AACL BIOFLUX
Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

Updates on the vaccination against bacterial diseases in tilapia, *Oreochromis* spp. and Asian seabass, *Lates calcarifer*

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Abstract. The use of vaccines in aquaculture is one of the widely accepted methods of preventing most pathogenic diseases. In warmwater aquaculture, various species of tilapia, *Oreochromis* spp., and the Asian seabass, *Lates calcarifer* are widely farmed in freshwater and brackishwater ponds and cages because of their high demand in the market both for local consumption and for export. However, farming of these fish species is hampered by the outbreaks of bacterial diseases that affect production and eventually revenues. This review provides updates on the different bacterial vaccines developed for these farmed fish. In tilapia, experimental trials have been done on the effectiveness of inactivated, attenuated and sub-unit vaccines against *Streptococcus iniae*, *S. agalactiae*, *Vibrio* spp., *Aeromonas hydrophila*, *Edwardsiella tarda* and *Francisella asiatica*. On the other hand, bacterial vaccines have been tested against *Vibrio anguillarum* in Asian seabass. The immune responses of the fish as a result of vaccination and the protective efficiency of these different vaccines are also discussed.
Key Words: aquaculture, bacteria, cage culture, freshwater, health management, vaccines.

Introduction. Aquaculture has grown rapidly over the last decades, and proof of this is the number of aquatic species, totalling to 600, that are being farmed worldwide (FAO 2012). Although most of the finfish production comes from the extensive production of carps, the industrialization of finfish farming is expanding for both high and low value species (Brudeseth et al 2013). In the Asia-Pacific region, the marine aquaculture industry has developed very rapidly over the last 10 years. Due to intensification in aquaculture activities, outbreaks of diseases are also becoming more rampant thereby imposing a significant limitation to the industry. Among the many infectious diseases caused by bacteria in marine fish, only a relatively small number are responsible of important economic losses in cultured fish worldwide. Moreover, the diseases that are classically considered to be typically found in freshwater aquaculture, including furunculosis (*Aeromonas salmonicida*), bacterial kidney disease (BKD) (*Renibacterium salmoninarum*) and some types of streptococcosis, these pathogenic diseases are also becoming more prevalent in marine culture (Toranzo et al 2005). Therefore, bacterial diseases of fish have a wide geographical scope.

The appearance and development of a fish disease is the result of the interaction among pathogen, host and environment. Studies that involve the characteristics of potential pathogenic microorganisms, aspects of the biology of the fish hosts as well as a better understanding of the environmental factors that affect aquaculture activities will allow the application of adequate measures to prevent and control the main diseases limiting the production in aquaculture. With the tremendous increase in aquaculture activity, the need for effective disease control measures is of prime concern. Indiscriminant use of antibiotics is not acceptable; thus safe and effective vaccines are critical for a sustainable development of the aquaculture industry (Evensen & Leong 2013). In the past, fish vaccines were made using a trial-and-error approach, which is the conventional vaccine design (Tafalla et al 2013). Whether intended for bacteria or viruses, vaccine design includes pathogen identification, pathogen cultivation, and

vaccine formulation containing whole cell preparation and oils. Because of this method, vaccines that are derived from whole inactivated extracellular bacterial pathogens were quite efficient, resulting in significant reductions in mortalities and antibiotic usage in the aquaculture industry (Hastein et al 2005). However, there are other infectious diseases, especially due to infections with intracellular pathogens, that are difficult to control even with vaccination. Thus, the need for effective vaccine design and strategies must be developed that are pathogen-specific.

Vaccines induce and build resistance in the host against a wide array of pathogenic diseases; and this remains as the most viable approach in the prevention of fish diseases. The most common vaccine in fish is the traditional inactivated vaccine (Sommerset et al 2005a, b). Although, the development of live attenuated and sub-unit vaccines is also gaining wide popularity. Generally, the route of administration for these vaccines in fish are through injection (both intraperitoneal and intramuscular injection), oral, direct immersion or spraying (Yang & Chen 1996; Sukenda & Wakabayashi 1999). Though there is no ideal method of administration, injection method remains to be the most popular method of choice when vaccinating fish. In most studies, injecting the vaccine in fish resulted in the best effect, although the operation could be excessive, time-consuming and difficult to administer to fry and the other small fishes. The other three methods of vaccination are very convenient for operation and suitable for inoculation of fry and small fishes, however, their protective effects are usually not as good. The vaccine might be partly degraded by the digestive fluids when given orally or the vaccine could not be sufficiently absorbed by the fish body using the immersion and spray methods. Therefore, the lack of a suitable inoculating method is one of the biggest problems in the administration of vaccines in fish (Ellis 1988; Yang & Chen 1996).

This paper provides an update of the various bacterial vaccines that have been developed and experimentally tested in tilapia, *Oreochromis* spp. and Asian seabass, *Lates calcarifer*. These two species of fish are cultured extensively in the Asia-Pacific region because both species supply the protein requirements of the growing population as well as a major source of income for most fishfarmers who are into the culture of these species. The different vaccine types, efficiency and the mechanisms of protection of these vaccines are briefly discussed in the succeeding sections.

Bacterial vaccines for tilapia. There are several vaccines that have been developed to protect tilapia against diseases caused by *Streptococcus* spp., *Vibrio* spp., *Aeromonas hydrophila*, *Francisella asiatica* and *Edwardsiella tarda*. These vaccines included the production of inactivated bacterial cells, live attenuated bacteria and sub-unit vaccines. Table 1 shows the different types of bacterial vaccines for tilapia. Based on published literature, the earliest vaccine that was experimentally tested on tilapia was a formalin-killed bacteria against *A. hydrophila* (Ruangpan et al 1986). The vaccine exhibited high protective efficiency in fish following challenge with the pathogen as well as resulted in high antibody titers in the vaccinated fish. Since then several vaccine formulations have been developed for bacterial diseases of this species of fish. Most vaccines have been tested against streptococcosis in tilapia. This disease is due to infection with *Streptococcus iniae* and *S. agalactiae*.

Streptococcal disease is emerging as one of the most fish diseases that has tremendous impact in worldwide aquaculture production. There are at least 27 fish species that are susceptible to this disease (Agnew & Barnes 2007) due to the global distribution of the *S. agalactiae* and *S. iniae*. This number is expected to increase as shown by a recent report that another newly cultured fish species, the red porgy *Pagrus pagrus* was also susceptible to this disease (El Aamri et al 2010).

Aside from streptococcosis, tilapias also are susceptible to infections with *Vibrio* spp. Vaccines to control vibriosis in this fish due to *Vibrio vulnificus* have been developed (Shoemaker et al 2011, 2012). *V. vulnificus* is an opportunistic human pathogen, and can cause disease in economically important aquaculture fish species (Austin 2010). Isolates are classified into three biotypes based on biochemical characteristics (Jones & Oliver 2009). Biotype 1 isolates are commonly associated with human infections; however, isolates from any of the three biotypes have the potential to cause disease in humans.

Biotype 3 is a recombinant clone that may have emerged as a result of hybridization of 2 *V. vulnificus* populations (Bisharat et al 2005). Most isolates that cause disease in fish belong to biotype 2 (Biosca & Amaro 1996; Fouz et al 2002).

Emerging pathogens including *Francisella* sp. and *Edwardsiella tarda* also cause diseases in tilapias. A live attenauted vaccine has been developed against francisellosis in tilapia due to *F. asiatica* (Soto et al 2011) and showed high protective ability in the fish. Similarly, the fish were also highly protected against edwardsiellosis when vaccinated with either formalin-killed bacteria, bacterial ghosts or with live attenuated bacteria (Kwon et al 2006; Pridgeon et al 2013).

Table 1

Bacterial vaccines experimentally tested in various species of tilapia

Pathogen	*Type of vaccine*	*Vaccine delivery*	*Vaccine efficiency*	*Reference*
Streptococcus difficile	Formalin-killed whole bacteria	Intraperitoneal injection	High protective ability in the vaccinated fish following bacterial challenge; increased specific agglutinins	Eldar et al (1995)
Aeromonas hydrophila	Formalin-killed bacteria	Intraperitoneal injection	High protective ability in the vaccinated fish follwing bacterial challenge; increased antibody titers	Ruangpan et al (1986)
A. hydrophila	Formalin-killed bacteria with ascogen feeding	Intramuscular (IM) injection and direct immersion (DI)	Moderate to high protection in IM-injected fish; whereas low to moderate protection in DI-vaccinated fish following challenge	Ramadan et al (1994)
A. hydrophila	Live attenuated bacteria (resistant to novobiocin and rifampicin)	Intraperitoneal injection	100% protection in Nile tilapia vaccinated with the mutant strain following challenge with the parental strains; increase agglutination titers	Pridgeon & Klesius (2011a)
Streptococcus agalactiae	Formalin-killed bacteria	Intraperitoneal injection and bath immersion	High protective ability in vaccinated fish (> 30 g) follwing challenge; reduction in protective ability in fish vaccinated through immersion	Evans et al (2004)
S. agalactiae	Serum from challenged fish	Passive immunization	Moderate to high protective ability in passively-immunized Nile tilapia	Pasnik et al (2006)
S. agalactiae	Live attenuated vaccines (resistant to sparfloxacin)	Intraperitoneal injection	Variable protective efficiency of the various attenuated isolates; polyvalent vaccines are recommended	Pridgeon & Klesius (2013)
S. agalactiae	Whole killed vaccine of various genotypes	Injection	Variable protective efficiency depending on genotype	Chen et al (2012)
S. agalactiae	DNA vaccine using Sip in live attenuated *Salmonella typhimurium*	Oral delivery	Increase antibody titer, protective effect upon challenge	Huang et al (2014)

S. agalactiae	Recombinant protein of cell wall surface anchor protein	Oral administration	High protective ability in vaccinated fish following challenge; increased IgM antibody response in serum, mucus and gut lavage fluids	Nur-Nazifah et al (2014)
S. agalactiae	Inactivated whole bacteria	Injection	High protective effect in vaccinated-Nile tilapia	Yi et al (2014)
S. agalactiae	Recombinant FbsA and a-enolase	Injection	Moderate protective effect in vaccinated-Nile tilapia; higher respiratory burst, lysozyme activity and antibody titer in vaccinated fish	Yi et al (2014)
S. iniae	Formalin-killed bacteria	Intraperitoneal and intramuscular injection	Variable protective ability in vaccinated fish depending on the serotype	Klesius et al (2000)
S. iniae	Lyophilized modified bacteria	Oral administration	Moderate to high protective ability in fish fed with the lyophilized bacteria using the Oralject™ technology	Shoemaker et al (2006)
S. iniae	Sub-unit vaccine (MtsB, a hydrophobic membrane protein)	Intraperitoneal injection	Possessed protective ability in vaccinated fish following challenge	Zou et al (2011)
S. iniae	Live attenuated bacteria (resistant to novobiocin)	Intraperitoneal injection and bath immersion	High protective efficiency when challenged with the parental strain and heterologous strains; increased antibody titers and cell-mediated immunity	Pridgeon & Klesius (2011b)
Vibrio vulnificus	Formalin killed, whole bacteria	Injection	Moderate to high protective effects in sex-reversed hybrid tilapia vaccinated with the inactivated bacteria following challenge with either the homologous or heterologous isolates	Shoemaker et al (2011)
S. iniae and *V. vulnificus*	Inactivated whole bacteria	Intraperitoneal injection	Sex-reversed hybrid tilapia vaccinated with the bivalent vaccines had high survival rates following challenge with either of the bacterial pathogens	Shoemaker et al (2012)
Edwardsiella tarda	Formalin-killed bacteria and bacterial ghosts	Injection	High protective ability, serum agglutination titers and bactericidal activity in both vaccines; higher effects observed in fish vaccinated with the bacterial ghosts	Kwon et al (2006)

E. tarda	Live attenuated vaccines (resistant to novobiocin)	Injection	High protective ability in vaccinated Nile tilapia following challenge	Pridgeon et al (2013)
Francisella asiatica	Live attenuated bacteria	Bath immersion and passive immunization	High protective ability in vaccinated fish as well as those that were passively immunizied with *F. asiatica* antibodies	Soto et al (2011)

Bacterial vaccines for Asian seabass. Vibriosis remains one of the most serious diseases, especially due to infection with *V. harveyi*, a major pathogen of marine fish in this region (Austin & Zhang 2006; Chabrillon et al 2005). *V. harveyi* infects both grouper, *Epinephelus* spp. (Harikrishnan et al 2011) and Asian seabass (Tendencia 2002; Caipang et al 2011).

The occurrence of vibriosis in fish is higher in the summer when there is increased water temperature and abundance of organic load (Kim et al 1990; Carli et al 1993). There were different components of the *V. harveyi* that have been tested as vaccines (Arijo et al 2005; Crosbie & Nowak 2004). The haemolysin gene from *V. harveyi* produced in yeast cells induced the production of specific antibodies and provided early protection in flounder, *Verasper variegatus*, however, no significant differences in the cumulative mortality were observed between the control and vaccinated group (Zhu et al 2006).

The outer membrane protein (OMP) of Gram-negative bacteria has an important role in the interaction between bacteria with hosts in terms of adherence, uptake of nutrients from the host, and subverting host defense mechanisms (Harikrishnan et al 2011). As such, recombinant OMP from *V. harveyi* and *V. parahaemolyticus* have been produced and were found to protect yellow croaker *Pseudosciaena crocea* from infection with virulent strains of both bacteria (Mao et al 2007; Zhang et al 2007). These vaccines were immunogenic, but no vaccine against *V. harveyi* is commercially available.

Inspite of the isolation of *V. harveyi* from Asian seabass (Tendencia 2002; Caipang et al 2011), there have been no studies done on the production of vaccines against this pathogen for this species of fish. However, vaccines against *V. anguillarum* and *S. iniae* have been tested experimentally in Asian seabass (Table 2).

DNA vaccines against *V. harveyi* using the OMP gene as an antigen have been tested in terms of their protective ability following bacterial challenge in Asian seabass. Whether injected intramuscularly or incorporated with chitosan nanoparticles and administered as feed, the relative efficiency of these DNA vaccines was moderate (Kumar et al 2007, 2008). On the other hand, formalin-killed bacterins of *S. iniae* provided protection in fish following challenge with the same strain of the pathogen but not the recombinant protein of its capsular polysaccharide (Aviles et al 2013). Furthemore, when a heterologous serotype of the bacterial pathogen was challenged in the vaccinated fish, the level of protection was reduced or was not significantly different from the non-vaccinated group.

Table 2

Bacterial vaccines experimentally tested in Asian seabass, *Lates calcarifer*

Pathogen	*Type of vaccine*	*Vaccine delivery*	*Vaccine efficiency*	*Reference*
Vibrio anguillarum	DNA vaccine using outer membrane protein	Intramuscular injection	Relative percentage survival (RPS) of 55.6% in vaccinated fish	Kumar et al (2007)
V. anguillarum	DNA vaccine with chitosan nanoparticles	Oral administration	RPS of 46% in fed fish following bacterial challenge	Kumar et al (2008)
Streptococcus iniae	Bacterin, formalin-killed	Intraperitoneal injection	100% protection in vaccinated fish when challenged with the same strain, but reduced protection when challenged by a heterologous strain	Aviles et al (2013)
S. iniae	Recombinant SiMA produced in Escherichia coli; SiMA is a capsular polysaccharide	Intraperitoneal injection	No siginficant protection from the control despite increase in antibody response	Aviles et al (2013)

Immunogenicity and protective effects of bacterial vaccines. The study of fish immune systems has been done for over 20 years. Advances in molecular biology enabled the cloning and identification of immune-related genes, including the immunoglobulin genes of catfish (Ghaffari & Lobb 1989) and the major histocompatibility complex (MHC) genes of carp (Hashimoto et al 1990). One important information that is needed towards the development of better finfish vaccines is to be able to elucidate how the fish immune systems recognize pathogens and target them for their B and T cells (Dixon 2012). This requires sufficient knowledge as to what particular fragment of a certain pathogen is recognized by the T and B cells of fish as well as the different accessory molecules that are involved in antigen presentation.

For vaccine development, it is necessary that the actual sequence of the protein products or the gene that produces that protein be known. This ensures that those such sequences are found within the vaccines, thereby triggering the activation of T or B cells that are specific for that pathogen (Dixon 2012). Once appropriate immune responses are initiated, the succeeding responses follow a series of cascades, which are mediated by cytokines that are produced in the early phases of the immune response. Depending on these initial signals, the immune response can modulate the appropriate mechanisms that respond to specific pathogens. Thus in developing vaccines for fish, it is important to ensure that the correct immune response pathway is triggered and this could be achieved by performing cytokine assays that are expressed during vaccination (Dixon 2012). These assays could detect specific amounts of cytokines in the blood, from immune-rich tissues of the fish, from the cells of peripheral blood or from primary cell culture.

The protective mechanisms of most bacterial vaccines for both tilapia and Asian seabass are due to the upregulation of specific antibodies as well some aspects of the innate immune systems including respiratory burst and lysozyme (Tables 1 and 2). Molecular studies on the mechanisms of gene expression as a consequences of vaccination are few although increasing over the years. There are a number of studies done on the immune responses in tilapia following vaccination but still limited on the immune responses of Asian seabass. Pridgeon & Klesius (2011c) analyzed the different

immune-related genes in Nile tilapia following vaccination with formalin-killed *S. iniae* using the expressed sequence tags (EST). Results showed that cytochrome c oxidase subunit II was highly upregulated in the vaccinated fish compared to that in non-vaccinated fish. They also found nine genes that were significantly upregulated, including five unknown or hypothetical proteins and four known proteins. These known proteins were: high density lipoprotein-binding protein vigilin, QM-like protein, ribosomal protein S13, and ribosomal protein L5. The upregulation of these genes induced by the *S. iniae* vaccine likely indicates that they might play important roles in the immune response of Nile tilapia against *S. iniae* infection. LaFrentz et al (2011) analyzed the production of specific antibodies to whole-cell lysate proteins of *S. iniae* using the immunoproteomic approach. Their results demonstrated that vaccination of tilapia with the *S. iniae* vaccine resulted in the elevation of specific antibody responses against proteins of the bacterium and the passive immunization of the fish with this serum also had protective effects. In addition, when whole-cell lysate proteins of *S. iniae* were separated by 2D-PAGE and were probed with a pooled serum sample from vaccinated tilapia, a total of eleven unique immunogenic proteins were positively identified by mass spectrometry. Of these immunogenic proteins, three of the identified proteins: enolase, glyceraldehyde-3-phosphate dehydrogenase, and fructose-bisphosphate aldolase, are likely involved in protection from streptococcosis caused by *S. iniae*. The results of these studies show that aside from the production of specific antibodies, there are various immune-related genes in tilapia that are triggered to produce their protein products that help the host in warding off the infection.

Concluding remarks. Disease prevention by vaccination is still considered as the most appropriate method in controlling pathogens in aquaculture. Traditionally, bacterial vaccines comprise either inactivated, live-attenuated or recombinant/sub-unit vaccines. Several bacterial vaccines have been developed and experimentally tested in two economically important fish species, tilapia and Asian seabass. Most of the bacterial vaccines showed protective effects in the host following pathogen challenge and the protection is likely due to the increased production of the specific antibodies. Although most bacterial vaccines have been found to be efficient, research efforts are still needed to make vaccines that are truly effective for teleost fish. This can be achieved through a thorough understanding of the immune processes in the host during vaccination. As more sequences of immune-related genes from fish are available, it would be logical to start the process of examining the individual genes and perform functional studies for each to better understand the fish immune system. Tliapias and Asian seabass are no exception to this research endeavor. A better understanding of the immune mechanisms of these species of fish during vaccination will lead to better vaccine design and formulation, which in turn result in higher protective efficiency when bacterial infections occur in the aquaculture facility.

Acknowledgements. The authors gratefully acknowledge the support provided by the administration and staff of the School of Applied Science, Temasek Polytechnic during the preparation of the manuscript.

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Received: 17 June 2014. Accepted: 28 June 2014. Published online: 29 June 2014.
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How to cite this article:
Caipang C. M. A., Lucanas J. B., Lay-yag C. M., 2014 Updates on the vaccination against bacterial diseases in tilapia, *Oreochromis* spp. and Asian seabass, *Lates calcarifer*. AACL Bioflux 7(3):184-193.