Fish and Shellfish Immunology 77 (2018) 298–303

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Short communication

Comparison of the susceptibility and resistance of four marine perciform fishes to *Cryptocaryon irritans* infection

Fei Yin[a,b,*], Wenchao Liu[b,c], Peibo Bao[b,c], Shan Jin[a], Dong Qian[a], Jiteng Wang[d], Baojun Tang[b,**]

[a] Key Laboratory of Applied Marine Biotechnology, Ministry of Education, Collaborative Innovation Center for Zhejiang Marine High-efficiency and Healthy Aquaculture, Ningbo University, Ningbo 315211, People's Republic of China

[b] Key Laboratory of East China Sea and Oceanic Fishery Resources Exploitation, Ministry of Agriculture, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, People's Republic of China

[c] College of Fisheries and Life Sciences, Shanghai Ocean University, Shanghai 201306, People's Republic of China

[d] Department of Aquaculture, Zhejiang Ocean University, Zhoushan, Zhejiang 316022, People's Republic of China

ARTICLE INFO

Keywords:
Cryptocaryon irritans
Nibea albiflora
Serum
Immobilization titer
Complement activity

ABSTRACT

Cryptocaryon irritans is a type of marine ectoparasitic ciliate that infects teleost fishes. To illustrate the susceptibility and innate immune mechanism of fishes to *C. irritans*, four species of marine perciform fishes were selected in Fujian Province, a high-prevalence area of cryptocaryoniasis in China. The survival, diameter/number of tomonts, and infection ratio among *Larimichthys crocea*, *Lateolabrax japonicus*, *Pagrus major*, and *Nibea albiflora* were compared after artificial infection. Meanwhile, the immobilization titers of four fish species with no *C. irritans* infection were detected. Results showed that survival and serum immobilization titer of *N. albiflora* were significantly higher than those of the other three fish species. A strong negative linear correlation was found between the survival/serum immobilization titer and the mean tomont diameter. In addition, the smallest *C. irritans* infection ratio was found in *N. albiflora*, implying that the serum of fishes especially that of *N. albiflora*, inhibited the development of parasitic *C. irritans* cells, and the smallest tomont size was directly related to the number of infective theronts corresponding to the highest survival of fish. Moreover, complement activity inhibition assays suggested that the alternative complement pathway might play a major role in *C. irritans* resistance.

1. Introduction

Cryptocaryon irritans, also known as *Ichthyophthirius marinus*, is a type of marine ectoparasitic ciliate that infects teleost fishes [1]. For several years since the first report of this parasite in aquaria of the Tokyo imperial university, institute for fisheries [2], basic biological research and explorations of curative strategies have been conducted [3,4]. *C. irritans* has a broad host range, including both wild and cultured fish species [5,6], and high mortality occurs in high-density cultured fish [7]. Previous studies have confirmed a low degree of host specificity of *C. irritans* for fish species [8–10], which is similar to that of its freshwater counterpart, *I. multifiliis* [11]. However, with the intensification of research on *C. irritans*, variability in the degree of susceptibility to *C. irritans* between fish species is a well-known phenomenon, and there have been several reports to this effect [6,12].

A previous field survey was performed on the host range in the Brisbane river estuary in Australia, indicating that the prevalence and intensity of *C. irritans* infections varied significantly among species [12]. In recent artificial infection assays, a remarkable difference also existed in resistance to *C. irritans* between *Trachinotus ovatus* [13], *Sebastiscus marmoratus* [14], *Epinephelus coioides* [15], *Siganus oramin* [16], and *Nibea albiflora* (unpublished data). However, empirical research directly comparing the susceptibility/resistance of different fish hosts is limited. Moreover, explanations of the mechanisms involved have been varied and confusing. Colorni and Burgess (1997) speculated that this phenomenon was attributed to a difference in the fish's adaptability to confinement, handling, and environmental conditions [8]. Moreover, it has also been suggested that wild fish are more susceptible to *I. multifiliis* infections than domestic fish, even when the interspecies difference between the above wild and domestic fish is not considered [17]. In some cases, the absence of a preliminary parasitological examination always caused confusion among authors by

* Corresponding author. Key Laboratory of Applied Marine Biotechnology, Ministry of Education, Collaborative Innovation Center for Zhejiang Marine High-efficiency and Healthy Aquaculture, Ningbo University, Ningbo 315211, People's Republic of China.

** Corresponding author. Key Laboratory of East China Sea and Oceanic Fishery Resources Exploitation, Ministry of Agriculture, East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, People's Republic of China.

E-mail addresses: Yinfei@nbu.edu.cn (F. Yin), Tangbj@ecsf.ac.cn (B. Tang).

https://doi.org/10.1016/j.fsi.2018.03.052
Received 25 December 2017; Received in revised form 23 February 2018; Accepted 29 March 2018
Available online 29 March 2018

suggesting that the unaffected fish species were possibly infected by other ciliates and obtained adequate immunity [6,18]. In fact, in vitro assays suggested that alternative complement activity could explain immobilization and lysis of *I. multifiliis* theronts in non-immune fish serum [11,19]. In this regards, it is interesting to show if, and to what extent, certain fish species are resistant to *C. irritans*. Moreover, it is of interest to understand the major underlying immune mechanism behind such phenomena.

In the areas with a high prevalence of cryptocaryoniasis in Fujian Province, China, some culturists found that *N. albiflora* did not die from cryptocaryoniasis, even during disease outbreaks. In contrast, *Larimichthys crocea*, a species of important farmed fish in China, is susceptible to *C. irritans* infection, with high mortality every year [20]. In order to further investigate the resistance and susceptibility of different fish species to *C. irritans*, the survival, mean tomont diameter, number of tomonts, and infection ratio between *L. crocea*, *Lateolabrax japonicus*, *Pagrus major*, and *N. albiflora* were compared after artificial infestation. The strain of *C. irritans* was collected from naturally infected *L. crocea* in Fuding City, China and propagated in the same fish species. Serum immobilization titers of the above four uninfected fish species to *C. irritans* were also performed; specifically, the serum of *N. albiflora* was treated with complement inhibitors to understand the major contributing complement pathway.

2. Materials and methods

2.1. Cryptocaryon irritans and experimental fish

Cryptocaryon irritans were collected from naturally infected *L. crocea* (100 ± 10 g) in Fuding city, Fujian Province, China, and the same fish was then used as the model to establish the passage system. Artificial propagation was performed in a 1062-L (φ_{bottom} = 130 cm; H = 80 cm) fiberglass aquarium with 5 L of water provided for each fish. The infection period lasted for 2 h in the dark, and then fresh seawater was added. Collection of tomonts and theronts was conducted as previously described [20].

L. crocea (Richardson, 1846), *L. japonicus* (Cuvier, 1828), *P. major* (Temminck & Schlegel, 1843), and *N. albiflora* (Richardson, 1846) of equal weight (mean = 150 g) were obtained in Fuding City, China. No outbreaks of cryptocaryoniasis had occurred around this breeding area during the year. A sample of 10 randomly selected fish was examined for each fish species, and no *C. irritans* were detected on the gills, fins, or skin of these fish. The fishes were overfed twice daily (8:00 and 15:00 Hrs) with commercial pellet feed. The salinity, water temperature, light intensity, and photoperiod for both aquaculture and experimental treatments were 29‰–31‰, 26 ± 1 °C, 1000 lx, and 12 L: 12 D, respectively.

2.2. Experimental methods

Active *C. irritans* theronts that had hatched for no more than 2 h were collected, and the concentration of parasites was determined as in a previously described protocol [21]. Forty healthy fish (10 fish of each species) were mixed and infected with *C. irritans* theronts at a dose of 330 theronts per gram fish in one tank, and this tank was replicated three times in parallel [14]. Infection was performed under the same conditions as described for *L. crocea*, in a 1062-L fiberglass aquarium with 5 L of water provided for each fish. The infection period lasted for 2 h in the dark, and then fresh seawater was added. Twelve hours after infection, the fishes were separated according to species and transferred to other clean aquaria (10 fish per aquarium) without parasites. In order to avoid artificial loss of tomonts, water was replaced gently and slowly with clean water twice daily (09:00 and 15:00 Hrs) without cleaning the bottom of the tank.

2.3. Measurement of survival, mean tomont diameter, relative tomont number, and infection ratio

Every day, we recorded the number of dead fish in each group and calculated the survival rate as follows: survival rate (%) = 100 × number of surviving fish/initial number of fish.

Every 24 h, we recorded the total number of tomonts in three petri dishes (φ = 8.5 cm) from each tank. The three petri dishes were lined up equidistantly from the center to the edge of the tank. Relative tomont number (RTN) = [S_{tank}/(3 × S_{dish})] × the total number of tomonts in three petri dishes/fish number/average weight of fish (g). S_{dish} = 56.75 cm^2; S_{tank} = 4300.84 cm^2. The diameter of 40 tomonts collected from four fish species was measured with an Olympus IX70 inverted microscope (Olympus Optical Co., Ltd., China).

Infection ratio = (RTN_{3dpi} + RTN_{4dpi} + $RTNd_{5dpi}$ + $RTNd_{6dpi}$)/ infection dose of theronts.

2.4. In vitro immobilization assay and complement inactivation

Non-infected fish were selected for the immobilization assay. The blood of three fish of each fish species was sampled. Blood was sampled from the tail vein with a sterile syringe. The blood was placed in a sterile centrifuge tube at room temperature for 2 h, kept at 4 °C overnight, centrifuged at 10,000 g for 10 min, and the upper serum was stored at −20 °C in a freezer.

The immobilization assay was performed using a method modified by Dan et al. (2008) [13]. Before incubation, the serum was treated with or without heating at 56 °C for 30 min or treated in the presence or absence of complement activity inhibitors [hydrazine dihydrochloride and ethylenediaminetetraacetic acid (EDTA) disodium salt, Sinopharm Group Co., Ltd., Shanghai, China; Ethylene glycol tetraacetic acid (EGTA) and L-lysine, Sangon Biotech Co., Ltd., Shanghai, China; Zymosan, Sigma-Aldrich Co., Ltd., Santa Clara, CA, USA] to block pathways of the complement cascade [22,23]. Two-fold serial dilutions of serum were prepared using sterilized seawater plus an equal volume of theront solution (100 μL, containing approximately 100 active theronts) in a 96-well plate. After incubation at room temperature for 1 h, the mixture was observed with an Olympus IX70 inverted microscope. Theronts that were killed or had stopped swimming and aggregated on the bottom of the well were considered to have been immobilized.

2.5. Data analysis and statistical methods

Statistical analyses were performed using SPSS 22. One-way analysis of variance (ANOVA) was used to compare the survival, mean tomont diameter, number of tomonts, infection ratio, and immobilization titer. If significant differences were detected, Tukey's post hoc multiple comparisons were performed. Arcsine transformation was conventionally utilised for survival and infection ratio analysis before ANOVA and multiple comparisons. Data were presented as the mean ± standard deviation (mean ± S.D.). Results were considered statistically significant at $P < 0.05$.

3. Results and discussion

Reports estimate that over 100 marine fish species have been infected naturally with *C. irritans* [2,3,9]. Nevertheless, different species of feral and cultured fish have different susceptibilities to *C. irritans*. In the field survey in Australia, the infection intensity of *Acanthopagrus australis* was much higher than that of *Rhabdosargus sarba*, *Gerres ovatus*, *Sillago maculata*, *Spheroides hamiltoni*, and *Lutjanus russellii* [12]. An investigation of naturally infected cultured marine fish in China showed that the susceptibility of *T. blochii* to *C. irritans* was the highest compared with that of *Seriola dumerili*, *Pseudosciaena crocea*, *E. coioides*, *Sparus macrocephalus*, *Diplodus sargus capensis*, *Plectorhinchus cinctus*, and *L. argentimaculatus*, whereas *Miichthys miiuy* and *Pomadasys hasta*