kg/m^3 to 50 kg/m^3. After 15 days, fish were challenged with Nodavirus (NNV) intraperitoneal (10^4 TCID50/ml) and with the bacteria *P. damselae* subsp. *piscicida*, by bath immersion (10^5 cfu/ml, 1 h) [5] and sampled 0 h as a control, 24 h and 72 h. No mortality was recorded during the experiment.

2.2. Serum and organs sampling

The fish were sacrificed with a lethal dose of 2-phenoxyethanol anesthetic (Merck, Fontenay-sous-Bois, France). From each fish, 1 ml of peripheral blood was withdrawn from the caudal vein. The blood samples were left to clot for 2 h and centrifuged (3200 rpm, 10 min, 4 °C) to obtain the serum. For the Q-PCR analysis livers were excised. The samples were kept at −80 °C until use.

2.3. Serum assays

For the bacteriolytic test [16] bacteria (*Escherichia coli*) were grown for 20 h in 20 ml of LB medium at 37 °C in an orbital incubator at 200 rpm. 1:100 of a bacterial suspension was chosen to give an optical reading of 0.5–0.6 at a wavelength of 540 nm when added to the serum dilution (1:1, bacterial suspension: serum dilution) and blank with sterile LB. The mixture was placed for 1 h at 37 °C on an orbital incubator at 200 rpm. To study the bactericidal kinetics of Gilthead seabream and European seabass serum, a 0.5 ml aliquot was withdrawn at intervals of 30 min, and read at 540 nm at the spectrophotometer (BioMate 3, USA). Results are given as fold increase of the absorbance. The measurement of the complement activity was performed using sheep red blood cells as antigen reactive or target [9]. Briefly, 10 ml of sheep blood was withdrawn and placed in a 50 ml falcon with 0.2 ml of heparin (DELTALAB, S.L.U., Rubí-Barcelona, Spain). Then, blood was washed with 3 times saline solution after centrifugation at 3000 rpm for 5 min, adding 4 volumes of saline solution per volume of blood. The final volume was adjusted to 2.8×10^8 cells/ml. The fish serum was then placed in an ELISA V bottom plate and subjected to two serial dilutions with the Test Buffer (0.1% gelatin, 5 mM sodium barbiturate, 0.13 M NaCl, 10 mM Mg^{2+}, 10 mM EGTA, pH 7.3) following the series: 1/32, 1/48, 1/64, 1/96, 1/128, 1/192. After this, 10 μl of diluted blood were added and the plate was incubated during 100 min, at room temperature. After 100 min, 150 μl of Stop Buffer (0.1% gelatin, 5 mM sodium barbiturate, 0.13 M NaCl, 20 mM EDTA, pH 7.3) were added to stop the lytic reaction, and 100 μl of supernatant were collected after centrifugation (2000 rpm, 2.5 min) and put into flat bottom plate to be read at 414 nm. Lysozyme activity assays were performed by a turbidimetric method that uses the lysis of *Micrococcus luteus* for determination of the lysozyme activity using egg-white lysozyme as a standard [1,23]. Briefly, 10 μl of serum was suspended with 200 μl of a *M. luteus* suspension, and read it at 540 nm each 10 min for 40 min and a last one measure at 60 min.

2.4. C3 expression analysis

Total liver RNA was isolated with Tri Reagent (Molecular Research Inc.) using the manufacturer's protocol. RNA concentration was quantified using a Nanodrop ND-1000 and RNA quality was assessed using a Bioanalyzer 2100 with the RNA 6000 Nano LabChip kit (Agilent). Complement C3 mRNA expression levels were analyzed by Quantitative-PCR and/or Real Time PCR (Q-PCR). PCR primers (Table 1) were designed against the cloned sequence for C3 from Gilthead seabream (Mauri et al., unpublished data) using Primer 3 version 0.4.0. For RT-PCR the following protocol was used: 5 min at 95 °C, followed by 30 cycles of 45 s denaturation at 95 °C, 45 s annealing at 55 °C, and 45 s extension at 72 °C. Samples

Table 1
Sequences of used primers in Q-PCR analysis.

Name	Sense	Sequence (5′-3′)
Complement component C3 of *Paralichthys olivaceus* (BAA88901) and *Anarhichas minor* (CAC29154.1)	Forward	5′-CCATGACAGACTAATTCCAGTG-3′
	Reverse	5′-GGGAGCAGAAGGAAACGATCC-3
18S (AF243428)	Forward	5′-CGAGCAATAACAGGTCTGTG-3′
	Reverse	5′-GGGCAGGGACTTAATCAA-3′

Download English Version:

https://daneshyari.com/en/article/2432720

Download Persian Version:

https://daneshyari.com/article/2432720

Daneshyari.com