GENERAL DISCUSSION
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*A. hydrophila* is a well recognized pathogen particularly of freshwater fish with an almost worldwide occurrence (Austin and Austin, 2007). However, the organism is not only associated with fish diseases, but has been implicated as possible cause of human gastroenteritis leading to diarrhea. In immunocompromised individuals, the organism may cause wound infections leading to septicaemia (Subashkumar *et al.*, 2007).

To prevent diseases caused by this organism, various measures have been adopted, of which the use of antibiotics is common one. To a lesser extent, vaccines have been considered, although the process of immunization and vaccine development is costly and relatively slow (Saitanu *et al.*, 1994 and Austin and Austin, 2007). Moreover, disease prevention in animals as food is slowly moving away from the dominant use of antimicrobial compounds as concerns about residues in tissues and the development and spread of antibiotic resistance have been increasingly voiced. Therefore, the adoption of immunoprophylactic agents in disease prevention and control has gained widespread acceptance especially as new and effective products enter the market place (Petrunov *et al.*, 2007 and Rajendiran *et al.*, 2008).

In addition, there is a widespread interest in probiotics and immunostimulants, both of which may enhance the immune state of the recipient animal (Leishman and Brundick, 2004). However, the use of live organisms as probiotics has raised concerns
about the possible acquisition of virulence factors due to plasmids. There is no evidence to prove the development of a pathogenic state in practice (Murray and Peeler, 2005 and Gibson et al., 1998). Justifiably, the use of immunostimulants remains an attractive option for use in disease control strategies in aquaculture. Certainly, their application is regarded as innovative in enhancing the innate defence mechanism of fish (Robertsen et al., 1994). As such, immunostimulants have been defined by Bricknell and Dalmo (2005) as “naturally occurring compounds that modulate the non-specific immune system by increasing the host resistance against diseases which under most circumstances are caused by pathogens”.

The kidney is an important haematopoietic organ in fish (Galindo-Villegas and Hosokawa, 2004). Yet, fish lack bone marrow and lymphoid nodes, which are present in their mammalian counterparts (Press and Evensen, 1999). Therefore, the kidney in addition to the spleen, the thymus and the liver serves as the site of formation of blood cells (Galindo-Villegas and Hosokawa, 2004). The posterior part of the kidney is concerned with blood filtration and excretory functions, whereas the anterior kidney, i.e. the head kidney, is the main site of blood cell differentiation involving the synthesis of immune cells (mostly monocytes) considered as immature macrophages (Takahashi, 2001). However, it is argued that monocytes represent the circulating macrophage population and should be considered as fully functional cell types (Stafford et al., 2001). The head kidney is composed of melanomacrophages and these are aggregates of macrophages, lymphocytes and plasma cells (Agius and Robert, 2003).
Melanomacrophages have been suggested to act in concerted efforts with the endothelial cells and spleen in trapping antigens from the bloodstream, and may play a role in immunogenic memory (Secombes et al., 1982). These all important immunocompetent organs in teleost fish have been shown to be influenced by dietary immunostimulants (Sakai, 1999; Dalmo et al., 2003; Galindo-Villegas and Hosokawa, 2004). In this study, the head kidney macrophages were found to be involved in phagocytic activity, respiratory burst and bacteriocidal activity. Phagocytic activity of the head kidney macrophage and other immune cells is an important defence mechanism against pathogenic organisms (Dalmo et al., 2003). Torrecillas et al., (2007) demonstrated high pathocytic activity in fish fed with 0.4% dietary mannan oligosaccharides (MOS). Results comparable to these also have been obtained in sea bass (Montero et al., 2005).

The emergence of antimicrobial resistance among bacteria is makes them among the most difficult bacteria to treat in aquaculture situations. As the efficacy of antibiotic therapy rapidly wanes, attention must be focused upon new approaches to controlling infection (Lim et al., 2003). Vaccines are one of the few protective measures that can potentially save money and improve output of farming operations. According to Klesius et al., (2000) research should be focused on determining the immunogenic components of bacteria and developing combination vaccines that may prevent future infections by mutated strains of the bacteria. The need for the development of alternatives to injectable and adjuvant based vaccines for example, orally and mucosally-delivered vaccines (Ototake and Yoshiura, 2000 and Nakanishi et al., 2002). The inductions of immune cells
against the optimal concentration of *A. hydrophila* were confirmed by comparing the several immunological assays in the normal fish. Immunomodulation to immune system was accessed directly by quantifying immunological factors that govern cell mediated and humoral immune response. All these tests indicate efficacy of immune complexes on immunomodulators. All immunoglobulin are antibodies and are produced in response to antigenic challenge. The synthesis of different types of immunoglobulin in normal fish and how far the heat killed pathogen and whole cell pathogen had impaired the immunoglobulin synthetic pathway were assessed. It can modulate the humoral and cell mediated immunity. Successful immunization requires the activation, replication and differentiation of T and B lymphocytes leading to the generation of memory cells. The vaccines in current use require multiple immunizations to maintain effective immunity. For a variety of bacterial and viral infections, there is a well defined threshold for the amount of antibody required for production. Live infections induce a greater frequency of antigen specific cells than immunizations with proteins and DNA vaccines encoding specific antigen.

The present study investigated different types of antigen produced from pathogen. It has also identified that the immune complex antigen as most suitable one for vaccine development against pathogens. Thus, in order for a vaccine to be effective, we must know when and where the virulence factors are expressed and utilize this knowledge when developing vaccine strategies. Aquaculture is an emerging industrial sector which requires continued research with scientific and technical developments and innovation. In particular, fish is a rich source of animal protein and its culture is an efficient protein food production system from aquatic environment (Rajakumar *et al.*, 2011).
A major challenge for health care in 21st century is the increase in level of resistance to effective vaccines by pathogens. Pathogens have been investigated for the potential as either therapeutic on prophylactic vaccines, in order to identify new antigens of diagnostic and vaccine potential. In the pathogen treated fishes, decrement in B-Lymphocyte was much pronounced in the first week compared to control. The present study, clearly confirms the decrement in B-cell number in fish exposed to whole and heat killed pathogens. Whole cell and heat killed pathogenic molecules have impact on the synthesis, proliferation and activation of lymphocytes. Gabel et al., (1997) and Dhasarathan et al., (2010) reported that differentiation of B-counts are affected by pathogens. Muller et al., (1997) reported that the immune suppressive drug inhibits cell proliferation and T-cell cytotoxicity. The enhancement in T and B-cell production is due to immune complex of antigens. The enhancement of this type of immune responses confirms the potential of immune complexes to be used as vaccines. Several workers such as Genestier et al., (1998) and Dhasarathan et al., (2010) reported that immuno enhancive drugs enrich cell proliferation. B cell proliferation modification depends on the exposure of antigens.

In the present investigation, heat killed bacterial antigen and whole cell bacterial antigen were tested for immunomodulation. The induction of immune cells against the optimal concentration of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, A. hydrophila*and *Klebsiella* were confirmed by comparing with the several immunological assays in the normal mice. Immunomodulation to immune system was accessed directly by quantifying immunological factors that govern cell mediated and
humoral immune response. All these indicate efficacy of immune complexes on immunomodulators.

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Successful immunization required the activation replication and differentiation of T and B lymphocytes were leading to the generation of memory cells. The vaccines in current use require multiple immunizations to maintain effective immunity. For a variety of bacterial and viral infections, there is a well defined threshold for the amount of antibody required for production. Live infections induce a greater frequency of antigen specific cells than immunizations with proteins and DNA vaccines encoding specific antigen. Thus, in order to manufacture an effective vaccine we must know when and where the virulence factors are expressed. These kinds of finding to be initiate develop newer kind of vaccine strategies.