



REVIEW

Development of *Streptococcus agalactiae* vaccines for tilapia

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ABSTRACT: Vaccination is a widely accepted and effective method to prevent most pathogenic diseases in aquaculture. Various species of tilapia, especially Nile tilapia *Oreochromis niloticus*, are farmed worldwide because of their high consumer demand. Recently, the tilapia-breeding industry has been hampered by outbreaks of *Streptococcus agalactiae* infection, which cause high mortality and huge economic losses. Many researchers have attempted to develop effective *S. agalactiae* vaccines for tilapia. This review provides a summary of the different kinds of *S. agalactiae* vaccines for tilapia that have been developed recently. Among the various vaccine types, inactivated *S. agalactiae* vaccines showed superior protection efficiency when compared with live attenuated, recombinant and DNA vaccines. With respect to vaccination method, injecting the vaccine into tilapia provided the most effective immunoprotection. Freund's incomplete adjuvant appeared to be suitable for tilapia vaccines. Other factors, such as immunization duration and number, fish size and challenge dose, also influenced the vaccine efficacy.

KEY WORDS: *Streptococcus agalactiae* · Nile tilapia · *Oreochromis niloticus* · Vaccine · Relative percent survival · Adjuvant

INTRODUCTION

Tilapia ranks as the second most cultivated fish in the world, after carp (Khan 2014). It is distributed in more than 135 countries worldwide, and is subject to high demand in the consumer market. Based on a survey by the Food and Agriculture Organization (FAO 2012), 72% of global tilapia production occurred in Asia (particularly in China and Southeast Asia), 19% in Africa, and 9% across North and South America. The most economically important species for tilapia aquaculture is the Nile tilapia *Oreochromis niloticus*, an Egyptian native fish (Mjoun et al. 2010). Although tilapia aquaculture has developed rapidly, it also faces great challenges from bacterial diseases

caused by *Streptococcus* spp., *Vibrio* spp. (Shoemaker et al. 2011), *Aeromonas hydrophila* (Ibrahim et al. 2008), *Flavobacterium* spp. (Shoemaker & LaFrentz 2015), *Lactococcus garvieae* (Anshary et al. 2014), *Francisella asiatica* (Hsieh et al. 2006), and *Edwardsiella tarda* (Thune et al. 1993). Currently, infections caused by *Streptococcus* spp., especially *S. agalactiae* and *S. iniae*, are the most common and cause huge economic losses to the tilapia industry. Their prevalence and severity depend on multiple environmental factors, including warm water temperatures (in the summer), increased ammonia levels, and low dissolved oxygen levels (caused by poor husbandry and high stocking density) (Bromage & Owens 2002).

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S. iniae is not only a major pathogen in fish (Agnew & Barnes 2007), but it is also an emerging human pathogen that can cause fulminant soft tissue infection resulting from the handling of diseased fish (Weinstein et al. 1997, Fuller et al. 2001). *S. agalactiae* is more commonly associated with diseases in human and bovine hosts. However, fish-pathogenic *S. agalactiae* were documented as early as 1966, when a non-hemolytic Group B *Streptococcus* was identified as the cause of 2 epizootics in golden shiners *Notemigonus crysoleucas* (Robinson & Meyer 1966). Today, *S. agalactiae* is more prevalent than *S. iniae* in tilapia. Epidemiological studies in the major tilapia-producing regions of Asia and Latin America from 2001 to 2009 showed that of the nearly 500 streptococcal isolates recovered from tilapia, 82% were identified as *S. agalactiae* and 18% were identified as *S. iniae* (www.thefishsite.com/articles/812/streptococcosis-in-tilapia-a-more-complex-problem/). In China, more than 90% of the clinical bacterial isolates from infected tilapia since 2009 have been *S. agalactiae* (Chen et al. 2012a). To date, *S. agalactiae* infection in tilapia outbreaks have been reported in several countries, including the USA (Evans et al. 2006), China (Liu et al. 2012), Kuwait (Evans et al. 2002), Israel (Eldar et al. 1994), Thailand (Suanyuk et al. 2008), Honduras (Delannoy et al. 2012), and Brazil (Mian et al. 2009), resulting in serious annual economic losses.

Vaccination is a widely accepted and effective method to control *S. agalactiae* infection and prevent mass tilapia mortalities. Here, we summarize the recent developments in *S. agalactiae* vaccines for tilapia and discuss how the vaccination methods, adjuvants, and other factors influence vaccine efficacy.

TYPES OF *STREPTOCOCCUS AGALACTIAE* VACCINES FOR TILAPIA

Since the 1930s, when the first vaccine against *S. agalactiae* in humans was developed (Lancefield 1938), an increasing number of reports have described safer and more effective vaccines against this pathogen (Baker & Kasper 1976, 1985, Baker et al. 1988, Paoletti et al. 1994, Baker & Edwards 2003). With the development of the human *S. agalactiae* vaccine, studies and use of *S. agalactiae* vaccines in reared tilapia have also advanced greatly in the past 2 decades. These vaccines include the production of inactivated bacterial cells, live attenuated bacteria, recombinant vaccines, and DNA vaccines. Table 1 shows a summary of the different types of vaccines against *S. agalactiae* for tilapia.

Whole-cell inactivated vaccine

As vaccines were developed in other fishes, traditional inactivated vaccines were used widely to provide protection for tilapia from *S. agalactiae* infection. During the early days of inactivated vaccine development, most products contained inactivated bacteria mixed with their extracellular products (Eldar et al. 1995, Evans et al. 2004, Pasnik et al. 2005), because several killed vaccines had been shown to be efficient against piscine bacterial disease caused by *S. iniae* (Eldar et al. 1997) and *Enterococcus* spp. (Toranzo et al. 1995). A formalin-killed *S. agalactiae* vaccine was tested successfully on tilapia for the first time in 1995 (Eldar et al. 1995). This formalin-killed *Streptococcus difficile* strain, now known as non-hemolytic, serotype Ib *S. agalactiae* (Vandamme et al. 1997), was able to protect tilapia against a challenge of 100× the median lethal dose (LD₅₀) when delivered via intraperitoneal (IP) injection. Since then, several inactivated vaccines have been used to control *S. agalactiae* infection in tilapia (Table 1). Previous studies showed that whole-cell inactivated *S. agalactiae* vaccines could provide protection to tilapia (weight >20 g), with a relative percent survival (RPS) of 46 to 100%, when challenged with homologous strains after IP immunization (Evans et al. 2004, Pasnik et al. 2005, Pretto-Giordano et al. 2010, Chen et al. 2012b). Using a whole-cell killed *S. agalactiae*, Merck Animal Health developed a commercial vaccine (AQUAVAC® Strep Sa), which has been available in Brazil, Indonesia, and Vietnam since 2011. This product is an inactivated oil-adjuvanted vaccine that protected 85% of tilapia (weight >15 g) for over 30 wk in laboratory tests (www.merck-animal-health.com/news/2015-03-10.aspx). Based on pulsed-field gel electrophoresis genotypes, Chen et al. (2012b) screened 85 candidate strains to identify the predominant epidemic genotype strains, and found that using a combination of 2 inactivated strains resulted in a wider protection scope and higher RPS values (65.52–100%) compared with either single strain when challenged by non-self genotype strains. Based on the above data, vaccines derived from inactivated *S. agalactiae* were quite efficient for tilapia (weight >5 g), resulting in significant reductions in mortalities when infected with the homologous virulent strain. For protection against homologous *S. agalactiae* infection in tilapia, the traditional inactivated vaccine is a reasonable and low cost choice when applied on a commercial scale. However, researchers should pay close attention to the serotype changes of the predominant epidemic

S. agalactiae strains to guarantee vaccine protection for the tilapia farm.

Live attenuated vaccines

Live attenuated vaccines are developed by weakening an infectious microbe such that it can still replicate without causing disease in the host. These vaccines can induce effective immune responses and often provide lifelong immunity at low doses. Despite their advantages, live attenuated vaccines are more difficult to create for bacteria because of their complexity. Only a few attenuated *S. agalactiae* vaccines have been developed. In 2013, a mixture of attenuated *S. agalactiae* strains were generated by selecting their resistance to sparflaxacin, a fluoroquinolone antibiotic (Pridgeon & Klesius 2013). This polyvalent vaccine provided 100% protection to both 3–5 g and 15–20 g tilapia via IP injection (Pridgeon & Klesius 2013), while the traditional formalin-killed *S. agalactiae* vaccine worked well in 15–20 g tilapia (RPS = 80%) but poorly in 3–5 g tilapia (RPS = 25%) (Evans et al. 2004). This kind of mixed attenuated vaccine is very promising for the fingerling tilapia industry; such fish are usually 1–2 wk old and weigh 3–5 g when obtained from the hatchery. In 2015, another live attenuated vaccine strain was generated via continuous passage (840 times) *in vitro*. It also displayed good protection for 30 g tilapia by IP injection (RPS = 96.88%), immersion (RPS = 67.22%), and oral administration (RPS = 71.81%) at 15 d post-vaccination (dpv) (Li et al. 2015). Thus, these studies developed stable and immunogenic attenuated *S. agalactiae* strains for tilapia, especially for fingerlings (3–5 g). Tilapia is among the most frequently consumed seafood and any live attenuated vaccines used must be safe for the consumers; therefore, the development of a commercial attenuated vaccine for tilapia against *S. agalactiae* might be hampered by safety concerns for human health.

Recombinant vaccines

Vaccination with a whole-cell *S. agalactiae* vaccine offers effective protection in tilapia when challenged with homologous serotype, but is ineffective against heterologous serotypes. To overcome this serotype specificity, many studies have used recombinant vaccines, which contain the antigens found in most *S. agalactiae* isolates and effectively stimulate the immune system (Heath 2011). In this respect, Eldar et

al. (1995) showed that only a few proteins could act as protective antigens in both the whole-cell vaccine and the streptococcal extract. With advances in new technologies, such as whole-genome sequencing and mass spectrometry based proteomics, researchers are gaining new opportunities to develop effective and globally relevant *S. agalactiae* recombinant vaccines (Johri et al. 2006). Based on comparative genome analysis and multiple genome screening, some surface proteins of *S. agalactiae*, such as surface immunogenic protein (Sip), CAMP factor, R5 protein, enolase, hyaluronidase, hemolysin (cylE), and pilus proteins have been suggested as potential vaccine candidates (Tettelin et al. 2002, Maione et al. 2005). Using the proteomics approach, Hughes et al. (2002) identified the main surface-exposed proteins of *S. agalactiae*. Sera directed against 2 of these proteins, ornithine carbamoyltransferase (OCT) and phosphoglycerate kinase (PGK), were protective against lethal doses of *S. agalactiae* infection in a neonatal-mouse model. Wang et al. (2014) later confirmed that the PGK protein enhanced the immunogenic effect of whole-cell *S. agalactiae* in tilapia. A feed-based recombinant vaccine of *S. agalactiae*, which includes a cell wall surface anchor family protein named pilus islands (PI)-1 ancillary protein 1 (Dramsai et al. 2006), was developed. This vaccine can stimulate high levels of mucosal and systemic immunity, and gave 70% protection to red hybrid tilapia following bacterial challenge (Nur-Nazifah et al. 2014). Moreover, using an immunoproteomics method, Liu et al. (2013) identified 4 immunoreactive proteins (serine-rich repeat glycoprotein 1, branched-chain alpha-keto acid dehydrogenase subunit E2, 5'-nucleotidase family protein, and OCT). These 4 proteins were conserved in multiple serotypes of *S. agalactiae* and are anticipated to act as protective antigens.

Although several antigenic proteins have been identified successfully as *S. agalactiae* recombinant vaccine candidates, few studies have directly addressed the protective efficacy of these targets in tilapia compared with that of whole-cell inactivated vaccine. Recent studies have shown that recombinant vaccines, such as PI, PGK, and Sip recombinant proteins, require booster immunization and must be mixed with suitable adjuvants to provide acceptable protection to tilapia (Table 1). Moreover, the recombinant vaccines still provide lower protection to tilapia than the whole-cell inactivated vaccines under the same conditions. For example, Yi et al. (2014) found that the recombinant α -enolase protein and fibrinogen-binding protein A (FbsA) conferred

Table 1. *Streptococcus agalactiae* vaccines tested experimentally in tilapia *Oreochromis* spp. RPS: relative percent survival; ECP: extracellular products; IP: intraperitoneal; BI: bath immersion; PFGE: pulsed-field gel electrophoresis; dpv: days post-vaccination

Vaccine description	Vaccination regime		Post-vacc. challenge		Tilapia weight (g)	Vaccine efficiency	Reference		
	Delivery	Adjuvant	Frequency	Interval (wk)				Delay (dpv)	Dose (cfu fish ⁻¹)
Inactivated vaccines									
Whole-cell formalin-inactivated <i>S. agalactiae</i>	IP	No	Twice	4	77	100 LD ₅₀ (10 ⁹ –10 ¹⁰)	150–180	100% protection, increased specific agglutinins	Eldar et al. (1995)
Cellular <i>S. agalactiae</i> extract	IP	Aluminum hydroxide (AH)	Twice	4	77	100 LD ₅₀ (10 ⁹ –10 ¹⁰)	150–180	92% protection when challenged with the homologous virulent strain, increased specific agglutinins	Eldar et al. (1995)
Formalin-killed cells with concentrated ECP added	IP and BI	No	Once	–	30 or 64	10 ³ –10 ⁶	5 and 30	High (RPS = 80%) protection in 30 g tilapia by IP, but low (25%) in 3–5 g tilapia under similar conditions; reduced protection (34%) in 5 and 30 g fish vaccinated through BI	Evans et al. (2004)
Formalin-killed cells with concentrated ECP added	IP	No	Once	–	47, 90, or 180	10 LD ₅₀ (2.0 × 10 ⁸)	45.5 ± 12.2	RPS = 61, 55, 47% at 47, 90, 180 dpv, respectively. Increased specific mean antibody	Pasnik et al. (2005)
Formalin-killed cells	IP	No	Once or twice	3	30	3.0 × 10 ⁶	20	RPS = 83.6 and 96.4% for a single and booster immunization, respectively	Pretto-Giordano et al. (2010)
Feed-based formalin-killed adjuvant vaccine	Oral	Freund's incomplete adjuvant (FIA)	Twice	2	28	1.7 × 10 ⁸	100 ± 10	High (100%) protection from higher antibody responses (humoral or mucosal)	Firdaus-Nawi et al. (2013)
Formalin-killed cells with concentrated ECP added	IP	No	Once	–	15	1.0 × 10 ⁸	51.57 ± 8.23	Variable protective efficiency depending on genotype, not only associated with serotypes. Combined vaccination (PFGE types G and B strains) had a wider protection scope and higher RPS (65.52–100%) when challenged by non-self genotype strains	Chen et al. (2012b)
Microwave-killed <i>S. agalactiae</i> cell vaccine	IP and BI	No	Once	–	30	1.2 × 10 ⁸	5.6 ± 1.5	Protective effects (RPS = 56%), but lower than formalin-killed cells (74%) by IP. BI vaccination showed limited efficacy (24–40%) on 5 g fish	Pasnik et al. (2014)
Live attenuated vaccines									
Live attenuated vaccines (resistant to sparflloxacin)	IP	No	Once	–	14 or 28	2 × 10 ⁷	3–5 and 15–20	Variable protective efficiency of attenuated isolates; polyvalent vaccines provide broader protection to both 3–5 g and 15–20 g tilapia	Pridgeon & Klesius (2013)
Live attenuated vaccine via continuous passage <i>in vitro</i>	IP, BI, and oral	No	Once	–	15 or 30	100 LD ₅₀ (1.0 × 10 ⁷)	30.15 ± 2.60	RPS = 96.88, 67.22, and 71.81% by IP, BI, and oral, respectively, at 15 dpv and 93.61, 60.56, and 53.16% at 30 d	Li et al. (2015)
Recombinant vaccines									
Recombinant pilus islands (PI)-1 ancillary protein 1	Oral	No	Twice	2	63	2.27 × 10 ⁸	100 ± 10	High level IgM antibody of both mucosal and systemic immunity, 70% protection following challenge	Nur-Nazifah et al. (2014)
Recombinant surface proteins phosphoglycerate kinase (PGK) and ornithine carbamoyl-transferase (OCT)	IP	Non-mineral oil adjuvant Montanide ISA 763 AVG	Twice	2	Unknown	4.26 × 10 ⁵	38 ± 6	Combinations of recombinant purified PGK and <i>S. agalactiae</i> whole cells provided greater protection (RPS = 82.4%) compared with individual bacterial components (0–58.8%)	Wang et al. (2014)
Recombinant fibrinogen-binding proteins A (Fbs A) and α-enolase	IP	Freund's complete adjuvant (FCA) and FIA	Twice	2	28	7.8 × 10 ⁷	30	Recombinant α-enolase protein conferred higher protection (62.50 ± 18.75%) than rFbsA (40.63 ± 17.21%). Both enhanced innate immune responses and increased specific antibody titer	Yi et al. (2014)
Recombinant truncated surface immunogenic protein (tSip)	IP	FIA and AH gel	Twice	2	42	50 LD ₅₀ (3 × 10 ⁷)	200	Recombinant tSip mixed with FIA gave stronger protection (RPS = 90%) than tSip alone (50%) or tSip + AH (55%)	He et al. (2014)
DNA vaccination									
DNA vaccine encoding Sip delivered by live attenuated <i>Salmonella typhimurium</i>	Oral	No	3×	1	21	50 LD ₅₀ (1 × 10 ⁸)	100 ± 5.0	Modest protective effect (RPS = 47–57%) after immunization 3× following challenge	Huang et al. (2014)

some protection (mean \pm SD RPS = 62.50 ± 18.75 and $40.63 \pm 17.21\%$, respectively) to tilapia against *S. agalactiae* infection, while the inactivated vaccine group provided higher protection (RPS = $93.75 \pm 5.41\%$). In this case, although increasing numbers of protective and conserved proteins have been identified using new technologies, the efficacies of these recombinant vaccines are not as good as those of the inactivated vaccines. Thus, recombinant vaccines should not only be improved in terms of their protective efficacy, but also the cost of their mass production should be controlled when applied as a commercial product for tilapia.

DNA vaccines

DNA vaccines and their effects against several viral and bacterial diseases in fish have been reported in the last decade (Hølvold et al. 2014); however, only a few DNA vaccine strategies have been successful in providing significant protection to fish against *S. agalactiae* infection. Huang et al. (2014) developed an oral DNA vaccine that encoded Sip. They used a live attenuated *Salmonella typhimurium* to deliver this DNA vaccine and then evaluated the safety and stability of the recombinant DNA vaccine *in vivo* and *in vitro*. This DNA vaccine provided a modest protective effect (RPS = 47–57%) after immunization 3 times following *S. agalactiae* challenge in tilapia. The development of DNA vaccines for tilapia is still in the early stages, and vaccine efficacy will be improved as we increase our understanding of the tilapia immune processes during infection.

VACCINATION METHODS IN TILAPIA

Vaccination in fish is more complicated compared with that in terrestrial livestock because of the aquatic environment. Many delivery methods, including IP injection, bath immersion (BI), and oral administration, have been studied to aid vaccination. For practical reasons, BI and oral vaccinations are convenient for fishes, especially for fry and small fishes. However, their protective efficiency is usually not as good as injection under the same conditions. The vaccine might be partially degraded by the digestive fluids when given orally, or the vaccine might not be sufficiently absorbed by the fish body using the immersion and spray methods (Noraini et al. 2013, Caipang et al. 2014). For example, the RPS

values of tilapia immunized with a live attenuated *Streptococcus agalactiae* via IP injection, BI, and oral administration were 96.88, 67.22, and 71.81%, respectively, at 15 dpv, but declined to 93.61, 60.56, and 53.16%, respectively, after 30 dpv (Li et al. 2015). These results indicate that vaccination by injection provided the strongest protection and that the protection period of oral vaccination was shorter than that of IP injection and BI. Although vaccination by IP injection resulted in a high level and long duration protective effect, the operation could be time-consuming and difficult to administer to small fishes (Caipang et al. 2014). Under the same conditions, tilapia obtained a high protection (RPS = 80%) from vaccination with inactivated *S. agalactiae* by IP injection at 30 dpv, while the protection was significantly reduced (RPS = 34%) using BI (Evans et al. 2004). With the appearance of dedicated vaccination teams and semi-automatic vaccination devices, vaccination by injection is becoming feasible and is practiced widely in modern tilapia aquaculture systems.

INFLUENCE OF ADJUVANTS

Adjuvants are defined as a group of structurally heterogeneous compounds that slow down the release and modulate the intrinsic immunogenicity of an antigen (Audibert & Lise 1993, Guy 2007). The traditional adjuvant, mineral oil, has been used in a commercial whole-cell killed *Streptococcus agalactiae* vaccine (AQUAVAC® Strep Sa). In addition to Freund's complete and incomplete adjuvants (FCA and FIA; He et al. 2014, Yi et al. 2014), many other types of adjuvants have been used in tilapia *S. agalactiae* vaccination, such as aluminum-based adjuvants (Eldar et al. 1995, He et al. 2014) and non-mineral oil adjuvant Montanide ISA 763 (Wang et al. 2014) (Table 1).

Based on published data, most inactivated *S. agalactiae* vaccines without adjuvants can provide good protection for tilapia (Table 1). However, *S. agalactiae* recombinant vaccines usually require the use of adjuvants to provide appropriate protection (Table 1). For instance, He et al. (2014) showed that recombinant truncated Sip (tSip) mixed with FIA and IP-injected provided very strong protection to tilapia (RPS = 90%) against Group B streptococcal infections, while tSip without adjuvant was only about half as effective (RPS = 50%). Similarly, Firdaus-Nawi et al. (2013) showed that a feed-based vaccine with FIA provided a significantly higher protection (RPS = 100%) in tilapia than that without FIA (RPS = 57%).

Based on current research, FIA appears to be a suitable adjuvant for tilapia vaccines. FIA promoted the induction of both humoral and cellular immune responses by producing higher humoral or mucosal antibody responses in red tilapia (Firdaus-Nawi et al. 2013). In a Japanese flounder model, vaccination with an FIA-adjuvanted recombinant protein enhanced the expression of a wide range of genes that are likely to participate in humoral immunity and innate cellular immunity mediated by activated natural killer cells and phagocytes (Jiao et al. 2010). Although FIA has been shown to be highly effective in vaccination of tilapia and provides a significant reduction in toxicity compared with FCA, some side effects still occur, such as granuloma and tissue necrosis (Gjessing et al. 2012). To the best of our knowledge, no study has been conducted to investigate the side effects of adjuvants used in tilapia. Thus, the search for effective adjuvants that maximize immunogenicity and minimize side effects for piscine vaccines needs to be intensified.

OTHER FACTORS INFLUENCING VACCINE EFFICACY

Some factors, such as temperature, immunization duration and number, fish size, and challenge dose, cannot be ignored when developing vaccines for tilapia. Evans et al. (2004) showed that water temperature (26 versus 32°C) did not appear to influence the RPS results of inactivated *Streptococcus agalactiae* vaccine; however, the size of the fish appeared to play an important role in the vaccine efficacy. Larger (30 g) tilapia had an RPS of 80%, while 5 g tilapia had an RPS of only 25% under similar conditions.

The production cycle of farmed tilapia in tropical regions takes 4 to 6 mo, and a desirable vaccine for tilapia should provide significant long-term protection against *S. agalactiae*. Thus, the duration of protection is also an important factor in the evaluation of vaccine efficacy. Table 1 lists the post-immunization challenge time for all types of vaccines. Most studies performed the challenge trials at 30 dpv; the longest delay between vaccination and challenge was recorded by Pasnik et al. (2005), wherein one inactivated vaccine could confer protection against *S. agalactiae* up to 180 dpv when challenged with $10 \times LD_{50}$.

Meanwhile, vaccine efficacy was also correlated with the number of immunizations: 1 or 2 booster vaccinations significantly improved the RPS (Pretto-Giordano et al. 2010, Huang et al. 2014, Li et al.

2015). The RPS of the booster immunization (96.4%) was significantly higher than that of a single immunization (83.6%; Pretto-Giordano et al. 2010). Recombinant and DNA vaccines usually require booster vaccinations to obtain satisfactory protection, while the inactivated and live attenuated vaccines only need single immunizations (Table 1).

In the published studies, researchers used different challenge doses, approximately 10 to 100 times the *S. agalactiae* LD_{50} . It is thought that lower challenge doses decrease vaccination-related mortality and provide better protection (Evans et al. 2004). Thus, these vaccines could provide higher protection for tilapia in the natural infection situation compared with laboratory IP injection.

CONCLUDING REMARKS AND PERSPECTIVES

Vaccines are among the most viable approaches to prevent fish diseases in aquaculture. Inactivated, live attenuated, recombinant, and DNA vaccines against *Streptococcus agalactiae* have been developed for tilapia, an economically important fish. Inactivated *S. agalactiae* vaccines showed superior protection efficiency when compared with live attenuated, recombinant, and DNA vaccines. Injecting the vaccine into tilapia remains the most effective vaccination method, resulting in very good immunoprotection. In addition, adjuvants and booster immunizations are necessary to increase the efficacy of vaccines, especially for recombinant vaccines. Many immune-related genes of tilapia during *S. agalactiae* infection have been identified (Nithikulworawong et al. 2012, Poochai et al. 2014, Shen et al. 2015); therefore, a full understanding of the immune processes in tilapia during infection would aid in vaccine development.

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