UNIVERSITI PUTRA MALAYSIA

APPLICATION OF BIOFILM-CHITIN ORAL VACCINE ON STREPTOCOCCOSIS INFECTED RED HYBRID TILAPIA (*Oreochromis* sp.)

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This Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Chairman: Assoc. Prof. Ina Salwany Md Yasin, PhD
Faculty: Agriculture

Malaysia has started developing tilapia culture and has become one of the top countries in Asia. However, disease such as streptococcosis is one of the obstacles in the aquaculture industry. Two main and renowned species are *Streptococcus agalactiae* and *Streptococcus iniae.* For prevention of streptococcosis, the use of antibiotics is discouraged. Therefore, developing vaccines to prevent streptococcosis is highly recommended. In this study, a feed-based oral biofilm-chitin vaccine was developed and tested in red hybrid tilapia to prevent streptococcosis. For identification, the results confirmed ten isolates of *S. agalactiae* and three isolates of *S. iniae* by API20 strep and 16SrRNA gene sequencing. This was the first report on the isolation of *S. iniae* from red hybrid tilapia in Malaysia.

The virulence of *S. agalactiae* and *S. iniae* was assessed by pathogenicity. The LD_{50} of *S. agalactiae* (MII) was determined to be 4.64 $\times 10^6$ CFU/ml, and *S. iniae* (TSK2) was 3.16 $\times 10^5$ CFU/ml. Clinical signs of streptococcosis were observed in infected fish such as erratic swimming, anorexia, loss of orientation, haemorrhage on the head and eye, exophthalmia, and corneal opacity.

In this study, feed-based oral biofilm-chitin (10^{10} CFU/g) and feed-based oral free-cell (10^{10} CFU/ml) vaccines were tested to determine the antibody level of serum, mucus, and gut lavage against *S. agalactiae* infection in red hybrid tilapia. In the vaccinated groups, the antibody levels of the serum, mucus and gut lavage were significantly higher (P = 0.000) than those of the control group. However, the antibody level of the feed-based biofilm-chitin group for mucus (P

= 0.039), serum (P = 0.042) and gut lavage (0.021) was significantly higher than those of the feed-based free-cell and control groups.

For determining Relative Percentage Survival (RPS), the experimental groups were challenged intraperitoneally by 0.15 mL of live virulent *S. agalactiae* with a concentration of 10^9 CFU/ml. At 14 days post-challenge, the RPS values for the feed-based biofilm-chitin, feed-based free cell and control groups were 87, 57 and 0%, respectively. Total mortality was observed in control group due to streptococcosis. The obtained results revealed that the survival rate in the biofilm-vaccinated group was significantly higher (P = 0.000) than those in other groups, which lead to high efficacy of biofilm-chitin vaccine compared with the other groups.

At the end of the experiment, the gut samples were subjected to histopathological test to evaluate the presence, size and number of lymphoid cells in GALT. As a result, the GALTs in the feed-based biofilm-vaccinated group were developed with higher intensity than those in the feed-based free-cell-vaccinated group. No GALT was observed in the control group. In addition, the size of GALTs and the number of lymphoid cells in the feed-based biofilm-vaccinated group were significantly higher (P = 0.000) than those in the feed-based free-cell-vaccinated group.

In conclusion, this study revealed that the feed-based oral biofilm-chitin of *S. agalactiae* stimulated the systemic and mucosal immunity in red hybrid tilapia more than feed-based free-cell vaccine and non-vaccinated groups. Moreover, the protection of the feed-based oral biofilm-chitin of *S. agalactiae* was higher against *S. agalactiae* infection compared with the other groups. Thus, the feed-based oral biofilm-chitin can be a preferable candidate for dealing with *S. agalactiae* infection in red hybrid tilapia due to its efficacy, low cost and ease of usage.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PANGGUNAAN VAKSIN ORAL SELAPUT BIOFILEM-KITIN PADA TILAPIA HIBRID MERAH (OREOCHROMIS SP.) YANG DIJANGKITI STREPTOCOCCOSIS

Oleh

MAHDI KAHIESHESFANDIARI

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Malaysia telah mula membangun kultur tilapia dan telah menjadi salah satu negara teratas di Asia. Walau bagaimanapun, penyakit seperti streptococcosis adalah salah satu halangan dalam industri akuakultur. Dua spesies utama adalah *Streptococcus agalactiae* dan *Streptococcus iniae.* Untuk mencegah streptococcosis, penggunaan antibiotik tidak digalakkan. Oleh itu, pengembangan vaksin untuk mencegah streptococcosis sangat disyorkan. Dalam kajian ini, vaksin biofilem oral berasaskan makanan telah dibangunkan dan diuji dalam tilapia hibrid merah untuk mencegah streptococcosis. Untuk pengenalpastian, keputusan mengesahkan sepuluh isolat *S. agalactiae* dan tiga isolat *S. iniae* oleh API20 strep dan analisis 16SrRNA gen. Ini adalah penemuan pertama mengenai isolat *S. iniae* dari tilapia hibrid merah di Malaysia.

Virulensi *S. agalactiae* dan *S. iniae* dinilai oleh ujian patogenik melalui suntikan intraperitoneal. Daripada hasil kajian, LD_{50} (kepekatan yang paling rendah yang boleh membunuh 50% ikan) *S. agalactiae* (MII) adalah 4.64 $\times 10^6$ CFU/ml, and *S. iniae* (TSK2) was 3.16 $\times 10^5$ CFU/ml. Tanda-tanda klinikal penting streptococcosis telah diperhatikan pada ikan yang diinfeksi seperti anoreksia, kehilangan orientasi, pendarahan pada kepala dan mata, exophthalmia dan kelegapan kornea.

Dalam kajian ini, vaksin oral biofilem-kitin oral berasaskan makanan (10^{10}CFU / g) dan vaksin sel-sel bebas oral (10^{10}CFU / ml) berasaskan makanan telah diuji untuk menilai paras antibodi bagi serum, lendir, dan usus lavage terhadap jangkitan *S. agalactiae* dalam tilapia hibrid merah. Dalam kumpulan yang divaksin, paras antibodi serum, lendir dan usus lavage adalah lebih tinggi (P = 0.000) daripada kumpulan kawalan. Walau bagaimanapun, tahap antibodi

kumpulan biofilem-kitin berasaskan makanan untuk lendir (P = 0.039), serum (P = 0.042) dan usus lavage (0.021) jauh lebih tinggi daripada kumpulan sel bebas berasaskan makanan dan kumpulan kawalan.

Untuk menentukan survival peratusan relatif (RPS), kumpulan eksperimen diinfeksi secara intraperitoneal menggunakan 0.15 mL kultur hidup *S. agalactiae* dengan kepekatan 10^9 CFU / ml. Pada 14 hari pasca cabaran, nilai RPS bagi kumpulan biofilem-kitin berasaskan makanan, kumpulan sel bebas berasaskan makanan dan kumpulan kawalan masing-masing adalah 87, 57 dan 0%. Jumlah kematian dijumpai dalam kumpulan kawalan kerana streptococcosis. Hasil yang diperolehi menunjukkan bahawa kadar kelangsungan hidup dalam kumpulan yang diberi vaksin biofilem-kitin jauh lebih tinggi (P = 0.000) berbanding dengan kumpulan lain, yang membawa kepada keberkesanan vaksin biofilem yang tinggi berbanding kumpulan lain.

Untuk mencapai Ketahanan Peratus Relatif (RPS), ikan yang digunakan dalam eksperimen telah disuntik dengan 0.15 mL *S. agalactiae* virulen hidup secara intraperitoneal dengan kepekatan 10^9 CFU/mL. Pada 14 hari pasca-cabaran, nilai RPS untuk biofilem berasaskan makanan, kumpulan sel dan kumpulan kawalan berasaskan makanan masing-masing adalah 87%, 57% dan 0%. Kesemua ikan dalam kumpulan kawalan telah mengalami kematian akibat streptococcosis. Keputusan yang diperolehi menunjukkan bahawa kadar ketahanan dalam kumpulan biofilem (P <0.05) adalah lebih tinggi daripada kumpulan lain, yang membawa kepada keberkesanan vaksin biofilem.

Pada akhir eksperimen, sampel usus telah dianalisis dengan ujian histopatologi untuk menilai kehadiran, saiz dan bilangan sel limfoid dalam GALT (*Gut Associated Lymphoid Tissue*) Secara amnya , GALT dalam kumpulan biofilem- kitin berasaskan makanan adalah dengan kadar intensiti yang lebih tinggi daripada kumpulan sel bebas makanan. Tiada GALT diperhatikan dalam kumpulan kawalan. Di samping itu, saiz GALT dan bilangan sel limfoid dalam kumpulan biofilem-kitin berasaskan makanan jauh lebih tinggi (P = 0.000) berbanding dengan kumpulan vaksin sel bebas berasaskan makanan.

Kesimpulannya, kajian ini menunjukkan bahawa vaksin biofilem-kitin berasaskan makanan merangsang imuniti sistemik dan mukosa dalam tilapia hibrid merah lebih tinggi daripada vaksin sel bebas berasaskan makanan dan kumpulan yang tidak divaksinasi. Selain itu, biofilem-kitin oral berasaskan makanan memberi perlindungan yang lebih baik lebih tinggi terhadap jangkitan *S. agalactiae* berbanding kumpulan lain. Oleh itu, biofilem-kitin oral berasaskan makanan boleh menjadi calon yang lebih baik untuk menangani jangkitan S. agalactiae dalam tilapia hibrid merah kerana keberkesanannya, kos rendah dan kemudahan penggunaan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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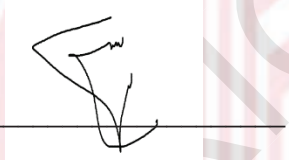
Signature:  Date: _____

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%	Percentage
$	Dollar
× g	Gravity at the earth's surface
α	Alpha
β	Beta
Y	Gamma
ºC	Degree Celcius
µg	Microgram
µl	Microliter
µm	Micrometer
µM	Micromolar
A_{260}nm/A_{280}nm	Ratio of absorbance measurements at 260 and 280 nanometer
A. hydrophila	*Aeromonas hydrophila*
API	Analytical profile index
A. salmonicida	*Aeromonas salmonicida*
BHI	Brain heart infusion
BHIB	Brain heart infusion broth
BALT	Bronchus associated lymphoid tissue
BSA	Bovine serum albumin
bp	Base pair
CFU	Colony forming units
CFU/ml	Colony forming units per milliliter
cm	Centimeter
cm^2	Square centimeter
CTL	Cytotoxic T lymphocyte
Cx	Control group
DNA	Deoxynucleotide acid
dNTP	Deoxynucleotide acid

DO	Dissolved oxygen
ECP	Extracellular products
ELISA	Enzyme-linked immunosorbent assay
FBV	Feed-based biofilm-chitin vaccine
FCV	Feed-based free-cell vaccine
g	Gram
GALT	Gut-associated lymphoid tissue
GBS	Group B streptococcus
GFP	Green fluorescent protein
GIALT	Gill-associated lymphoid tissue
h	Hour
ha	Hectare
H&E	Hematoxylin and eosin
HGNNV	Humpback grouper nervous necrosis
IHNV	Infectious hematopoietic necrosis virus
IgA	Immunoglobin A
IgD	Immunoglobin D
IgE	Immunoglobin E
IgG	Immunoglobin G
IgM	Immunoglobin M
IM	Intramuscular
IP	Intraperitoneal
IPNV	Infectious pancreatic necrosis virus
Kb	Kilobase pair
L	Liter
LCDV	Lymphocyctis virus disease
LD_{50}	Lethal dose 50%
L. garvieae	*Lactococcus garvieae*
M	Molar
MALT	Mucosa-associated lymphoid tissue

$MgCl_2$	Magnesium chloride
mg	Milligram
mg/L	Milligram per liter
min	Minute
ml	Milliliter
mm	Millimeter
MMC	Melanomacrophage centre
mmol	Millimole
mRNA	Messenger ribonucleic acid
MT	Metric tonnes
ng	Nanogram
nm	Nanometer
O. niloticus	*Oreochromis niloticus*
OD	Optical density
OMP	Outer membarane protein
p. aeruginosa	*Pseudomonas aeruginosa*
PBS	Phosphate-buffered saline
PBST 20	Phosphate-buffered saline + 0.05% Tween
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PH	Puissance hydrogen
pmol	Picomole
RAPD	Randomly amplified polymorphic DNA
RM	Malaysian ringgit
RNA	Ribonucleic acid
rpm	Revolutions per minute
RPS	Relative percent survival
RNase	Ribonuclease
rRNA	Ribosomal ribonucleic acid

SALT	Skin-associated lymphoid tissue
S. agalactiae	*Streptococcus agalactiae*
S. difficile	*Streptococcus difficile*
Sec	Second
SEM	Scanning electron microscopy
S. iniae	*Streptococcus iniae*
SOP	Surface outer protein
Taq	Thermus aquaticus
THB	Todd Hewitt broth
TM	Trade mark
TMB	Tetramethylbenzidine
TSA	Trypticase soy agar
TSB	Trypticase soy broth
U	Unit
USA	United States of America
USD	United States Dollar
VHSV	Viral haemorrhage septicemia virus
V. anguillarum	*Vibrio anguillarum*
w/v	weight per volume

CHAPTER 1

INTRODUCTION

Tilapia is the most cultured fish worldwide and is second to carp as a farmed fish food (Fitzsimmons, 2015). The production of tilapia in 2000 was more than 1.5 million MT compared with that in 1980 (28,260 million MT). This information shows that tilapia is the most important species for aquaculture in the 21st century. The introduction of tilapia in international markets in the first half of 2015 was 200,000 MT. Moreover, more than 75,000 MT of whole tilapia were exported to the international market from Asian countries (FAO, 2015). The global production of tilapia is estimated to exceed 5 million MT with a growth rate of 6% compared with that in 2014 (FAO, 2015). In Malaysia, tilapia products are second most produced in the aquaculture industry (13.5%) with a wholesale value of RM 99,167,000 in 2010. In addition, in a recent report by Department of fisheries Malaysia (DOF), tilapia production has reached 36,299.01 million MT with a value of RM 302,839,050 in 2015 (DOF, 2016). This value increased from USD 154 million to 1800 million globally from 1981 to 2002.

One of the serious threats in aquaculture industry is streptococcosis (Haghighi et al., 2010). Streptococcosis is a vital disease in aquaculture which can make significant mortality (up to 50%) in fresh and marine fishes (Agnew and Barnes, 2007), such as striped bass (*Morone saxatilis*) (Baya et al., 1990), bull minnows (*Fundulus grandis*) (Rasheed and Plumb, 1984), menhaden (*Berevoortia patronus*) (Plumb et al., 1974), mullet (*Liza klunzingeri*) and tilapia (*Oreochromis niloticus*) (Evans et al., 2002). Streptococcosis is caused by *Streptococcus agalactiae and Streptococcus iniae* (Soltani et al., 2005). Tilapia is exposed more to streptococcosis compared with other cultured fish species; this disease results in huge economic losses (Intervet, 2006; Soltani et al., 2005; Pasnik et al., 2005). Recently, Streptococcosis outbreak has occurred in different countries, such as Israel, the USA, Brazil and Thailand, and caused huge economic losses (Wang and Lu, 2016) estimated to reach USD 150 million annually (Zamri-Saad et al., 2015).

The streptococcosis outbreak in Malaysia is frequently reported in red tilapia, especially during dry and hot seasons. Streptococcosis also causes economic losses in tilapia industry in Malaysia (Amal et al., 2010 a,b; Zamri-Saad et al., 2010; Najiah et al., 2008). Recent studies have also reported that streptococcosis outbreak occurs more in cage cultures of red tilapia in rivers and lakes than in examining pools, earthen ponds and irrigation canals (Amal, 2011). Antibiotics are ineffective for preventing streptococcosis due to environmental pollution, residue in fish and antibiotic resistance, thereby increasing the concern on antibiotic use (Liaghat et al., 2011; Klesius et al.,

2000a). Moreover, antibiotics that can cure streptococcosis remain unavailable to date (Agriculture Research Service, 2010). In the farm, pathogens are transmitted from fish to fish, in which the sources of infection are dead and dying fishes (Kitao, 1993).

Vaccination is the most important and feasible way to prevent this zoonotic disease and losses in the aquaculture industry (Soltani et al., 2009). Vaccines are obtained from inactive or killed pathogens which stimulate the immune system in inoculated animals (Klesius et al., 2002). Vaccination is a helpful method for countering infectious diseases (Gudding et al., 1999). This method can be applied through several routes, such as injection, immersion and oral vaccination (Moore et al., 1998). These vaccines have high protective properties (Lillehaug, 1989), but they have certain limitations in the aquaculture industry (Lamer et al., 1985). In addition, injection of these vaccines is often conducted on fishes which weigh more than 30 g. One of the commercialised vaccines which is administered by IP injection is Aquavac® strep vaccine, which provides long-term and good protection against *S. agalactiae* and *S. iniae*. The injection route is expected to be effective and provide long-term protection, but some of its limitations include stressful factors for fishes, such as handling, injection and anesthetising; in addition, this method is more costly compared with other routes. Vaccine administration through injection is mostly used for precious species such as Atlantic salmon (Plant and Lapatra, 2011).

The most applied route for farms is oral vaccination rather than immersion and injection routes. In oral vaccination, the vaccines are incorporated in feeds. To date, oral vaccination is the ideal route of vaccination for fish, especially from an economic stand point (Muktar et al., 2016; Firdaus-Nawi et al., 2013). The advantages of vaccination are minimum stress of handling, simplicity, suitability for fishes of all sizes and greater cost effectiveness compared with other methods. This method also reduces time and labour costs. The first publication about oral vaccines in aquaculture was the inactivated vaccine against *Aeromonas salmonicida*, which was extremely successful (Duff 1942). In addition to the advantages highlighted above, oral vaccines can enhance mucosal immunity and can also cause the development of antigen-specific antibodies in the intestine, bile and skin. The first barrier of the fish against pathogen attack is the mucosal layer. Immunisation by oral vaccination acts on antigen-specific antibodies in the intestine, mucus and blood in several fish species (Fletcher and White, 1972; Roumbout et al., 1986). The remarkable response via the mucosal immune system renders oral vaccination applicable because the first contact between the pathogen and the fish body occurs in the mucosal surface (Manganaro et al., 1994). To date, oral vaccines, such as Garvetil™ and Aquavac™, are the most popular (Firdaus-Nawi et al., 2013; Evans et al., 2002) against streptococcosis. However, these vaccines are specifically developed against *S. iniae* and *Lactococcus garvieae* (Ming et al., 2012; Intervet, 2010; Naraid et al., 2008).

The major problem of oral vaccination is the destruction of antigen in the stomach by enzymes before reaching the hindgut. Most antigens are destructed before intake, affecting the immune responsiveness of lymphoid organs. Given this problem, the efficacy of oral vaccination is lower than that of injection vaccines. Natural bacterial populations benefit from food concentration and are protected against toxic agents and predators, which prefer to assemble in a polymeric glycocalyx matrix called biofilm. This natural strategy of bacteria used for developing biofilm vaccines can prevent destruction in the stomach. This protective characteristic of bacteria was established to develop a helpful oral vaccine which can resist gastric destruction, thereby facilitating improved antigen delivery (Siriyapagouder et al., 2014; Davey et al., 2000). Compared with free-cell vaccines (FCVs), biofilm vaccines remain in larger quantities and are retained longer in the tissues of hindgut, kidney and spleen after a single delivery (Nayak et al., 2004). Biofilm requires a substrate for bacteria to attach and grow on. One of the suitable substrates is chitin which is a natural material that highly found in insects, crustacean shells and fungi. For commercial use, this natural polymer is obtained from shells of crabs and shrimps. In aquaculture, chitin serves immunostimulatory and immunomodulatory activities to protect fishes against bacterial diseases (Anderson and Siwicki, 1994; Siwicki et al., 1994). It can also be used as dietary and immersion supplements (Anderson and Siwicki, 1994). Chitin can improve humoral defence and non-specific cellular mechanism in animals (Gopalakannan and Arul, 2006; Dautremepuits et al., 2004; Esteban et al., 2001). Thus, for developing biofilm vaccine, chitin which can play substrate or stimulatory roles to enhance the innate immune system of the fish, is important. Chitin can also act as dietary supplement (Vinay et al., 2013).

However, researchers have concentrated on the prevention of digesting antigens and denaturing while passing through the stomach and gut. To our best knowledge, no research was reported on the application of biofilm oral vaccine using chitin against streptococcosis in tilapia. Thus, this study aims to develop a feed-based biofilm-chitin vaccine against streptococcosis which can protect antigens against destruction caused by stomach enzymes, enhance the immune system and increase the antibody titre of tilapia for protection against *S. agalactiae* infection.

The specific objectives of this study were:

1) To isolate and identify *Streptococcus* species from cage-cultured red hybrid tilapia
2) To develop feed-based biofilm-chitin and free-cell oral vaccines of streptococcosis.

3) To evaluate efficacy of the biofilm-chitin vaccine against streptococcosis.

Hypothesis:

H_0: The feed-based biofilm-chitin vaccine of *S. agalactiae* could not induce the immune system of the skin mucosal layer, gut lavage and serum against *S. agalactiae* infection in red hybrid tilapia (*Oreochromis* sp).

H_A: The feed based biofilm-chitin vaccine of *S. agalactiae* could induce the immune system of the skin mucosal layer, gut lavage and serum against *S. agalactiae* infection in red hybrid tilapia (*Oreochromis* sp).

REFERENCES

Abdullah, S., Misri, S., Padilah, B., Zulkafli, R., Kua, B., Azila, A., & Rimatulhana, R. (2004). *Pre-disposing factors associated with outbreak of Streptococcal infection in floating cage-cultured red tilapia in reservoirs.* Paper presented at the 7th Asian Fisheries Forum, The Triennial Meeting of The Asian Fisheries Society, (p. 129), Penang, Malaysia.

Abdullah, S., Padilah, B., Azila, A., Rimatulhana, R., & Shahidan, H. (2005). *Multiple streptococcal species infection in cage cultured red tilapia , but showing similar clinical signs.* Paper presented at the Proceeding of the sixth symposium on disease in Asian aquaculture, Combo, Srilanka. PP. 332-339.

Abdullah, S., Padilah, B., Azila, A., Rimatulhana, R., & Shahidan, H. (2008). Multiple streptococcal species infection in cage-cultured red tilapia but showing similar clinical signs. *Diseases in Asian aquaculture VI*, 313-320.

Abdullah, S., Zamri-Saad, M., Zulkafli, A., Sabri, M., Amal, M., Misri, S., . . . Nur-Nazifah, M. (2009). *Prevalence and significant of Streptococcosis in cage cultured red tilapia of West Malaysia.* Paper presented at the Abstract of the Asian Pacific Aquaculture Conference, (p. 49), PWTC Kuala Lumpur.

Abdullah, S., Omar, N., Yusoff, S. M., Obukwho, E. B., Nwunuji, T. P., Hanan, L., & Samad, J. (2013). Clinicopathological features and immunohistochemical detection of antigens in acute experimental *Streptococcus agalactiae* infection in red tilapia (*Oreochromis* sp.). *SpringerPlus, 2*(1), 286.

Abuseliana, A. F., Daud, H. H. M., Aziz, S. A., Bejo, S. K., & Alsaid, M. (2011). Pathogenicity of *Streptococcus agalactiae* isolated from a fish farm in Selangor to juvenile red tilapia (*Oreochromis* sp.). *Journal of Animal and Veterinary Advances, 10*(7), 914-919.

Abuseliana, A. F., Hassan, D., Saleha, A. A., Siti, K. B., & Milud, A. (2010). *Streptococcus agalactiae* the etiological agent of mass mortality in farmed red tilapia (*Oreochromis* sp.). *Journal of Animal and Veterinary Advances, 9*(20), 2640-2646.

Abutbul, S., Golan-Goldhirsh, A., Barazani, O., & Zilberg, D. (2004). Use of Rosmarinus officinalis as a treatment against *Streptococcus iniae* in tilapia (*Oreochromis* sp.). *Aquaculture, 238*(1), 97-105.

Agnew, W., & Barnes, A. C. (2007). Streptococcus iniae: an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. *Veterinary microbiology, 122*(1), 1-15.

Agriculture research service (ARS) United Stetes department of agriculture (USDA). (2010). new streptococcal vaccine. Retrieved from http://www.ars.usda.gov/research/patents/patents.htmserialnum=10807575

Akhlaghi, M. (2000). Immunogenicity of *Aeromonas hydrophila* in common carp (*Cyprinus carpio, L.*). *Journal of the Faculty of Veterinary Medicine, University of Tehran, 55*(1), 56-62.

Al-Harbi, A. H. (1994). First isolation of Streptococcus sp. from hybrid tilapia (*Oreochromis niloticus*× *O. aureus*) in Saudi Arabia. *Aquaculture, 128*(3), 195-201.

Al-Harbi, A. H. (2011). Molecular characterization of Streptococcus iniae isolated from hybrid tilapia (*Oreochromis niloticus*× *Oreochromis aureus*). *Aquaculture, 312*(1), 15-18.

Alagappan, K., Deivasigamani, B., Kumaran, S., & Sakthivel, M. (2009). Histopathological alterations in estuarine catfish (*Arius maculatus*; Thunberg, 1792) due to *Aeronomas hydrophila* infection. *World Journal of Fish and Marine Sciences, 1*(3), 185-189.

Alcamo, I. (1997). Environment and industrial microbiology. *Fundamentals of microbiology, 5th editon. Addison Wesley Longman, Menlo Park, California*, 766.

Alitheen, N. B., McClure, S., & McCullagh, P. (2010). B-cell development: one problem, multiple solutions. *Immunology and cell biology, 88*(4), 445-450.

Alsaid, M., Mohd, H., Mohamed, N., Khairani, S., Mohamed, Y., Farag, A., & Hayati, R. (2013a). *Pathological findings of experimental Streptococcus agalactiae infection in red hybrid tilapia (Oreochromis* sp.*)*. Paper presented at the International Conference on Chemical, Agricultural and Medical Sciences (CAMS-2013). (PP 70-73), Kuala Lumpur, Malaysia.

Alsaid, M., Daud, H., Mohamed, N., Bejo, S. K., Mohamed, Y., & Abuseliana, A. (2013b). Environmental factors influencing the susceptibility of red hybrid tilapia (Orechromis sp.) to Streptococcus agalactiae infection. Advanced Science Letters, 19(12), 3600-3604.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research, 25*(17), 3389-3402.

Amal, A., Abdullah, S., Zulkafli, R., Misri, S., Ramley, A., & Zamri-Saad, M. (2008). *The effect of water temperature on the incidence of Streptococcus agalactiae infection in cage-cultured tilapia.* Paper presented at the International Seminar on Management Strategies on

Animal Health and Production Control in Anticipation of Global Warming.

Amal, A., Zamri-Saad, M., Abdullah, S., Zulkafli, R., Misri, S., Nur-Nazifah, M., & Shahidan, H. (2010a). Prevalence of *Streptococcus agalactiae* in tilapia in flowing water, ponds, rivers and reservoirs. *Online Journal of Veterinary Research, 14*(2), 153-162.

Amal, M., Zamri-Saad, M., Zulkafli, A., Abdullah, S., A., Misri, S., Ramley, B., . . . Sabri, M. (2010b). Water thermocline confirms susceptibility of tilapia cultured in lakes to *Streptococcus agalactiae*. *Journal of Animal and Veterinary Advances, 9*(22), 2811-2817.

Amal, M. (2011). Prevalence, risk factors and transmission of *Streptococcus agalactiae* in the red hybrid tilapia *(Oreochromis sp.).* PhD thesis. Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor,

Amal, M., & Zamri-Saad, M. (2011). Streptococcosis in tilapia (*Oreochromis niloticus*): a review. *Pertanika J Trop Agric Sci, 34*(2), 195-206.

Amal, M., Zamri-Saad, M., Iftikhar, Abdullah, S., A., Aziel, S., & Fahmi, S. (2012). An outbreak of *Streptococcus agalactiae* infection in cage-cultured golden pompano, *Trachinotus blochii* (Lacépède), in Malaysia. *Journal of Fish Diseases, 35*(11), 849-852.

Amal, M., Zamri-Saad, M., Abdullah, S., & Zulkafli, A. (2013a). Transmission of *Streptococcus agalactiae* from a hatchery into a newly established red hybrid tilapia, *Oreochromis niloticus* (L.)× *Oreochromis mossambicus* (Peters), farm. *Journal of Fish Diseases, 36*(8), 735-739.

Amal, M., Zamri-Saad, M.,Abdullah, S., Zulkafli, A., & Nur-Nazifah, M. (2013b). Molecular characterization of *Streptococcus agalactiae* strains isolated from fishes in Malaysia. *Journal of applied microbiology, 115*(1), 20-29.

Amal, M. N. A., Zamri-Saad, M., Abdullah, S., & Zulkafli, A. R. (2015). Water quality influences the presence of *Streptococcus agalactiae* in cage cultured red hybrid tilapia, *Oreochromis niloticus*× *Oreochromis mossambicus*. *Aquaculture Research, 46*(2), 313-323.

Amann, R. I., Ludwig, W., & Schleifer, K.-H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological reviews, 59*(1), 143-169.

Amend, D. F. (1981). Potency testing of fish vaccines. Fish biologics: serodiagnostics and vaccines, 447-454.

Anderson, D. P. (1992). Immunostimulants, adjuvants, and vaccine carriers in fish: applications to aquaculture. *Annual Review of Fish Diseases, 2*, 281-307.

Anderson, D. P., & Siwicki, A. K. (1994). Duration of protection against Aeromonas salmonicida in brook trout immunostimulated with glucan or chitosan by injection or immersion. *The Progressive Fish-Culturist, 56*(4), 258-261.

Anderson, E. D., Mourich, D. V., Fahrenkrug, S., LaPatra, S., Shepherd, J., & Leong, J. (1996). Genetic immunization of rainbow trout (Oncorhynchus mykiss) against infectious hematopoietic necrosis virus. *Molecular marine biology and biotechnology, 5*(2), 114-122.

Asha, A., Nayak, D., Shankar, K., & Mohan, C. (2004). Antigen expression in biofilm cells of *Aeromonas hydrophila* employed in oral vaccination of fish. *Fish & Shellfish Immunology, 16*(3), 429-436.

Austin, B. (1984). The future of bacterial fish vaccines. *Vaccine, 2*(4), 249-254.

Austin, B., & Austin, D. (2007). Characteristics of the diseases. *Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish*, 15-46.

Azad, I., Al-Marzouk, A., James, C., Almatar, S., Al-Gharabally, H., & Qasem, J. (2012). Outbreak of natural Streptococcosis in hatchery produced silver pomfret (*Pampus argenteus Euphrasen*) larvae in Kuwait. *Aquaculture, 330-333*, 15-20.

Azad, I., Shankar, K., & Mohan, C. (1997). Evaluation of biofilm of *Aeromonas hydrophila* for oral vaccination of carps. *Diseases in Asian aquaculture III. Fish Health Section, AFS, Manila*, 181-186.

Azad, I., Shankar, K., Mohan, C., & Kalita, B. (1999). Biofilm vaccine of *Aeromonas hydrophila*–standardization of dose and duration for oral vaccination of carps. *Fish & Shellfish Immunology, 9*(7), 519-528.

Azad, I., Shankar, K., Mohan, C., & Kalita, B. (2000a). Protective response of common carp orally vaccinated with biofilm and free cells of *Aeromonas hydrophila* challenged by injection and immersion routes. *Journal of Aquaculture in the Tropics, 15*(1), 65-70.

Azad, I., Shankar, K., Mohan, C., & Kalita, B. (2000b). 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*(2), 103-108.

Baccrach,G., Zlotkin,A., Hurvitz,A., Evans, D.L., Eldar A. (2001). Recovery of *Streptococcus iniae* from diseased fish previously vaccinated with a *Streptoccus* vaccine. Apply Environment Microbiology, 67, 3756-3758.

Bader, J. A., & Shotts Jr, E. B. (1998a). Determination of phylogenetic relationships of Flavobacterium psychrophilum (Flexibacter psychrophilus), Flavobacterium columnare (Flexibacter columnaris), and Flexibacter maritimus by sequence analysis of 16s ribosomal RNA genes amplified by polymerase chain reaction. *Journal of Aquatic Animal Health, 10*(4), 320-327.

Bader, J. A., & Shotts Jr, E. B. (1998b). Identification of Flavobacterium and Flexibacter species by species-specific polymerase chain reaction primers to the 16s ribosomal RNA gene. *Journal of Aquatic Animal Health, 10*(4), 311-319.

Badgett, M. R., Auer, A., Carmichael, L. E., Parrish, C. R., & Bull, J. J. (2002). Evolutionary dynamics of viral attenuation. *Journal of virology, 76*(20), 10524-10529.

Balarin, J. D., & Hatton, J. P. (1979). Tilapia: A guide to their biology and culture in Africa. FAO.

Ballesteros, N. A., Alonso, M., Saint-Jean, S. R., & Perez-Prieto, S. I. (2015). An oral DNA vaccine against infectious haematopoietic necrosis virus (IHNV) encapsulated in alginate microspheres induces dose-dependent immune responses and significant protection in rainbow trout (Oncorrhynchus mykiss). *Fish & Shellfish Immunology, 45*(2), 877-888.

Barham, W., Schoonbee, H., & Smit, G. (1979). The occurrence of Aeromonas and Streptococcus in rainbow trout, Salmo gairdneri Richardson. *Journal of Fish Biology, 15*(4), 457-460.

Barnes, A. C., Young, F. M., Horne, M. T., & Ellis, A. E. (2003). S*treptococcus iniae*: serological differences, presence of capsule and resistance to immune serum killing. *Diseases of Aquatic Organisms, 53*(3), 241-247.

Barry, T., Powell, R., & Gannon, F. (1990). A general method to generate DNA probes for microorganisms. *Nature Biotechnology, 8*(3), 233-236.

Baya, A., Lupiani, B., Hetrick, F., Roberson, B., Lukacovic, R., May, E., & Poukish, C. (1990). Association of Streptococcus sp. with fish mortalities in the Chesapeake Bay and its tributaries. *Journal of Fish Diseases, 13*(3), 251-253.

Bengtén, E., Quiniou, S. M.-A., Stuge, T. B., Katagiri, T., Miller, N. W., Clem, L. W., . . . Wilson, M. (2002). The IgH locus of the channel catfish, *Ictalurus punctatus*, contains multiple constant region gene sequences: different genes encode heavy chains of membrane and secreted IgD. *The Journal of Immunology, 169*(5), 2488-2497.

Bercovier, H., Ghittino, C., & Eldar, A. (1996). Immunization with bacterial antigens: infections with streptococci and related organisms. *Developments in biological standardization, 90*, 153-160.

Beveridge, T. J., Makin, S. A., Kadurugamuwa, J. L., & Li, Z. (1997). Interactions between biofilms and the environment. *FEMS Microbiology reviews, 20*(3-4), 291-303.

Boddey, J. A., Flegg, C. P., Day, C. J., Beacham, I. R., & Peak, I. R. (2006). Temperature-regulated microcolony formation by Burkholderia pseudomallei requires pilA and enhances association with cultured human cells. Infection and immunity, 74(9), 5374-5381.

Bonar, C. J & Wagner, R. A. (2003). A third report of golf ball disease in an Amazon River dolphin (*Inia geoffrensis*) associated with *Streptococcus iniae*. Journal of Zoo and Wildlife Medicine, 34, 296-301.

Boomker, J., Imes Jr, G., Cameron, C., Naude, T., & Schoonbee, H. (1979). Trout mortalities as a result of Streptococcus infection. *The Onderstepoort journal of veterinary research, 46*(2), 71-77.

Bordi, C., & de Bentzmann, S. (2011). Hacking into bacterial biofilms: a new therapeutic challenge. Annals of intensive care, 1(1), 19.

Borges, S., Silva, J., & Teixeira, P. (2012). Survival and biofilm formation by Group B streptococci in simulated vaginal fluid at different pHs. Antonie Van Leeuwenhoek, 101(3), 677-682.

Bowater, R., Forbes-Faulkner, J., Anderson, I., Condon, K., Robinson, B., Kong, F., . . . McPherson, G. (2012). Natural outbreak of *Streptococcus agalactiae* (GBS) infection in wild giant Queensland grouper, *Epinephelus lanceolatus* (Bloch), and other wild fish in northern Queensland, Australia. *Journal of Fish Diseases, 35*(3), 173-186.

Bragg, R. R., Oosthuizen, J., & Lordan, S. M. (1989). The leech Batracobdelloides tricarinata (Blanchard, 1897)(Hirudinea: Glossiphoniidae) as a possible reservoir of the rainbow trout pathogenic Streptococcus species.

Bromage, E., Thomas, A., & Owens, L. (1999). *Streptococcus iniae*, a bacterial infection in barramundi Lates calcarifer. *Diseases of Aquatic Organisms, 36*(3), 177-181.

Bromage, E., & Owens, L. (2002). Infection of barramundi *Lates calcarifer* with *Stereptococcus iniae* : effects of differrent routes of exposure. Dis. Aquat. Org. 52, 199- 205.

Brudeseth, B. E., Wiulsrød, R., Fredriksen, B. N., Lindmo, K., Løkling, K.-E., Bordevik, M., . . . Gravningen, K. (2013). Status and future perspectives of vaccines for industrialised fin-fish farming. *Fish & Shellfish Immunology, 35*(6), 1759-1768.

Buchanan, J. T., Stannard, J. A., Lauth, X., Ostland, V. E., Powell, H. C., Westerman, M. E., & Nizet, V. (2005). *Streptococcus iniae*

phosphoglucomutase is a virulence factor and a target for vaccine development. *Infection and immunity, 73*(10), 6935-6944.

Bunch, E. C., & Bejerano, I. (1997). The effect of environmental factors on the susceptibility of hybrid tilapia *Oreochromis niloticus*× *Oreochromis aureus* to streptococcosis. *Israeli Journal of Aquaculture –Bamidgeh 49*, 67-76.

Chalmers, L., Thompson, K. D., Taylor, J. F., Black, S., Migaud, H., North, B., & Adams, A. (2016). A comparison of the response of diploid and triploid Atlantic salmon (Salmo salar) siblings to a commercial furunculosis vaccine and subsequent experimental infection with Aeromonas salmonicida. Fish & shellfish immunology, 57, 301-308

Chen, M., Li, L.-P., Wang, R., Liang, W.-W., Huang, Y., Li, J., . . . Gan, X. (2012a). PCR detection and PFGE genotype analyses of streptococcal clinical isolates from tilapia in China. *Veterinary microbiology, 159*(3), 526-530.

Chen, M., Wang, R., Li, L.-P., Liang, W.-W., Li, J., Huang, Y., . . . Gan, X. (2012b). Screening vaccine candidate strains against *Streptococcus agalactiae* of tilapia based on PFGE genotype. *Vaccine, 30*(42), 6088-6092.

Chiu, T.-H., Chen, T.-R., Hwang, W.-Z., & Tsen, H.-Y. (2005). Sequencing of an internal transcribed spacer region of 16s–23S rRNA gene and designing of PCR primers for the detection of Salmonella spp. in food. *International journal of food microbiology, 97*(3), 259-265.

Colorni, A., Diamant, A., Eldar, A., Kvitt, H., & Zlotkin, A. (2002). *Streptococcus iniae* infections in Red Sea cage-cultured and wild fishes. *Diseases of Aquatic Organisms, 49*(3), 165-170.

Cook, D. W., & Lofton, S. R. (1975). Pathogenicity studies with a Streptococcus sp. isolated from fishes in an Alabama-Florida fish kill. *Transactions of the American Fisheries Society, 104*(2), 286-288.

Corbeil, S., LaPatra, S., Anderson, E., & Kurath, G. (2000). Nanogram quantities of a DNA vaccine protect rainbow trout fry against heterologous strains of infectious hematopoietic necrosis virus. *Vaccine, 18*(25), 2817-2824.

Costerton, J. W., Geesey, G. G., & Cheng, G. K. (1978). How bacteria stick. Scientific American, 238, 86-95.

Coykendall, A. L. (1989). Classification and identification of the viridans streptococci. *Clinical Microbiology Reviews, 2*(3), 315-328.

Crother, T. R., Champion, C. I., Wu, X.-Y., Blanco, D. R., Miller, J. N., & Lovett, M. A. (2003). Antigenic composition of Borrelia burgdorferi during infection of SCID mice. *Infection and immunity, 71*(6), 3419-3428.

Dalmo, R., Ingebrigtsen, K., & Bøgwald, J. (1997). Non-specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). *Journal of Fish Diseases, 20*(4), 241-273.

Dalmo, R., Leifson, R. M., & Bøgwald, J. (1995). Microspheres as antigen carriers: studies on intestinal absorption and tissue localization of polystyrene microspheres in Atlantic salmon, Salmo salar L. *Journal of Fish Diseases, 18*(1), 87-91.

Dang, H., Park, H., Myung, S., & Kim, W. (2012). Development of a novel PCR assay based on the 16s–23S rRNA internal transcribed spacer region for the detection of Lactococcus garvieae. *Journal of Fish Diseases, 35*(7), 481-487.

Dautremepuits, C., Paris-Palacios, S., Betoulle, S., & Vernet, G. (2004). Modulation in hepatic and head kidney parameters of carp (Cyprinus carpio L.) induced by copper and chitosan. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 137*(4), 325-333.

Davey, M. E., & O'toole, G. A. (2000). Microbial biofilms: from ecology to molecular genetics. *Microbiology and molecular biology reviews, 64*(4), 847-867.

Davidson, G., Ellis, A., & Secombes, C. (1993). Route of immunization influences the generation of antibody secreting cells in the gut of rainbow trout (Oncorhynchus mykiss). *Developmental & Comparative Immunology, 17*(4), 373-376.

De Las Heras, A., Saint-Jean, S. R., & Pérez-Prieto, S. I. (2010). Immunogenic and protective effects of an oral DNA vaccine against infectious pancreatic necrosis virus in fish. *Fish & Shellfish Immunology, 28*(4), 562-570.

Department of fisheries Malaysia (DOF), Ministry of agriculture and agro-based industry (2016). Fisheries statistic. Annual fisheries statistics. Retrieved http://www.dof.gov.my/index.php/pages/view/2614

Dodson, S., Maurer, J., & Shotts, E. (1999). Biochemical and molecular typing of *Streptococcus iniae* isolated from fish and human cases. *Journal of Fish Diseases, 22*(5), 331-336.

Donlan, R. M., & Costerto, J. W. (2002) Biofilm: survival mechanism of clinically relevent microorganism. Clinical Microbiology Reviewers, 15 (2), 167-193.

Duff, D. (1942). The oral immunization of trout against Bacterium salmonicida. *J. Immunol., 44*, 87-94.

Dumrongphol, Y., Hirota, T., Kondo, H., Aoki, T., & Hirono, I. (2009). Identification of novel genes in Japanese flounder (Paralichthys olivaceus) head kidney up-regulated after vaccination with

Streptococcus iniae formalin-killed cells. *Fish & Shellfish Immunology, 26*(1), 197-200.

Duremdez, R., Al-Marzouk, A., Qasem, J., Al-Harbi, A., & Gharabally, H. (2004). Isolation of *Streptococcus agalactiae* from cultured silver pomfret, Pampus argenteus (Euphrasen), in Kuwait. *Journal of Fish Diseases, 27*(5), 307-310.

D'Urzo, N., Martinelli, M., Pezzicoli, A., De Cesare, V., Pinto, V., Margarit, I., . . . Group, M. o. D. S. (2014). Acidic pH strongly enhances *in vitro* biofilm formation by a subset of hypervirulent ST-17 Streptococcus agalactiae strains. Applied and environmental microbiology, AEM. 03627-03613.

Effendy, A., Zamri-Saad, M., Maswati, M., Ismail, M., & Jamil, S. (1998). Stimulation of the bronchus-associated lymphoid tissue of goats and its effect on *in vitro* colonization by Pasteurella haemolytica. *Veterinary research communications, 22*(3), 147-153.

Eide, D. M., Linder, R. D., Strømsheim, A., Fjalestad, K., Larsen, H. J. S., & Røed, K. H. (1994). Genetic variation in antibody response to diphtheria toxoid in Atlantic salmon and rainbow trout. *Aquaculture, 127*(2-3), 103-113.

El-Sayed, A.-F. M. (2006). *Tilapia culture*: CABI, Oxfordshire, United Kingdom

Eldar, A., Bejerano, Y., Bercovier, H. (1994). Streptococcus shiloi and Streptococcus difficile; two new Streptococcal species causing a meningoencephalitis in fish. Curr. Microbiol, 28, 139-143.

Eldar, A., Bejerano, Y., Livoff, A., Horovitcz, A., & Bercovier, H. (1995a). Experimental streptococcal meningo-encephalitis in cultured fish. *Veterinary microbiology, 43*(1), 33-40.

Eldar, A., & Ghittino, C. (1999). Lactococcus garvieae and *Streptococcus iniae* infections in rainbow trout Oncorhynchus mykiss: similar, but different diseases. *Diseases of Aquatic Organisms, 36*(3), 227-231.

Eldar, A., Horovitcz, A., & Bercovier, H. (1997b). Development and efficacy of a vaccine against *Streptococcus iniae* infection in farmed rainbow trout. *Veterinary Immunology and Immunopathology, 56*(1-2), 175-183.

Eldar, A., Lawhon, S., Frelier, P. F., Assenta, L., Simpson, B. R., Varner, P. W., & Bercovier, H. (1997a). Restriction fragment length polymorphisms of 16s rDNA and of whole rRNA genes (ribotyping) of *Streptococcus iniae* strains from the United States and Israel. *FEMS microbiology letters, 151*(2), 155-162.

Eldar, A., Shapiro, O., Bejerano, Y., & Bercovier, H. (1995b). Vaccination with whole-cell vaccine and bacterial protein extract protects tilapia against Streptococcus difficile meningoencephalitis. *Vaccine, 13*(9), 867-870.

Eldridge, J. H., Hammond, C. J., Meulbroek, J. A., Staas, J. K., Gilley, R. M., & Tice, T. R. (1990). Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the Peyer's patches. *Journal of Controlled Release, 11*(1-3), 205-214.

Elliott, J. A., Facklam, R. R., & Richter, C. B. (1990). Whole-cell protein patterns of nonhemolytic group B, type Ib, streptococci isolated from humans, mice, cattle, frogs, and fish. *Journal of clinical microbiology, 28*(3), 628-630.

Embregts, C. W., & Forlenza, M. (2016). Oral vaccination of fish: Lessons from humans and veterinary species. *Developmental & Comparative Immunology, 64*, 118-137.

Erfanmanesh, A., Soltani, M., Pirali, E., Mohammadian, S., & Taherimirghaed, A. (2012). Genetic characterization of *Streptococcus iniae* in diseased farmed rainbow trout (*Onchorhynchus mykiss*) in Iran. *The Scientific World Journal, 2012*.

Esteban, M., Cuesta, A., Ortuno, J., & Meseguer, J. (2001). Immunomodulatory effects of dietary intake of chitin on gilthead seabream (Sparus aurata L.) innate immune system. *Fish & Shellfish Immunology, 11*(4), 303-315.

Evans, D. J., Allison, D. G., Brown, M. R. W., & Gilbert, t. p. (1990). Effect of growth rate on resistance of gram-negative biofilms to cetrimide. Journal of Antimicrobial Chemotherapy, 26 (4), 473-478.

Evans, J., Klesius, P., Gilbert, P., Shoemaker, C., Al Sarawi, M., Landsberg, J., . . . Al Zenki, S. (2002). Characterization of β-haemolytic Group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus L*., and wild mullet, *Liza klunzingeri* (Day), in Kuwait. *Journal of Fish Diseases, 25*(9), 505-513.

Evans, J., Klesius, P., & Shoemaker, C. (2006a). An overview of Streptococcus in warmwater fish. *Aquaculture Health International, 7*, 10-14.

Evans, J., Klesius, P., Shoemaker, C., & PASNIK, D. (2006b). Identification and epidemiology *of Streptococcus iniae* and *Streptococcus agalactiae* in tilapias *Oreochromis* spp. Paper presented at the Proceedings 7th International Symposium on Tilapia in Aquaculture.

Evans, J. J., Bohnsack, J. F., Klesius, P. H., Whiting, A. A., Garcia, J. C., Shoemaker, C. A., & Takahashi, S. (2008). Phylogenetic relationships among *Streptococcus agalactiae* isolated from piscine, dolphin, bovine and human sources: a dolphin and piscine lineage associated with a fish epidemic in Kuwait is also associated with human neonatal infections in Japan. *Journal of medical microbiology, 57*(11), 1369-1376.

Evans, J. J., Klesius, P. H., Pasnik, D. J., & Bohnsack, J. F. (2009). Human *Streptococcus agalactiae* isolate in Nile tilapia (*Oreochromis niloticus*). *Emerging infectious diseases, 15*(5), 774-776.

Evans, J. J., Klesius, P. H., & Shoemaker, C. A. (2004). Efficacy of *Streptococcus agalactiae* (group B) vaccine in tilapia (*Oreochromis niloticus*) by intraperitoneal and bath immersion administration. *Vaccine, 22*(27), 3769-3773.

Evans, J. J., Pasnik, D. J., Klesius, P. H., & Al-Ablani, S. (2006). First report of *Streptococcus agalactiae* and Lactococcus garvieae from a wild bottlenose dolphin (*Tursiops truncatus*). *Journal of wildlife diseases, 42*(3), 561-569.

Evans, J. J., Shoemaker, C. A., & Klesius, P. H. (2000). Experimental *Streptococcus iniae* infection of hybrid striped bass (*Morone chrysops*× *Morone saxatilis*) and tilapia (*Oreochromis niloticus*) by nares inoculation. *Aquaculture, 189*(3), 197-210.

Evelyn, T. (1997). A historical review of fish vaccinology. *Developments in biological standardization, 90*, 3-12.

Evensen, O. (2009). Development in fish vaccinology with focus on delivery methodologies, adjuvants and formulations. *Options Méditerranéennes, 86*, 177-186.

Facklam, R., Elliott, J., Shewmaker, L., & Reingold, A. (2005). Identification and characterization of sporadic isolates of *Streptococcus iniae* isolated from humans. *Journal of clinical microbiology, 43*(2), 933-937.

Fanrong, K., Lin, M., & Gwendolyn, L. G. (2005). Simultaneous detection and serotype identification of *Streptococcus agalactiae* using multiplex PCR and reverse line blot hybridization. *Journal of medical microbiology, 54*(12), 1133-1138.

FAO. (2004). Fishatat plus.FAO.Rome.

FAO. (2016). *Year book of Fishery and Aquaculture statistics*. Rome : Food and agriculture organization of the United nations.

FAO. (December 2015). *GLOBEFISH - Analysis and information on world fish trade*. Rome: Food and Agriculture Organization of the united nation.

FAO. (September 2015). *GLOBEFISH - Analysis and information on world fish trade*. Rome: Food and Agriculture Organization of the united nation.

Feng, J., Liu, H.-C., Chu, J.-F., Zhou, P.-J., Tang, J.-A., & Liu, S.-J. (2006). Genetic cloning and functional expression in Escherichia coli of an archaerhodopsin gene from Halorubrum xinjiangense. *Extremophiles, 10*(1), 29-33.

Ferguson, H., Morales, J., & Ostland, V. (1994). Streptococcosis in aquarium fish. *Diseases of Aquatic Organisms, 19*(1), 1-6.

Ferguson, H., St John, V., Roach, C., Willoughby, S., Parker, C., & Ryan, R. (2000). Caribbean reef fish mortality associated with *Streptococcus iniae*. *Veterinary Record, 147*(23), 662-664.

Filho, C. I., Muller, E., Pretto-Giordano, L., & Bracarense, A. (2009). Histological findings of experimental *Streptococcus agalactiae* infection in Nile tilapias (*Oreochromis niloticus*). *Brazilian Journal of Veterinary Pathology, 2*, 12-15.

Firdaus-Nawi, M., Noraini, O., Sabri, M., Abdullah, S., Zamri-Saad, M., & Latifah, H. (2011). The effects of oral vaccination of *Streptococcus agalactiae* on stimulating gutassociated lymphoid tissues (GALTs) in tilapia (*Oreochromis spp.*). *Pertanika Journal of Tropical Agricultural Sciences, 34*, 137-143.

Firdaus-Nawi, M., Yusoff, S. M., Yusof, H., Abdullah, S. Z., & Zamri-Saad, M. (2013). Efficacy of feed-based adjuvant vaccine against *Streptococcus agalactiae* in *Oreochromis* spp. in Malaysia. *Aquaculture Research, 45*(1), 87-96.

Fitzsimmon, K. (2015). *Market stability:why tilapia supply and demand have avoided boom and bust of other commodities* Paper presented at the 4th International trade and technical conference and exposition on tilapia, Malaysia, Kuala Lampur.

Fletcher, T. C., & White, A. (1972). Antibody production in the plaice (*Pleuronectes platessa L.*) after oral and parenteral immunization with *Vibrio anguillarum* antigens. *Aquaculture, 1*, 417-428.

Forchielli, M. L., & Walker, W. A. (2005). The role of gut-associated lymphoid tissues and mucosal defence. *British Journal of Nutrition, 93*(S1), S41-S48.

Foss, G. S. (2003). Regulation of DNA vaccines and gene therapy on animals. *The Norwegian Biotechnology Advisory Board. Website: www. bion. no/publikasjoner/regulation_ of_DNA_vaccines. pdf (accessed on 4 March 2005)*.

Fox, G. E., Magrum, L. J., Balch, W. E., Wolfe, R. S., & Woese, C. R. (1977). Classification of methanogenic bacteria by 16s ribosomal RNA characterization. *Proceedings of the National Academy of Sciences, 74*(10), 4537-4541.

Fuglem, B., Jirillo, E., Bjerkås, I., Kiyono, H., Nochi, T., Yuki, Y., . . . Koppang, E. O. (2010). Antigen-sampling cells in the salmonid intestinal epithelium. *Developmental & Comparative Immunology, 34*(7), 768-774.

Fujino, Y., Ono, S.-I., & Nagai, A. (1987). Studies on uptake of rabbit's immunoglobulin into the columnar epithelial cells in the gut of rainbow trout Salmo gairdneri. *Nippon Suisan Gakkaishi, 53*(3), 367-370.

Fuller, J. D., Bast, D. J., Nizet, V., Low, D. E., & de Azavedo, J. C. (2001). *Streptococcus iniae* virulence is associated with a distinct genetic profile. *Infection and immunity, 69*(4), 1994-2000.

Geng, Y., Wang, K., Huang, X., Chen, D., Li, C., Ren, S., . . . Du, Z. (2012). *Streptococcus agalactiae*, an Emerging Pathogen for Cultured Ya-Fish, Schizothorax prenanti, in China. *Transboundary and emerging diseases, 59*(4), 369-375.

Genmoto, K., Nishizawa, T., Nakai, T., & Muroga, K. (1996). 16s rRNA targeted RT-PCR for the detection of Vibrio penaeicida, the pathogen of cultured kuruma prawn Penaeus japonicus. *Diseases of Aquatic Organisms, 24*(3), 185-189.

Georgopoulou, U., Sire, M., & Vernier, J. (1986a). Immunological demonstration of intestinal absorption and digestion of protein macromolecules in the trout (Salmo gairdneri). *Cell and Tissue Research, 245*(2), 387-395.

Georgopoulou, U., & Vernier, J.-M. (1986b). Local immunological response in the posterior intestinal segment of the rainbow trout after oral administration of macromolecules. *Developmental & Comparative Immunology, 10*(4), 529-537.

Ghittino, P., & Prearo, M. (1992). Report of Streptococcosis in rainbow trout(Oncorhynchus mykiss) in Italy: Preliminary note. *Bollettino Societa Italiana di Patologia Ittica*(8), 4-9.

Glaser, P., Rusniok, C., Buchrieser, C., Chevalier, F., Frangeul, L., Msadek, T., . . . Poyart, C. (2002). Genome sequence of *Streptococcus agalactiae*, a pathogen causing invasive neonatal disease. *Molecular microbiology, 45*(6), 1499-1513.

Gopalakannan, A., & Arul, V. (2006). Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. *Aquaculture, 255*(1), 179-187.

Gudding, R., Lillehaug, A., & Evensen, Ø. (1999). Recent developments in fish vaccinology. *Veterinary Immunology and Immunopathology, 72*(1), 203-212.

Gupta, R., Lanter, J. M., & Woese, C. R. (1983). Sequence of the 16s ribosomal RNA from Halobacterium volcanii, an archaebacterium. *Science, 221*(4611), 656-659.

Haghighi, K. S., Soltani, M., Nikbakhat-Brojeni, G., Ghasemi, M., & Skall, H. F. (2010). Molecular epidemiology of zoonotic streptococcosis/lactococcosis in rainbow trout (Oncorhynchus mykiss) aquaculture in Iran. *Iranian journal of microbiology, 2*(4), 198-209.

Hansen, E., Fernandes, K., Goldspink, G., Butterworth, P., Umeda, P. K., & Chang, K.-C. (1991). Strong expression of foreign genes following direct injection into fish muscle. *FEBS letters, 290*(1-2), 73-76.

Hanson, B. R., Runft, D. L., Streeter, C., Kumar, A., Carion, T. W., & Neely, M. N. (2012). Functional analysis of the CpsA protein of *Streptococcus agalactiae*. *Journal of Bacteriology, 194*(7), 1668-1678.

Hart, S., Wrathmell, A., Harris, J., & Grayson, T. (1988). Gut immunology in fish: a review. *Developmental & Comparative Immunology, 12*(3), 453-480.

Hashim, M. B. (2015). *Industry and Market Status of Tilapia in Malaysia* Paper presented at the 4th INTERNATIONAL TRADE AND TECHNICAL CONFERENCE AND EXPOSITION ON TILAPIA (TILAPIA 2015) Aquaculture Development Division Department of Fisheries Malaysia

Heath, S., Pak, S., Marshall, S., Prager, E., & Orrego, C. (2000). Monitoring Piscirickettsia salmonis by denaturant gel electrophoresis and competitive PCR. *Diseases of Aquatic Organisms, 41*(1), 19-29.

Heppell, J., & Davis, H. L. (2000). Application of DNA vaccine technology to aquaculture. *Advanced drug delivery reviews, 43*(1), 29-43.

Heppell, J., Lorenzen, N., Armstrong, N. K., Wu, T., Lorenzen, E., Einer-Jensen, K., . . . Davis, H. L. (1998). Development of DNA vaccines for fish: vector design, intramuscular injection and antigen expression using viral haemorrhagic septicaemia virus genes as model. *Fish & Shellfish Immunology, 8*(4), 271-286.

Hernández, E., Figueroa, J., & Iregui, C. (2009). Streptococcosis on a red tilapia, *Oreochromis* sp., farm: a case study. *Journal of Fish Diseases, 32*(3), 247-252.

Ho, Y.-R., Li, C.-M., Yu, C.-H., Lin, Y.-J., Wu, C.-M., Harn, I.-C., . . . Lu, C.-Y. (2013). The enhancement of biofilm formation in Group B streptococcal isolates at vaginal pH. Medical microbiology and immunology, 202(2), 105-115.

Hoffmann, M., Brown, E. W., Feng, P. C., Keys, C. E., Fischer, M., & Monday, S. R. (2010). PCR-based method for targeting 16s-23s rRNA intergenic spacer regions among Vibrio species. *BMC microbiology, 10*(1), 90.

Hølvold, L. B., Myhr, A. I., & Dalmo, R. A. (2014). Strategies and hurdles using DNA vaccines to fish. *Veterinary research, 45*(1), 21.

Hoseinifar, S. H., Esteban, M. Á., Cuesta, A., & Sun, Y.-Z. (2015). Prebiotics and fish immune response: a review of current knowledge and future perspectives. *Reviews in Fisheries Science & Aquaculture, 23*(4), 315-328.

Hoshina, T., Sano, T., & Morimoto, Y. (1958). A Streptococcus pathogenic to fish. *J. Tokyo Univ. Fish, 44*, 57-68.

Houpikian, P., & Raoult, D. (2001). 16s/23s rRNA intergenic spacer regions for phylogenetic analysis, identification, and subtyping ofBartonella species. *Journal of clinical microbiology, 39*(8), 2768-2778.

Huang, L., Wang, K., Xiao, D., Chen, D., Geng, Y., Wang, J., . . . Xiao, G. (2014). Safety and immunogenicity of an oral DNA vaccine encoding Sip of *Streptococcus agalactiae* from Nile tilapia *Oreochromis niloticus* delivered by live attenuated *Salmonella typhimurium*. Fish & shellfish immunology, 38(1), 34-41

Hughes, M. J., Moore, J. C., Lane, J. D., Wilson, R., Pribul, P. K., Younes, Z. N., . . . Redfern, J. M. (2002). Identification of major outer surface proteins of *Streptococcus agalactiae*. *Infection and immunity, 70*(3), 1254-1259.

Humphrey, J., Lancaster, C., Gudkovs, N., & Copland, J. (1987). The disease status of Australian salmonids: bacteria and bacterial diseases. *Journal of Fish Diseases, 10*(5), 403-410.

Iida, H., & Yamamoto, T. (1985). Intracellular transport of horseradish peroxidase in the absorptive cells of goldfish hindgut *in vitro*, with special reference to the cytoplasmic tubules. *Cell and Tissue Research, 240*(3), 553-560.

Ingolotti, M., Kawalekar, O., Shedlock, D. J., Muthumani, K., & Weiner, D. B. (2010). DNA vaccines for targeting bacterial infections. *Expert review of vaccines, 9*(7), 747-763.

Inocente Filho, C., Müller, E. E., Pretto-Giordano, L. G., & Bracarense, A. P. F. (2017). Histological findings of experimental *Streptococcus agalactiae* infection in Nile tilapias (*Oreochromis niloticus*). *Brazilian Journal of Veterinary Pathology, 2*(1), 12-15.

Intervet. (2006). Streptococcosis in tilapia. Retrieved http://aqua.intervet.com/news/2006-06-20-streptococcosis-in-tilapia.asp

Intervet. (2010). New advances for warmwater Aquaculture. Retrieved October 10 2010 http://aqua.intervet.com/binaries/aguirre_tcm127-207376.pdf

Irie, T., Watarai, S., Iwasaki, T., & Kodama, H. (2005). Protection against experimental Aeromonas salmonicida infection in carp by oral

immunisation with bacterial antigen entrapped liposomes. *Fish & Shellfish Immunology, 18*(3), 235-242.

Isiaku, A., Sabri, M., Ina-Salwany, M., Hassan, M., Tanko, P., & Bello, M. (2017). Biofilm is associated with chronic streptococcal meningoencephalitis in fish. *Microbial pathogenesis, 102*, 59-68.

Ismail, M., Syafiq, M., Abdullah, S., Fahmi, S., Shahidan, H., Hanan, Y., . . . Zamri-Saad, M. (2017). The effect of feed-based vaccination on tilapia farm endemic for streptococcosis. Fish & shellfish immunology, 60, 21-24.

Iwama, G., & Nakanishi, T. (1996). *The fish immune system: organism, pathogen, and environment*: Academic Press, San Diego. 1-380.

Izumi, S., & Wakabayashi, H. (1997). Use of PCR to detect Cytophaga psychrophila from apparently healthy juvenile ayu and coho salmon eggs. *Fish pathology, 32*(3), 169-173.

Izumi, S., & Wakabayashi, H. (2000). Sequencing of gyrB and their application in the identification of Flavobacterium psychrophilum by PCR. *Fish pathology, 35*(2), 93-94.

Jantrakajorn, S., Maisak, H., & Wongtavatchai, J. (2014). Comprehensive investigation of streptococcosis outbreaks in cultured Nile tilapia, *Oreochromis niloticus*, and red tilapia, *Oreochromis* sp., of Thailand. *Journal of the World Aquaculture Society, 45*(4), 392-402.

Jenkins, P., Wrathmell, A., Harris, J., & Pulsford, A. (1994). The effects of different adjuvants on intestinal antigen absorption and subsequent immune responses of the tilapian *Oreochromis mossambicus*. *Fish & Shellfish Immunology, 4*(3), 167-177.

Jimenez, A., Rey, A., Penagos, L., Ariza, M., Figueroa, J., & Iregui, C. (2007). *Streptococcus agalactiae*: up To date the only pathogenous streptococcus of cultured tilapias in Colombia. *Revista de la Facultad de Medicina Veterinaria y de Zootecnia, 54*(II), 285-294.

Jiménez, A., Tibatá, V., Junca, H., Ariza, F., Verjan, N., & Iregui, C. (2011). Evaluating a nested-PCR assay for detecting *Streptococcus agalactiae* in red tilapia (*Oreochromis* sp.) tissue. *Aquaculture, 321*(3), 203-206.

Johnson, K., & Amend, D. (1983). Efficacy of Vibrio anguillarum and Yersinia ruckeri bacterins applied by oral and anal intubation of salmonids. *Journal of Fish Diseases, 6*(5), 473-476.

Joosten, P. H., Aviles-Trigueros, M., Sorgeloos, P., & Rombout, J. (1995). Oral vaccination of juvenile carp (Cyprinus carpio) and gilthead seabream (Sparus aurata) with bioencapsulatedVibrio anguillarumbacterin. *Fish & Shellfish Immunology, 5*(4), 289-299.

Kaur, H., Kumar, P., Ray, P., Kaur, J., & Chakraborti, A. (2009). Biofilm formation in clinical isolates of group B streptococci from north India. Microbial pathogenesis, 46(6), 321-327.

Kayansamruaj, P., Dong, H. T., Pirarat, N., Nilubol, D., & Rodkhum, C. (2017). Efficacy of α-enolase-based DNA vaccine against pathogenic *Streptococcus iniae* in Nile tilapia (*Oreochromis niloticus*). *Aquaculture, 468*, 102-106.

Kim, M. S., Choi, S. H., Lee, E. H., Nam, Y. K., Kim, S. K., & Kim, K. H. (2007). α-enolase, a plasmin (ogen) binding and cell wall associating protein from a fish pathogenic *Streptococcus iniae* strain. *Aquaculture, 265*(1), 55-60.

Kimura, H., & Kusuda, R. (1979). Studies on the pathogenesis of streptococcal infection in cultured yellowtails Seriola spp.: effect of the cell free culture on experimental streptococcal infection. *Journal of Fish Diseases, 2*(6), 501-510.

Kimura, H., & Kusuda, R. (1982). Studies on the pathogenesis of streptococcal infection in cultured yellowtails, Seriola spp.: effect of crude exotoxin fractions from cell-free culture on experimental streptococcal infection. *Journal of Fish Diseases, 5*(6), 471-478.

Kitao, T. (1993). streptococcal infection In V. Inglis, R. J. Roberts, & N. R. Bromage (Eds.), *Bacterial diseases of fish* (pp. 196-210): Blackwell, Oxford, United Kingdom.

Kitao, T., Aoki, T., & Sakoh, R. (1981). Epizootic caused by β-haemoltytic Streptococcus species in cultured freshwater fish. *Fish pathology, 15*(3-4), 301-307.

Kiyono, H. (1992). Mucosal immune system: Features of inductive and effector sites to consider in mucosal immunization and vaccine development. *Reg Immunol, 4*, 52-62.

Klesius, D. P. H., Evans, J. J., Shoemaker, C. A., & Lim, C. (2006b). A US perspective on selected biotechnological advancements in fish health. *Aquaculture Health International, 5*, 10-12.

Klesius, P., Shoemaker, C., & Evans, J. (2000b). *VACCINATION: A health management practice for preventing diseases caused by streptococcus in tilapia and other cultured fish* Paper presented at the 5th International Symposium on Talipia in Aquaculture. 558-559, Rio Dejaneiro. Brazil

Klesius, P., Shoemaker, C., & Evans, J. (2008). *Streptococcus: A worldwide fish health problem.* Paper presented at the 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt. vol. 1, 83-107.

Klesius, P. H., Evans, J. J., & Shoemaker, C. A. (2007). The macrophage chemotactic activity of *Streptococcus agalactiae* and *Streptococcus iniae* extracellular products (ECP). *Fish & Shellfish Immunology, 22*(5), 443-450.

Klesius, P. H., Evans, J. J., Shoemaker, C. A., & Pasnik, D. J. (2006a). A vaccination and challenge model using calcein marked fish. *Fish & Shellfish Immunology, 20*(1), 20-28.

Klesius, P. H., Shoemaker, C., & Evans, J. (1999). Efficacy of a killed *Streptococcus iniae* vaccine in tilapia (*Oreochromis niloticus*). *Bulletin-European Association Of Fish Pathologists, 19*, 39-41.

Klesius, P. H., Shoemaker, C. A., & Evans, J. J. (2000a). Efficacy of single and combined *Streptococcus iniae* isolate vaccine administered by intraperitoneal and intramuscular routes in tilapia (*Oreochromis niloticus*). *Aquaculture, 188*(3), 237-246.

Klesius, P. H., Shoemaker, C. A., & Evans, J. J. (2002). *Streptococcus iniae* vaccine. In: United State Patent. US 6379677.

Komar, C., Enright, W. J., Grisez, L., & Tan, Z. (2004). Understanding fish vaccination. *Aqua Culture Asia Pacific Magazine*, 27-29.

Konto-Ghiorghi, Y., Mairey, E., Mallet, A., Duménil, G., Caliot, E., Trieu-Cuot, P., & Dramsi, S. (2009). Dual role for pilus in adherence to epithelial cells and biofilm formation in Streptococcus agalactiae. PLoS pathogens, 5(5), e1000422.

Koprowski, H., & Weiner, D. B. (2012). *DNA vaccination/genetic vaccination* (Vol. 226): Springer Science & Business Media.

Kumar, S. R., Ahmed, V. I., Parameswaran, V., Sudhakaran, R., Babu, V. S., & Hameed, A. S. (2008). Potential use of chitosan nanoparticles for oral delivery of DNA vaccine in Asian sea bass (Lates calcarifer) to protect from Vibrio (Listonella) anguillarum. *Fish & Shellfish Immunology, 25*(1), 47-56.

Kusuda, R., & Kimura, H. (1978). Studies on the pathogenesis of streptococcal infection in cultured yellowtails Seriola spp.: the fate of Streptococcus sp. bacteria after inoculation. *Journal of Fish Diseases, 1*(1), 109-114.

Kusuda, R., Toyoshima, T., Komatsu, I., & Kawai, K. (1976). A new pathogenic bacterium belonging to the genus Streptococcus, isolated from an epizootic cultured yellowtail. *Bulletin of the Japanese Society of Scientific fisheries, 42*, 1345-1352.

Kvitt, H., & Colorni, A. (2004). Strain variation and geographic endemism in *Streptococcus iniae*. *Diseases of Aquatic Organisms, 61*(1-2), 67-73.

LaFrentz, B. R., Shoemaker, C. A., & Klesius, P. H. (2011). Immunoproteomic analysis of the antibody response obtained in Nile tilapia following vaccination with a *Streptococcus iniae* vaccine. *Veterinary microbiology, 152*(3), 346-352.

Lamers, C., Haas, M., & Muiswinkel, W. (1985). The reaction of the immune system of fish to vaccination: development of immunological memory in carp, *Cyprinus carpio L*., following direct immersion in *Aeromonas hydrophila* bacterin. *Journal of Fish Diseases, 8*(3), 253-262.

Lau, S. K., Woo, P. C., Luk, W.-k., Fung, A. M., Hui, W.-t., Fong, A. H., . . . Yuen, K.-y. (2006). Clinical isolates of *Streptococcus iniae* from Asia are more mucoid and β-hemolytic than those from North America. *Diagnostic microbiology and infectious disease, 54*(3), 177-181.

Lau, S. K., Woo, P. C., Tse, H., Leung, K.-W., Wong, S. S., & Yuen, K.-Y. (2003). Invasive *Streptococcus iniae* infections outside north america. *Journal of clinical microbiology, 41*(3), 1004-1009.

Le Breton, A. (2009). Vaccines in Mediterranean aquaculture: practice and needs. *Options Méditerranéennes, 86*, 147-154.

Levine, M. M., & Sztein, M. B. (2004). Vaccine development strategies for improving immunization: the role of modern immunology. *Nature immunology, 5*(5), 460-464.

Li, L., Wang, R., Liang, W., Huang, T., Huang, Y., Luo, F., . . . Gan, X. (2015). Development of live attenuated *Streptococcus agalactiae* vaccine for tilapia via continuous passage *in vitro*. Fish & shellfish immunology, 45(2), 955-963

Li, P., & Gatlin, D. M. (2003). Evaluation of brewers yeast (Saccharomyces cerevisiae) as a feed supplement for hybrid striped bass (Morone chrysops× M. saxatilis). *Aquaculture, 219*(1), 681-692.

Liaghat, M., Akhlaghi, M., Hoseini, A., Nematollahi, A., & Hoseini, S. M. (2011). Humoral and non-specific immune responses in rainbow trout (*Oncorhynchus mykiss*) naturally exposed to and immunized with *Streptococcus iniae*. *Iranian Journal of Veterinary Medicine, 5*(4), 218-224.

Lillehaug, A. (1989). Oral immunization of rainbow trout, Salmo gairdneri Richardson, against vibriosis with vaccines protected against digestive degradation. *Journal of Fish Diseases, 12*(6), 579-584.

Liu, H., Wang, Q., Liu, Q., Cao, X., Shi, C., & Zhang, Y. (2011). Roles of Hfq in the stress adaption and virulence in fish pathogen *Vibrio alginiolyticus* and its potencial application as target for live attenuated vaccine. Applied Microbiology and Biotechnology, 91, 353-364.

Lock, J. B., Colvin, K. M., Datta, A. K., Patel, S. K., Naidu, N. N., Neely, M. N., Nizet, V., & Buchanan, J. T. (2007). *Streptococcus iniae* capsule impairs phagocytic clearance and contributes to virulence in fish. Jpurnal of Bacteriology, 189, 1279-1287.

Locke, J. B., Aziz, R. K., Vicknair, M. R., Nizet, V., & Buchanan, J. T. (2008). *Streptococcus iniae* M-like protein contributes to virulence in fish and is a target for live attenuated vaccine development. *PLoS One, 3*(7), e2824.

Locke, J. B., Vicknair, M. R., Ostland, V. E., Nizet, V., & Buchanan, J. T. (2010). Evaluation of *Streptococcus iniae* killed bacterin and live attenuated vaccines in hybrid striped bass through injection and bath immersion. *Diseases of Aquatic Organisms, 89*(2), 117-123.

Lorenzen, N., & LaPatra, S. (2005). DNA vaccines for aquacultured fish. *Revue Scientifique Et Technique-Office International Des Epizooties, 24*(1), 201.

Lorenzen, N., Lorenzen, E., Einer-Jensen, K., Heppell, J., Wu, T., & Davis, H. (1998). Protective immunity to VHS in rainbow trout (Oncorhynchus mykiss, Walbaum) following DNA vaccination. *Fish & Shellfish Immunology, 8*(4), 261-270.

Lovshin, L. (1997). *Worldwide tilapia culture.* Paper presented at the Anais do 1 Workshop International de Aquicultura, October.

Lovshin, L., & Cyrino, J. (1998). Status of commercial fresh water fish culture in Brazil. *World Aquaculture-Baton Rouge-, 29*, 23-41.

Lovshin, L. L. (2000). Criteria for selecting Nile tilapia and red tilapia for culture. *Tilapia Aquaculture in the 21st Century. Rio de Janeiro, Brazil: American Tilapia Association and Departamento de Pesca e Aqüicultura*.

Lowe, B. A., Miller, J. D., & Neely, M. N. (2007). Analysis of the polysaccharide capsule of the stwmic pathogen *Streptococcus iniae* and its implications in virulence. Infection and immunity, 75, 1255-1264.

Magnadottir, B. (2010). Immunological control of fish diseases. *Marine biotechnology, 12*(4), 361-379.

Mah, T.-F. C., & O'Toole, G. A. (2001). Mechanisms of biofilm resistance to antimicrobial agents. *Trends in microbiology, 9*(1), 34-39.

Manganaro, M., Ogra, P. L., & Ernst, P. B. (1994). Oral immunization: turning fantasy into reality. *International archives of allergy and immunology, 103*(3), 223-233.

Marsden, M., Vaughan, L., Fitzpatrick, R., Foster, T., & Secombes, C. (1998). Potency testing of a live, genetically attenuated vaccine for salmonids. *Vaccine, 16*(11-12), 1087-1094.

Marsden, M. J., Vaughan, L. M., Foster, T. J., & Secombes, C. J. (1996). A live (delta aroA) Aeromonas salmonicida vaccine for furunculosis preferentially stimulates T-cell responses relative to B-cell responses in rainbow trout (Oncorhynchus mykiss). *Infection and immunity, 64*(9), 3863-3869.

Marshall, S., Heath, S., Henríquez, V., & Orrego, C. (1998). Minimally Invasive Detection ofPiscirickettsia salmonis in Cultivated Salmonids via the PCR. *Applied and environmental microbiology, 64*(8), 3066-3069.

McAndrew, B., Roubal, F. R., Roberts, R. J., Bullock, A. M., & McEwen, I. (1988). The genetics and histology of red, blond and associated colour variants in *Oreochromis niloticus*. *Genetica, 76*(2), 127-137.

McKeever, D., & Rege, J. (1999). Vaccines and diagnostic tools for animal health: the influence of biotechnology. *Livestock Production Science, 59*(2), 257-264.

MCMILLAN, D. N., & SECOMBES, C. J. (1997). Isolation of rainbow trout (Oncorhynchus mykiss) intestinal intraepithelial lymphocytes (IEL) and measurement of their cytotoxic activity. *Fish & Shellfish Immunology, 7*(8), 527-541.

Meiri-Bendek, I., Lipkin, E., Friedmann, A., Leitner, G., Saran, A., Friedman, S., & Kashi, Y. (2002). A PCR-based method for the detectionof *Streptococcus agalactiae* in milk. *Journal of dairy science, 85*(7), 1717-1723.

Membrebe, J. D., Yoon, N.-K., Hong, M., Lee, J., Lee, H., Park, K., . . . Kim, Y.-C. (2016). Protective efficacy of *Streptococcus iniae* derived enolase against Streptococcal infection in a zebrafish model. *Veterinary Immunology and Immunopathology, 170*, 25-29.

Mian, G., Godoy, D., Leal, C., Yuhara, T., Costa, G., & Figueiredo, H. (2009). Aspects of the natural history and virulence of *Streptococcus agalactiae* infection in Nile tilapia. *Veterinary microbiology, 136*(1), 180-183.

Miller, J. D., & Neely, M. N. (2005). Large-scale screen highlights the importance of capsule for virulence in the zoonotic pathogen *Streptococcus iniae*. *Infection and immunity, 73*(2), 921-934.

MINAMI, T., NAKAMURA, M., IKEDA, Y., & OZAKI, H. (1979). A beta-hemolytic Streptococcus isolated from cultured yellowtail. *Fish pathology, 14*(1), 33-38.

Ming, C., Wang, R., Li, L.-P., Liang, W.-W., Li, J., Huang, Y., . . . Gan, X. (2012). Screening vaccine candidate strains against *Streptococcus*

agalactiae of tilapia based on PFGE genotype. *Vaccine, 30*(42), 6088-6092.

Miyazaki, T., Kubota, S. S., Kaige, N., & Miyashita, T. (1984). A histopathological study of streptococcal disease in tilapia. *Fish pathology, 19*(3), 167-172.

Mohammadi, F., Mousavi, S. M., & Rezaie, A. (2012). Histopathological study of parasitic infestation of skin and gill on Oscar (Astronotus ocellatus) and discus (Symphysodon discus). *Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society (AACL Bioflux), 5*(2).

Moore, J. D., Ototake, M., & Nakanishi, T. (1998). Particulate antigen uptake during immersion immunisation of fish: the effectiveness of prolonged exposure and the roles of skin and gill. *Fish & Shellfish Immunology, 8*(6), 393-408.

Mora, D., Ricci, G., Guglielmetti, S., Daffonchio, D., & Fortina, M. G. (2003). 16s–23s rRNA intergenic spacer region sequence variation in Streptococcus thermophilus and related dairy streptococci and development of a multiplex ITS-SSCP analysis for their identification. *Microbiology, 149*(3), 807-813.

Moyle, P. M., & Toth, I. (2013). Modern subunit vaccines: development, components, and research opportunities. *ChemMedChem, 8*(3), 360-376.

Muktar, Y., Tesfaye, S., & Tesfaye, B. (2016). Present status and future prospects of fish vaccination: A review. *J Veterinar Sci Technol, 7*(299), 2.

Musa, N., Wei, L. S., Musa, N., Hamdan, R. H., Leong, L. k., Wee, W., . . . Abdullah, S. (2009). Short communication: Streptococcosis in red hybrid tilapia (*Oreochromis niloticus*) commercial farms in Malaysia. *Aquac Res, 40*, 630-632.

Mutoloki, S., Munang'andu, H. M., & Evensen, Ø. (2015). Oral vaccination of fish–antigen preparations, uptake, and immune induction. Frontiers in immunology, 6, 519.

Najiah, M., Aqilah, N., Lee, K., Khairulbariyyah, Z., Mithun, S., Jalal, K., . . . Nadirah, M. (2012). Massive mortality associated with *Streptococcus agalactiae* infection in cage-cultured red hybrid tilapia *Oreochromis niloticus* in Como River, Kenyir Lake, Malaysia. *Journal of Biological Sciences, 12*(8), 438.

Najiah, M., Lee, S. W., Nadirah, M., Ruhil, H., Lee, K. L., Wendy, W., . . .Abdullah, S. (2009). Streptococcosis in red hybrid tilapia (*Oreochromis niloticus*) commercial farms in Malaysia. *Aquac Res, 40*, 630-632.

Nakanishi, T., Kiryu, I., & Ototake, M. (2002). Development of a new vaccine delivery method for fish: percutaneous administration by immersion with application of a multiple puncture instrument. *Vaccine, 20*(31), 3764-3769.

Nakanishi, T., & Ototake, M. (1997). Antigen uptake and immune responses after immersion vaccination. *Developments in biological standardization, 90*, 59-68.

Naraid, S., Kong, F., Ko, D., Gilbert, G. L., & Supamattaya, K. (2008). Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia *Oreochromis* sp. and Nile tilapia *O. niloticus* in Thailand—Relationship to human isolates? *Aquaculture, 284*(1), 35-40.

Navot, N., Kimmel, E., & Avtalion, R. (2005). Immunisation of fish by bath immersion using ultrasound. *Developments in biologicals, 121*, 135-142.

Nayak, D., Asha, A., Shankar, K., & Mohan, C. (2004). Evaluation of biofilm of *Aeromonas hydrophila* for oral vaccination of *Clarias batrachus*—a carnivore model. *Fish & Shellfish Immunology, 16*(5), 613-619.

Nguyen, H., & Kanai, K. (1999). Selective agars for the isolation of *Streptococcus iniae* from Japanese flounder, Paralichthys olivaceus, and its cultural environment. *Journal of applied microbiology, 86*(5), 769-776.

Nguyen, H. T., Kanai, K., & Yoshikoshi, K. (2002). Ecological investigation of *Streptococcus iniae* in cultured Japanese flounder (*Paralichthys olivaceus*) using selective isolation procedures. *Aquaculture, 205*(1), 7-17.

Nho, S. W., Shin, G. W., Park, S. B., Jang, H. B., Cha, I. S., & Ha, M. A. (2009). Phenotypical characteristics of *Streptococcus iniae* and *Streptococcus parauberis* isolated from olive flounder (*Paralichthys oliviceus*). FEMS Microbiol Lett, 293, 20-27.

Nikoskelainen, S., Ouwehand, A., Salminen, S., & Bylund, G. (2001). Protection of rainbow trout (Oncorhynchus mykiss) from furunculosis by Lactobacillus rhamnosus. Aquaculture, 198(3-4), 229-236.

Nilsson, W., Noonan, P., & Strom, M. (1998). *Rapid identification of fish pathogens using RFLP analysis of bacterial 16s ribosomal RNA.* Paper presented at the Abstract, 3rd international symposium on aquatic animal health.

Noraini, O., Sabri, M., & Abdullah, S. (2013). Efficacy of spray administration of formalin-killed *Streptococcus agalactiae* in hybrid Red Tilapia. *Journal of Aquatic Animal Health, 25*(2), 142-148.

Nur-Nazifah, M., Sabri, M., Amal, M., Latifah, H., & Abdullah, S. (2009). *Isolation and identification of Streptococcus agalactiae from Oreochromis* spp. *at Broga, Semenyih, Malaysia.* Paper presented at the Proceeding of the 21st Malaysian Veterinary Annual Conference,(p 360-362). 8th-9th August.

Nur-Nazifah, M., Sabri, M., & Abdullah, S. (2014). Development and efficacy of feed-based recombinant vaccine encoding the cell wall surface anchor family protein of *Streptococcus agalactiae* against streptococcosis in *Oreochromis* sp. *Fish & Shellfish Immunology, 37*(1), 193-200.

Ohnishi, K., & Jo, Y. (1981). Studies on streptococcal infection in pond-cultured fishes, 1: Characteristics of a beta-hemolytic streptococcus isolated from cultured ayu [Plecoglossus altivelis] and amago [Oncorhynchus rhodurus var. macrostomus] in 1977-1978. *Fish pathology, 16*, 63-67.

Olivares-Fuster, O., Klesius, P., Evans, J., & Arias, C. (2008). Molecular typing of *Streptococcus agalactiae* isolates from fish. *Journal of Fish Diseases, 31*(4), 277-283.

Othman, F., Islam, M., Sharifah, E., Shahrom-Harrison, F., & Hassan, A. (2015). Biological control of streptococcal infection in Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) using filter-feeding bivalve mussel Pilsbryoconcha exilis (Lea, 1838). *Journal of applied ichthyology, 31*(4), 724-728.

Park, S. E., Jiang, S., & Wessels, M. R. (2012). CsrRS and environmental pH regulate group B streptococcus adherence to human epithelial cells and extracellular matrix. Infection and immunity, IAI. 00699-00612.

Pasnik, D., Evans, J., & Klesius, P. (2007). Development of skeletal deformities in a *Streptococcus agalactiae*-challenged male Nile tilapia (*Oreochromis niloticus*) broodfish and in its offspring. *BULLETIN-EUROPEAN ASSOCIATION OF FISH PATHOLOGISTS, 27*(5), 169-176.

Pasnik, D., Evans, J., Panangala, V., Klesius, P., Shelby, R., & Shoemaker, C. (2005a). Antigenicity of *Streptococcus agalactiae* extracellular products and vaccine efficacy. *Journal of Fish Diseases, 28*(4), 205-212.

Pasnik, D. J., Evans, J. J., & Klesius, P. H. (2005b). Duration of protective antibodies and correlation with survival in Nile tilapia *Oreochromis niloticus* following *Streptococcus agalactiae* vaccination. *Diseases of Aquatic Organisms, 66*(2), 129-134.

Pasnik, D. J., Evans, J. J., & Klesius, P. H. (2009). Fecal strings associated with *Streptococcus agalactiae* infection in nile tilapia, *Oreochromis niloticus*. *Open veterinary science journal, 3*, 6-8.

Peaudecerf, L., & Rocha, B. (2011). Role of the gut as a primary lymphoid organ. *Immunology letters, 140*(1), 1-6.

Pedersen, K., Kühn, I., Seppänen, J., Hellström, A., Tiainen, T., Rimaila-Pärnänen, E., & Larsen, J. (1999). Clonality of Vibrio anguillarum strains isolated from fish from the Scandinavian countries, Sweden, Finland and Denmark. *Journal of applied microbiology, 86*(2), 337-347.

Pereira, U., Mian, G., Oliveira, I., Benchetrit, L., Costa, G., & Figueiredo, H. (2010). Genotyping of *Streptococcus agalactiae* strains isolated from fish, human and cattle and their virulence potential in Nile tilapia. *Veterinary microbiology, 140*(1), 186-192.

Perera, R. P., Johnson, S. K., Collins, M. D., & Lewis, D. H. (1994). *Streptococcus iniae* associated with mortality of Tilapia nilotica× T. aurea hybrids. *Journal of Aquatic Animal Health, 6*(4), 335-340.

Perera, R. P., Johnson, S. K., & Lewis, D. H. (1997). Epizootiological aspects of *Streptococcus iniae* affecting tilapia in Texas. *Aquaculture, 152*(1-4), 25-33.

PIER, G. B., & MADIN, S. H. (1976). *Streptococcus iniae* sp. nov., a beta-hemolytic Streptococcus isolated from an Amazon freshwater dolphin, Inia geoffrensis. *International Journal of Systematic and Evolutionary Microbiology, 26*(4), 545-553.

Pier, G. B., Madin, S. H., & Alnakeeb, S. (1978). Isolation and characterization of a second isolate of *Streptococcus iniae.* J Syst: Bacteriol, 28, 311-314.

Piganelli, J. D., Zhang, J. A., Christensen, J. M., & Kaattari, S. L. (1994). Enteric coated microspheres as an oral method for antigen delivery to salmonids. *Fish & Shellfish Immunology, 4*(3), 179-188.

Plant, K. P., & LaPatra, S. E. (2011). Advances in fish vaccine delivery. *Developmental & Comparative Immunology, 35*(12), 1256-1262.

Plumb, J., Schachte, J., Gaines, J., Peltier, W., & Carroll, B. (1974). Streptococcus sp. from marine fishes along the Alabama and northwest Florida coast of the Gulf of Mexico. *Transactions of the American Fisheries Society, 103*(2), 358-361.

Press, C. M., & Evensen, Ø. (1999). The morphology of the immune system in teleost fishes. *Fish & Shellfish Immunology, 9*(4), 309-318.

Pretto-Giordano, L. G., Müller, E. E., Klesius, P., & Da Silva, V. G. (2010a). Efficacy of an experimentally inactivated *Streptococcus agalactiae* vaccine in Nile tilapia (*Oreochromis niloticus*) reared in Brazil. *Aquaculture Research, 41*(10), 1539-1544.

Pretto-Giordano, L. G., Müller, E. E., Freitas, J. C. d., & Silva, V. G. d. (2010b). Evaluation on the Pathogenesis of *Streptococcus agalactiae* in Nile Tilapia (*Oreochromis niloticus*). *Brazilian Archives of Biology and Technology, 53*(1), 87-92.

Pretto-Giordano, L. G., Scarpassa, J. A., Barbosa, A. R., Altrão, C. S., Ribeiro, C. G. G., & Vilas-Boas, L. A. (2015). *Streptococcus iniae*: an unusual important pathogen fish in Brazil. *Journal of Aquaculture Research & Development, 6*(9), 1.

Pridgeon, J. W., Aksoy, M., Klesius, P. H., Li, Y., Mu, X., Srivastava, K., & Reddy, G. (2011a). Identification and expression profiles of multiple genes in Nile tilapia in response to bacterial infections. *Veterinary Immunology and Immunopathology, 144*(1), 111-119.

Pridgeon, J. W., & Klesius, P. H. (2011b). Development and efficacy of a novobiocin-resistant *Streptococcus iniae* as a novel vaccine in Nile tilapia (*Oreochromis niloticus*). *Vaccine, 29*(35), 5986-5993.

Pullin, R. S., & Lowe-McConnell, R. H. (1982). *The Biology and Culture of Tilapias: Proceedings of the International Conference on the Biology and Culture of Tilapias, 2-5 September 1980 at the Study and Conference Center of the Rockefeller Foundation, Bellagio, Italy* (Vol. 7): WorldFish.

Quentel, C., & Vigneulle, M. (1997). Antigen uptake and immune responses after oral vaccination. *Developments in biological standardization, 90*, 69-78.

Rasheed, V., & Plumb, J. A. (1984). Pathogenicity of a non-haemolytic group B Streptococcus sp. in gulf killifish (Fundulus grandis Baird and Girard). *Aquaculture, 37*(2), 97-105.

Rattanachaikunsopon, P., & Phumkhachorn, P. (2009a). Prophylactic effect of Andrographis paniculata extracts against *Streptococcus agalactiae* infection in Nile tilapia (*Oreochromis niloticus*). *Journal of bioscience and bioengineering, 107*(5), 579-582.

Rattanachaikunsopon, P., & Phumkhachorn, P. (2009b). Protective effect of clove oil-supplemented fish diets on experimental Lactococcus garvieae infection in tilapia. Bioscience, biotechnology, and biochemistry, 73(9), 2085-2089.

Rattanachaikunsopon, P., & Phumkhachorn, P. (2010). Potential of cinnamon (Cinnamomum verum) oil to control *Streptococcus iniae* infection in tilapia (*Oreochromis niloticus*). *Fisheries Science, 76*(2), 287-293.

Rinaudo, C. D., Rosini, R., Galeotti, C. L., Berti, F., Necchi, F., Reguzzi, V., . . . Maione, D. (2010). Specific involvement of pilus type 2a in biofilm formation in group B Streptococcus. PLoS One, 5(2), e9216.

Ristow, S., De Avila, J., Lapatra, S. E., & Lauda, K. (1993). Detection and characterization of rainbow trout antibody against infectious hematopoietic necrosis virus. *Diseases of Aquatic Organisms, 15*, 109-114.

Roach, J. C. M., Levett, P. N., & Lavoie, M. C. (2006). Identification of *Streptococcus iniae* by commercial bacterial identification systems. *Journal of microbiological methods, 67*(1), 20-26.

Robinson, J. A., & Meyer, F. P. (1966). Streptococcal fish pathogen. *Journal of Bacteriology, 92*(2), 512.

Romalde, J. L., & Toranzo, A. E. (2002). Molecular approaches for the study and diagnosis of salmonid streptococcosis. In *Molecular diagnosis of salmonid diseases* (pp. 211-233): Springer.

Romalde, J. L., Toranzo, A. E., & OLIVIER, G. (1999). *Streptococcosis of marine fish*: ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish. No. 56. International Council for the Exploration of the Sea. Copenhagen, Denmark, pp. 1 –8.

Rombout, J., & Berg, A. (1989). Immunological importance of the second gut segment of carp. I. Uptake and processing of antigens by epithelial cells and macrophages. *Journal of Fish Biology, 35*(1), 13-22.

Rombout, J., Berg, A., Berg, C., Witte, P., & Egberts, E. (1989). Immunological importance of the second gut segment of carp. III. Systemic and/or mucosal immune responses after immunization with soluble or particulate antigen. *Journal of Fish Biology, 35*(2), 179-186.

Rombout, J., Lamers, C., Helfrich, M., Dekker, A., & Taverne-Thiele, J. (1985). Uptake and transport of intact macromolecules in the intestinal epithelium of carp (Cyprinus carpio L.) and the possible immunological implications. *Cell and Tissue Research, 239*(3), 519-530.

Rombout, J. H., Abelli, L., Picchietti, S., Scapigliati, G., & Kiron, V. (2011). Teleost intestinal immunology. *Fish & Shellfish Immunology, 31*(5), 616-626.

Rombout, J. H., Taverne-Thiele, A. J., & Villena, M. I. (1993). The gut-associated lymphoid tissue (GALT) of carp (Cyprinus carpio L.): an immunocytochemical analysis. *Developmental & Comparative Immunology, 17*(1), 55-66.

Rombout, J. W., Blok, L. J., Lamers, C. H., & Egberts, E. (1986). Immunization of carp (Cyprinus carpio) with a Vibrio anguillarum bacterin: indications for a common mucosal immune system. *Developmental & Comparative Immunology, 10*(3), 341-351.

Rosini, R., & Margarit, I. (2015). Biofilm formation by Streptococcus agalactiae: influence of environmental conditions and implicated virulence factors. Frontiers in cellular and infection microbiology, 5, 6.

Saad, T. T., Abd El-Latif, G., Helmy, A. T., & Atallah, S. T. (2015). Bacteriological and Molecular Studies on the Enterococcus Species Isolated From Diseased Fish and Its Effect on Fish Farm Profits. *Journal of Life Sciences Research, 2*(1), 5-14.

Salinas, I., Zhang, Y.-A., & Sunyer, J. O. (2011). Mucosal immunoglobulins and B cells of teleost fish. *Developmental & Comparative Immunology, 35*(12), 1346-1365.

Salvador, R., Muller, E. E., Freitas, J. C. d., Leonhadt, J. H., Pretto-Giordano, L. G., & Dias, J. A. (2005). Isolation and characterization of Streptococcus spp. group B in Nile tilapias (*Oreochromis niloticus*) reared in hapas nets and earth nurseries in the northern region of Parana State, Brazil. *Ciência Rural, 35*(6), 1374-1378.

Schroeder, H. W., & Cavacini, L. (2010). Structure and function of immunoglobulins. *Journal of Allergy and Clinical Immunology, 125*(2), S41-S52.

Secombes, C., & Wang, T. (2012). The innate and adaptive immune system of fish. *Infectious disease in aquaculture: prevention and control, 231*, 3-68.

Sharma, S.R.K., Shankar, K. M., Sathyanarayana, M. L., Sahoo, A. K., Patil., R., Narayanaswamy, h. d., Rao, s. (2010). Evaluation of immune response and diseases in tiger shrimp *Penaeus monodon* fed with biofilm of *Vibrio alginolyticus*. Fish and shelfish immunology, 29 (5), 724-732.

Shen, Z., Qian, D., Xu, W., Gu, J., & Shao, J. (2005). Isolation, identification and pathogenicity of *Streptococcus iniae* isolated from red drum Sciaenops ocellatus. *Acta Hydrobiologica Sinica, 29*(6), 683.

Shin, G.-W., Palaksha, K., Kim, Y.-R., Nho, S.-W., Kim, S., Heo, G.-J., . . . Jung, T.-S. (2007). Application of immunoproteomics in developing a *Streptococcus iniae* vaccine for olive flounder (*Paralichthys olivaceus)*. *Journal of Chromatography B, 849*(1), 315-322.

Shoemaker, C. A., Evans, J. J., & Klesius, P. H. (2000). Density and dose: factors affecting mortality of *Streptococcus iniae* infected tilapia (*Oreochromis niloticus*). Aquaculture, 188(3), 229-235.

Shoemaker, C. A., Klesius, P. H., & Evans, J. J. (2001). Prevalence of *Streptococcus iniae* in tilapia, hybrid striped bass, and channel catfish on commercial fish farms in the United States. *American journal of veterinary research, 62*(2), 174-177.

Shoemaker, C. A., LaFrentz, B. R., Klesius, P. H., & Evans, J. J. (2010). Protection against heterologous *Streptococcus iniae* isolates using a modified bacterin vaccine in Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Fish Diseases, 33*(7), 537-544.

Shoemaker, C. A., Vandenberg, G. W., Désormeaux, A., Klesius, P. H., & Evans, J. J. (2006). Efficacy of a *Streptococcus iniae* modified bacterin delivered using Oralject™ technology in Nile tilapia (*Oreochromis niloticus*). *Aquaculture, 255*(1), 151-156.

Simon, B. E. (2001). *Recombinant vaccines against infectious hematopoietic necrosis virus: bacterial systems for vaccine production and delivery.* (PhD), Oregon State University, USA,

Siriyappagouder, P., Shankar, K., Kumar, B. N., Patil, R., & Byadgi, O. V. (2014). Evaluation of biofilm of *Aeromonas hydrophila* for oral vaccination of *Channa striatus*. *Fish & Shellfish Immunology, 41*(2), 581-585.

Siwicki, A. K., Anderson, D. P., & Rumsey, G. L. (1994). Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Veterinary Immunology and Immunopathology, 41*(1-2), 125-139.

Smith, G. L., & Moss, B. (1983). Infectious poxvirus vectors have capacity for at least 25 000 base pairs of foreign DNA. *Gene, 25*(1), 21-28.

Smith, M., Golding, P., Eddleston, A., Mitchell, C., Kemp, A., & Williams, R. (1972). Cell-mediated immune responses in chronic liver diseases. *Br Med J, 1*(5799), 527-530.

Soler, E., Parez, N., Passet, B., Dubuquoy, C., Riffault, S., Pillot, M., . . . Schwartz-Cornil, I. (2007). Recombinant rotavirus inner core proteins produced in the milk of transgenic rabbits confer a high level of protection after intrarectal delivery. *Vaccine, 25*(34), 6373-6380.

Soltani, M., Jamshidi, S., & Sharifpour, I. (2005). Streptococcosis caused by *Streptococcus iniae* in farmed rainbow trout (*Oncorhynchys mykiss*) in Iran: biophysical characteristics and pathogenesis. *Bulletin of the European Association of Fish Pathologists, 25*(3), 95-106.

Soltani, M., Mousavi, H., & Mirzargar, S. (2009). *Status of aquaculture health management in the Islamic Republic of Iran.* Paper presented at the Proceedings of the 1th International Congress on Aquatic Animal.

Sommerset, I., Krossøy, B., Biering, E., & Frost, P. (2005). Vaccines for fish in aquaculture. *Expert review of vaccines, 4*(1), 89-101.

Soto, E., Hawke, J., Fernandez, D., & A Morales, J. (2009). Francisella sp., an emerging pathogen of tilapia, *Oreochromis niloticus* (L.), in Costa Rica. *Journal of Fish Diseases, 32*(8), 713-722.

Stroband, H., & Kroon, A. G. (1981). The development of the stomach in Clarias lazera and the intestinal absorption of protein macromolecules. *Cell and Tissue Research, 215*(2), 397-415.

Suanyuk, N., Kanghear, H., Khongpradit, R., & Supamattaya, K. (2005). *Streptococcus agalactiae* infection in tilapia (*Oreochromis niloticus*). *Warasan Songkhla Nakharin (Sakha Witthayasat Iae Technology)*(27), 307-319.

Suanyuk, N., Kong, F., Ko, D., Gilbert, G. L., & Supamattaya, K. (2008). Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia *Oreochromis sp*. and Nile tilapia *O. niloticus* in Thailand—Relationship to human isolates? *Aquaculture, 284*(1), 35-40.

Suanyuk, N., Sukkasame, N., Tanmark, N., Yoshida, T., Itami, T., Thune, R. L., . . . Supamattaya, K. (2010). *Streptococcus iniae* infection in cultured Asian sea bass (*Lates calcarifer*) and red tilapia (*Oreochromis* sp.) in southern Thailand. *Songklanakarin Journal of Science & Technology, 32*(4), 341-348.

Sumanas, S., Zhang, B., Dai, R., & Lin, S. (2005). 15-zinc finger protein Bloody Fingers is required for zebrafish morphogenetic movements during neurulation. *Developmental biology, 283*(1), 85-96.

Sun, Y., & Hu, Y.-h. (2015). Cell-penetrating peptide-mediated subunit vaccine generates a potent immune response and protection against *Streptococcus iniae* in Japanese flounder (*Paralichthys olivaceus*). *Veterinary Immunology and Immunopathology, 167*(3), 96-103.

Sun, Y., Hu, Y.-h., Liu, C.-s., & Sun, L. (2010). Construction and analysis of an experimental *Streptococcus iniae* DNA vaccine. *Vaccine, 28*(23), 3905-3912.

Sun, Y., Hu, Y.-H., Liu, C.-S., & Sun, L. (2012). Construction and comparative study of monovalent and multivalent DNA vaccines against *Streptococcus iniae*. *Fish & Shellfish Immunology, 33*(6), 1303-1310.

Suwannasang, A., Suanyuk, N., Issaro, A., Phromkunthong, W., Tantikitti, C., Itami, T., & Yoshida, T. (2017). Growth, immune responses and protection of Nile tilapia *Oreochromis niloticus* immunized with formalin-killed Streptococcus agalactiae serotype Ia and III vaccines. Songklanakarin Journal of Science & Technology, 39(4).

Suwannasang, A., Dangwetngam, M., Issaro, A., Phromkunthong, W., & Suanyuk, N. (2014). Pathological manifestations and immune responses of serotypes Ia and III Streptococcus agalactiae infections in Nile tilapia (*Oreochromis niloticus*). Songklanakarin J. Sci. Technol, 36(5), 499-506.

Tafalla, C., Bøgwald, J., & Dalmo, R. A. (2013). Adjuvants and immunostimulants in fish vaccines: current knowledge and future perspectives. *Fish & Shellfish Immunology, 35*(6), 1740-1750.

Talaat, A. M., Reimschuessel, R., & Trucksis, M. (1997). Identification of mycobacteria infecting fish to the species level using polymerase chain reaction and restriction enzyme analysis. *Veterinary microbiology, 58*(2-4), 229-237.

Tobar, J. A., Jerez, S., Caruffo, M., Bravo, C., Contreras, F., Bucarey, S. A., & Harel, M. (2011). Oral vaccination of Atlantic salmon (Salmo salar) against salmonid rickettsial septicaemia. Vaccine, 29(12), 2336-2340.

Tompkins, D. M., Carver, S., Jones, M. E., Krkošek, M., & Skerratt, L. F. (2015). Emerging infectious diseases of wildlife: a critical perspective. *Trends in Parasitology, 31*(4), 149-159.

Tonheim, T. C., Bøgwald, J., & Dalmo, R. A. (2008). What happens to the DNA vaccine in fish? A review of current knowledge. *Fish & Shellfish Immunology, 25*(1), 1-18.

Toranzo, A., Devesa, S., Heinen, P., Riaza, A., Nunez, S., & Barja, J. (1994). Streptococcosis in cultured turbot caused by an Enterococcus-like bacterium. *BULLETIN-EUROPEAN ASSOCIATION OF FISH PATHOLOGISTS, 14*, 19-19.

Toranzo, A. E., Magarinos, B., & Romalde, J. L. (2005). A review of the main bacterial fish diseases in mariculture systems. *Aquaculture, 246*(1), 37-61.

Vandamme, P., Devriese, L., Pot, B., Kersters, K., & Melin, P. (1997). Streptococcus difficile is a nonhemolytic group B, type Ib Streptococcus. *International Journal of Systematic and Evolutionary Microbiology, 47*(1), 81-85.

Vandenberg, G. W. (2004). Oral vaccines for finfish: academic theory or commercial reality? *Animal Health Research Reviews, 5*(2), 301-304.

Villumsen, K. R., Neumann, L., Ohtani, M., Strøm, H. K., & Raida, M. K. (2014). Oral and anal vaccination confers full protection against enteric redmouth disease (ERM) in rainbow trout. *PLoS One, 9*(4), e93845.

Vinay, T., Patil, R., Suresh Babu, P., Rajesh, R., & Shankar, K. (2013). Evaluation of the Efficacy of *Aeromonas hydrophila* Biofilm Vaccine in *Labeo rohita* Employing Monoclonal Antibody Based ELISA. . *Open Access Scientific Reports, 2*(3), 684-687.

Vinay, T., Girisha, S., D'souza, R., Jung, M.-H., Choudhury, T., & Patil, S. (2016). Bacterial biofilms as Oral Vaccine Candidates in Aquaculture. Indian J. Comp. Microbiol. Immunol. Infect. Dis, 37(2), 57-62.

Wagener, C. (1997). Molecular diagnostics. *Mol. Med, 75*(10), 728– 744.

Wang, J., Wang, K., Chen, D., Geng, Y., Huang, X., He, Y., . . . Yang, Q. (2015). Cloning and characterization of surface-localized α-Enolase of *Streptococcus iniae*, an effective protective antigen in mice. *International journal of molecular sciences, 16*(7), 14490-14510.

Wang, K.-y., Chen, D.-f., Huang, L.-y., Lian, H., Wang, J., Xiao, D., . . . Lai, W.-m. (2013). Isolation and characterization of Streptococcus agalactiae from Nile Tilapia *Oreochromis niloticus* in China. African Journal of Microbiology Research, 7(4), 317-323.

Wang, L., & Coppel, R. L. (2008). Oral vaccine delivery: can it protect against non-mucosal pathogens? *Expert review of vaccines, 7*(6), 729-738.

Wang, X., Xiao, M., Kong, F., Sintchenko, V., Wang, H., Wang, B., . . . Chen, S. (2010). Reverse line blot hybridization and DNA sequencing studies of the 16s–23s rRNA gene intergenic spacer regions of five emerging pathogenic Nocardia species. *Journal of medical microbiology, 59*(5), 548-555.

Weinstein, M. R., Litt, M., Kertesz, D. A., Wyper, P., Rose, D., Coulter, M., . . . Willey, B. M. (1997). Invasive infections due to a fish pathogen, *Streptococcus iniae*. *New England Journal of Medicine, 337*(9), 589-594.

Wiklund, T., Madsen, L., Bruun, M. S., & Dalsgaard, I. (2000). Detection of Flavobacterium psychrophilum from fish tissue and water samples by PCR amplification. *Journal of applied microbiology, 88*(2), 299-307.

Xu, D. H., & Klesius, P. (2003). Protective effect of cutaneous antibody produced by channel catfish, Ictalurus punctatus (Rafinesque), immune to Ichthyophthirius multifiliis Fouquet on cohabited non-immune catfish. *Journal of Fish Diseases, 26*(5), 287-291.

Xu, D. H., Shoemaker, C., & Klesius, P. (2007). Evaluation of the link between gyrodactylosis and streptococcosis of Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Fish Diseases, 30*(4), 233-238.

Xu, Z., Parra, D., Gómez, D., Salinas, I., Zhang, Y.-A., von Gersdorff Jørgensen, L., . . . Sunyer, J. O. (2013). Teleost skin, an ancient mucosal surface that elicits gut-like immune responses. *Proceedings of the National Academy of Sciences, 110*(32), 13097-13102.

Yang, Q., Porter, A. J., Zhang, M., Harrington, D. J., Black, G. W., & Sutcliffe, I. C. (2012). The impact of pH and nutrient stress on the growth and survival of Streptococcus agalactiae. Antonie Van Leeuwenhoek, 102(2), 277-287.

Yanong, R. P., & Francis-Floyd, R. (2002). Streptococcal infections of fish. *Circular 57. Florida Cooperative Extension Service. IFAS, University of Florida*, 1-5.

Yanong, R. P. E., & Francis-Floyd, R. (2006). Streptococcal infections of fish. http://edis.ifas.ufl.edu/pdffiles/FA/FA05700.pdf

Ye, X., Li, J., Lu, M., Deng, G., Jiang, X., Tian, Y., . . . Jian, Q. (2011). Identification and molecular typing of *Streptococcus agalactiae* isolated from pond-cultured tilapia in China. *Fisheries Science, 77*(4), 623-632.

Yoshida, T., Endo, M., Sakai, M., & Inglis, V. (1997). A cell capsule with possible involvement in resistance to opsonophagocytosis in Enterococcus seriolicida isolated from yellowtail Seriola quinqueradiata. *Diseases of Aquatic Organisms, 29*(3), 233-235.

Yuasa, K., Kitancharoen, N., Kataoka, Y., & Al-Murbaty, F. A. (1999). *Streptococcus iniae*, the causative agent of mass mortality in rabbitfish Siganus canaliculatus in Bahrain. *Journal of Aquatic Animal Health, 11*(1), 87-93.

Yuniarti, A. (2005). Serological characterization of *Streptococcus iniae* isolates from differrent parts of Australia. Masters. University of Queensland, St. Lucia. Queensland.

Zamri-Saad, M., Amal, M., & Siti-Zahrah, A. (2010). Pathological changes in red tilapias (*Oreochromis* spp.) naturally infected by *Streptococcus agalactiae. Journal of Comparative Pathology, 143*(2), 227-229.

Zamri-Saad, M., Amal, M., Siti-Zahrah, A., & Zulkafli, A. (2014). Control and Prevention of Streptococcosis in Cultured Tilapia in Malaysia: A Review. *Pertanika Journal of Tropical Agricultural Science, 37*(4), 389-410.

Zhang, D., Li, A., Guo, Y., Zhang, Q., Chen, X., & Gong, X. (2013). Molecular characterization of *Streptococcus agalactiae* in diseased farmed tilapia in China. *Aquaculture, 412*, 64-69.

Zhang, Y.-A., Salinas, I., Li, J., Parra, D., Bjork, S., Xu, Z., . . . Sunyer, J. O. (2010). IgT, a primitive immunoglobulin class specialized in mucosal immunity. *Nature immunology, 11*(9), 827-835.

Zhou, F., Shefer, A., Weinbaum, C., McCauley, M., & Kong, Y. (2007). Impact of hepatitis A vaccination on health care utilization in the United States, 1996–2004. *Vaccine, 25*(18), 3581-3587.

Zhou, S., Xie, M., Zhu, X., Ma, Y., Tan, Z., & Li, A. (2008). Identification and genetic characterization of *Streptococcus iniae* strains isolated from diseased fish in China. *Journal of Fish Diseases, 31*(11), 869-875.

Zhu, L.-y., Nie, L., Zhu, G., Xiang, L.-x., & Shao, J.-z. (2013). Advances in research of fish immune-relevant genes: a comparative overview of innate and adaptive immunity in teleosts. *Developmental & Comparative Immunology, 39*(1), 39-62.

Zlotkin, A., Chilmonczyk, S., Eyngor, M., Hurvitz, A., Ghittino, C., & Eldar, A. (2003). Trojan horse effect: phagocyte-mediated *Streptococcus iniae* infection of fish. *Infection and immunity, 71*(5), 2318-2325.

Zlotkin, A., Eldar, A., Ghittino, C., & Bercovier, H. (1998a). Identification of Lactococcus garvieae by PCR. *Journal of clinical microbiology, 36*(4), 983-985.

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