Frontiers in Aquaculture, Pages 319–330
Edited by: Sundaray, Sukham, Mohanty and Otta

20

RECENT ADVANCES IN VACCINE DEVELOPMENT IN AQUACULTURE

Sujeet Kumar, R. Ananda Raja and P.S. Shyne Anand

Kakdwip Research Center
Central Institute of Brackishwater Aquaculture, South 24 Paragans, West Bengal-743347

INTRODUCTION

Recent techniques of intensive and super intensive culture practices have substantially enhanced the incidence of disease outbreaks to rapidly growing aquaculture industry. This has resulted in severe economic losses through mortality and growth retardation of aquatic animals. Infectious agents such as bacteria, viruses, fungi & parasites are responsible for these outbreaks in culture systems. Therapeutics and prophylactics play pivotal role in the disease control of human and animals. But, the scenario is completely different as far as aquatic animals are concerned. Use of chemicals and antibiotics for the treatment of aquatic animals often prove harmful, as it lead to development of antibiotic resistant strains apart from imparting a serious risk to human health and sustainability of aquatic environment itself. In comparison, prophylactic measures like vaccination, control infectious diseases cost effectively and reduces indiscriminate use of antibiotics. For example, In Norway, introduction of oil adjuvant vaccine against Furunculosis decreased the antibiotics application from 47 tons to around one ton (Markestad and Grave, 1997). Vaccination or immunoprophylaxis is based on the principle that when an animal's immune system encounters a pathogen it gets primed to respond against same pathogen later. This is referred as memory immune response or adaptive immunity.

The first commercial bacterial vaccine for aquaculture was developed against Enteric Redmouth (ERM) followed by Vibriosis and Furunculosis while the first viral vaccine was produced against spring viremia of carp (SVC) in 1982 (Sommerset *et al.*, 2005). The first adjuvanted vaccine was prepared against *Aeromonas salmonicida* to improve its immunogenicity (Krantz *et al.*, 1963). First live vaccine was licensed against bacterial kidney disease using *Arthrobacter davidneili* as attenuated strain (Salonius *et. al.*, 2005), while, first DNA vaccine came in aquaculture industry against Infectious Hematopoietic Necrosis (IHN) Virus in 2005 (Meeusen *et al.*, 2007). Detailed history of vaccination has been reviewed by Muiswinkel (2008).

Type of Vaccine

Success of any vaccination program depends upon types of vaccine available, its efficacy, safety, duration of immunity as well as its final cost. Currently, in aquaculture five types of vaccines are available (Hanson, 2000).

- Killed Vaccine
- Live Vaccine (Attenuated vaccine)
- Subunit Vaccine
- DNA Vaccine
- Inducers of non-specific defence (Immunostimulants)

Killed Vaccine

This is the most commonly used vaccines in aquaculture. Heat or formalin is commonly employed for inactivation of bacteria, while, formaldehyde, glutaraldehyde, binary ethylenimine and â-propiolactone are used as inactivating agent for virus.

Killed vaccine is very effective in inducing humoral antibody response, but is less effective in stimulating cell mediated immunity or inducing a mucosal response. Storage and safety are of less concern, as it is killed and cannot revert to virulence. The common drawbacks are need of adjuvants and sometimes booster dose is required to prime the humoral immune response. Another concern is the presence of inactivating agents which may alter the form of a critical antigen, and therefore reduce vaccine effectiveness. Also, against intracellular pathogen (like a virus), the killed vaccine will not induce as strong a cell-mediated immune response as would a live vaccine.

Successful commercially available killed vaccines include vaccines for enteric red mouth disease for salmonids, furunculosis for salmon, mono- and multivalent *Vibrio* vaccines for salmon and other marine fish and spring viremia of carp (Hanson, 2000)

LIVE VACCINES

Live vaccines or attenuated vaccines are generally mutated strains of an infectious agent that have a reduced or no ability to cause disease. These vaccines replicate in the host. Thus, they produce long lasting immunity even in small doses. And, this is particularly effective in stimulating cellular immunity and inducing a memory response. Cellular immunity is well desired for protection against intracellular pathogen such as viruses. The major disadvantages are capacity of vaccine strains to revert to a virulent form and potential to cause disease in immuno compromised host. By methods of genetic engineering now it is possible to irreversibly attenuate the microbes by removing the virulent genes. Other major challenge with the live vaccines is risk of transmission to non-farmed fish in the surrounding water for which the vaccine may be virulent. As live vaccine must be kept alive, improper storage or application can result in vaccine failure.

First live vaccine was licensed against bacterial kidney disease using related bacterium *Arthrobacter davidneili*. Live vaccine is also available against Edwardsillosis (ESC) using Rifampicin resistant *Edwardsiella ictaluri* strain (RE-32 strain) (Klesius *et al.*, 2006) and aroA and purA deficient *E. Ictaluri* strain (Thune, 2002).

Subunit Vaccine

Subunit vaccine includes immune response against the purified protein, synthetic peptides and recombinant protein (recombinant vaccine). For the subunit recombinant vaccines, the gene(s) encoding a particular antigen, which must be immunogenic, are cloned and subsequently introduced into a permissive host, e.g. bacterium, yeast or insect cells which then synthesize the recombinant antigen. Gilmore *et al.* (1988) were the first to exploit *Escherichia coli* for expression of the IHN virus glycoprotein. Several groups investigated baculovirus system to synthesize recombinant IHN or VHS virus G protein in insect cells (Cain *et al.*, 1999). The rationale for using this system is that insect cells have the ability to glycosylate proteins which generate better immune response compared to unglycosylated protein.

These types of vaccines have the benefit of being well-defined, non-infectious and inexpensive to produce in large quantities. Also, the differentiation of vaccinated from potential carrier fish is possible by serological tests, because fish is not exposed to the entire array of antigens that a pathogen expresses. Recombinant antigen vaccines are particularly useful for pathogens which are difficult to propagate, such as viruses, Piscrickettsia or *Renibacterium salmoninarum*. Disadvantages are similar to killed vaccine. Larger dose, need for booster immunization, shorter duration of immunity, need of adjuvant and vaccine delivery system and inefficiency in inducing cell-mediated immune responses are some major disadvantages.

Licensed recombinant vaccine for fish is available for infectious pancreatic necrosis (IPN) in Norway using VP2 gene of the virus (Frost and Ness, 1997). Glycoprotein of infectious hematopoietic necrosis (IHN) and viral hemorrhagic septicemia (VHS) virus have shown good results in experimental trials (Lapatra, 2004).

DNA Vaccines

For a range of viral diseases in aquaculture, there is no efficient vaccine available based on either killed or live attenuated or recombinant protein. DNA vaccines hold good prospects to protect fish and shrimp against such viral diseases (Myhr and Dalmo, 2005). DNA vaccination involves injection of naked DNA directly into the skeletal muscle of the fish where it is expressed extra chromosomally. Many methods are employed for enhancing cell uptake of DNA vaccine, such as gene gun, electroporation, encapsulation of DNA in liposomes or polylactide-L-glycolide (PLG) microparticles (Babiuk, 2008). Incorporation

of sufficient and appropriate CpG motifs, cytokines, defensins, or other immune stimulator molecules to plasmid expressing DNA vaccine creates a cytokine microenvironment conducive for developing an adaptive immune response.

Advantages of DNA Vaccine

Easy to manufacture: DNA vaccine is comprised of a plasmid containing various regulatory elements such as an origin of replication, a selectable marker and the gene of interest under a strong promoter. This single platform makes DNA vaccines very attractive from the prospective of manufacturing. Because, once the protocol is standardised for one plasmid-based vaccine, the same process can be used for production and purification of a variety of different vaccines.

Safety: DNA vaccines are considered safe since it lack extraneous materials and are non-infectious. Initially, there was concern regarding the potential of the introduced plasmids to integrate into somatic or germ line cells. However, studies have suggested that the probability of this occurring is 3 times lower than spontaneous mutations (Kanellos, 1999). There was another concern that introduction of large amounts of DNA might lead to the generation of anti-DNA antibodies. But, a number of studies designed to test this possibility have demonstrated that this is likely to be a rare event if it would occur at all (Mor *et al.*, 1997). Thus, it is widely believed that DNA-based vaccines are relatively safe.

Multicomponent vaccines: Combining many plasmids encoding different genes of interest (like four plasmids encoding the four different viral genes in Dengue fever) or introduction of two different genes in a single plasmid, encoding two different proteins either from the same or different pathogens is possible (Hew and Fletcher, 2002). This paves the way for vaccination against many diseases at single time in single stroke.

Vaccine Dose: The majority of fish DNA vaccines have been tested at single doses ranging from 5 μg to 50 μg. For, Fish rhabdoviruses such as Infectious hematopoietic necrosis virus (IHNV), Viral hemorrhagic septicemia virus (VHSV) and Hirame rhabdovirus (HIRRV), a single intra muscular injection of a 1.0 μg with no adjuvant or boosters, was found sufficient to provide a high level of protection (Kurath, 2008).

Immune Response: DNA vaccine is capable to induce both humoral and cellular immune response. It is also potent to induce immunity in neonates in absence as well as presence of maternal antibodies.

Disadvantages of DNA Vaccine

Sub-optimal immunity: Possibly the greatest challenge for adopting DNA vaccination as a routine is the poor efficiency of transfection, leading to suboptimal induction of immunity. Due to this reason, DNA vaccine currently employed to prime the immune system followed by protein boost.

Licensing of DNA vaccines: Commercial development of a DNA vaccine is very difficult due to public perception, who confuse it with genetically modified organisms, and stringent law. For instance, Norwegian Directorate for nature management has stated that a DNA vaccinated animal is to be considered as genetically modified as long as the added DNA is present in the animal (Fos and Rogne, 2003).

Environmental release: In aquatic environments, vaccinated DNA may be distributed to vast areas and phyla as a result of the relative lack of physical and physiological barrier. In addition, DNA is much more resistant to breakdown in ecosystem so may prove hazardous (Tappeser *et al.*, 2002).

Recently, a DNA vaccine was licensed to immunize fish against infectious hematopoietic necrosis virus for commercial use in Canada (Babiuk, 2008). Among many viral diseases of fish tried, the DNA vaccines against the Glycoprotein (G protein) of salmonid rhabdoviruses, IHNV and VHSV, remain the most efficient and also the most extensively studied (Lorenzen and LaPatra, 2005).

Immunostimulants

Immunostimulants enhances the ability of aquatic animals to resist infection by the activation of cellular and humoral arms of innate immune system. Many substances such as β-glucan and other cell wall componenet of yeast, chitin, lipopolysacharide of gram negative bacteria, peptidoglycan of gram positive bacteria, lactoferrin, levamisole, and nutritional factors like vitamins B and C, growth hormone and prolactin are immunostimulatory because of their direct positive influence on non-specific immune elements. Nucleotide supplementation in fish diet was observed to protect against many infectious agents (Burrells *et al.*, 2001).

Methods of Vaccine Delivery

Three different methods of administration are commonly used to vaccinate fish, namely injection, immersion and oral.

Injection Administration

Vaccines can be delivered either by intramuscular or intra peritoneal injection. Injection administration elicits a good immune response in vaccinated fish which is better than that obtained with immersion or oral vaccination (Evelyn, 1997). This happens because maximum amount of vaccine is retained in the body. However, procedure is more labor-intensive and stressful to fish compared to other two methods of administration. Also, injection vaccination is suitable only for larger fishes like broodstock and not fit for fish below 15 grams.

Immersion Administration

Vaccination by immersion (and also spraying) provides intermediate levels of protection compared to injection and oral administration (Evelyn, 1997). The method is less labor intensive and less stressful to fish compared to injection method. It can easily be used to vaccinate small fish, while larger fish can also be vaccinated by spraying.

Lateral line, gills, gut and skin are the antigen uptake sites in immersion vaccination. Duration of immunity and antigen uptake is largely dependent upon antigen concentration and duration of immersion. Dos Santos *et al.*, (2001) observed that immunity generated by immersion vaccination is of mucosal type with little systemic immune response.

Oral Administration

Oral administration is "the ideal method" for administering vaccines to fish where the vaccine is incorporated into fish feed. It is least labour-intensive, avoid handling stress and can be used to vaccinate large numbers of fish of all sizes. This way of vaccine delivery may not be fruitful for sick fishes which refuge feed. The major disadvantage is lower levels of protection with shorter duration which may be due to degradation of antigen by the gastric fluid in the stomach and anterior gut of the fish and inefficient transport of antigen across the gut wall. Microencapsulation of the antigen is used to protect the antigen. Bio encapsulation using live artemia has also shown good results (Gomez-Gil *et al.*, 1998).

Adjuvants

Adjuvants are substances that are used in combination with a specific antigen to provide more immunity than the antigen used alone. Adjuvants have been used extensively in human and veterinary vaccines to strengthen the immune response of weak antigens and to increase the duration of protection. Its role is critical for enhancing immune response against killed vaccine, recombinant vaccine and DNA vaccine. Aluminum salts, mineral oil, Freund complete adjuvant (contain killed *Mycobacterium phlei*) are commonly employed adjuvants. Muramyl dipeptide (MDP) from *Mycobacterium tuberculosis*, Lipopolysaccharide of gram-negative bacteria, peptidoglycan, and â-glucan are also observed to enhance the immune response in fish.

Adjuvants work either by depot effect, emulsified antigen in oil droplets gradually release the antigen over long period of time, or by causing inflammation at the site of injection leading to attraction of leucocytes and better antigen presentation to lymphocytes or by creating the required cytokine micro environment.

Initially, adjuvanted vaccines were composed of water-in-oil emulsions (antigen exist in aqueous droplets and dispersed throughout the oil phase), using mineral oil. Water-in-oil emulsion is viscous and poses difficulty in injection. Also, there is a chance of breakdown, if not emulsified properly, leading to incorrect dose of antigen administration and vaccine

failure. Higher levels of mineral oil increases the risk of side effects such as granuloma at the site of injection, adhesions within the abdominal cavity and melanin deposition (Smith, 2001). Use of alternative oil based adjuvant systems such as oil in water emulsion (antigen is contained in oil droplets, dispersed throughout an aqueous phase) and vegetable oil reduces the side effects seen with mineral oil adjuvants.

Current Status of Vaccine Against Bacterial Diseases of Fish

Licensed vaccines are commercially available against many bacterial pathogens such as *Aeromonas salmonicida, Photobacterium damselae* subsp. *piscicida, Vibrio anguillarum* (serotypes 1 and 2), *V. ordalii, V. salmonicida, V. viscosus (Moritella viscosa), Yersinia ruckeri, Flavobacterium columnare, Edwardsiella ictaluri, Streptococcus iniae and Lactococcus garvieae* (Thompson and Adams, 2004). Many of these vaccines are multivalent, offering protection against a variety of pathogens.

Vibriosis: Vibriosis is considered as one of the most economically devastating diseases in aquaculture. The most significant *Vibrio* species to cause disease in aquaculture includes *Vibrio anguillarum (Listonella anguillarum), V. ordalii, V. salmonicida* and *V. vulnificus* biotype 2. Potent killed vaccines are commercially available against Vibriosis. Most vaccines include only serotype *Vibrio anguillarum* O1 in their formulations or a mixture of serotypes O1 and O2á. Different polyvalent oil-adjuvanted vaccines including different combinations of *V. anguillarum* with other pathogens such as *Vibrio ordalii, Vibrio salmonicida, Aeromonas salmonicida,* and infectious pancreatic necrosis virus (IPN) are also available.

Pasteurellosis: Pasteurellosis or photobacteriosis is caused by the halophilic bacterium *Photobacterium damselae* subsp. *piscicida* (formerly *Pasteurella piscicida*). Many commercial vaccines against *Ph. damselae* subsp. *piscicida* are available in the market and their efficacy is dependent on the fish species, fish size, vaccine formulation and use of immunostimulants. Recently, a toxoid enriched-whole cell bacterin, DI vaccine, has been patented by the University of Santiago (Hipra Laboratories, Spain).

Furunculosis: *Aeromonas salmonicida* is an important fish pathogen causing significant economic losses to aquaculture worldwide. Oil-adjuvant vaccine is commercially available which appear to give a long lasting protection against the disease, but its side effects such as induced formation of granulomatous lesion, adhesion to the viscera and reduction in weight gain are main constraint (Midtlyng *et al.*, 1996). To avoid these drawbacks non mineral oil based vaccine is now available in the market. Polyvalent vaccines including different *Vibrio* species and *A. salmonicida* as antigens are also available for salmonids, which seems to be more effective than monovalent furunculosis bacterins.

Bacterial Kidney Disease: The causative agent of bacterial kidney disease (BKD) is *Renibacterium salmoninarum.* Whole cell inactivated *Renibacterium salmoninarum* does not produce consistent level of protection because, the bacteria express large amount immunosuppressive protein, p57, on its cell surface. Recently, a live vaccine for bacterial kidney disease has been licensed in Canada using *Arthrobacter davidanieli*, a non-pathogenic Gram-variable bacterium related but taxonomically distinct from *Renibacterium*

salmoninarum (Salonius, 2005). A protection up to 23 months was recorded by long term field trials in Atlantic salmon. The same vaccine strain also proved effective against piscirickettsiosis in experimental challenge as well as field trial in coho salmon (*Oncorhynchus kisutch*) in Chile. The vaccine strain is unique in that it is the first live organism to be licensed as a vaccie for use in aquaculture.

Edwardsiellosis: Two major species of bacteria are responsible for Edwardsiellosis in fish, namely, *Edwardsiella tarda* and *E. ictaluri.* Vaccine containing *E. ictaluri* whole-cell bacterin is less effective. A live vaccine based on Rifampicin resistant *E. ictaluri* isolates (RE-33 strain) has been licensed in USA. Single immersion of this vaccine stimulates strong acquired immunity against many isolates of *E. ictaluri* without the need for booster immunization (Shoemaker, 1999). Another live-attenuated vaccine has been developed against *E. ictaluri* by deletion mutation in virulent aroA gene, the purA gene, or both (Thune, 2002). The interest also lies to use these mutated *E. ictaluri* strains as vectors to present antigens from other fish pathogens.

Yersiniosis: Yersiniosis *or e*nteric red mouth (ERM) is caused by *Yersinia ruckeri.* This represents one of the first fish disease, which was successfully controlled with commercial vaccination. Most vaccines are bacterin preparations using whole cell preparations of serovar 1 (the Hagerman strain and the major cause of disease outbreaks). Bacteria are generally inactivated with formalin and sometimes pH lysed (pH 9.8).

***Aeromonas Hydrophilla*:** Diseases caused by *A. hydrophila* (hemorrhagic septicemia, fin-tail rot, and epizootic ulcerative syndrome) have a major impact in aquaculture. At present, no commercial vaccines for the protection of farmed fish against *A. hydrophila* infections are available. The production of commercial vaccine is hampered by great phenotypic and serological heterogeneity existing within the group of mesophillic motile Aeromonas species.

Experimental biofilm vaccine for oral vaccination in Indian major carps was formulated. The glycocalyx of the biofilm vaccine is believed to protect the antigen against destruction in the gut, thus facilitating its transport in intact condition to the immune responsive areas (Azad *et al.,* 2000). The *aroA* gene deleted mutants attenuated live vaccine have also been prepared and evaluated in various species of fish (Moral, 1998).

Current Status of Vaccines Against Viral Diseases

Lack of effective viral vaccines is one of the major constraints of aquaculture industry. But, in last five years some major breakthrough in recombinant and DNA viral vaccines have taken place which has raised great hope for control of viral diseases.

Infectious Hematopoietic Necrosis (IHN) Virus: IHN virus is a member of the family *Rhabdoviridae* and the genus *Novirhabdovirus*. This virus has the distinction, as it is first microbes against which a DNA vaccine is licensed. This DNA vaccine was licensed in 2005 in Canada to protect Atlantic Salmon and being marketed by Novartis (Aqua Health) in the brand name of Apex-IHN (Meeusen *et al.,* 2007). This vaccine encodes surface glycoprotein and administered intramuscularly.

Viral Hemorrhagic Septicemia Virus: Glycoprotein based recombinant and DNA vaccines have been constructed and have shown very good results on experimental trial (Lorenzen *et al.*, 1999; Lapatra, 2004). A small-scale field-testing of a VHS DNA vaccine was performed in Denmark in 2004-2005 with rainbow trout. Although a high cage-to-cage variability was seen, the overall result indicated that the vaccine was effective. The experimental fish were not approved for human consumption and therefore vaccine was not licensed (Salonius, 2007).

Infectious Pancreatic Necrosis Virus: Vaccines based on inactivated virus as well as recombinant antigens are commercially available. Most IPNV vaccines exist as polyvalent oil adjuvant vaccine where IPNV antigen is mixed with several bacterins; this appears to improve the efficacy compared to monovalent vaccine (Sommerset, 2005). The first commercial recombinant vaccine in aquaculture was introduced against IPNV in Norway in 1995. The vaccine is based upon VP2 viral protein and has come into use throughout the industry.

Viral Nervous Necrosis: The disease also known as viral encephalopathy and retinopathy, a devastating disease of many marine cultured species worldwide, caused by betanodavirus. No commercial vaccine against nodavirus is currently available (Thiery, 2006). Partial protection has been observed by recombinant vaccine or DNA vaccine using viral coat protein construct. Recently, viral like particles (VLPs) were constructed in baculovirus expression system, which showed good protection (Thiery, 2006).

White Spot Syndrome Virus in Shrimp (WSSV): White spot syndrome virus and other viral diseases became serious threat for shrimp industry since ninety. Crustaceans largely depend upon the innate arm of immune system, comprising hemocytes (blood cells) and humoral factors, for protection against pathogens. It is generally thought that shrimp lack the immunoglobulin based adaptive immune system (Kimbrell and Beutler, 2001). However, studies on *Penaeus japonicus* shrimps exhibited the presence of 'quasi-immune response' in which naturally or experimentally WSSV survived animals showed the resistance to subsequent challenge (Venegas, 2000). Also, Plasma from the surviving infected shrimp could neutralize WSSV from 20 days up to 2 months after infection (Wu *et al.*, 2002). Now, work is underway to make recombinant protein and DNA vaccines taking viral structural protein, VP28 (Witteveldt *et al.*, 2004; Kumar *et al.*, 2008) which has shown good results in preliminary experimental trial. Another potential means of limiting WSSV infection is the RNA interference induced by dsRNA (Westenberg *et al.*, 2005).

Conclusion

The list of fish diseases that can be controlled through vaccination is steadily increasing. It proved very successful in reducing mortalities, and in turn, the use of antibiotics in aquaculture. Fish vaccines have become much more sophisticated in recent years with a trend for the development of subunit recombinant vaccines and DNA vaccine in preference

to the original killed whole-cell preparations. Recent licensing of IHNV DNA vaccine in Canada has stimulated active research efforts with many other pathogens. Results on experimental as well as field trial with DNA vaccine of viral hemorrhagic septicemia virus are encouraging and expected to get licensed soon. For DNA vaccine, better delivery mechanism and use of new adjuvant systems are tried to improve the transfection efficiency. The application of Proteomics in vaccine development is also an exciting new development as vaccine antigens can be characterized with great precision. In last two decades, shrimp industry is plagued by emergence of many viral diseases, especially WSSV. Finding of some level of adaptive immune response (quasi immune phenomenon) in shrimp has generated sensation and propelled the momentum for development of vaccine against lethal viral infection.

REFERANCES

Azad, I.S., Shankar, K.M., Mohan, C.V., Kalita, B., 2000. Uptake and processing of biofilm and free-cell vaccines of *Aeromonas hydrophila* in Indian major carps and common carp following oral vaccination antigen localization by a monoclonal antibody. *Diseases of Aquatic Organisms* 43: 103–108.

Babiuk, S., Babiuk, L.A., 2008. DNA vaccine. In: Mahy, B.W.J., M.H.V. Van Regenmortel, (Eds), *Encyclopedia of Virology* (3rd edition): pp. D51-D55.

Burrells, C., Williams, P.D., Forno, P.F., 2001. Dietary nucleotides: a novel supplement in fish feeds: 1. Effects on resistance to disease in salmonids. *Aquaculture* 199(1-2): 159-169.

Cain, K.D., LaPatra, S.E., Shewmaker, B., Jones, J., Byrne, K.M., Ristow, S.S., 1999. Immunogenicity of a recombinant infectious hematopoietic necrosis virus glycoprotein produced in insect cells. *Diseases of Aquatic Organisms.* 36(1): 67-72.

Dos Santos, N.M.S., Taverne-Thiele, J.J., Barnes, A.C., Van-Muiswinkel, W.B., Ellis, A.E., Rombout, J.H.W.M., 2001. The gill is a major organ for antibody secreting cell production following direct immersion of sea bass (*Dicentrarchus labrax*, L.) in a *Photobacterium damselae* ssp. *piscicida* bacterin: an ontogenetic study. *Fish & Shellfish Immunology* 11(1): 65–74.

Evelyn, T.P.T., 1997. A historical review of fish vaccinology. In: Gudding R., A. Lillehaug, P.J. Midtlyng, and F. Brown (eds.), *Fish Vaccinol. Dev. Biol. Stand.* Karger, Basel, pp. 3–12.

Foss, G.S., Rogne, S., 2003. Gene modification or genetic modification? The devil is in its details. *Nature biotechnology* 21: 1280-1281.

Frost, P., Ness, A., 1997. Vaccination of Atlantic salmon with recombinant VP2 of infectious pancreatic necrosis virus (IPNV), added to a multivalent vaccine, suppresses viral replication following IPNV challenge. *Fish & Shellfish Immunology.* 7(8): 609-619.

Gilmore, R.D.J., Engelking, H.M., Manning, D.S., Leong, J.C., 1988. Expression in Escherichia Coli of an epitope of the glycoprotein of infectious hematopoietic necrosis virus protects against viral challenge. Bio/Technology *6: 295–300.*

Gomez-Gill, B., Herrera-Vega, M.A., Abreu-Grobois, F.A., Roque, A., 1998. Bioencapsulation of two different vibrio species in nauplii of the brine shrimp (*Artemia franciscana*). *Applied Environmental microbiology* 64: 2318-2322.

Hanson, L.A., 2000. Vaccines. In: Stickney, R.R. (Ed.), *Encyclopedia of Aquaculture*. pp: 945-949.

Hew, C.L., Fletcher, G.L., 2002. The role of aquatic biotechnology in aquaculture. *Aquaculture* 197: 191-204.

Kanellos T., Sylvester, I.D., Ambali, A.G., Howard, C.R., Russell, P.H., 1999. The safety and longevity of DNA vaccines for fish. *Immunology* 96: 307-313.

Kimbrell, D. A., Beutler, B., 2001. The evolution and genetics of innate immunity. *Nature Review of Genetics* 2: 256-267.

Klesius, P.H., Evans, J.J., Shoemaker, C.A., 2006. Advancements in fish vaccine development. *Aquaculture Health International* 4 (Feb.): 20-21.

Krantz, G.E., Reddecliff, J.M., Heist, C.E., 1963. Development of Antibodies against Aeromonas *salmonicida* in Trout1. *Journal of Immunology* 91: 757–760.

Kumar, R. S, Ishaq-Ahamed, V.P., Sarathi, M., Nazeer-Basha, A., Sahul-Hameed, A.S., 2008. Immunological responses of *Penaeus monodon* to DNA vaccine and its efficacy to protect shrimp against white spot syndrome virus (WSSV). *Fish & Shellfish Immunology* 24(4): 467-78.

Kurath, G., 2008. Biotechnology and DNA vaccines for aquatic animals. *Rev. Sci. Tech. Off. Int. Epiz.*, 27(1):175- 196

Laptra, S., 2004. Current trends in immunotherapy and vaccine development for viral diseases of fish. In: Leung Ka Yin (Ed.), Current trends in the study of bacterial and viral fish and shrimp diseases (Molecular aspects of fish and marine biology; v. 3): pp. 363-389.

Lorenzen, N., LaPatra, S.E., 2005. DNA vaccines for aquacultured fish. *Rev. Sci. Tech. Off. Int. Epiz.*, 24(1): 201-213.

Lorenzen, N., Olesen, N.J., Koch, C., 1999. Immunity to VHS virus in rainbow trout. *Aquaculture* 172(1-2): 41-61.

Markestad, A., Grave. K., 1997. Reduction of antibacterial drug use in Norwegian fish farming due to vaccination. *Developments of Biologicals Standardisation* 90: 365-369.

Meeusen, E.N.T., Walker, J., Peters, A., Pastoret, P.P., Jungersen, G., 2007. Current status of veterinary vaccines. *Clinical Microbiological Review* 20(3): 489–510.

Midtlyng, P.J., Reitan, U., Speilberg, L., 1996. Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (*Salmo salar*L.) against furunculosis. *Fish & Shellfish Immunology* 6: 335–350.

Mor, G., Singla, M., Steinberg, A.D., Hoffman, S.L., Okuda, K., Klinman, D.M., 1997. Do DNA vaccine induce autoimmune disease? *Human Gene Therapy* 8(3): 293-300.

Moral, C.H., Castillo, E.F., Fierro, P.L., Cortés, A.V., Castillo, J.A., Soriano, A.C., Salazar, M.S., Peralta, B.R., Carrasco, G.N., 1998. Molecular characterization of the *Aeromonas hydrophila aroA* gene and potential use of an auxotrophic *aroA* mutant as a live attenuated vaccine. *Infection and Immunity* 66(5): 1813–1821.

Muiswinkel, W.B.V., 2008. A history of fish immunology and vaccination I. The early days, *Fish & Shellfish Immunology* 25(4): 397-408.

Myhr, A.I., Dalmo, R.A., 2005. Introduction to genetic engineering in aquaculture: Ecological and ethical implication for science and governance. *Aquaculture* 250: 542-554.

Salonius K., Simard, N., Harland, R., Ulmer, J.B., 2007. The road to licensure of a DNA vaccine. *Current Opinion in investigational Drugs,* 8: 635-641.

Salonius, K., Siderakis, C., Mackinnon, A. M., Griffiths, S. G, 2005. Use of *arthrobacter davidanieli* as a live vaccine against *Renibacterium salmoninarum* and *Piscirickettsia salmonis* in salmonids. In: International Symposium on Progress in Fish Vaccinology, Bergen , Norvege (09/04/2003) 121: 189-197.

Shoemaker, C.A., Klesius, P.H., Bricker, J.M., 1999. Efficacy of a modified live *Edwardsiella ictaluri* vaccine in channel catfish as young as seven days post hatch *Aquaculture* 176(3-4): 189-193.

Smith, P.D., 2001. Adjuvants and their side effects. *Fish Farmer* 24(6): 44–45.

Sommerset, I., Krossoy, B., Biering, E., Frost, P., 2005. Vaccines for fish in aquaculture. *Expert Review in Vaccines* 4(1): 89-101.

Tappesser, B., Jager, M., Eckelkamp, C., 2002. Survival, persistence, transfer: the rate of genetically modified microorganisms and recombinant DNA in different environments. In: Letourneau D.K., and B.E. Burrows (Eds). Genetically engineered organisms; Assessing environmental and human health aspects. CRC Press, Boca Raton, FL, pp. 223-251.

Thiéry, R., Cozien, J., Cabon, J., Lamour, F., Baud, M., Schneemann, A., 2006. Induction of a protective immune response against viral nervous necrosis in the European Sea Bass *Dicentrarchus labrax* by using betanodavirus virus-like particles. *Journal of Virology* 80 (20): 10201-10207.

Thompson, K.D., Adams, A., 2004. Current trends in immunotherapy and vaccine development for bacterial diseases of fish, In: Leung Ka yin (Ed.), Current trends in the study of bacterial and viral fish and shrimp diseases (Molecular aspects of fish and marine biology; v. 3): pp. 313-362.

Thune, R.L., 2002. Attenuated *Pasteurella piscicida* vaccine for fish. US Patent: 6350454.

Venegas, C.A., Nonaka, L., Mushiake, K., Nishizawa, T., Muroga, K., 2000. Quasi-immune response of *Penaeus japonicas* to penaeid rod-shaped DNA virus (PRDV). *Disease of Aquatic Organisms* 42: 83–89.

Westenberg, M., Heinhuis, B., Zuidema, D., Vlak, J.M., 2005. siRNA injection induces sequence-independent protection in *Penaeus monodon* against white spot syndrome virus. *Virus Research* 114(1-2): 133-139.

Witteveldt, J., Vlak, J.M., Van- Hulten, M.C.W., 2004. Protection of *Penaeus monodon* against white spot syndrome virus using a WSSV subunit vaccine. *Fish & Shellfish Immunology* 16(5): 571-579.

Wu, J.L., Nishioka, T., Mori, K., Nishizawa, T., Muroga, K. A., 2002. Time-course study on the resistance of *Penaeus japonicus* induced by artificial infection with white spot syndrome virus. *Fish and Shellfish Immunology* 13: 391-403.