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Bacillus as potential probiotics for use in aquaculture: its importance and future perspectives

Mona, Abd El khalek Salem*; Reham, A. Abd El-Wahab and Samar, A. El Gamal***

*Department of Fish Diseases, Animal Health Research Institute (AHRI), Mansoura Branch, Agriculture Research Center, (ARC), Giza POB,12618,Egypt.
**Biochemistry Department, Animal Health Research Institute (AHRI), Mansoura Branch, Agriculture Research Center (ARC), 246 Dokki, Giza 12618, Egypt.

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Abstract

Probiotics are beneficial live microbes when given in the accurate quantities, improve benefits for the host. By incorporating probiotics into feed or water, farmers can stimulate beneficial bacteria, which helps to control pathogens and increase nutrient absorption.
The utilization of probiotics can also increase illness resistance, improve growth performances and improve water quality.
Bacterial infections pose an enormous challenge in fish, thus promoting significant losses in economic for the aquaculture. Standard managements for bacterial pathogens including antibiotics and chemicals that have been extensively used for the purpose of preventing and controlling fish diseases. The efficacy of probiotics can be depend on various factors, including the probiotic strain consumed, the dose of administration of probiotic, and the method of application in aquaculture.
The utilization of Bacillus species in tilapia aquaculture is a prevalent and beneficial practice, principally as supplements in diet or water. A common approach to delivering fish probiotics is through their integration into the meal. Feeding Nile tilapia with *Bacillus pumilus* resulted in significant progress in their overall health, immune defenses, and gut flora. The growth performance of *Oreochromis Niloticus* was greatly enhanced by the administration of a Bacillus strain. The findings indicated that the fish had an increase in weight, were provided with more food, and exhibited a higher specific growth rate. It was found that different species of Bacillus can effectively enhance the host's growth performance, possibly owning to the digestive enzymes generated by the probiotic bacteria.
Bacillus beneficial bacteria enhance gastrointestinal function by increas-

Corresponding author: Mona Abd El khalek Salem, Department of Fish Diseases, Mansoura Branch, Animal Health Research Institute (AHRI) Dokki, Giza Agriculture Research Center (ARC), Nadi El-Seid Street, Dokki P.O., Giza 12618, Egypt.
Email address: salemmona144@yahoo.com

ing the synthesis of digestive enzymes, namely protease, amylase and lipase. Commercial probiotics enhanced blood biochemistry and immunological measures, including white blood cells, phagocytic activity and lysozyme activity.
The inclusion of *Bacillus amyloliquefaciens* in fish feed enhanced the immune response of mucosa in intestine in *Oreochromis Niloticus* by stimulating villous height enhancement mainly in the proximal and middle sections of the intestines.
Bacillus might potentially reduce gut inflammation by decreasing the expression of TLR and can also generate compounds that may activate cytokines by stimulating NF-κB signaling in enterocytes. Bacillus probiotics enhance intestinal health by promoting the growth of villi in both length and depth, thereby improving nutrient uptake. Bacillus subtilis, when used as a dietary supplement, can significantly boost the growth and overall health of tilapia, with optimal results achieved at specific dosage levels.

Introduction

Global fish aquaculture production has increased from fewer than 1 million tons in 1950s, to 82 million tons in 2018. Impact of aquaculture to global fish production raised from 25.7% in the 2000 to reach 46% in 2018 **(Fishery and Statistics, 2023)**.

Therefore, aquaculture production practices must be developed and intensified to meet the growing need for fish-based protein sources. This is faced by several challenges such as diseases, brood stock improvement and water quality management. Therefore, probiotic is an ideal alternative to antibiotics, additional supplements and other alternative ingredients **(Rahayu *et al.,* 2024)**.

Preventive measures in aquaculture include creating and utilizing vaccines, products derived from medicinal plants, broad spectrum immunostimulants and probiotics. Probiotics provide multiple advantages and have been effectively employed in farming various species, such as mollusks, fish and crustaceans.

Probiotics may be used to improve growth, reproduction, gut microbiota, immunity, water quality of culture system and food utilization, also control physiological stress and microbial diseases **(Sharifuzzaman and Austin, 2017, Carnevali *et al.,* 2017)**.

Probiotics are widely utilized to promote economic growth, enhance digestion and prevent the spread of infectious diseases **(Balcázar *et al.,* 2006, Nayak, 2010)**. Probiotics have recognized more attention by preventing pathogen , improving health and increasing the resistance of aquatic animals **(Dimitroglou *et al.,* 2011)**.

Bacterial diseases in fish:

Aquaculture involves the farming of aquatic species such as mollusks, fish, crustaceans and aquatic plants. It has emerged as a main source of animal protein and a vital economic resource for several countries. Increasing of aquaculture practices lead to more incidence of diseases that has become the most important challenge for the industry **(Aly *et al.,* 2023)**.

The occurrence of diseases in fish is one of the most important challenges opposite the aquaculture, which can lead to significant economic damage. Bacterial diseases are highly prevalent and cause severe losses in fish farming. Primary bacterial threats to cultured fish species comprise *Aeromonas hydrophila*, *Flavobacterium columnare*, *Photobacterium damselae*, *Streptococcus agalactiae* and *Edwardsiella tarda* **(Austin *et al.,* 2012)**.

Pathogenic bacteria could be transmitted by many routes as water, feed, workers and equipment. Practices like high stocking densities, poor. water quality management, and antibiotic .misuse in aquaculture may promote the proliferation and transmission of harmful .bacteria **(Austin *et al.,* 2007, Stephens, 2016)**.

<u>Traditional methods of treating bacterial fish diseases: their limits and potential environmental hazards:</u>

Bacterial pathogens are a main problem in aquaculture industry, producing massive economic losses. Traditional approaches to treatment bacterial infections, including the application of antibiotics. and chemical .treatments, have been widely used in disease control **(Aly *et al.,***

2023).

Antibiotics have been widely employed in aquaculture to prevent and treat bacterial infections in fish. However, their excessive use has contributed to the emergence and spread of antibiotic-resistant bacteria, creating substantial risks for both human and animal health **(Cabello *et al.,* 2013)** .

Probiotics, which are beneficial bacteria have been recommended as an alternate treatment for bacterial pathogens in fish and can enhance immunity against fish pathogens and also improve the gut health of fish **(El-Saadony *et al.,* 2021, Wang *et al.,* 2021)**.

In aquaculture using of probiotics, either directly or with other materials such as microalgae, fermented products, plant protein diets, vitamins, has been shown to improve the growth, health of aquatic animals and offer considerable benefits to the sustainability of the aquaculture industry **(Rahayu *et al.,* 2024)**.

Research has demonstrated that probiotics can effectively prevent and manage bacterial diseases in fish **(Nayak, 2010)**. However, their efficacy varies depending on factors such as probiotic strain, dosage, fish species and duration of treatment **(El-Saadony *et al.,* 2021)**.

<u>Probiotic strains</u>

Probiotics have important effect on utilization of feed, growth rate and immune response of finfish and shrimp. Probiotics demonstrate the capacity to prevent pathogenic colonization while improving aquatic conditions and water quality through the biodegradation of organic materials and residual feed **(Shefat, 2018)**.

Briefly, Probiotics are dead or live microorganisms or part of them or their extract which converses health benefits to the host, enhance disease resistance, feed conversion ratio and growth performance when administered at suitable dose **(Hoseinifar *et al.,* 2016).**

Recently, a wide range of bacteria have described as potential probiotics in fish farming sectors. *Lactobacillus* sp. and *Bacillus* sp. gain special attention owing to their high antagonistic activities, availability and production of extracellular enzyme **(Banerjee and Ray, 2017).**

During early phases of fish cultivation, live feed supplementation is essential but poses risks of pathogen introduction to the closed aquaculture system. This challenge can be mitigated through probiotic application, which serves dual functions: (1) degrading complex compounds that larval fish cannot metabolize, and (2) enhancing growth parameters through stimulation of key digestive enzymes including lipase, amylase and protease **(Mohammadi *et al.,* 2022)**.

<u>Administration technique of probiotics in aquaculture:</u>

<u>1- Administration in diet</u>

Application of probiotic in pelleted feed is considered an important and commonly appropriate administration technique. Probiotics are commonly administered through feed supplementation, typically incorporated into pellets as spore-forming microbial preparations **(Assefa and Abunna, 2018)**.

At this time, most scientists exploring the properties of using probiotics as dietary supplementation in aquaculture. But, slight consideration has been given to the possible positive effects of their administrating in water **(Jahangiri and Esteban, 2018)**.

A fish's diet can be supplemented with probiotics in one of two ways: either by encapsulating them within the feed or by supplying them to the water **(Shija *et al.,* 2023)**.

<u>2- Administration in water</u>

Probiotics administration in water is the most suitable method for all ages of fish, beginning from larvae to the marketable size **(Lauzon *et al.,* 2014)**.

Water supplementation with probiotics demonstrated major improvements in growth performance, feed conversion efficiency, and overall production yield when compared to control groups **(Mohamed *et al.,* 2024)**.

Numerous agents may affect the probiotics efficacy supplemented through water, such as source of probiotics, salinity, water temperature, inoculation period, dose and age of fish **(Jahangiri and Esteban, 2018)**.

<u>Forms of probiotics</u>

<u>1-**Microencapsulation:**</u>

Micro-encapsulation, nano-encapsulation, and co-encapsulation techniques enhance the viability and persistence of probiotics in fish diets, promoting sustained maintenance in the intestines, facilitating interaction with the immune system, and providing considerable beneficial effects for aquatic life **(Puri *et al.,* 2023)**.

Probiotic strains are encapsulated in a colloidal matrix at high density by alginate, carboxymethylcellulose, chitosan and pectin to chemically and physically protect the microbes **(Martínez Cruz *et al.,* 2012)**.

The strain *Shewanella putrefaciens* was immobilized within calcium alginate microcapsules, demonstrating enhanced survivability during intestinal transit in .Solea senegalensis. The alginate. encapsulation matrix. provided effective protection against both enzymatic. degradation and low. pH conditions in the digestive.tract **(Kumar *et al.,* 2016)**.

<u>Mechanism of actions</u>

Probiotics mode of actions were not fully understood. Probiotics can entering the digestive tract of host and expel out harmful pathogens, the beneficial microorganism manufacture inhibitory substance, compete with pathogens for energy, nutrient, binding site and inhibit pathogen activities **(Irianto and Austin, 2002, Balcázar *et al.,* 2006)**.

Adding *Lactobacillus fermentum, L lactis and L. plantarum.* to rainbow trout diet decreased *Vibrio anguillarum Aeromonas salmonicida* and *Aeromonas hydrophila* in the intestinal tract. Probiotic are able to prevent growth of pathogen on the gut surface **(Balcázar *et al.,* 2006)** .

A- Competition for binding sites:

Probiotics can provide beneficial effects by competitively avoiding harmful bacteria, contributing nutrients and enzymes to fish absorption and exhibiting antiviral properties, and enhancing the immunity of fish **(Ringø, 2020)**.

B- Synthesis of antimicrobial compounds

Probiotic bacteria yield many substances such as hydrogen peroxide and bacteriocins, which possess antibacterial properties against pathogenic microorganisms. In addition, they generate organic acids that decrease the pH of the intestine, so inhibiting the proliferation of different pathogens and the emergence of specific Lactobacillus sp. **(Shefat, 2018)**. Probiotics represent a promising method to suppress the harmful effects of infections and manage diseases in aquatic animals in aquaculture. Probiotics have the capacity to produce chemical molecules that can kill or stop the growth of bacteria **(C De *et al.,* 2014)**.

C- Immune system stimulation: Certain probiotic bacteria have a direct impact on immunity stimulation by promoting the generation of immunoglobulin, activating macrophages, T-cell activation and interferon production **(Shefat, 2018)**.

<u>The impact of probiotics on fish</u>

Extended probiotic therapy improved the rate of growth, health status, resistance to disease, and promoted the immune responses of the host. When four probiotic strains were given to *Penaeus monodon*, they effectively suppressed the growth of harmful microorganisms, improved the survival rate and enhanced resistance to diseases caused by *Vibrio parahaemolyticus* and *Vibrio harveyi* **(Swain *et al.,* 2009)**.

Litopenaeus vannamei larvae treated with Bacillus probiotics showed enhanced growth rate, higher shrimp survival, elevated levels of favorable bacteria and improved water parameters **(Nimrat *et al.,* 2012)**.

Probiotics could be used as a water additive for improving blood biochemistry, immunity, expression of growth-related gene in *Oreochromis Niloticus*, water quality and hence fish productivity **(El-Kady *et al.,* 2022)**

Supplementation of *Bacillus licheniformis* SB3086 to the *Oreochromis mossambicus* diet improved the growth efficiency, immunity, intestinal histology and challenge infections **(Yaqub *et al.,* 2021)**.

Supplementation of probiotic microbial feed additives into fish diets enhances fish health by regulating the existing intestinal microbiota and introducing beneficial microbiota from external sources, which can combat pathogens, improve nutrient uptake, and promote growth and survival **(Puri *et al.,* 2023)**.

<u>(1) Enhancing of water quality</u> through the reduction of ammonium and other pollutants, as well as the utilization of bioremediation methods **(Aly *et al.,* 2016, Thurlow *et al.,* 2019)**.

The inclusion of probiotics leads to an apparent improvements in water quality, demonstrated by a major decrease in total and harmful ammonia levels in groups supplemented with probiotic **(El-Kady *et al.,* 2022)**.

Supplementation of *Bacillus licheniformis* into fish rearing water resulted in a decrease in hazardous compounds such as TAN and NH3, while simultaneously enhancing the breakdown of carbohydrates and protein in residual feed. Bacillus species facilitate the nitrification and denitrification process, which involves the conversion of nitrogenous byproducts into mineralized wastes. This process ultimately leads to an enhancement in water parameters **(Zheng *et al.,* 2011)**. Probiotics as *Bacillus subtilis*, a gram-positive bacterium, exhibits a remarkable ability to transform organic materials into carbon dioxide. Gram-negative bacteria possess the capability to transform organic substances into bacterial biomass, unlike other types of bacteria **(Zorriehzahra *et al.,* 2016)**.

Addition of probiotics to water affect positively on the ecology and the number of microbes in aquatic environments. Consequently, improve the immune system and the quality of water **(Wang *et al.,* 2017)**.

(2) <u>Increasing growth rate</u> according to **(Lotfy, 2015)** addition of probiotic increase growth rate and could be linked to the useful function that good bacteria perform in improving the digestive system. In addition to feed consumption, probiotics significantly improved tilapia growth performance **(El-Kady *et al.,* 2022)**.

Enhancing the balance of intestinal microorganisms can improve appetite, create vitamins, break down indigestible foods, and eliminate pathogenic microorganisms in the gastrointestinal tract **(Irianto and Austin, 2002, Lotfy, 2015)** and so the feed absorption will be improved **(Tovar *et al.,* 2002)**.

(3) <u>Enhancing the immune system's condition</u>

Administration of commercial probiotic formulations significantly improved hematological and serum biochemical parameters in Nile tilapia (*Oreochromis niloticus*) **(Wang *et al.,* 2008, Sutthi et al., 2018)**.

(Sutthi *et al.,* 2018) investigated how the performance and biochemical parameters of Nile tilapia were affected by the introduction of probiotics, specifically bacteria (*Bacillus spp*., including *B. subtilis*, *B. licheniformis* and *B. megaterium*) and yeast (*Saccharomyces cerevisiae*) in water.

Probiotics improve gut health and immune responses, resulting in increased productivity and survival rates in fish. Sustained-release effects from feed enhance probiotic accumulation in intestines, facilitating beneficial interactions with the immune system **(Pardosi *et al.,* 2024)**.

Fish grown water supplemented with probiotic had a synergistic impact that considerably reduced AST and ALT levels. Additionally, *Cyprinus carpio* receiving probiotics showed increased catalase, lysozyme, and superoxide dismutase activity, according to **(Gupta *et al.,* 2016)**.

<u>(4)- Improving the composition of microbiota in gut</u> **(Tan *et al.,* 2019)**.

<u>(5)- Boosting growth, nutritional metabolism, and immunological genes</u> **(Yi *et al.,* 2019)**.

A limited number of research publications have examined the effects of probiotics on gene expression in the spleen, liver and intestine of *Oreochromis niloticus*. There is a deficiency of research concerning the genes IGF1, GHR, TNF-α, and IL-1B. The present study demonstrated a major increase in the expression of the specified genes in the probiotic-treated groups **(El-Kady *et al.,* 2022).**

The administration of the probiotic Prime TM in the water for Yellow Perch significantly enhanced the expression of growth-regulated genes relative to the other control group **(Eissa, 2014)**.

Tumor necrosis factor-alpha (TNF-α) purposes as a biomarker for immunoregulation. It is essential in the immunological response of fish to bacterial infections. *Bacillus subtilis* increases the expression of intestinal cytokines, including TNF-α and IL-1B in hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus* **(He *et al.,* 2013)**.

<u>Popular Bacillus probiotics widely applied to Nile tilapia.</u>

The application of *Bacillus* probiotic in *Oreochromis niloticus* aquaculture is a common and effective practice, mostly as additives in feed or water. The greatest common technique for giving probiotics to fish is through feeding **(Galagarza *et al.,* 2018, Opiyo *et al.,* 2019, Cavalcante *et al.,* 2020)**.

Nile tilapia feed efficiency, growth rates, and immunological responses were all much improved by the addition of *Bacillus coagulans*. It improved gut health, increased serum immune markers, and upregulated immune-related genes, suggesting its potential as a prophylactic substitute to antibiotics in aquaculture (**Omar *et al.,* 2024**).

The Microbiota of the Intestinal Tract and Bacillus Probiotics

The gut microbiota of Nile tilapia mostly consists of the phyla Fusobacteria, Proteobacteria, and Bacteroidetes **(Egerton *et al.,* 2018, Yu *et al.,* 2019)** .

The gut microbiome regulates the structure, function and immunological responses of the intestinal epithelium, hence affecting digestion and preserving internal equilibrium **(Tarnecki *et al.,* 2017)**.

Incorporating *Bacillus subtilis* into the diet of Nile tilapia significantly enhances their immune system **(El-Ezabi *et al.,* 2011)**. Inclusion of Bacillus strains in tilapia feed formulations demonstrates three key benefits: increased beneficial gut microbiota, optimized feed conversion through improved digestion, and strengthened disease resistance via microbial competition in the intestinal tract **(Jahangiri and Esteban, 2018)**.

Dietary consumption of *Bacillus cereus* led to an increase in useful bacteria, including *Bifidobacterium,* Lactobacillus, and *Enterococcus* species, while lessening the prevalence of pathogenic microorganisms as *Vibrio* and *Aeromonas species* **(Wang *et al.,* 2017).**

The administration of Bacillus probiotics, mainly *B. cereus* and *B. velezensis*, has demonstrated an enhancement in the chain fatty acids production, This confers significant beneficial effects on gastrointestinal health **(Chen *et al.,* 2020)**.

Bacillus pumilus significantly improved the growth, immune response and gastrointestinal microbiota of Nile tilapia. The growth rate of Nile tilapia was significantly improved by the introduction of a probiotic Bacillus strain. The results shown that the fish demonstrated an increase in weight, consumed a larger quantity of food and experienced a heightened specified growth rate **(Hassaan *et al.,* 2021)**.

The consumption of *Bacillus coagulans* significantly raises the levels of immunoglobulin E (IgE), serum protein concentration and lysozyme activity in *Oreochromis Niloticus* **(Patel *et al.,* 2015)**.

The Theory of Action of Bacillus Probiotics in *Oreochromis niloticus*

The research indicated that certain Bacillus species markedly improve the growth performance of the host. This effect is probably attributable to the release of digestive enzymes by the probiotic bacteria **(Ringø *et al.,* 2016)**. Bacillus probiotics (*B. licheniformis and B. subtilis*) improve feed digestibility by enhancing the synthesis of digestive enzymes, such as amylase, lipase and protease **(Yang *et al.,* 2021)**.

Bacillus clausii synthesizes many inhibitory chemicals, including proteases, which could be helpful in inhibiting pathogen adherence to the mucosal epithelium **(C De *et al.,* 2014)**.

Numerous Bacillus species (*B. cereus, B. subtilis,* and *B. amyloliquefaciens*) may compete for adhesion sites with pathogens, thereby defense Nile tilapia from infections **(Tabassum *et al.,* 2021)**.

Bacillus subtilis is a popular probiotic used in aquaculture. It helps protect in many ways, including through competitive exclusion, immune stimulation, and synergistic and antagonistic effects **(Nayak, 2021)**. The incorporation of *B. subtilis* and *B. cereus* into the tilapia diet augmented the density of intestinal cells and microvilli, hence improving disease resistance via modifications in the microbiota of the intestine **(Xia *et al.,* 2020)**.

One of the indirect processes that is referred to as "exploitation competition" is the restriction of the access of micro-organisms for nutrients. According to **(Verschuere *et al.,* 2000)**, the ability of any microbial population to compete with other organisms in the same environment for available resources and nutrients is essential to the population's ability to survive. Exploitation competition was discovered through the examination of natural infection in pilot or large-scale experiments that the mechanisms of action of the probiotic strain WFLU12 are utilized when it inhibits fish disease (such as streptococcosis) and influences fish growth. These mechanisms include antimicrobial secretion and immune parameter stimulation **(Nguyen *et al.,* 2017)**.

Interference competition is a direct method that inhibits the colonization and growth of other bacteria by precolonizing and synthesizing antimicrobial compounds **(Knipe *et al.,* 2021)**. A crucial criterion for identifying promising probiotic candidates is their in vitro antagonistic efficacy against aquatic pathogens. In the digestive tract, certain bacteria produce antimicrobial peptides known as bacteriocins, which may facilitate the settlement of potentially harmful pathogens or inhibit the propagation of specific bacteria **(Austin, 2002)**.

Probiotic bacillus and fish immunology

Nile tilapia is a significant fish species owing to its advanced innate immunity **(Saurabh and Sahoo, 2008).** This system comprises physical barriers, humoral elements, plus cellular components **(Gómez and Balcázar, 2008)**. The Nile tilapia has physical defenses, including its multi-layered skin that act as a protective barrier against pathogens. The mucus coating serves to trap foreign elements that could get inside the organism **(Mohammadi *et al.,* 2020b)**.

The Nile tilapia's humoral components of immune system consist of proteins and other compounds synthesized by the fish to counteract possible pathogenic viruses or bacteria **(Pirarat *et al.,* 2011)**. In addition, its immune system has cellular components consist of many cell types that are tasked with identifying and combating any foreign particles that may invade the organism **(Van Doan *et al.,* 2019, Mokhtar *et al.,* 2023)**.

Probiotics improve host health by enhancing immune responses. The preliminary investigation on fish focused mainly on the developing and disease-preventing properties of probiotic bacteria **(Picchietti *et al.,* 2009, Salinas *et al.,* 2008)**.

Many immune cells in the Nile tilapia's gut are affected by probiotic Bacillus species like *B. amyloliquefaciens* and *B. subtilis*. Pattern recognition receptors (PRRs) recognize molecular patterns specific to infectious agents, enabling hosts to detect harmful pathogens **(Al-Deriny *et al.,* 2020)**.

Pathogenic and nonpathogenic microorganisms possess molecular patterns recognizable by the host. Nonpathogenic bacteria can be influenced by PAMPs, like Bacillus probiotics (*B. fragilis subtilis, B. subtilis*) **(Akhter *et al.,* 2015)**.

Probiotic bacteria, especially *Bacillus subtilis*, can interact with cells of the immune system. Some of the cells that make up the immune system include monocytes, natural killer cells, granulocytes, lymphocytes, and macrophages **(Ashraf and Shah, 2014)**.

Toll-like receptors (TLRs) have been extensively investigated pattern recognition receptors capable of detecting microbial constituents, including lipopolysaccharides (LPS), peptidoglycans, nucleic acids and flagella **(Hoseinifar *et al.,* 2018)**.

Multiple research **(Azimirad *et al.,* 2016, Modanloo *et al.,* 2017, Plaza-Diaz *et al.,* 2019)** indicate that probiotics may decrease gut inflammation by suppressing TLR expression. Adding *Bacillus amyloliquefaciens* to the diet of Nile tilapia fish enhanced the immune system and gut function by raising the villous, particularly in the middle and near the end of the intestines **(Reda and Selim, 2015)**.

There are a number of different Bacillus probiotics, including *B. amyloliquefaciens* and Lactobacillus sp. are supposed to promote level of immunoglobulin and leukocyte counts. These impacts have been noted with the administration of probiotics either singularly or in conjunction **(Ridha and Azad, 2012, Reda and Selim, 2015)**.

1- **Bacillus probiotics control phagocytosis.**

The research demonstrated that *Bacillus subtilis* probiotics enhanced the phagocytic index of *Oreochromis Niloticus*, under conditions with an intensive rearing density. This suggests that Bacillus probiotics may alleviate stress associated with difficult raising techniques **(Telli *et al.,* 2014)**. The addition of *Bacillus coagulans* to the diets of Nile tilapia increased their phagocytic activity **(Fath El-Bab *et al.,* 2022)**.

Phagocytosis were enhanced in tilapia fish feed diets supplemented with *Bacillus safensis NPUST*1 alongside *Aeromonas hydrophila* **(Wu *et al.,* 2021)**.

The incorporation of *Enterococcus faecium* in tilapia diets led to an improved immune response when challenged with *A. hydrophila*, indicating increased immunological tolerance **(Tachibana *et al.,* 2020)**.

2-Bacillus probiotics modulate lysozyme activity.

An increase in either the number of phagocytes releasing the protein or the number of cells generating it could help explain the higher lysozyme levels in tilapia **(Saurabh and Sahoo, 2008)**. The addition of Bacillus probiotics, specifically *B. velezensis H3.1*, can influence lysozyme activity. The impact is determined by factors like the type, amount, and timing of the administered probiotics **(Butt and Volkoff, 2019)**.

Bacillus amyloliquefaciens increased lysozyme concentrations in fish challenged with the *Streptococcus agalactiae* **(Saputra *et al.,* 2016).** Numerous plants, animals, and microbes contain this multifunctional enzyme. Chitin, a crucial component of fungal cell walls, is its main target **(Saurabh and Sahoo, 2008)**.

Commercial probiotics improve specific blood biochemical and immunological parameters, such as phagocytic activity, lysozyme activity and white blood cells (WBCs) **(El-Kady *et al.,* 2022)**.

Bacillus probiotics significantly increased lysozyme activity in Nile tilapia, with the highest levels observed in fish fed the supplemented diet. This enhancement in immune parameters indicates the beneficial effects of probiotics on fish health and immunity **(Ridha *et al.,* 2023)**.

Including *B. amyloliquefaciens* and *B. coagulans,* Bacillus probiotics raise lysozyme levels, so vital for fish defense against infections. In Nile tilapia, this modulation is linked to improved growth performance and disease resistance. Probiotics included in the diet cause activation of immune-related genes, hence improving lysozyme production and general immunological activity **(Omar *et al.,* 2024)**.

3-The immunomodulatory effects of Bacillus probiotics include regulation of respiratory burst mechanisms:

Bacillus probiotic supplementation enhances the respiratory burst activity in Nile tilapia (*Oreochromis niloticus*), potentiating this innate immune response against viral pathogens and various environmental challenges **(Sookchaiyaporn *et al.,* 2020).**

Furthermore, by producing molecules called antioxidants, it can alter the respiratory burst process. These compounds reduce oxidative stress by preventing cells from producing reactive oxygen species (ROS) **(Saputra *et al.,* 2016)**. After 51 days of dietary supplementation with *Bacillus subtilis* strain NZ86, Nile tilapia (*Oreochromis niloticus*) exhibited significantly enhanced respiratory burst activity relative to control groups receiving no probiotic treatment **(Galagarza *et al.,* 2018)**.

Including *Bacillus amyloliquefaciens* AV5 in the Nile tilapia diet significantly increased serum superoxide dismutase and reactive oxygen species levels, indicating a potential regulatory effect on the respiratory burst process and overall immune response **(Shija *et al.,* 2024)**.

4-Probiotics from Bacillus Improve Complement Function

Researchers have recorded that complement activity increased *Oreochromis niloticus* that fed diet supplemented with Bacillus probiotics by promoting the production of proteins called complement factors. The elimination of foreign substances is greatly facilitated by these proteins **(Mohammadi *et al.,* 2020a)**. An increase in complement-mediated hemolysis was seen in Nile tilapia when the following bacteria were added to their diet: *Bacillus amyloliquefaciens, B. pumilus, B. licheniformis, Pediococcus pentosaceus* and *B. subtilis* **(Han *et al.,* 2015, Sookchaiyaporn *et al.,* 2020)**.

5-Bacillus probiotics boost gene expression that affects the immune system.

Various studies have looked into examining the immunomodulatory effects of Bacillus probiotics on immune gene regulation in tilapia (*Oreochromis spp*.) **(Van Doan *et al.,* 2018)**. Administering *Bacillus* probiotics, particularly *B. amyloliquefaciens, B. licheniformis*, and *B. subtilis* to Nile tilapia fish resulted in amarked elevation in pro-inflammatory signaling molecules, including key chemokines and cytokines **(Ismail *et al.,* 2021)**. When Nile tilapia exposed to different infections, genes relevant to the immune system are activated. The synthesis of cytokines, including IL-1, IFN, IL-8, IL-10, and TNF which can either promote or inhibit inflammation, is part of this process **(Foysal *et al.,* 2020)**.

6-Bacillus probiotics control the activity of antioxidant enzymes

Antioxidant enzymes like glutathione peroxidase, catalase, and superoxide dismutase are present in Nile tilapia and other fish species **(Dawood *et al.,* 2020, Won *et al.,* 2020).** A functional metric for evaluating the immune responses of Nile tilapia is the antioxidant enzyme activities **(Wang *et al.,* 2017).**

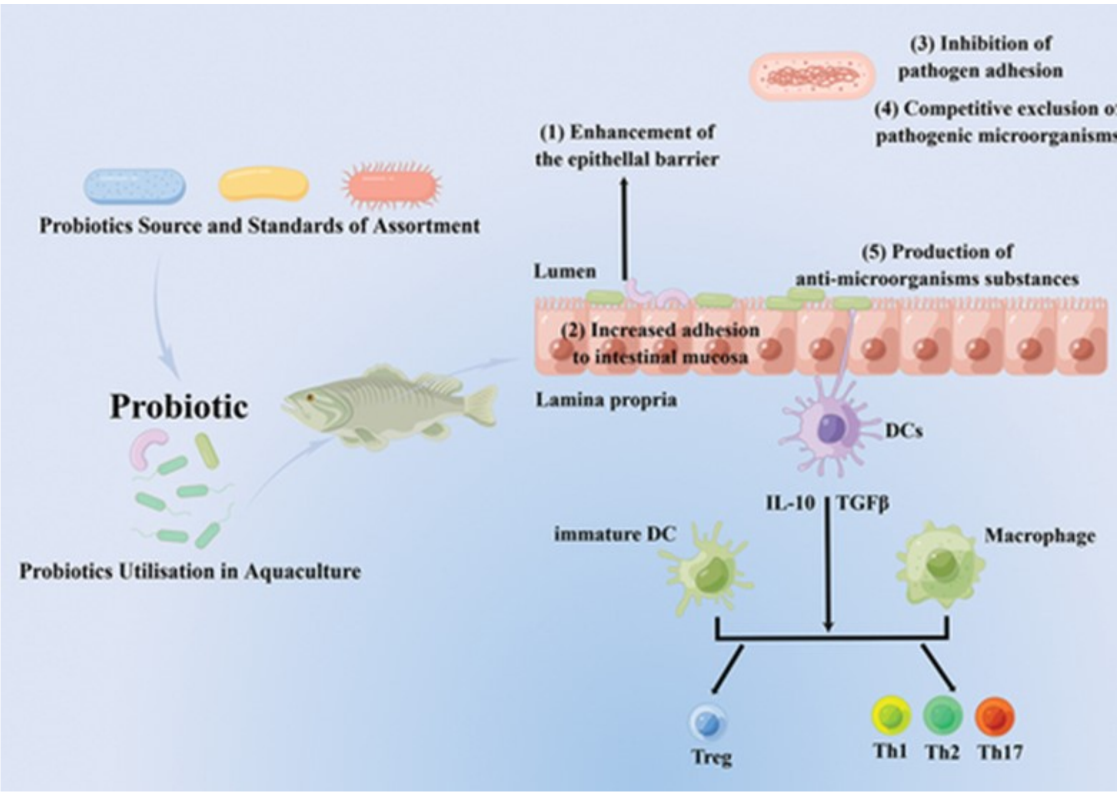
Antioxidant enzyme activity in Nile tilapia fish farms can be modulated with the help of Bacillus probiotics, such as *B. subtilis*. Antioxidant enzymes found in fish help in the breakdown of toxic substances and the prevention of free radical damage **(Tang *et al.,* 2020)**. Supplementation with Bacillus probiotics demonstrates significant .antioxidant effects modifying oxidative damage that causes several pathological conditions **(Esteban *et al.,* 2014)**.

Supplementation of Bacillus probiotic in Nile tilapia revealed significantly increased activities of antioxidant enzyme specifically superoxide dismutase and catalase while reducing tissue malondialdehyde levels, indicating improved antioxidant capacity and reduced oxidative stress after exposure to stressors like heat shock **(Elbahnaswy *et al.,* 2024)**.

Bacillus supplementation not only improves SOD but also enhances overall immune responses, including increased serum total protein and other immune markers **(Shija *et al.,* 2024, Omar *et al.,* 2024)**.

Supplementation of *Bacillus paralicheniformis* SO1 in tilapia diets revealed increased their superoxide dismutase (SOD) activity ***(*Avella *et al.,* 2010)**. Intestinal and liver levels of GPx, SOD, and CAT are all higher in fish, which may indicate that they are better able to fight off illnesses. Several fish species, including tilapia, have been found to have elevated levels of antioxidant enzymes when given fish probiotics, including Bacillus spp **(Esteban *et al.,* 2014, Zhou *et al.,* 2010)**.

Bacillus probiotics help moderate stress-induced oxidative damage, as demonstrated by lower serum biochemical stress indicators and improved antioxidant enzyme activities **(El-Son *et al.,* 2022, Elbahnaswy *et al.,* 2024).**



Mechanism of probiotic in fish **(Amenyogbe, 2023)**

Conclusion

Probiotics have gained interest as they can enhance the growth performance, survival, immune system of aquatic species and general health of fish and shellfish. It is important to develop alternative eco-friendly ways for disease prevention and treatment. Despite promising results from earlier studies, local species have only been examined in a small number of aquaculture trials. To completely comprehend the ways in which probiotics work and the impact they have on the environment, however, additional research is required. For example, additional study on the probable ecological hazards of probiotics in aquaculture is necessary and our current understanding of how probiotics affect aquatic microbial communities is limited. More research is required to improve their use in aquaculture. Probiotics have significant potential to improve aquaculture sustainability, but more research is required to determine advantages and hazards of them. Lacks in research can be addressed to boost probiotics' potential for sustainable and eco-friendly aquaculture techniques.

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