Past, current, and future trends in fish vaccines development

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Trend in aquaculture production

Global aquaculture production in million tonnes, 1950-2010, FAO

Intensification of fish production and consequences

- Maximize production
- Limited space
- High density
- Complete diet
- High water exchange

Fish diseases

- Common aquatic diseases in Thailand (1999)

What causes fish disease?

1. Environmental disorders:
   - Temperature
   - Oxygen level
   - pH
   - Environmental toxins e.g. NH₃, NO₂⁻, heavy metals, chlorine, chloramine
   - Fish farming condition
   - etc.
2. Heredity and individual defects
3. Pathogens: Bacteria, parasites, virus

What causes fish disease?

Fish diseases are the result of interaction between a pathogen, a fish (host), and a stressful environment (Figure 1). Even if the pathogen is present, a disease outbreak will not occur unless the environment becomes too stressful for the fish (Figure 2).
Control of fish diseases

1. Husbandry
2. Chemotherapy and chemoprophylaxis
3. Vaccination

Possible consequences of fish diseases intervention by medications

The use of antibiotics in fish farming is associated with new hazards in fishery products that are not encountered in wild captured species. The main hazards are antibiotic residues and the development of antimicrobial resistance in bacteria that may be transferred to consumers of farmed fish.

Chemoprophylaxis

Advantages:
- can provide a useful means of helping to control some diseases e.g. bacteria and parasites (both curative and preventive measure, independent of physiological status)

Disadvantages:
- have no direct effect to viral infection
- residue problems and increasing consumer concern
- development of drug resistance among bacterial pathogens
- work only as long as they are present in appropriate concentration in the target organ

Diseases prevention: vaccination vs chemoprophylaxis

Vaccination:

Advantages:
- a preventative measure
- can act against bacterial, viral and, at least experimentally, parasitic infections and they will usually act specifically against only the targeted pathogens

Disadvantages:
- vaccines are not “cure all” remedies but are an integral part of comprehensive health management programs

Vaccine development: from idea to licensed product

Ideally, a vaccine should be:
1. Safe for both the fish, the administrator and the consumer
2. Have a broad strain or pathogen coverage
3. Provide 100% protection
4. Give a long-lasting protection, preferable as long as the production cycle
5. Be easy to apply
6. Be applicable in various species
7. Be cost effective and
8. Be readily licensed or registered.
Vaccine development

- Of principal importance in the entire vaccine development process is the precise identification of the causative organism, including the existence and significance of serotypes, and a full understanding of the epidemiology of the disease.
- Knowledge on the prevailing diseases, their economic significance and the pathogens associated are key information required to support a vaccination program.
- Unfortunately such information are still lacking for most pathogens involved.
- Far too often, disease outbreaks are described based on disease signs and not on the isolation and characterization of the pathogen.

Fish diseases or their causative agents against which the development of vaccines has been investigated (Ellis, 1988)

- Bacterial diseases
  - Aeromonas hydrophila
  - Bacterial kidney disease (Renibacterium salmoninarum)
  - Edwardsiella tarda
  - Edwardsiella ictaluri
  - Vibrio salmonicida (cold water vibriosis)
- Viral diseases
  - Infectious pancreatic necrosis (IPN)
  - Viral haemorrhagic septicemia (VHS)
  - Infectious haematopoietic necrosis (IHN)
  - Channel catfish virus
  - Spring viraemia of carp (SVC)
- Parasites
  - Ichthyopthiriasis
  - Costiasis
  - Trypanosomiasis
  - Diplostomiasis

Commercially available vaccines

- Bacterial diseases
  - Yersinia ruckeri
  - Aeromonas salmonicida
  - Vibriosis (multiple strains and species)
  - Edwardsiella ictaluri (Enteric septicemia of catfish)
  - Morantella viscosus
  - Flavobacterium columnare
- Viral diseases
  - Infectious pancreatic necrosis
  - Infectious salmon anemia
  - Infectious haematopoietic necrosis
  - Koi herpes virus
- Parasites
  - Piscirickettesiae

Successful vaccination and the reduction of antibiotics usage

Fish vaccines development: in the past and current

- In the past and present, vaccines have relied on the isolation of fish whole pathogens
- Of the types currently in use, the most common are described below:
  - Killed or inactivated vaccines
  - Live, attenuated vaccines
  - Toxoids (inactivated toxins)

Some methods of producing killed and attenuated vaccines

**Inactivation**
- Heat
- Formalin
- Other "micro-toxins" E.g. ß-propiolactone
- Pressure (French press)
- Irradiation

**Attenuation**
- Non-specific:
  - Mutagen culture passing
  - Natural strains
  - Aging cultures
- Specific
  - Genetically-engineered

Fish vaccines development: in the past and current

**Killed vaccine**

**Advantages**
- Safe and generally quick to develop
- Most common in human vaccines
- Usually excellent protection if humoral response is primary

**Disadvantages**
- Little residual effect
- Adjuvant generally needed
- Inactivation substance can be a concern
- May not be seen like live organism to immune system
- Not great for stimulation of cell-mediated response

**Live, attenuated vaccine**

**Advantages**
- Mimics natural infection and immune response

**Disadvantages**
- Danger to reversion to virulence
- Shelf-life, storage conditions and reviving can be critical to efficacy
- Adjuvant difficult
Fish vaccines development: trend in the future

Current new type of vaccine

1. Subunit vaccine
2. DNA vaccine

Subunit vaccine

- Subunit vaccines are defined as those containing one or more pure or semi-pure antigens. In order to develop subunit vaccines, it is critical to identify the individual components out of a myriad of proteins and glycoproteins of the pathogen that are involved in inducing protection.
- Indeed, some proteins if included in the vaccine may be immunosuppressive whereas in other cases immune responses to some proteins may actually enhance disease.
- Thus, it is critical to identify those proteins that are important for inducing protection and eliminate the others.
- Combining genomics with our understanding of pathogenesis, it is possible to identify specific proteins from most pathogens that are critical in inducing the immune responses.

The potential advantages of using subunits as vaccines are the increased safety, less antigenic competition, since only a few components are included in the vaccine, ability to target the vaccines to the site where immunity is required, and the ability to differentiate vaccinated animals from infected animals (marker vaccines).

One of the disadvantages of subunit vaccines is that they generally require strong adjuvants and these adjuvants often induce tissue reactions. Secondly, duration of immunity is generally shorter than with live vaccines.

Identification of *P. salmonis* proteins as potential vaccine candidates

1. Cloning and expression of recombinant *P. salmonis* proteins
   - Cloning of *P. salmonis* antigen coding regions
   - Isolation of genomic DNA from *P. salmonis*
   - Predicted coding regions of selected antigens were isolated by PCR amplification
   - PCR products were ligated to pGEMT and used to transform Novoblue competent cells
   - Positive clones were selected by blue/white screening using lacZ α-complementation
   - DNA analysis and sequencing

2. Expression of the selected proteins in *P. salmonis*

Recombinant vaccine trial

Analysis of immune responses of salmonids against recombinant proteins

http://www.britannica.com/EBchecked/media/128149/
A genomic DNA library is a collection of DNA fragments.
https://www.fishersci.ca/browseResults.do?cid=2643427
Subunit vaccine

3. Expression of the selected proteins in P. salmonis

It was of interest to study if the antigen candidates are expressed by the pathogen during infection. Therefore extracts of P. salmonis growing in CHSE-214 cells were analyzed by Western blot for the presence of the native proteins. Specific monoclonal antibodies were used to detect the presence of the selected proteins in these extracts.

4. Recombinant vaccine trial

Three different vaccine formulations:

- **Vaccine 1**: Trx-Hsp70 (a) Trx-Hsp60 (b) Trx-FlgG (c)
- **Vaccine 2**: Trx-TbpB-N (d) Trx-MltB-C (e) Trx-MltB-N (f)
- **Vaccine 3**: Trx-FlaA-C (g) Trx-Omp-C (h)

5. Analysis of immune response of salmonids against recombinant proteins

DNA vaccine

- The most recent development in vaccinology is immunization with polynucleotides. This technology has been referred to as genetic immunization or DNA immunization.
- The basic idea of this approach to immunization is that cells can take up plasmid DNA and express the genes within the transfected cells.
- Thus, the animal acts as a bioreactor to produce the vaccine.
- This makes the vaccine relatively inexpensive to produce.
- Some of the advantages of polynucleotide immunization is that it is extremely safe, induces a broad range of immune responses, cellular and humoral responses, long-lived immunity, and most importantly can induce immune responses in the presence of maternal antibodies.
- Although this is one of the most attractive developments in vaccinology, there is a great need to develop better delivery systems to improve the transfection efficiency in vivo.
DNA vaccine

Methodology

1. Cells and bacterial strains
2. Plasmid construction
3. In vitro transfection of recombinant plasmid pEGFP-N1/m-empA7; in cell lines
4. Detection of m-empA7 expressed in transfected eukaryotic cells
5. Prechallenge of V. anguillarum W 1 in Japanese flounder
6. Immunization and experimental challenge
7. Detection of injected DNA in fish tissues
8. Detection of in vivo transcription of m-empA7 gene by RT-PCR
9. Detection of in vivo expression of m-empA7 by Western blot
10. Analysis of antibody response
11. Statistical analysis

DNA vaccine: Methods and results

Mutated pET24d(+)/m-empA7 (Glu374Lys mutation) was inserted in frame with EGFP reporter gene of pEGFP-N1 to make plasmid pEGFP-N1/m-empA7 (recombinant plasmid). Gene cloning, isolation of plasmid, DNA sequence and PCR amplification was done using primers: (Glu374, CCGCAGCAGCAATCGTCCGC; Lys374, GGGTAGTAGGGTTTCAGTCT). PCR products were cloned into pET24d(+) vector and sequenced. Green fluorescence was observed in eukaryotic cell lines (CHO and HEK293T) transfected with pEGFP-N1/m-empA7. pEGFP-N1 was used as negative control.

In vitro transfection of pEGFP-N1/m-empA7 in cell lines

Chinese hamster ovary (CHO) and human embryonic kidney (HEK293T) cell lines were seeded in 6-well plates at 75-80% confluency. Purified pEGFP-N1/m-empA7 was transfected to cell lines. pEGFP-N1 was used as negative control (4 µl of plasmid in 100 µl transfection buffer). Expression of EGFP was determined by SDS-PAGE and observation of green fluorescence from the EGFP fusion protein was done under a fluorescent microscopy.

In vivo evaluation of m-empA7 as immunogen

18 Japanese flounders (9 g BW) were randomly divided to 3 groups. Bacterial suspension (0.1 ml) was injected (IP) in fish (3.5x10^7, 3.5x10^6, 3.5x10^5 cfu ml^-1). Disease progress was followed for 14 days. Randomly sampled dead fish were recorded in order to determine median lethal dose (LD50). LD50 = 3.5x10^7 cfu ml^-1.
DNA vaccine: Methods and results

In vivo evaluation of m-empA7 as immunogent

**Immunization and experimental challenge**

- **70 fish** (mean weight = 9.38 g) were divided to 5 groups
- **5, 20, 50 µg of p-EGFP-N1/m-empA7**
- **50 µg of p-EGFP-N1**
- 0.1 ml of PBS
- Immunization (IM)
- 4 wks later
- Each group was challenged with *V. Anguillarum* (3.5x10^7 cfu ml^-1)

**Cumulative mortality and CS were recorded daily until the mortality progression decreased in all groups at 10 dpc**

**Relative percent survival (RPS)**

Survived fish were collected and examined for histopathological changes.

**Muscle tissue**

Genomic DNA

Total RNA

Extracted protein

**sera were collected (protective efficiency at different day post vaccination)**

**PCR**

RT-PCR

Western blot

In direct ELISA

Ab response to DNA vaccination

Persistence of p-EGFP-N1/m-empA7 in muscle tissue

In vivo transcription of m-empA7 gene

In vivo expression of m-empA7 gene

Ab response to DNA vaccination
Thank you for your attention

Questions and suggestions are welcome.