



## Full length article

# CpG ODN mimicking CpG rich region of myxosporean *Myxobolus supamattayai* stimulates innate immunity in Asian sea bass (*Lates calcarifer*) and defense against *Streptococcus iniae*



Kittichon U-taynapun<sup>a, b</sup>, Nion Chirapongsatongkul<sup>a, c</sup>, Toshiaki Itami<sup>d</sup>,  
Chutima Tantikitti<sup>b, \*</sup>

<sup>a</sup> Aquatic Animal Health Management Research Unit, Department of Fisheries, Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, 80110, Thailand

<sup>b</sup> Aquatic Animal Health Research Center, Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Songkhla, 90110, Thailand

<sup>c</sup> Department of Biochemistry, Faculty of Science, Prince of Songkla University, Songkhla, 90110, Thailand

<sup>d</sup> Department of Marine Biology and Environmental Sciences, Faculty of Agriculture, University of Miyazaki, 889-2192, Japan

## ARTICLE INFO

## Article history:

Received 13 June 2016

Received in revised form

8 September 2016

Accepted 10 September 2016

Available online 11 September 2016

## Keywords:

CpG ODN

*Lates calcarifer*

Immunostimulant

Innate immunity

*Streptococcus iniae*

## ABSTRACT

Oligodeoxynucleotides (ODNs) containing unmethylated cytosine-phosphate-guanine CpG dinucleotides within specific sequence contexts (CpG motifs) have been reported as pathogen-associated molecular patterns (PAMPs). Its immunostimulatory effects have been demonstrated in diverse vertebrate models. CpG ODN is typically found in bacterial or viral genome and recognized by a non-self recognition receptor Toll-like receptor9 (TLR9). Here, a new CpG ODN 1013 which mimics sequence of SSU rDNA of early eukaryotic organism myxosporidia, *Myxobolus supamattayai*, was employed to stimulate the immune responses of Asian sea bass *Lates calcarifer*. Its immunostimulant potentiality was comparatively compared with that of CpG ODN 1668, a widely used as functional immunostimulant. Both unmethylated CpG ODNs with some modified phosphorothioated positions were intraperitoneally injection (5 µg/fish). Hematological examination, immunological assays and immune-related genes expression were evaluated 12 h, 1, 3 and 5 d after post CpG ODN challenge. The immunostimulatory effect of these CpG ODNs on fish immunity to protect the bacterial pathogen *Streptococcus iniae* was also determined. The results demonstrated that these two CpG ODNs could induce immune responses in Asian sea bass including the significant ( $P < 0.05$ ) increase level of WBC, peroxidase activity and oxidative radicals in head kidney (HK) leukocyte, serum innate immune parameters and up-regulation of four immune responsive genes compared with the control group. Most of immune responses induced by ODN 1668 were strong within 1 d but lesser extended while ODN 1013 prolonged the stimulatory effects during the whole experimental period. After challenge with *S. iniae*, the survival proportion in ODN 1013-treated fish was apparently higher than that treated with ODN 1668 and PBS, respectively. The results together suggested that CpG ODN 1013 enhanced innate immune responses, including humoral and cellular responses, through TLR9 mediated signaling pathway which is mainly contribute to the protective immunity in Asian sea bass against *S. iniae* infection. These findings can lead to a new approach in immunostimulant development by using the novel CpG ODN originating from the parasite *M. supamattayai*, besides those from bacterial and viral genomes, for disease control in fish host.

© 2016 Elsevier Ltd. All rights reserved.

## 1. Introduction

The Asian sea bass is a carnivorous, euryhaline species

originating in seawater but can be reared in freshwater and at high densities. They are a high potential aquaculture candidate species because of a high consumer demand and market value, and its fast

\* Corresponding author. Aquatic Animal Health Research Center, Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90110, Thailand.

E-mail address: [chutima.t@psu.ac.th](mailto:chutima.t@psu.ac.th) (C. Tantikitti).

growth under the low farm management cost. However, the control of diseases caused by bacteria, virus and parasites are complicated. An application of antibiotics and chemicals is reported as an effective tool although improper uses of these substances can cause the accumulation in the environment and the emergence of drug resistant strains. Moreover, their residues in fish lead to consumer's reluctance [1]. One of the most promising methods to control infectious diseases in aquaculture has been focusing on strengthening the defense mechanisms of fish through enhancing nonspecific or innate defense mechanisms to increase resistance. In this regards, several approaches including immunostimulants application has been adopted [2].

Varieties of immunostimulants including extracts from traditional herbs and molecules classified as pathogen-associated molecular patterns (PAMPs) have been used in aquaculture. Among PAMPs, microbial and viral biopolymers, such as double stranded RNA, lipopolysaccharide,  $\beta$ -glucan, DNA fragment with specific motifs and unmethylated cytosine-phosphate-guanine oligodeoxynucleotides (CpG ODNs) have proved their immunostimulatory actions in mammals and fish [3]. These PAMPs allow the immune system to make the distinction between self and non-self recognition [4]. Specific DNA motifs from bacteria and virus fit this definition of PAMPs because of the presence of unmethylated ODNs containing CpG motifs flanked by certain nucleotide sequences that are abundant in bacterial and viral genomes and rare in vertebrate genomes [5]. CpG is recognized by the Toll-like receptor9 (TLR9) belonging to one of pattern recognition receptors (PRR) [6]. Based on their structural differences, CpG ODNs are classified into three classes (A, B and C), which induce different immune responses [6,7]. Class A – CpG ODNs contain a central palindromic phosphodiester CpG motif and phosphorothioated poly G motifs at the 5' and 3' ends that is known to induce the secretion of massive type I interferon (IFN) and the activation of natural killer (NK) cell. Class B – CpG ODNs contain one or more CpG motifs, which are constructed on phosphorothioate (PS) backbones. This class promotes monocyte maturation and B cell activation. Class C – CpG ODNs are known to possess structural and functional features of class A and B [8].

Recently synthetic ODNs containing effective unmethylated CpG motifs are well known to stimulate immune responses and are indeed potent adjuvant and, in some cases, protect against bacterial and viral challenge in higher vertebrates including fish [7,9,10]. To date, CpG ODNs have been reported for its immunostimulatory effects in a number of economic fish species for example Atlantic salmon (*Salmo salar* L.) [11,12], rainbow trout (*Oncorhynchus mykiss*) [13,14], Japanese flounder (*Paralichthys olivaceus*) [8,15–19], catfish (*Ictalurus punctatus*) [20], and Pacific red snapper (*Lutjanus peru*) [21]. Cárdenas-Reyna et al. [21] and Shen et al. [22] have reported the mode of action of synthetic CpG ODNs sequence in stimulating immune responses in fish through the induction of multi-responsible parameters of innate immune including humoral and cellular responses. Moreover, these synthetic CpG ODNs act as a strong adjuvant increasing the leukocyte immune activity to antigens [12,18,19].

All of the synthetic unmethylated CpG ODNs that have been used as the effective immunostimulants to enhance the *in vivo* disease resistance are mimicked from or designed according to the bacterial DNA. However, synthetic CpG ODNs mimicking other pathogen genomes such as protozoan and metazoan parasite are not yet verified. In this study, the effects of CpG ODN 1013 mimicking small subunit (SSU) rDNA gene of the myxosporean parasite, *Myxobolus supamattayai*, triggering immune responses of Asian sea bass were demonstrated and compared with those induced by the well known and widely used CpG ODN 1668. These two tested CpG ODNs are classified as class B based on their

structure. Since the mechanism and the defense responses involving in the immunity induced by CpG ODNs are variety, the humoral and cellular responses in CpG ODNs treated fish were investigated by analyzing systemic innate immune parameters and the expression of four immune relevant genes. In addition the protective effects of CpG ODNs in Asian sea bass against *Streptococcus iniae*, a causing agent of an important disease streptococcosis in fish culture, were also evaluated. To the best of our knowledge, this is the first time to prove the apparent immunostimulatory effect of CpG ODN, which synthesized based on the sequence originated from parasite, on fish defense responses. This may provide a novel approach for immunostimulant development used in aquaculture.

## 2. Materials and methods

### 2.1. Fish

Asian sea bass, mean weight of 25 g, maintained in seawater (30 ppt), were obtained from Trang Coastal Aquaculture Station (Department of Fisheries, Thailand). Following routine practices of freshwater culture for Asian sea bass, salinity had been reduced at the rate of 5 ppt per day in plastic aquarium tank containing 3000 L of water under laboratory condition (DO > 6.0 ppm, pH 6–7.5 and temperature at  $29 \pm 2$  °C) at Aquatic Animal Health Research Center, Prince of Songkla University. Fish were fed twice daily with commercial marine fish pellets (Thaiunion Feedmill, Thailand). Fish were cultured to gain 50 g mean weight (approximately 6 weeks) after completed retainable in freshwater and environmental condition described above.

### 2.2. CpG ODNs

Nuclease-resistant phosphorothioate ODNs were synthesized by Sigma (Sigma, Singapore). The sequence of CpG ODN 1668, the classical effective CpG ODNs inducing the immune responses in fish and mammal, is 5' TC\*CA\*TGACGTTCC T\*GAT\*GCT 3' (phosphorothioate modifications are marked with \* and CpG motif is underlined) with minor modification of phosphorothioate positions from previous study [23]. CpG ODN 1013 mimicking SSU rDNA of *M. supamattayai* was synthesized according to the sequence of 5' CT\*CAC\*TATCGTTC\*TTG\*ATT 3'. Synthetic ODNs were dissolved in DNase/RNase-free distilled water (Promega, US).

### 2.3. Experimental design

Fish were randomly divided into 9 groups; 12 fish per group were fed in a plastic aquarium tank (200 L). All fish were acclimated for 2 weeks prior to being used in the experiment. The feeding tanks were randomly divided into 3 groups in order to exposure to different CpG ODNs (3 tanks/treatment); group A (PBS, control), group B (5  $\mu$ g of 1668 CpG ODN/fish) and group C (5  $\mu$ g of 1013 CpG ODN/fish). Feed was withheld for 24 h then one fish was randomly sampling from each tank (3 fish were pool to one sample). The final 3 samples were thus used as a control at 0 h for all parameters assayed. The fish were anaesthetized with clove oil before being challenged with immunostimulants or PBS by intraperitoneal (i.p.) injection.

At 12 h and 1, 3, 5 d interval, 2 fish were randomly picked from each tank and pooled from 3 tanks in each treatment. Blood and HK were collected from all picked fish after anaesthetized with clove oil.

Download English Version:

<https://daneshyari.com/en/article/5540599>

Download Persian Version:

<https://daneshyari.com/article/5540599>

[Daneshyari.com](https://daneshyari.com)