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Effects of Probiotics and Current Scenario of Fish Vaccination

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ABSTRACT

Food consumption is increasing due to rapid population growth. Fish is one of the most important animal foods, providing a high amount of energy in the form of proteins, essential amino acids, nutrients, vitamins, and minerals. Antibiotics are used to control several pathogens that harm fish. However, the use of antibiotics in fish causes numerous infections like tissue damage and immunological suppression. So, due to these side effects of antibiotics, probiotics and vaccines were introduced to protect fish from various pathogens. Probiotics are beneficial bacteria that have numerous positive effects on fish, while vaccines are precautionary measures that can prevent the fish from disease if administered to healthy fish. Several gram-positive and gram-negative bacteria are used as probiotics in fish, while different types of vaccines are used to control fish diseases, including both vibriosis as well as infectious hematopoietic necrosis. The dose as well as the mode of administration of both probiotics and vaccines are also significant factors that affect fish health. Vaccines and probiotics, if used in appropriate amounts, provide health benefits to fish. Although the effects of probiotics and vaccines may differ in different fish species, they are more useful than antibiotics. This review article discusses the beneficial effects of probiotics on fish as well as vaccines that are currently used to protect fish from disease.

INTRODUCTION

The need for animal-based food (particularly aquaculture) is increasing with the increase in the human population **(FAO, 2020)**. Antibiotics are applied to protect aquaculture from various pathogens. However, antibiotic resistance is rising on a daily basis. Moreover, for nearly thirty years, antibiotics were the popular and conventional agents of bacterial control until evidence of the risks of antibiotics was not made available to the environment and consumers **(Cabello, 2006; Hektoen *et al.*, 1995)**.

Antibiotic use in aquaculture also leads to numerous different disorders and complications, such as health problems in humans, the reduction of beneficial microbiota, tissue damage in fish, deposition in fish body, antibiotic resistance in bacteria, and immune suppression in fish **(Bondad-Reantaso *et al.*, 2005; Li & Gatlin III, 2005; Muñoz de la Peña & Espinosa-Mansilla, 2009; Sapkota *et al.*, 2008)**. In addition, antibiotics disturb the natural environment of the ecosystem, which ultimately affects the physiology, nutrition, and immunity of fish **(Maynard *et al.*, 2012; Rawls *et al.*, 2007; Rekecki *et al.*, 2009)**.

Recently, the use of chemicals as well as antibiotics to control diseases in aquaculture has been discouraged due to side effects on public health **(Okocha *et al.*, 2018)**. As a result of the threat associated with antibiotic use, the improvement of an eco-friendly agent (non-antibiotic) is considered to be one of the most important factors for better health care in aquaculture. Therefore, many aquaculture experts have proposed a variety of alternative approaches for developing environmentally friendly aquaculture. The use of probiotics (eco-friendly agents) and bio-control agents instead of chemotherapeutics is also one of these approaches **(Gatesoupe, 2005; Robertson *et al.*, 2000)**. The replacement of antibiotics with probiotics could be an alternative and effective method to control fish diseases and enhance pond health **(Steenbergen *et al.*, 2015)**.

The word “probiotics” is derived from two Greek words, “pro” and “bios”. “Pro” means “before” and “bios” means “life”. Probiotics are living microorganisms that provide health benefits to the host (fish) when used in appropriate amounts **(Steenbergen *et al.*, 2015)**. Probiotics also have the ability to colonize and multiply in the host’s intestine. As they can multiply in the host’s intestine, they have many beneficial effects by modulating different biological systems in the body of the host **(Cross, 2002)**. Probiotics or the products secreted by them are found to be used in aquaculture to replace chemotherapeutic agents and to control different fish diseases. Microalgae, yeast, and a large number of gram-negative as well as gram-positive bacteria have been assessed as probiotics **(Akter *et al.*, 2016)**.

Probiotic use is considered a promising strategy against various fish diseases. In addition, the wide acceptance of the use of probiotics in aquaculture has been demonstrated in many research studies over the past ten years **(Balcázar *et al.*, 2006; Irianto & Austin, 2002; Merrifield *et al.*, 2010; Wang *et al.*, 2008)**. Probiotics are beneficial to aquaculture as they enhance the immunity of fish, improve the digestive system and protect the fish from various pathogens **(Gatesoupe, 2005; Robertson *et al.*, 2000)**.

1. Probiotics

1.1 Characteristics of Probiotics

The characteristics of good probiotics as described by **Fuller (1989)** are:

1. The strain should be capable of colonizing the gut of fish. The bacteria that are able to colonize the gut of fish are categorized as autochthonous bacteria.

2. The strain should have the capability to metabolize and survive in the environment of the gut, e.g., should be resistant to low pH and bile of fish due to enrichment of organic acids.
3. It should have the ability to survive under normal storage conditions.
4. The strain should be capable of multiplying in the intestine of fish with the ability to adhere to the gut.
5. The strain should have antagonistic and inhibiting properties so that it could inhibit the growth of the pathogen.
6. The strain should be less toxic and non-pathogenic for fish.
7. The strain should have no adverse effects on other aquatic animals or human consumers.

The strain should be beneficial for host e.g., by providing disease resistance or increased growth.

1.2 Dose

The probiotic dose is an important factor for the achievement of maximum beneficial effects **(Minelli & Benini, 2008)**. The optimal dose of probiotics is required not only for beneficial effects such as immunostimulatory activity, but also for establishment and proliferation in the gut. The probiotic dose is determined by the ability of bacteria to increase the protection and growth of the host **(Nayak, 2010)**. The probiotic dose more often fluctuates from 10^6 to 10^{10} CFU/g feed in aquaculture, i.e., the dose of *Lactobacillus* is 10^7 CFU/g **(Zuo *et al.*, 2019)**. The type of host and immune parameters are the two crucial factors due to which the optimum dose of probiotics varies. **Panigrahi *et al.* (2004)** discovered that when fed 10^{11} CFU/g feed for 30 days with the strain of *L. rhamnosus, O. mykiss* showed phagocytic activity of head kidney leucocyte, high serum lysozyme, and complement activities but did not show any such activity when fed 10^9 CFU/g feed. Moreover, the stimulation of a specific immune response in the context of an organ or tissue also depends on the dose. For example, in the case of *M. miiuy*, the increase of lysozyme activity in skin and serum is observed at two varied doses, i.e., 10^9 and 10^7 CFU of *C. butyricum*/g feed, respectively **(Song *et al.*, 2006)**.

1.3 Mode of Supplementation

There are different methods of supplementation; for example, oral/feed, bath, and suspension methods. The most reliable method of these is the feed method, through which the probiotics settle in the gut and it will be easy for them to activate the immunity of the fish **(Gildberg *et al.*, 1997; Moriarty, 1998)**. Oral administration is suggested because it is considered effective in elevating subsequent protection and immunity **(Taoka *et al.*, 2006)**. The feeding mode of supplementation has limitations during the larval stage of fish development due to the immature gastrointestinal tract of fish during this stage. In addition, injection is also not used during the larval stage of fish as it causes a high degree of stress to the larvae. In contrast to these modes of supplementation, probiotics can be added directly to the water in incubators from the first day of hatching. It is recommended that probiotic combinations should be supplemented with water and live feed like rotifers, as it is a better way to apply the probiotics during the larval stage **(Sveinsdóttir *et al.*, 2009)**. Some results revealed that supplementation of probiotics in water has better effects on fish health than other protocols. This might be due to continuous drinking in aquaculture **(Villamil *et al.*, 2010)**.

1.4 Feeding duration

To stimulate innate immunity, the time duration of probiotic feeding may vary among the various probiotic strains of the same family **(Choi & Yoon, 2008)**. The difference in the activation of particular immune parameters also depends on the duration of feeding. For instance, **P Díaz-Rosales *et al.* (2009)** reported a clear increase in respiratory burst activity when probiotics are fed for 60 days. But unfortunately, at the initial stages, **Patricia Díaz-Rosales *et al.* (2006)** failed to observe any substantial enhancement of such activity when the inactivated form of the probiotic was fed for almost 28 days.

2. Beneficial effects of probiotics in aquaculture

2.1 Effects on immune response

Innate immunity (non-specific immunity) and adaptive immunity (highly specific immunity) are two kinds of immunity in fish **(Sakai, 1999)**. By stimulating the innate immune response, probiotics such as *B. subtilis* and *L. acidophilus* can be used to enhance health conditions in Nile tilapia, boosting their ability to resist infection and improving their developmental response **(Aly *et al.*, 2008)**. Feeding probiotics to Cobia (a fish species) may improve immunological response and growth rate by increasing feed utilization, as well as survival against a harmful bacteria known as *Vibrio harveyi* **(Geng *et al.*, 2012)**.

Antimicrobial peptides are found in fish blood serum, the most significant of which are lysozyme and immunoglobulin. They can serve as the first line of defense against a variety of diseases. As a result, they have the potential to prevent numerous pathogens from colonizing and thereby prevent diseases **(Alexander & Ingram, 1992)**. Lysozyme is an enzyme found in fish and various vertebrates. It has the most proactive activity against the attack of gram-positive and some gram-negative bacteria. It breaks down the β-1,4 glycosidic bonds between N-acetylglucosamine and N-acetylmuramic acid present in the peptidoglycan, a layer of the bacterial cell wall. The concentration of lysozymes can be enhanced by feeding the supplemented diets of probiotics **(Alexander & Ingram, 1992; Balcázar *et al.*, 2007)**.

Immunoglobulin is another significant immune factor in fish. It also performs an important function in preventing pathogens **(Alexander & Ingram, 1992)**. By improving the production of immunoglobulin, probiotics have shown the ability to improve both innate and adaptive immunity. In African catfish serum, higher immunoglobulin content was reported when fed *L. acidophilus* **(Al- Dohail *et al.*, 2009; Nikoskelainen *et al.*, 2003)**.

2.2 Effects on the intestinal ecosystem

The gastrointestinal system has long been thought to be a critical habitat for a variety of microbial communities, including allochthonous (exogenous) and autochthonous (indigenous) species **(Nayak, 2010)**. Autochthonous microbes have many significant effects on the development and improvement of the gastrointestinal tract, including the development and maturity of the immune system and intestine. These microorganisms also play a vital role in resistance to various disease-causing agents in fish **(Birkbeck & Ringø, 2005; Ringø *et al.*, 2007)**.

Additionally, these microbes have the capability to inhibit the colonization of various bacteria using a variety of mechanisms, including competition for food and space, the presence of receptors on mucosal surfaces, and the secretion of antimicrobial compounds **(Nayak, 2010)**. In this manner, the species of probiotics have extremely favorable effects on the body of the host by altering the microbial balance of their intestines **(Aires & Butel, 2011)**.

2.3 Effects on digestive enzymes

The organs of the digestive system have a profound effect on the food composition and are responsible for instant changes in the activities of digestive enzymes **(Shan *et al.*, 2008)**, which are simultaneously linked with the growth and health of fish. It was reported that feeding probiotics to Nile tilapia increased growth rate as well as food utilization by increasing the rate of fat, starch, and protein digestion **(Essa *et al.*, 2010)**. Furthermore, when fed three different probiotics (*Lactococcus lactis*, *Saccharomyces cerevisiae*, and *Bacillus subtilis*), the activities of digestive enzymes such as lipase, protease, and amylase increased in another fish species, *Labeo rohita* **(Mohapatra *et al.*, 2013)**.

Bacteria and enzymes secreted by them have a significant effect on the digestion processes **(Munilla-Moran & Stark, 1990)** by increasing the activity of digestive enzymes in the intestine of fish **(Wang, 2007)**. Probiotics secrete various enzymes that are called exogenous enzymes. Evidence suggests that exoenzymes secreted by probiotics have the potential to increase the digestive usage of feed **(Mohapatra *et al.*, 2013)**. Probiotics have been shown to have beneficial effects not only on the fish digestive system but also on the absorption of components of digested food **(Irianto & Austin, 2002)**.

2.4 Effects on intestinal morphology

Nayak (2010) explains that probiotics can colonize and apply their beneficial effects in the intestinal tract of animals, which is considered a complicated harbor for pathogenic and non-pathogenic microorganisms. Some pathogenic microorganisms that remain within the gastrointestinal tract of fish can change the shape of the intestinal epithelial layer by increasing nutrient absorption and reducing mucosal damage. **Merrifield *et al.* (2010)** described how probiotics maintain a healthy intestinal state by lowering the number of harmful microorganisms. An additional dose of *L. acidophilus* (probiotic) resulted in a significant increase in the length of villus in Senegalese sole, which indicates that a wider surface area capable of better absorption of digested food **(Barroso *et al.*, 2016; Caspary, 1992)**.

The possible mechanism behind the increase in the length of the villus is that during passing through the stomach, probiotics can spread in the fish intestine and can use the sugar present in the hindgut, resulting in the formation of components like SCFAs (short chains of fatty acids) during the process of fermentation, which can play an important role in increasing the height of the villus **(Pelicano *et al.*, 2005)**.

2.5 Effects on growth and food

The most anticipated result of the use of probiotics is that they have a direct effect on the growth of fish, either by providing necessary nutrients or by directly increasing the amount of nutrients **(Kolndadacha *et al.*, 2011; Sakata, 1990)**. Numerous studies have shown the beneficial effects of the most significant group of probiotics, i.e., *Lactobacillus* species, on the growth of African catfish, Nile tilapia, gilthead sea bream, beluga fry, and Persian sturgeon **(Al- Dohail *et al.*, 2009; Askarian *et al.*, 2011; Irianto & Austin, 2002; Lara-Flores *et al.*, 2003; Suzer *et al.*, 2008)**. Improvements in the growth of cultivated fish after eating probiotics may be due to better food quality and efficiency **(Al- Dohail *et al.*, 2009)**, which ultimately increases the appetite of fish **(Irianto & Austin, 2002)**.

2.6 Effects on haematological parameters

It is reported that the physiological state is strongly affected by various haematological parameters, which also provide a clue about the health of fish **(Pelicano *et al.*, 2005)**. Numerous studies have described the promising results of probiotics on haematological parameters like hemoglobin content, PCV (Packed Cell Volume), RBC (Red Blood Cell) count, and WBC (White Blood Cell) count in fish **(Olayinka & Afolabi, 2013)**. PCV, different WBC types, and total WBC counts are haematological parameters that also provide a significant indication of the health condition of fish **(Sampath *et al.*, 1998)**. The total amount of RBC increased immensely after feeding gram-negative as well as gram-positive probiotics at a diet of 10^7 CFU/g **(Irianto & Austin, 2002)**. Several studies have also found that probiotics have the potential to actively increase the production of T and B lymphocytes in fish bodies, which play an important role in identifying antigens through the production of specific antibodies or can destroy infected cells **(Al- Dohail *et al.*, 2009)**.

2.7 Effects on larval and gamete quality

The reproductive health of a fish is the most important thing in the context of their well-being, along with reproduction, and mainly for the health of embryos and gametes. In the case of the fish farming industry, the quality of fish gametes matters a lot and is one of the biggest issues for the industry, primarily for intensively propagated species. The control of gamete quality in captivated fish is considered one of the most critical hindrances in the development of commercial fish farming as a large portion of them are equipped with poor quality gametes.

One thing that can help in this regard is probiotics, because these can promote fecundity and ovulation and also have an impact on gamete quality. Probiotics also assist in supporting the survival and hatching of larvae and embryos. The most prominent and potent probiotic that is emerging these days for the quality of both larvae and gametes is *L. rhamnosus* **(Carnevali *et al.*, 2013; Vilchez *et al.*, 2015)**. This probiotic is well-known for a substantial increase in egg quality and fecundity at the physiological level. The use of *Bacillus subtilis* helps in boosting the fertility and viability of larvae and egg quality, as investigated in female ornamental fish **(Ghosh *et al.*, 2007)**.

3. The current scenario of fish vaccination

Microorganisms are the cause of a variety of infections in fish, which is one of the most significant challenges in the fish industry **(Mahida *et al.*, 2014)**. The main causative agents for various diseases of fish in aquaculture include bacteria (up to 54.9%), viruses (22.6 %), parasites (19.4%), and fungi (3.1%) **(Dhar *et al.*, 2014)**. Vaccination and probiotics are two alternatives to antibiotics for combating bacterial and viral diseases, particularly in aquaculture **(Mahida *et al.*, 2014)**. The first report on fish vaccination was given by David C. B. Duff (the father of fish vaccination). In 1942, he reported the prolonged use of inactivated bacteria with the help of chloroform, which could protect the trout from bacterial illness. Vaccination is believed to say "Prevention is better than cure" **(Mahida *et al.*, 2014)**. One of the best ways to prevent the disease outbreak is to immunize the fish against common microbes or pathogens. Therefore, vaccination is becoming a more vital part of aquaculture **(Mahida *et al.*, 2014)**.

3.1 Properties of an ideal vaccine

The properties of an effective vaccine, as described by **Grisez and Tan (2005)** are:

1. It should provide 100% protection against different strains.
2. It should be readily registered or licensed.
3. It should be able to protect the organism for a long time.
4. It should be safe for the fish and the administrator.

3.2 Types of fish vaccines

According to **Dadar *et al.* (2017)**, vaccines can be categorized as attenuated, killed, synthetic peptides, DNA, recombinant vector, subunit, or genetically modified vaccines (**Fig.1, Table 1**).

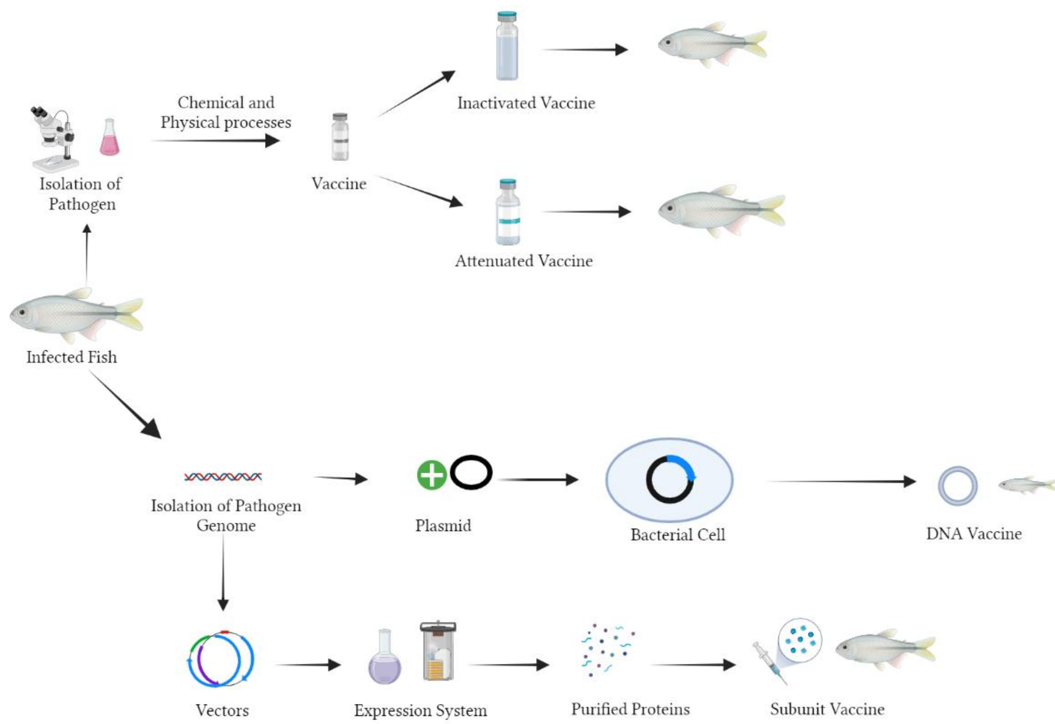

Figure 1. Different types of vaccines used for fish

Table 1. Different types of fish vaccines with their pathogens and host fishes

Vaccine Type	Pathogen	Fish Species	Disease	Reference
Inactivated	*F. columnare*	*Oreochromis* spp.	Columnaris	**(Kitiyodom *et al.*, 2019)**
	Vibrio vulnificus	*Anguilla Anguilla/*European eel	Vibriosis	**(Esteve-Gassent *et al.*, 2004)**
	Y. ruckeri	Rainbow trout	Yersiniosis	**(Jaafar *et al.*, 2018)**
	Yersinia ruckeri	*Salmo salar*/Atlantic salmon	Yersiniosis	**(Bridle *et al.*, 2012)**
Live attenuated	*E. tarda*	*Pangasius hypophthalmus*	Edwardsiellosis	**(Triet *et al.*, 2019)**
	F. psychrophilum	Rainbow trout	Rainbow trout fry syndrome (RTFS)	**(Ma *et al.*, 2019)**
Subunit	ISKNV	*Siniperca chuatsi*/Mandarin fish	Kidney necrosis and infectious spleen	**(Zhao *et al.*, 2019)**
DNA	VHSV	Rainbow trout	Viral hemorrhagic septicemia	**(Fernandez-Alonso *et al.*, 2001)**

3.2.1 Inactivated/killed vaccines

Inactivated vaccines are produced by replication or multiplication of disease-causing agents in bulk amounts and then subjected to deactivating agents, which kill all the microbes without compromising the ability of vaccines to activate the immune system of the body. The biosafety as well as effectuality of these vaccines depend on the conditions of cultivation, such as temperature range and type of media **(Toranzo *et al.*, 2009)**. Some of the diseases that can be prevented by using inactivated vaccines include *V. salmonicida, A. salmonicida,* and infectious hematopoietic necrosis **(Monaghan *et al.*, 2016)**. In aquaculture, mostly inactivated vaccines are used. The benefits of using killed vaccines as described by **Pridgeon and Klesius (2012)** are:

- These can be designed easily.
- These are less expensive.
- These vaccines do not have virulence issues.
- These are stable to store.

3.2.2. Live attenuated vaccines

Attenuated vaccines are living but weekend pathogens, genetically or chemically, so these vaccines can persuade an immune response for a short time **(Adams *et al.*, 2008)**. Attenuated vaccines are produced by losing the virulence of the pathogens without killing them by physical or chemical processes. **Shoemaker *et al.* (2009)** have shown that these vaccines are effective against various fish diseases. These vaccines can induce cellular, mucosal, and humoral immunity **(Shoemaker *et al.*, 2009)**. Weakened organisms do not show clinical signs during replication in the host **(Lillehaug, 2014)**. These are used to prevent various diseases in humans and in animals **(Munang'andu *et al.*, 2014)**.

3.2.3. Subunit vaccines

If the organism's culturing is difficult, the immunogenic part of the organism is used in the development of subunit vaccines **(Dadar *et al.*, 2017)**. The first tool used for the identification of appropriate pathogens to design a subunit vaccine was molecular identification. After this, reverse vaccinology was also one of the effective tools that were used to design a subunit vaccine by identifying the antigenic components of the pathogen by using genomics as well as proteomics analysis. This type of vaccine can be developed by using either a single antigen or multiple antigens to produce a wide range of vaccines. It is necessary to prove whether the antigenic components (used for developing subunit vaccines) can induce an immune response before testing the efficacy of the vaccine in animal models **(Unajak *et al.*, 2022)**. Subunit vaccines are not very useful because of their poor immunogenicity. However, for safe use, adjuvants can be added that may help in improving the immunogenicity of such vaccines **(Dadar *et al.*, 2017)**.

3.2.4 Synthetic peptide vaccines

The amino acid short sequences are used to prepare synthetic peptide-based vaccines that act as an antigen or as a viable antigenic site **(Lillehaug, 2014; Pridgeon & Klesius, 2012)**. Such vaccines have been used for various viral diseases, including rhabdoviruses, septicemia, nodavirus, and birnavirus **(Dadar *et al.*, 2017)**. The fish immune responses against antigens are not fully understandable. Carrier molecules may be required. However, peptide-based vaccination of fish is not yet recommended by researchers **(Lillehaug, 2014)**.

3.2.5 Recombinant vector vaccines

In biotechnology, advanced techniques have been implemented to prepare recombinant vector vaccines. The heterologous host is used to insert the pathogen's immunogenic region and its expressions to carriers are noted. The purified protein's large quantities are utilized for in vitro vaccine development. In vector selection, antigen expressions are considered leading factors because of their ability to produce large quantities of proteins very easily. In salmonid infections, necrosis, and salmon anemia, the vectors are being expressed as vaccine candidates to fight against salmonid rhabdoviruses **(Dadar *et al.*, 2017)**.

3.2.6 DNA vaccines

DNA vaccines are developed with specific genes that code for specific antigenic proteins and are expressed in the plasmid, which contains very strong immune responses in host cells. The plasmids are produced within the bacteria-containing cells. The gene expression is carried out with the help of promotors and terminators of the gene of interest **(Kurath, 2008)**. Cellular and humoral immune responses play an important role in DNA vaccines. If the protective antigen is found, the development of vaccines goes rapid. Such vaccines are effective against fish rhabdoviruses because of their favorable environment against viral infections, and they also prevent fish and intracellular bacteria exposure. The DNA vaccines use the same cellular pathway by which the virus first enters the host cell **(Hølvold *et al.*, 2014)**.

In fish, DNA vaccines have been extensively studied by researchers for the diagnostic purpose of both VHSV and IHNV salmonid rhabdoviruses. The DNA vaccines used in the salmon aquaculture industry are thought to be beneficially effective in reducing the effects of viruses because they contain a single gene in purified plasmid DNA of the pathogens. Moreover, in the host, DNA vaccines do not transfer or replicate, thus having noninfectious effects even with the actual disease.

3.2.7 RNA vaccines

In these vaccines, RNA is used because of its numerous advantages. The degradation of RNA is done by a natural process, so it is considered safe and non-infectious for usage in vaccines **(Pardi *et al.*, 2018)**. Also, these types of vaccines are non-infectious as they are not made up of inactivated pathogens or pathogenic particles **(Bedekar & Kole, 2022)**.

RNA stimulates and regulates immunity, so it is considered an effective candidate for advancements in RNA-based vaccinology **(Brito *et al.*, 2015; Geall & Blagbrough, 2000; Pardi *et al.*, 2018; Restifo *et al.*, 2000; Ulmer *et al.*, 2012)**. Humoral as well as cell-mediated immunity can be elicited by RNA vaccines. RNA vaccination is a very new technology, so the experimentation of RNA vaccines is limited in fish vaccinology **(Bedekar & Kole, 2022)**.

3.2.8 Polyvalent vaccines

These vaccines are considered ideal candidates for protecting the majority of susceptible fish species against diseases **(Busch, 1997)**. The polyvalent vaccines have been extensively used on salmonids and turbot. These vaccines must be handled carefully to avoid antigen competition in the formulation, especially when used as infusions **(Busch, 1997; Toranzo *et al.*, 2009)**.

CONCLUSION

It is concluded that although probiotic use is an important way of protecting fish from various infections, the amount of probiotic dose given to fish varies among different fish species. Therefore, it requires further investigation to know what quantity of a probiotic dose and its feed duration are highly effective for a particular fish species. Probiotics have enormous beneficial effects on fish, including positive effects on immune response, intestinal ecosystem, digestive enzymes, intestinal morphology, haematological parameters, and quality of gametes. Moreover, use of probiotics also results in increased fish growth with immunity to various pathogens. Control of fish diseases is a significant challenge for the fish industry. Vaccines of various types are also used to protect fish, depending on the pathogen or disease. However, inactivated or killed vaccines are mostly used or recommended for fish.

REFERENCES

Adams, A.; Aoki, T.; Berthe, C.; Grisez, L. and Karunasagar, I. (2008). Recent technological advancements on aquatic animal health and their contributions toward reducing disease risks-a review. Diseases in Asian Aquaculture VI. Colombo, Sri Lanka: Fish Health Section, Asian Fisheries Society: 71-88.

Aires, J. and Butel, M. J. (2011). Proteomics, human gut microbiota and probiotics. Expert Rev Proteomics, 8(2): 279-288. doi: https://doi.org/10.1586/epr.11.5

Akter, M. N.; Parvez, I. and Patwary, Z. P. (2016). Beneficial effects of probiotics in aquaculture. International Journal of Fisheries and Aquatic Studies, 4(5): 494-499.

Al- Dohail, M. A.; Hashim, R. and Aliyu- Paiko, M. (2009). Effects of the probiotic, Lactobacillus acidophilus, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (Clarias gariepinus, Burchell 1822) fingerling. Aquaculture Research, 40(14): 1642-1652. doi: https://doi.org/10.1111/j.1365-2109.2009.02265.x

Alexander, J. B. and Ingram, G. A. (1992). Noncellular nonspecific defence mechanisms of fish. Annual Review of Fish Diseases, 2: 249-279. doi: https://doi.org/10.1016/0959-8030(92)90066-7

Aly, S. M.; Ahmed, Y. A.-G.; Ghareeb, A. A.-A. and Mohamed, M. F. (2008). Studies on Bacillus subtilis and Lactobacillus acidophilus, as potential probiotics, on the immune response and resistance of Tilapia nilotica (Oreochromis niloticus) to challenge infections. Fish Shellfish Immunology, 25(1-2): 128-136. doi: https://doi.org/10.1016/j.fsi.2008.03.013

Askarian, F.; Kousha, A.; Salma, W. and Ringø, E. (2011). The effect of lactic acid bacteria administration on growth, digestive enzyme activity and gut microbiota in Persian sturgeon (Acipenser persicus) and beluga (Huso huso) fry. Aquaculture Nutrition, 17(5): 488-497. doi: https://doi.org/10.1111/j.1365-2095.2010.00826.x

Balcázar, J. L.; De Blas, I.; Ruiz-Zarzuela, I.; Cunningham, D.; Vendrell, D. and Múzquiz, J. L. (2006). The role of probiotics in aquaculture. Veterinary Microbiology, 114(3-4): 173-186. doi: https://doi.org/10.1016/j.vetmic. 2006. 01.009

Balcázar, J. L.; De Blas, I.; Ruiz-Zarzuela, I.; Vendrell, D.; Gironés, O. and Muzquiz, J. L. (2007). Enhancement of the immune response and protection induced by probiotic lactic acid bacteria against furunculosis in rainbow trout (Oncorhynchus mykiss). FEMS Immunology & Medical Microbiology, 51(1): 185-193. doi: https://doi.org/10.1111/j.1574-695X.2007.00294.x

Barroso, C.; Ozorio, R. O.; Afonso, A.; Moraes, J. R. and Costas, B. (2016). Immune responses and gut morphology in Senegalese sole (Solea senegalensis) fed dietary probiotic supplementation and following exposure to Photobacterium damselae subsp. piscicida. Aquaculture Research, 47(3): 951-960. doi: https://doi.org/10.1111/are.12553

Bedekar, M. K. and Kole, S. (2022). Fundamentals of Fish Vaccination. In "Vaccine Design" Springer, pp. 147-173.

Birkbeck, T. and Ringø, E. (2005). Pathogenesis and the gastrointestinal tract of growing fish. In "Biology of Growing Animals" Elsevier, pp. 208-234.

Bondad-Reantaso, M. G.; Subasinghe, R. P.; Arthur, J. R.; Ogawa, K.; Chinabut, S.; Adlard, R.; Tan, Z. and Shariff, M. (2005). Disease and health management in Asian aquaculture. Vet Parasitol, 132(3-4): 249-272. doi: https://doi. org/10.1016/j.vetpar.2005.07.005

Bridle, A. R.; Koop, B. F. and Nowak, B. F. (2012). Identification of surrogates of protection against yersiniosis in immersion vaccinated Atlantic salmon. PLoS One, 7(7): e40841. doi: https://doi.org/10.1371/journal.pone.0040841

Brito, L. A.; Kommareddy, S.; Maione, D.; Uematsu, Y.; Giovani, C.; Berlanda Scorza, F.; Otten, G. R.; Yu, D.; Mandl, C. W.; Mason, P. W.; Dormitzer, P. R.; Ulmer, J. B. and Geall, A. J. (2015). Self-amplifying mRNA vaccines. Adv Genet, 89: 179-233. doi: https://doi.org/10.1016/bs.adgen.2014.10.005

Busch, R. A. (1997). Polyvalent vaccines in fish: the interactive effects of multiple antigens. Dev Biol Stand, 90: 245-256.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environmental microbiology, 8(7): 1137-1144. doi: https://doi.org/10.1111/j.1462-2920. 2006. 01054.x

Carnevali, O.; Avella, M. A. and Gioacchini, G. (2013). Effects of probiotic administration on zebrafish development and reproduction. Gen Comp Endocrinol, 188: 297-302. doi: https://doi.org/10.1016/j.ygcen.2013.02.022

Caspary, W. F. (1992). Physiology and pathophysiology of intestinal absorption. Am J Clin Nutr, 55(1 Suppl): 299S-308S. doi: https://doi.org/10.1093/ajcn/55.1.299s

Choi, S.-H. and Yoon, T.-J. (2008). Non-specific immune response of rainbow trout (Oncorhynchus Mykiss) by dietary heat-inactivated potential probiotics. Immune Network, 8(3): 67-74.

Cross, M. L. (2002). Microbes versus microbes: immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens. FEMS Immunology Medical Microbiology, 34(4): 245-253. doi: https://doi.org/ 10.1111/j.1574-695X.2002.tb00632.x

Dadar, M.; Dhama, K.; Vakharia, V. N.; Hoseinifar, S. H.; Karthik, K.; Tiwari, R.; Khandia, R.; Munjal, A.; Salgado-Miranda, C. and Joshi, S. K. (2017). Advances in aquaculture vaccines against fish pathogens: global status and current trends. Reviews in Fisheries Science Aquaculture, 25(3): 184-217. doi: https://doi.org/10.1080/23308249.2016.1261277

Dhar, A. K.; Manna, S. K. and Thomas Allnutt, F. C. (2014). Viral vaccines for farmed finfish. Virusdisease, 25(1): 1-17. doi: https://doi.org/10.1007/s13337-013-0186-4

Díaz-Rosales, P.; Arijo, S.; Chabrillón, M.; Alarcón, F.; Tapia-Paniagua, S.; Martínez-Manzanares, E.; Balebona, M. and Moriñigo, M. (2009). Effects of two closely related probiotics on respiratory burst activity of Senegalese sole (Solea senegalensis, Kaup) phagocytes, and protection against Photobacterium damselae subsp. piscicida. Aquaculture, 293(1-2): 16-21. doi: https://doi.org/10.1016/j.aquaculture.2009.03.050

Díaz-Rosales, P.; Salinas, I.; Rodríguez, A.; Cuesta, A.; Chabrillon, M.; Balebona, M. C.; Morinigo, M. A.; Esteban, M. A. and Meseguer, J. (2006). Gilthead seabream (Sparus aurata L.) innate immune response after dietary administration of heat-inactivated potential probiotics. Fish Shellfish Immunology, 20(4): 482-492. doi: https://doi.org/10.1016/j.fsi.2005.06.007

Essa, M. A.; El-Serafy, S.; El-Ezabi, M. M.; Daboor, S. M.; Esmael, N. A. and Lall, S. P. (2010). Effect of different dietary probiotics on growth, feed utilization and digestive enzymes activities of Nile tilapia, Oreochromis niloticus. Journal of the Arabian Aquaculture Society, 5(2): 143-162.

Esteve-Gassent, M. D.; Fouz, B. and Amaro, C. (2004). Efficacy of a bivalent vaccine against eel diseases caused by Vibrio vulnificus after its administration by four different routes. Fish Shellfish Immunol, 16(2): 93-105. doi: https://doi.org/10.1016/S1050-4648(03)00036-6

FAO. (2020). The State of World Fisheries and Aquaculture 2020: Sustainability in action, Food and Agriculture Organization of the United Nations, Rome, Italy.

Fernandez-Alonso, M.; Rocha, A. and Coll, J. M. (2001). DNA vaccination by immersion and ultrasound to trout viral haemorrhagic septicaemia virus. Vaccine, 19(23-24): 3067-3075. doi: https://doi.org/10.1016/s0264-410x(01)00046-9

Fuller, R. (1989). A review: probiotics in man and animals. J Appl Bacteriol, 66(5): 365-378.

Gatesoupe, J. (2005). Probiotics and prebiotics for fish culture, at the parting of the ways. Aqua Feeds: Formulation Beyond, 2(3): 3-5.

Geall, A. J. and Blagbrough, I. S. (2000). Rapid and sensitive ethidium bromide fluorescence quenching assay of polyamine conjugate–DNA interactions for the analysis of lipoplex formation in gene therapy. Journal of Pharmaceutical and Biomedical Analysis, 22(5): 849-859. doi: https://doi.org/10.1016/S0731-7085(00)00250-8

Geng, X.; Dong, X. H.; Tan, B. P.; Yang, Q. H.; Chi, S. Y.; Liu, H. Y. and Liu, X. Q. (2012). Effects of dietary probiotic on the growth performance, non- specific immunity and disease resistance of cobia, Rachycentron canadum. Aquaculture Nutrition, 18(1): 46-55. doi: https://doi.org/10.1111/j.1365-2095.2011.00875.x

Ghosh, S.; Sinha, A. and Sahu, C. (2007). Effect of probiotic on reproductive performance in female livebearing ornamental fish. Aquaculture Research, 38(5): 518-526. doi: https://doi.org/10.1111/j.1365-2109.2007.01696.x

Gildberg, A.; Mikkelsen, H.; Sandaker, E. and Ringø, E. (1997). Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (Gadus morhua). Hydrobiologia, 352(1): 279-285. doi: https://doi.org/10.1023/A:1003052111938

Grisez, L. and Tan, Z. (2005). Vaccine development for Asian aquaculture. Paper presented at the Diseases in Asian Aquaculture V Fish health section, Proceedings of the Fifth Symposium in Asian Aquaculture.

Hektoen, H.; Berge, J.; Hormazabal, V. and Yndestad, M. (1995). Persistence of antibacterial agents in marine sediments. Aquaculture, 133(3-4): 175-184. doi: https://doi.org/10.1016/0044-8486(94)00310-K

Hølvold, L. B.; Myhr, A. I. and Dalmo, R. A. (2014). Strategies and hurdles using DNA vaccines to fish. Veterinary Research, 45(1): 1-11. doi: https://doi.org/10.1186/1297-9716-45-21

Irianto, A. and Austin, B. (2002). Probiotics in aquaculture. Journal of Fish Diseases, 25(11): 633-642. doi: https://doi.org/10.1046/j.1365-2761.2002.00422.x

Jaafar, R. M.; Al-Jubury, A.; Chettri, J. K.; Dalsgaard, I.; Kania, P. W. and Buchmann, K. (2018). Secondary immune response of rainbow trout following repeated immersion vaccination. J Fish Dis, 41(1): 117-123. doi: https://doi.org/10.1111/jfd.12682

Kitiyodom, S.; Kaewmalun, S.; Nittayasut, N.; Suktham, K.; Surassmo, S.; Namdee, K.; Rodkhum, C.; Pirarat, N. and Yata, T. (2019). The potential of mucoadhesive polymer in enhancing efficacy of direct immersion vaccination against Flavobacterium columnare infection in tilapia. Fish Shellfish Immunol, 86: 635-640. doi: https://doi.org/10.1016/j.fsi.2018.12.005

Kolndadacha, O.; Adikwu, I.; Okaeme, A.; Atiribom, R.; Mohammed, A. and Musa, Y. (2011). The role of probiotics in aquaculture in Nigeria-a review. Continental Journal of Fisheries Aquatic Science, 5(1): 8-15.

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

Lara-Flores, M.; Olvera-Novoa, M. A.; Guzmán-Méndez, B. E. and López-Madrid, W. (2003). Use of the bacteria Streptococcus faecium and Lactobacillus acidophilus, and the yeast Saccharomyces cerevisiae as growth promoters in Nile tilapia (Oreochromis niloticus). Aquaculture, 216(1-4): 193-201. doi: https://doi.org/10.1016/S0044-8486(02)00277-6

Li, P. and Gatlin III, D. M. (2005). Evaluation of the prebiotic GroBiotic®-A and brewers yeast as dietary supplements for sub-adult hybrid striped bass (Morone chrysops× M. saxatilis) challenged in situ with Mycobacterium marinum. Aquaculture, 248(1-4): 197-205. doi: https://doi.org/10.1016/j.aquaculture. 2005. 03.005

Lillehaug, A. (2014). Vaccination strategies and procedures. In "Fish Vaccination", pp. 140-152.

Ma, J.; Bruce, T. J.; Sudheesh, P. S.; Knupp, C.; Loch, T. P.; Faisal, M. and Cain, K. D. (2019). Assessment of cross-protection to heterologous strains of Flavobacterium psychrophilum following vaccination with a live-attenuated coldwater disease immersion vaccine. J Fish Dis, 42(1): 75-84. doi: https://doi.org/10.1111/jfd.12902

Mahida, P.; Lende, S.; Shrivastava, V.; Jha, A. and Yusufzai, S. (2014). Recent Development of Vaccination in Fish. Indian Farmer, 1(4): 243-255.

Maynard, C. L.; Elson, C. O.; Hatton, R. D. and Weaver, C. T. (2012). Reciprocal interactions of the intestinal microbiota and immune system. Nature, 489(7415): 231-241. doi: https://doi.org/10.1038/nature11551

Merrifield, D. L.; Dimitroglou, A.; Foey, A.; Davies, S. J.; Baker, R. T.; Bøgwald, J.; Castex, M. and Ringø, E. (2010). The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture, 302(1-2): 1-18. doi: https://doi.org/10.1016/j.aquaculture.2010.02.007

Minelli, E. B. and Benini, A. (2008). Relationship between number of bacteria and their probiotic effects. Microbial Ecology in Health and Disease, 20(4): 180-183. doi: https://doi.org/10.1080/08910600802408095

Mohapatra, S.; Chakraborty, T.; Kumar, V.; DeBoeck, G. and Mohanta, K. N. (2013). Aquaculture and stress management: a review of probiotic intervention. J Anim Physiol Anim Nutr (Berl), 97(3): 405-430. doi: https://doi.org/10.1111/ j.1439-0396.2012.01301.x

Monaghan, S. J.; Thompson, K. D.; Smith, P. D. and Adams, A. (2016). Potential of DIVA Vaccines for Fish. In "Fish Vaccines" Springer, pp. 143-173.

Moriarty, D. (1998). Control of luminous Vibrio species in penaeid aquaculture ponds. Aquaculture, 164(1-4): 351-358. doi: https://doi.org/10.1016/S0044-8486 (98) 00199-9

Munang'andu, H. M.; Mutoloki, S. and Evensen, Ø. (2014). Non-replicating vaccines. Fish Vaccination: 22-32.

Munilla-Moran, R. and Stark, J. (1990). Metabolism in marine flatfish—VI. Effect of nutritional state on digestion in turbot, Scophthalmus maximus (L.). Comparative Biochemistry Physiology Part B: Comparative Biochemistry, 95(3): 625-634. doi: https://doi.org/10.1016/0305-0491(90)90032-O

Muñoz de la Peña, A. and Espinosa-Mansilla, A. (2009). Analysis of antibiotics in fish samples. Analytical and bioanalytical chemistry, 395(4): 987-1008.

Nayak, S. K. (2010). Probiotics and immunity: a fish perspective. Fish Shellfish Immunol, 29(1): 2-14. doi: https://doi.org/10.1016/j.fsi.2010.02.017

Nikoskelainen, S.; Ouwehand, A. C.; Bylund, G.; Salminen, S. and Lilius, E. M. (2003). Immune enhancement in rainbow trout (Oncorhynchus mykiss) by potential probiotic bacteria (Lactobacillus rhamnosus). Fish Shellfish Immunol, 15(5): 443-452. doi: https://doi.org/10.1016/s1050-4648(03)00023-8

Okocha, R. C.; Olatoye, I. O. and Adedeji, O. B. (2018). Food safety impacts of antimicrobial use and their residues in aquaculture. Public Health Rev, 39(1): 1-22. doi: https://doi.org/10.1186/s40985-018-0099-2

Olayinka, A. S. and Afolabi, O. O. (2013). Evaluation of the effects of Lactobacillus acidophilus on the haematological parameters of Clarias gariepinus. International Journal of Research in Fisheries Aquaculture, 3(2): 38-41.

Panigrahi, A.; Kiron, V.; Kobayashi, T.; Puangkaew, J.; Satoh, S. and Sugita, H. (2004). Immune responses in rainbow trout Oncorhynchus mykiss induced by a potential probiotic bacteria Lactobacillus rhamnosus JCM 1136. Vet Immunol Immunopathol, 102(4): 379-388. doi: https://doi.org/10.1016/j.vetimm. 2004.08. 006

Pardi, N.; Hogan, M. J.; Porter, F. W. and Weissman, D. (2018). mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov, 17(4): 261-279. doi: https://doi.org/10.1038/nrd.2017.243

Pelicano, E. R. L.; Souza, P.; Souza, H.; Figueiredo, D.; Boiago, M.; Carvalho, S. and Bordon, V. (2005). Intestinal mucosa development in broiler chickens fed natural growth promoters. Brazilian Journal of Poultry Science, 7(4): 221-229. doi: https://doi.org/10.1590/S1516-635X2005000400005

Pridgeon, J. W. and Klesius, P. H. (2012). Major bacterial diseases in aquaculture and their vaccine development. Cab Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 7: 1-16. doi: https://doi.org/10.1079/ PAVSNNR20127048

Rawls, J. F.; Mahowald, M. A.; Goodman, A. L.; Trent, C. M. and Gordon, J. I. (2007). In vivo imaging and genetic analysis link bacterial motility and symbiosis in the zebrafish gut. Proc Natl Acad Sci U S A, 104(18): 7622-7627. doi: https://doi.org/10.1073/pnas.0702386104

Rekecki, A.; Dierckens, K.; Laureau, S.; Boon, N.; Bossier, P. and Van Den Broeck, W. (2009). Effect of germ-free rearing environment on gut development of larval sea bass (*Dicentrarchus labrax* L.). Aquaculture, 293(1-2): 8-15. doi: https://doi.org/10.1016/j.aquaculture.2009.04.001

Restifo, N. P.; Ying, H.; Hwang, L. and Leitner, W. W. (2000). The promise of nucleic acid vaccines. Gene Ther, 7(2): 89-92. doi: https://doi.org/10.1038/sj.gt.3301117

Ringø, E.; Myklebust, R.; Mayhew, T. M. and Olsen, R. E. (2007). Bacterial translocation and pathogenesis in the digestive tract of larvae and fry. Aquaculture, 268(1-4): 251-264. doi: https://doi.org/10.1016/j.aquaculture. 2007. 04.047

Robertson, P. A. W.; O'Dowd, C.; Burrells, C.; Williams, P. and Austin, B. (2000). Use of Carnobacterium sp. as a probiotic for Atlantic salmon (Salmo salar L.) and rainbow trout (Oncorhynchus mykiss, Walbaum). Aquaculture, 185(3-4): 235-243. doi: https://doi.org/10.1016/s0044-8486(99)00349-x

Sakai, M. (1999). Current research status of fish immunostimulants. Aquaculture, 172(1-2): 63-92. doi: https://doi.org/10.1016/S0044-8486(98)00436-0

Sakata, T. (1990). Microflora in digestive tract of fish and shell fish. Microbiology in Poeciloterms.

Sampath, K.; James, R. and Ali, K. A. (1998). Effects of copper and zinc on blood parameters and prediction of their recovery in Oreochromis mossambicus (Pisces: Cichlidae). Indian Journal of Fisheries, 45(2): 129-139.

Sapkota, A.; Sapkota, A. R.; Kucharski, M.; Burke, J.; McKenzie, S.; Walker, P. and Lawrence, R. (2008). Aquaculture practices and potential human health risks: current knowledge and future priorities. Environ Int, 34(8): 1215-1226. doi: https://doi.org/10.1016/j.envint.2008.04.009

Shan, X.; Xiao, Z.; Huang, W. and Dou, S. (2008). Effects of photoperiod on growth, mortality and digestive enzymes in miiuy croaker larvae and juveniles. Aquaculture, 281(1-4): 70-76. doi: https://doi.org/10.1016/j.aquaculture. 2008. 05.034

Shoemaker, C. A.; Klesius, P. H.; Evans, J. J. and Arias, C. R. (2009). Use of modified live vaccines in aquaculture. Journal of the World Aquaculture Society, 40(5): 573-585. doi: https://doi.org/10.1111/j.1749-7345.2009.00279.x

Song, Z. F.; Wu, T. X.; Cai, L. S.; Zhang, L. J. and Zheng, X. D. (2006). Effects of dietary supplementation with clostridium butyricum on the growth performance and humoral immune response in Miichthys miiuy. J Zhejiang Univ Sci B, 7(7): 596-602. doi: https://doi.org/10.1631/jzus.2006.B0596

Steenbergen, L.; Sellaro, R.; van Hemert, S.; Bosch, J. A. and Colzato, L. S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. Brain Behav Immun, 48: 258-264. doi: https://doi.org/10.1016/j.bbi.2015.04.003

Suzer, C.; Çoban, D.; Kamaci, H. O.; Saka, Ş.; Firat, K.; Otgucuoğlu, Ö. and Küçüksari, H. (2008). Lactobacillus spp. bacteria as probiotics in gilthead sea bream (Sparus aurata, L.) larvae: effects on growth performance and digestive enzyme activities. Aquaculture, 280(1-4): 140-145. doi: https://doi.org/10.1016/j.aquaculture.2008.04.020

Sveinsdóttir, H.; Steinarsson, A. and Gudmundsdóttir, Á. (2009). Differential protein expression in early Atlantic cod larvae (Gadus morhua) in response to treatment with probiotic bacteria. Comp Biochem Physiol Part D Genomics Proteomics, 4(4): 249-254. doi: https://doi.org/10.1016/j.cbd.2009.06.001

Taoka, Y.; Maeda, H.; Jo, J.-Y.; Kim, S.-M.; Park, S.-I.; Yoshikawa, T. and Sakata, T. (2006). Use of live and dead probiotic cells in tilapia Oreochromis niloticus. Fish Sci, 72(4): 755-766. doi: https://doi.org/10.1111/j.1444-2906.2006.01215.x

Toranzo, A.; Romalde, J.; Magariños, B. and Barja, J. (2009). Present and future of aquaculture vaccines against fish bacterial diseases. Options Mediterraneennes, 86: 155-176.

Triet, T. H.; Tinh, B. T.; Hau, L. V.; Huong, T. V. and Binh, N.-Q. (2019). Development and potential use of an Edwardsiella ictaluri wzz mutant as a live attenuated vaccine against enteric septicemia in Pangasius hypophthalmus (Tra catfish). Fish & Shellfish Immunology, 87: 87-95. doi: https://doi.org/10. 1016/j.fsi.2019.01.005

Ulmer, J. B.; Mason, P. W.; Geall, A. and Mandl, C. W. (2012). RNA-based vaccines. Vaccine, 30(30): 4414-4418. doi: https://doi.org/10.1016/j.vaccine.2012.04.060

Unajak, S.; Pumchan, A.; Roytrakul, S.; Sawatdichaikul, O. and Areechon, N. (2022). Novel Vaccine Development for Fish Culture Based on the Multiepitope Concept. In "Vaccine Design" Humana, New York, NY, pp. 219-240.

Vilchez, M. C.; Santangeli, S.; Maradonna, F.; Gioacchini, G.; Verdenelli, C.; Gallego, V.; Penaranda, D. S.; Tveiten, H.; Perez, L.; Carnevali, O. and Asturiano, J. F. (2015). Effect of the probiotic Lactobacillus rhamnosus on the expression of genes involved in European eel spermatogenesis. Theriogenology, 84(8): 1321-1331. doi: https://doi.org/10.1016/j.theriogenology.2015.07.011

Villamil, L.; Figueras, A.; Planas, M. and Novoa, B. (2010). Pediococcus acidilactici in the culture of turbot (Psetta maxima) larvae: Administration pathways. Aquaculture, 307(1): 83-88. doi: https://doi.org/10.1016/j.aquaculture. 2010. 07.004

Wang, Y.-B. (2007). Effect of probiotics on growth performance and digestive enzyme activity of the shrimp Penaeus vannamei. Aquaculture, 269(1-4): 259-264. doi: https://doi.org/10.1016/j.aquaculture.2007.05.035

Wang, Y.-B.; Li, J.-R. and Lin, J. (2008). Probiotics in aquaculture: challenges and outlook. Aquaculture, 281(1-4): 1-4. doi: https://doi.org/10.1016/j.aquaculture. 2008.06.002

Zhao, Z.; Zhang, C.; Jia, Y. J.; Qiu, D. K.; Lin, Q.; Li, N. Q.; Huang, Z. B.; Fu, X. Z.; Wang, G. X. and Zhu, B. (2019). Immersion vaccination of Mandarin fish Siniperca chuatsi against infectious spleen and kidney necrosis virus with a SWCNTs-based subunit vaccine. Fish Shellfish Immunol, 92: 133-140. doi: https://doi.org/10.1016/j.fsi.2019.06.001

Zuo, Z. H.; Shang, B. J.; Shao, Y. C.; Li, W. Y. and Sun, J. S. (2019). Screening of intestinal probiotics and the effects of feeding probiotics on the growth, immune, digestive enzyme activity and intestinal flora of Litopenaeus vannamei. Fish Shellfish Immunol, 86: 160-168. doi: https://doi.org/10.1016/j.fsi.2018.11.003