Development of an idiotypic vaccine against Vibrio harveyi in grouper

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(1)Introduction

Aquaculture in Taiwan has become an important industry. Grouper, a farmed fish species with high economic value, has rapidly developed and become an important flagship product of Taiwan's top five high-quality agriculture. However, the grouper aquaculture is concentrated in the southern Taiwan and the intensification of fish culture has also been accompanied by the outbreaks of many infectious diseases, causing dramatic reduction in grouper production.

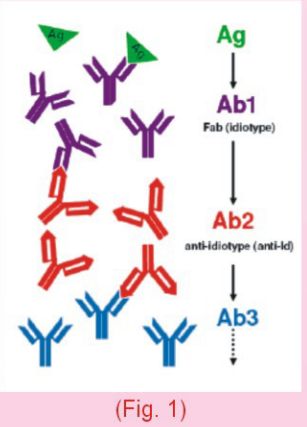
The grouper aquaculture has suffered several pathogens [1]. Among them, *Vibrio* bacteria leading to vibriosis are major bacterial pathogens causing a massive death of group and resulting in a significant economic loss [2]. According to the previous data in 2003 year, Gram-negative bacteria isolated from the grouper farms was 75%, in which the detection rate of *Vibrio* was up to 88% [3]. An additional data show that 73% vibriosis was detected from disease cases caused by Gram-negative bacteria in 2004 year [3]. In addition, *Vibrio harveyi*, mostly isolated from diseased grouper, has been described in numerous studies. Therefore, the impact initiated by *V. harveyi* in Taiwan grouper aquaculture must not be depreciated.

Antimicrobial substances, including tetracycline, oxytetracycline, oxolinic acid and flumequine, have been used to control vibriosis, but they have some drawbacks, such as emergence of resistant strains of pathogens and problems of food safety. Vaccination with effect vaccines is an alternative and safer strategy for controlling vibriosis in fish. Since *V. harveyi* bacteria uses multiple virulent antigens to maintain its infection, immunization with a multi-antigenic compound vaccine that induces a strong immune response to a broad array of *Vibrio* antigens is likely to be more effective than immunization with a single antigen.

(2)Design Concept

The Aim of This study is to use the immunological network theory proposed by Jerne in 1974 to develop a grouper idiotypic vaccine against *V. harveyi*. The immunological network theory concerning the immune surveillance system enables Jerne to win the Nobel Prize in 1985. The basic concept of the theory is described as below.

Generally, the immune system in the body has an ability to regulate and control itself in a stable status. For example, the first wave of antibodies, termed Ab-1, is initiated when an antigen enters into the host body (Fig. 1). Afterward, the immune system in the body also produces the second wave of antibodies, termed Ab-2, responsible for the Ab-1. The specific conformations presented by Ab-1 idiotype (Id), which is also called antigen-binding fragment (Fab), are considered as epitopes to result in the production of Ab-2, whose Fab is called anti-idiotype (anti-Id). Through such interactions between Id with anti-Id, a series of network recognition system is generated to become the main architecture of the immunological network theory.

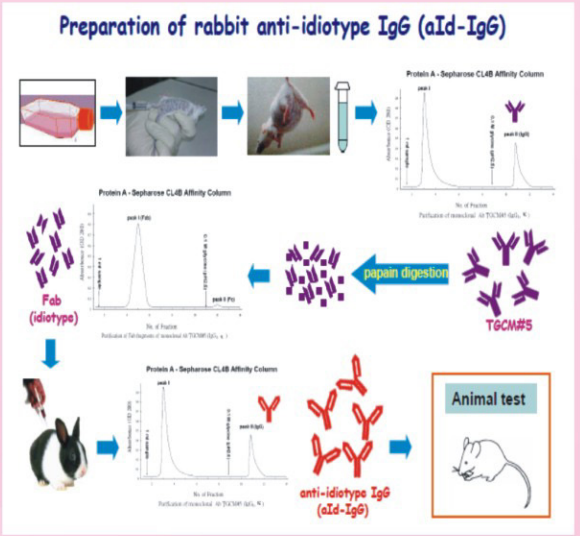


(Fig. 1)

The intensity of the immune response can be precisely regulated by the interaction of these antibodies. According to the complementary relationship presented by an antigen and an antibody, the Ab-2 anti-Id resembles the original antigenic epitope. In other words, anti-id of Ab-2 can show an internal image of the original antigen, thereby inducing an immune response against the original antigen. In addition, if more immunodorment epitopes are presented by Ab-2 anti-Id, induced immune responses are more specific and stronger. More importantly, the vaccine antigen prepared by the immunological network theory is not directly derived from the original pathogen so that the preparation of the idiotypic vaccine is safe for use in farmed animals. To our knowledge, however, the idiotypic vaccines are so far rarely applied in controlling aquatic diseases. In the present study, therefore, we make a brave attempt to use the immunological network theory to produce an idiotypic vaccine against *V. harveyi* in grouper.

(3)Technical Development

V. harveyi-infected sera from grouper infected with *V. harveyi* BCRC13812 strain purchased from Bioresource Collection and Research Center (BCRC) are obtained to collect the anti-*V. harveyi* antibodies by a protein A column. The resulting purified antibodies are used as the Ab-1, the first wave of antibodies. New Zealand rabbits are then subcutaneously immunized with the purified Ab-1 to produce the second wave of antibodies (Ab-2), anti-idiotype antibodies that possess the internal image of *V. harveyi* antigens. The purified Ab-2, the IgG portion of sera, is also collected by a protein A column and then used as the idiotypic vaccine in the present study[6]. So, such a vaccine can be designed and prepared by the simple procedures for protein purification. In the animal experiment, we examined the ability of idiotypic vaccine to induce an effective immune response compared to an inactivated vaccine. Fish immunized with idiotypic vaccine or inactivated vaccine elicit a humoral immune response (Fig. 3) [6]. However, only idiotypic vaccine can generate a 2-folded higher cell-mediated immune response than the inactivated vaccine and, more importantly, the enhanced response is maintained after the boosting immunization (Fig. 4) [6]. Finally, the immunized fish are challenged with the BCRC13812 *V. harveyi* strain (2.5×10^6 CFU / fish). The cumulative mortality rate of fish inoculated with idiotypic vaccine is 27.3%, while that of inactivated vaccine group is up till 54.5% (Fig. 5) [6]. Taken together, we predict that the powerful protective immunity against an experimental challenge observed in the idiotypic vaccine group is correlated to humoral and cell-mediated immune responses elicited by the idiotypic vaccine [6].



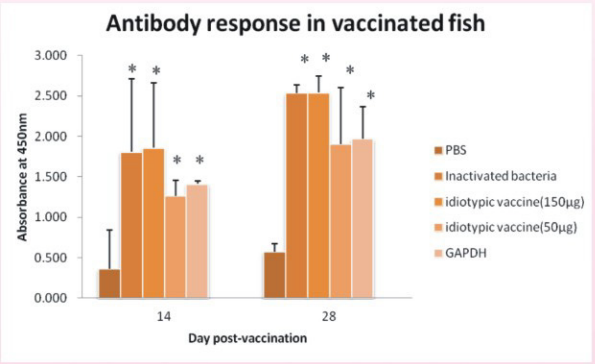
(Fig. 2)

(4)Technological Competitiveness

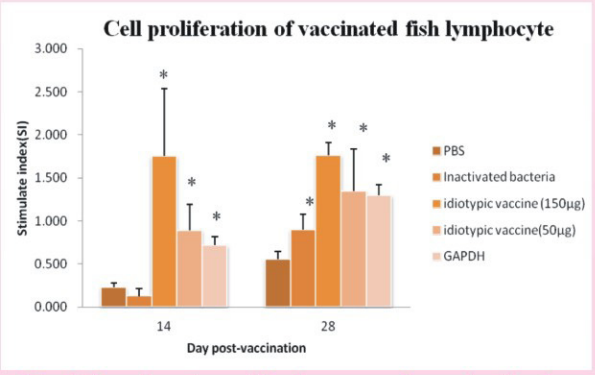
1.In the present study, the idiotypic vaccine prepared from animal antibodies does not contain components of the original pathogen. The situation eliminates biohazard concerns from the idiotypic vaccine.

2.Using a single virulence factor as a vaccine can not elicit a sufficient protection. Therefore, more virulence factors have to introduce into the vaccines prepared. Most of the previous procedures for producing idiotypic vaccines are based on monoclonal antibodies. However, idiotypic vaccines generated by these procedures only present the epitopes of a single antigen. In order to resemble all *Vibrio* proteins capable of inducing immunity, infected serum antibodies are being substituted for monoclonal antibodies and are used as Ab-1 for presenting epitopes of numerous virulence factors.

3.Clearance of pathogens in various fish infections is critically dependent on the cell-mediated immune response, which is essential for achieving an effective immune memory. Increase of the cellular immune response is an important point of the vaccine design. Results of this study show that using idiotypic vaccine can stimulate a specific cell-mediated immune response (T-cell responses) against *V. harveyi*. Therefore, the idiotypic vaccine prepared in the present study possesses the advantage described above.



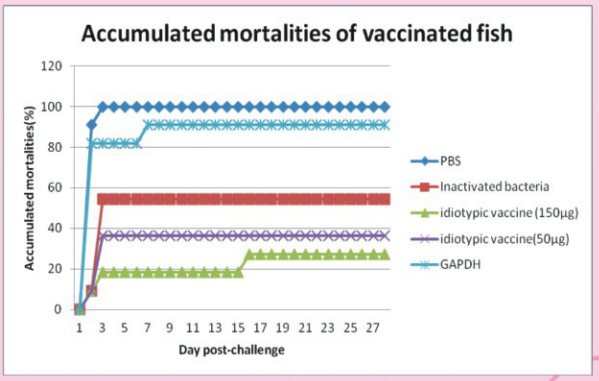
(Fig. 3) Antibody assay for evaluating the humoral immune response



(Fig 4) Lymphocyte proliferation assay for evaluating the cell-mediated immune response

(5)R&D Result

Overuse of conventional antibiotics induces the growing emergence of resistant strains of pathogenic microorganisms. Side effects are also usually observed in humans due to use of restrained chemical drugs for controlling fish diseases. Therefore, prophylactic vaccination is a most attractive strategy in the future. We predict that effective protection of idiotypic vaccine against *V. harveyi* in the present study will reduce overuse of conventional antibiotics. Most of the previous fish vaccines are prepared by formalin-inactivated bacteria. However, formalin treatment causes conformational changes of bacterial protein antigens so that formalin-inactivated bacteria induce insufficient protection as shown in our study. In addition, the idiotypic vaccine truly reflects the internal images of all antigens capable of inducing an immune response, thereby resembling whole *V. harveyi* antigenic epitopes to induce an effective immune response. A strong cell-mediated immune response can be observed in grouper immunized with the idiotypic vaccine. This indicates that the idiotypic vaccine appears to can be designed as a single-dose vaccine without the need for booster doses. Although the feasibility of a single-dose vaccine needs to be assessed in future studies, this possible potential derived from the idiotypic vaccine makes us excited.



(Fig. 5) Cumulative mortality rates of immunized grouper.

Acknowledges

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