Aquaculture and Fisheries xxx (2017) 1–6

Contents lists available at ScienceDirect

Aquaculture and Fisheries

journal homepage: www.keaipublishing.com/en/journals/aquaculture-and-fisheries/

Monoclonal antibody based Dot-ELISA and indirect fluorescence antibody technique for detecting *Edwardsiella ictaluri* infection in yellow catfish (*Pelteobagrus fulvidraco*)

Ruijun Li [a], Hua Huang [a], Xianyu Zhang [a], Shigen Ye [a], Qiang Li [a, b, *]

[a] Dalian Key Laboratory of Marine Animal Disease Control and Prevention, Agriculture Department Key Laboratory of Mariculture & Stock Enhancement in North China's Sea, Dalian Ocean University, Dalian 116023, China
[b] Department of Ocean Technology, College of Marine and Biology Engineering, Yancheng Institute of Technology, Yancheng 224051, China

ARTICLE INFO

Article history:
Received 1 April 2017
Received in revised form
10 October 2017
Accepted 10 October 2017
Available online xxx

Keywords:
Edwardsiella ictaluri
Monoclonal antibody
Dot-ELISA
IFAT

ABSTRACT

Edwardsiella ictaluri is known as the etiological agent of Red-head disease of yellow catfish (*Pelteobagrus fulvidraco*), and is the cause of heavy economic losses in the aquaculture industry. In this study, a Dot-Enzyme Linked Immunosorbent Assay (Dot-ELISA) and an Indirect Fluorescence Antibody technique (IFAT) for detecting *Edwardsiella ictaluri* were developed by using a monoclonal antibody, 5D11. For Dot-ELISA, the working dilutions of 5D11 and the secondary antisera (enzyme-labeled goat anti-mouse Ig) were 1:320 dilution and 1:3000 dilution, respectively. For IFAT, the working dilutions of 5D11 and goat-*anti*-mouse Ig conjugated with fluorescein isothiocyanate were as respectively 1:80 and 1:256. Both of the methods established were highly sensitivity (Minimum detectable concentration, 5×10^7 CFU/mL) and had a high degree of accuracy (positive rate was 100% for both artificial infected fish and spontaneous diseased *Pelteobagrus fulvidraco*). The two reliable methods developed have high potential for quick and efficient detection of *Edwardsiella ictaluri* in aquaculture production units.

1. Introduction

Red-head disease (RHD) caused by *Edwardsiella ictaluri* is one of the most serious diseases of yellow catfish (*Pelteobagrus fulvidraco*) in the Chinese aquaculture industry, and causes a 50%–60% cumulative mortality during disease outbreaks (Liu, Li, Zhou, Wen, & Ye, 2010; Ye, Hua, Guo, & Li, 2009). RHD has also been reported in a number of other species, mainly in channel catfish, as well as *Pangasianodon hypophthalmus*, Walking catfish (*Clarias batrachus*), European catfish (*Silurus glanis*), Chinook salmon (*Oncorhynchus tshawytscha*) and Rainbow trout (*Oncorhynchus mykiss*) (Crumlish, Dung, Turnbull, Ngoc, & Ferguson, 2002; Inglis, Roberts, & Bromage, 2012; Liu et al., 2010; Plumb & Hanson, 2011; Tu et al., 2009). For the prevention and control of RHD, several detection methods have been established, including, physiological and biochemical identification, and molecular biology techniques (PCR) (Bader, Shoemaker, Klesius, Connolly, & Barbaree, 1998; Bilodeau, Waldbieser, Terhune, Wise, & Wolters, 2003; Panangala, Santen, Shoemaker, & Klesius, 2005). However, the current diagnostic methods tend only to be used in a laboratory setting and are rarely used on aquaculture farms. In recent years, immunological detection techniques (Bai, Lan, Wang, Han, & Zhang, 2009; Plumb & Vinitnantharat, 1989) have become the approache for clinical diagnosis because of their high specificity, sensitivity, simplicity and accuracy. The Dot enzyme-linked immunoassay (Dot-ELISA) (Klesius, 1993; Waterstrat, Ainsworth, & Capley, 1989) and Immunofluorescent test (IFAT) (Panangala et al., 2006) that use monoclonal antibody (mAbs) are now the most widely applied techniques for the rapid clinical detection of diseases. Therefore, in this study, we attempted to develop a fast and reliable method to detect the *Edwardsiella ictaluri* in aquaculture producers.

2. Materials and methods

2.1. Bacterial strains

A total of 11 reference strains (Table 1) were stocked in culture broth supplemented with 30% glycerol at −80 °C in our laboratory. After activation the strains were cultured in 2 mL of LB broth

* Corresponding author. 50 Heishijiao Street, Shahekou District, Dalian Ocean University, Dalian 116023, China.
E-mail address: fish790608@163.com (Q. Li).

https://doi.org/10.1016/j.aaf.2017.10.003

Table 1
List of reference strains used in cross-reaction test.

Bacteria	Annotations
Edwardsiella ictaluri ATCC 33202	Standard strain
E. tarda SU 100	Isolated from Turbot, *Scophthalmus maximus*
Escherichia coli ATCC 29532	Standard strain
Aeromonas hydrophila BSK-70	Isolated from *Carassius auratus*
Streptococcus iniae KCTC 3657	Obtained from Pukyong National University
A. salmonicida MT 004	Isolated from Rainbow trout, *Oncorhynchus mykiss*
Vibrio anguillarum HUFP 5001	Isolated from Turbot, *Scophthalmus maximus*
V. alginolyticus KCCM 40513	Obtained from Pukyong National University
V. parahaemolyticus KCTC 2471	Obtained from Pukyong National University
V. vulnificus ATCC 29306	Standard strain
V. harveyi ATCC 14126	Standard strain

(HiMedia, India) overnight at 28 °C until the experiments.

2.2. Antibodies

The hybridoma cells secreting mAbs (5D11, prepared and stored in our lab) against *E. ictaluri* A86 were taken from liquid nitrogen, transferred into 25 cm^2 cell culture flasks containing RPMI-1640 medium (Hyclone, USA), and maintained at37 °C under 5% CO_2 in an incubator. Several days later, collected culture supernatants were centrifuged at 1000×*g* for 10 min to remove the cell debris. MAb 5D11 were secreted in culture solution and stored at −20 °C. Goat anti-mouse Ig conjugated with alkaline phosphatase (GAM-AP, Sigma, USA) was prepared for Dot-ELISA and goat-*anti*-mouse Ig conjugated with fluorescein isothiocyanate (GAM-FITC, Sigma, USA) was prepared for IFAT.

2.3. Dot-Enzyme Linked Immunosorbent Assay (Dot-ELISA)

For the Dot-ELISA, nitrocellulose membrane (NCM) with 6 mm diameter was loaded into 96-well cell culture plates (Costar, USA). 2 μL of bacterial cultures were added dropwise to the center of each NCM, respectively. Blank, positive and negative controls were also set-up using the same approach. After drying at room temperature, the wells were blocked with 200 μL blocking buffer (PBS containing 3% BSA) for 1 h at 37 °C. The plates were incubated with mAb 5D11 for 1 h and then washed with PBS-T (PBS containing 0.05% Tween-20) 3 times (5 min for each time). After incubation with mAb 5D11 and washed. Goat anti-mouse Ig conjugated with alkaline phosphatase was added. The 100 μL goat anti-mouse lg conjugated with alkaline phosphatase (GAM-AP, Sigma) was added to each well and the plate was incubated for 1 h at 37 °C. At the end of the incubation the plate was washed well with PBS-T and then washed before developing in 100 μL of BCIP/NBT substrate solution for 5 min. The reaction was stopped after approximately 20 min by rinsing the plate with PBS-T. The samples with clear and obvious violet dots were judged as positive reaction.

In the Dot-ELISA, the optimum coating concentration of the antigen and the incubated concentration of antibodies were determined using a chessboard assay. The antigen was coated at 5×10^8 CFU/mL. A dilution series of MAb 5D11 was prepared using a two-fold dilution with PBS from 1:5 to 1:5120, and GAM-AP was diluted with PBS to 1:1000, 1:2000 to 1:16000, respectively. The samples with clear and obvious violet dots were judged to be positive reactions. The strains listed in Table 1 were used to check the specificity of the Dot-ELISA, while *E. ictaluri* A86 served as a positive control and PBS served as a negative control. Different antigen dilutions were used to test assaysensitivity.

2.4. Indirect fluorescence antibody technique (IFAT)

Monolayers of *E. ictaluri* A86 were prepared by allowing cells to settle onto glass slides and then incubating them in a wet chamber at room temperature for 45 min, and then air dried and fixed in acetone for 15 min. Subsequently, the slides were incubated with 20 μL mAb 5D11 in a moist chamber at 37 °C for 45 min. After washings with PBS three times, the cells were incubated with goat-*anti*-mouse Ig conjugated with fluorescein isothiocyanate (GAM-FITC) at the dilution of 1:256 at 37 °C for 45min in the dark. After slides were briefly washed they were air-dried and mounted using buffered glycerin. Slides were examined using a fluorescence microscope (Nikon). SP20 cell culture supernatants were used as a negative control.

2.5. Application of two methods for the experimental infection test

Pelteobagrus fulvidraco (15–20 g) was used for the infection experiment. In the infection group, each fish was intraperitoneally (IP) infected with 0.2 mL *E. ictaluri* A86 at 5×10^8 CFU/mL, and the none-infection group were injected with 0.2 mL TSB. During the experiment, all fish were kept in 57 L tanks at 25 ± 1 °C. Mortality was monitored for up to 10 days after the challenge. The brains of fish were collected and examined with the established IFAT and Dot-ELISA.

2.6. Application for natural infection test

Tissues (brain and kidney) were collected from infected *P. fulvidraco of* spontaneous which have “Red-head” disease from Liaoning and Guangdong Provinces of China. 11 strains (Table 2) of *P. fulvidraco* were isolated from infected tissues and identity confirmed using PCR. All the isolated strains were then used to test the IFAT and Dot-ELISA developed.- The methods of IFAT and Dot-ELISA were the same as those described previously.

Table 2
E. ictaluri isolated strains of from diseased *P. fulvidraco*.

Sampling site	No.	Date	Origin of bacteria
Liaoning	A51	2005–05	brain
	A52	2009–08	kidney
	A53	2009–08	heart
	A54	2009–08	brain
	A55	2009–08	brain
Guangdong	A86	2009–05	brain
	A89	2009–08	brain
	A90	2009–08	brain
	A92	2009–08	brain
	A95	2009–08	brain
	A96	2009–08	brain

Please cite this article in press as: Li, R., et al., Monoclonal antibody based Dot-ELISA and indirect fluorescence antibody technique for detecting *Edwardsiella ictaluri* infection in yellow catfish (*Pelteobagrus fulvidraco*), Aquaculture and Fisheries (2017), https://doi.org/10.1016/j.aaf.2017.10.003