

## Construction of a recombinant plasmid containing multi-copy CpG motifs and its effects on the innate immune responses of aquatic animals

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Received 28 March 2006; revised 11 December 2006; accepted 9 January 2007

Available online 20 January 2007

### Abstract

Bacterial DNA and synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG dinucleotides (CpG motifs) have been shown to induce potential immune responses. In this study, we designed a recombinant plasmid containing multi-copy CpG motifs, and observed its effects on innate immune responses of fish and prawn. The results showed that such plasmid DNA, compared to the vacant vector, can highly induce the activation of head kidney macrophages and the proliferation of peripheral blood leukocytes in *Carassius auratus* and *Lateolabrax japonicus* *in vitro*, as well as the activity of humoral defense proteins and the antibacterial activity of haemolymph in *Litopenaeus vannamei* *in vivo*. It implies that the multi-copy CpG motifs harboured in plasmid could contribute to these innate immunostimulatory effects. Therefore, the study suggested that the plasmid containing multi-copy CpG motifs might have its potential application in improving host resistance to pathogen insults in aquaculture, and have its notable advantages of high efficacy, economical cost and application to a broad range of aquatic species.

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**Keywords:** CpG motif; Recombinant plasmid; Innate immune responses; Crucian carp; Japanese seabass; Pacific white shrimp

### 1. Introduction

In commercial aquaculture, considerable effort is directed towards preventing the onset of disease caused by pathogens. One approach to prevention is the use of immunostimulants which are naturally occurring compounds that modulate the immune system by increasing the host's defence to pathogens. Immunostimulants are also defined in view of recent discoveries of the pathogen-associated molecular patterns (PAMPs) including cell wall products such as lipopolysaccharide (LPS), glucans, alginate, and nucleic acids [1].

Recent research found that the immunostimulatory response observed in mammalian cells has been shown to arise by the way of the recognition of the unmethylated CpG dinucleotides within a certain base context (CpG motif) present in bacterial DNA or synthetic oligonucleotides (ODNs) [2,3]. B cells [2], macrophages [4], natural killer cells [3,5], and dendritic cells [6] subsequently identified as direct cellular targets of CpG motifs require Toll-like receptor

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